LAB ANALYST

CONTINUING EDUCATION
PROFESSIONAL DEVELOPMENT COURSE

Technical Learning College

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Internet Link to Assignment…
http://www.abctlc.com/downloads/PDF/LabAnalystAss.pdf

State Approval Listing Link, check to see if your State accepts or has pre-approved this course. Not all States are listed. Not all courses are listed. Do not solely trust our list for it may be outdated. It is your sole responsibility to ensure this course is accepted for credit. No refunds.

Hyperlink to the Glossary and Appendix
http://www.abctlc.com/downloads/PDF/WTGlossary.pdf

State Approval Listing URL…
http://www.abctlc.com/downloads/PDF/CEU%20State%20Approvals.pdf

You can obtain a printed version from TLC for an additional $189.95 plus shipping charges.

All downloads are electronically tracked and monitored for security purposes.

Contributing Editors
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Jack White, Environmental, Health, Safety expert, City of Phoenix. Art Credits.

Some States and many employers require the final exam to be proctored.

Do not solely depend on TLC's Approval list for it may be outdated.

Most of our students prefer to do the assignment in Word and e-mail or fax the assignment back to us. We also teach this course in a conventional hands-on class. Call us and schedule a class today.

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

Technical Learning College's Scope and Function

Welcome to the Program,

Technical Learning College (TLC) offers affordable continuing education for today's working professionals who need to maintain licenses or certifications. TLC holds several different governmental agency approvals for granting of continuing education credit.

TLC's delivery method of continuing education can include traditional types of classroom lectures and distance-based courses or independent study. TLC's distance based or independent study courses are offered in a print - based distance educational format. We will beat any other training competitor's price for the same CEU material or classroom training.

Our courses are designed to be flexible and for you to finish the material at your convenience. Students can also receive course materials through the mail. The CEU course or e-manual will contain all your lessons, activities and instruction to obtain the assignments. All of TLC's CEU courses allow students to submit assignments using e-mail or fax, or by postal mail. (See the course description for more information.)

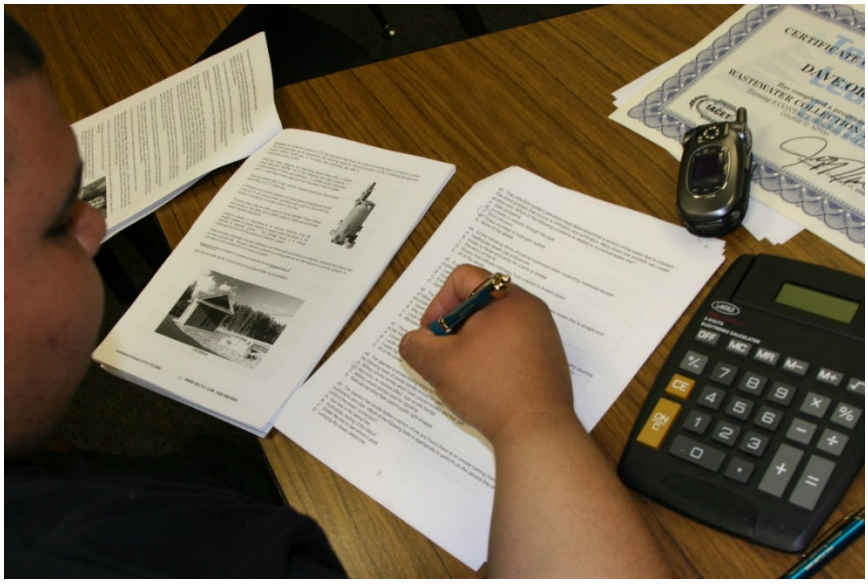
Students have direct contact with their instructor—primarily by e-mail or telephone. TLC's CEU courses may use such technologies as the World Wide Web, e-mail, CD-ROMs, videotapes and hard copies. (See the course description.) Make sure you have access to the necessary equipment before enrolling; i.e., printer, Microsoft Word and/or Adobe Acrobat Reader. Some courses may require proctored closed-book exams, depending upon your state or employer requirements.

Flexible Learning
At TLC, there are no scheduled online sessions or passwords you need contend with, nor are you required to participate in learning teams or groups designed for the "typical" younger campus based student. You will work at your own pace, completing assignments in time frames that work best for you. TLC's method of flexible individualized instruction is designed to provide each student the guidance and support needed for successful course completion.

Course Structure
TLC's online courses combine the best of online delivery and traditional university textbooks. You can easily find the course syllabus, course content, assignments, and the post-exam (Assignment). This student-friendly course design allows you the most flexibility in choosing when and where you will study.

Classroom of One
TLC offers you the best of both worlds. You learn on your own terms, on your own time, but you are never on your own. Once enrolled, you will be assigned a personal Student Service Representative who works with you on an individualized basis throughout your program of study. Course specific faculty members (S.M.E.) are assigned at the beginning of each course providing the academic support you need to successfully complete each course. Please call or email us for assistance.

Satisfaction Guaranteed
We have many years of experience, dealing with thousands of students. We assure you, our customer satisfaction is second to none. This is one reason we have taught more than 20,000 students.



We welcome you to do the electronic version of the assignment and submit the answer key and registration to us either by fax or e-mail.

If you need this assignment graded and a certificate of completion within a 48-hour turn around, prepare to pay an additional rush charge of $50.

Contact Numbers

Fax (928) 468-0675
Email Info@tlch2o.com
Telephone (866) 557-1746

Lab Analyst CEU Training Course
CEU Course Introduction

A water laboratory analyst is a person who performs collection of samples and assists in carrying out laboratory chemical and biological tests on water, wastewater, and industrial wastes. The analyst also carries out field investigations to determine and minimize various water or wastewater problems. This distance learning CEU training course will examine various aspects of conventional water/wastewater laboratory analyst in understanding sampling rules, methods and procedures and examination of common waterborne pathogens. This course was designed to provide continuing education credit to water and/ or wastewater treatment operators.

Course Purpose
The main purpose of this course is to provide continuing education in understanding various water related laboratory procedures utilized in determining various water quality related testing and waterborne pathogen determination.

Target Audience
The target audience for this course is primarily for operators who work inside a water laboratory but includes water treatment operators, and wastewater operators. Also included are people interested in working in a water treatment/wastewater treatment or distribution facility and/or wishing to maintain CEUs for a certification license or to learn how to perform their job safely and effectively, and/or to meet education needs for promotion. There are no prerequisites, and no other materials are needed for this course.

Instructions for Written Assignments
The *Lab Analyst* distance learning course uses a multiple-choice style answer key. You can find the answer key in the front section of the assignment.

Feedback Mechanism (Examination Procedures)
Each student will receive a feedback form as part of his or her study packet. You will be able to find this form in the front of the course assignment or lesson.

Security and Integrity
All students are required to do their own work. All lesson sheets and final exams are not returned to the student to discourage sharing of answers. Any fraud or deceit and the student will forfeit all fees and the appropriate agency will be notified. A random test generator will be implemented to protect the integrity of the assignment.

Grading Criteria
TLC will offer the student either pass/fail or a standard letter grading assignment. If TLC is not notified, you will only receive a pass/fail notice. In order to pass your final assignment, you are required to obtain a minimum score of 70% on your assignment.

Required Texts
The ***Lab Analyst*** course comes complete with a short summary of the EPA's Rules and Regulations and related drinking water standards. If you need more information or a complete set of Rules, you can download them off the EPA's web page, www.epa.gov or contact your local state environmental agency. You may need to contact a laboratory or state agency for certain sampling information.

Recordkeeping and Reporting Practices
TLC will keep all student records for a minimum of seven years. It is the student's responsibility to give the completion certificate to the appropriate agencies. TLC will not release any records to any party, except to the student.

ADA Compliance
TLC will make reasonable accommodations for persons with documented disabilities. Students should notify TLC and their instructors of any special needs. Course content may vary from this outline to meet the needs of this particular group. There is an option course assignment available, please contact an Instructor for further assistance.

Mission Statement
Our only product is educational service. Our goal is to provide you with the best education service possible. TLC will attempt to make your learning experience an enjoyable opportunity.

Student Verification
The student shall submit a driver's license for signature verification and track their time worked on the assignment. The student shall sign an affidavit verifying they have not cheated and worked alone on the assignment. All student attendance is tracked on the student attendance database.

Feedback Mechanism (Examination Procedures)
A feedback form is included in the front of each study packet.

Environmental Terms, Abbreviations, and Acronyms
TLC provides a glossary in the rear of this manual that defines, in non-technical language, commonly used environmental terms appearing in publications and materials, as well as abbreviations and acronyms used throughout the EPA and other governmental agencies.

Record Keeping and Reporting Practices
TLC keeps all student records for a minimum of five years. It is the student's responsibility to give the completion certificate to the appropriate agencies.

Educational Mission

The educational mission of TLC is:
To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

To provide TLC student's opportunities to apply and understand the theory and skills needed for operator certification,

To provide opportunities for TLC students to learn and practice environmental educational skills with members of the community for the purpose of sharing diverse perspectives and experience,

To provide a forum in which students can exchange experiences and ideas related to environmental education,

To provide a forum for the collection and dissemination of current information related to environmental education, and to maintain an environment that nurtures academic and personal growth.

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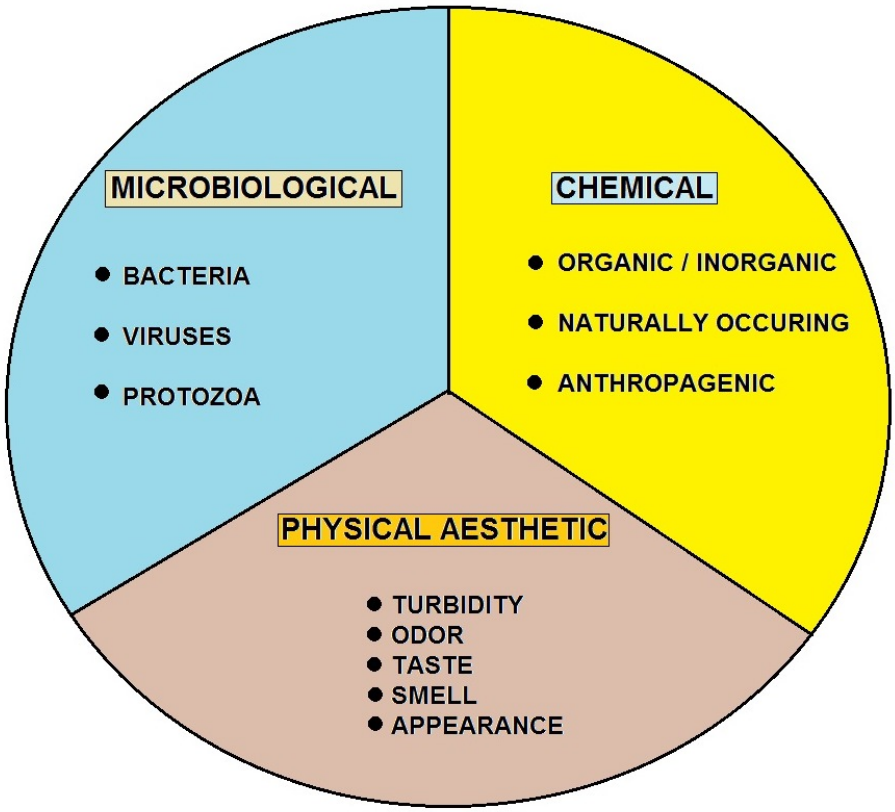
Hyperlink to the Glossary and Appendix

http://www.abctlc.com/downloads/PDF/WTGlossary.pdf



WATER QUALITY BROKEN DOWN INTO 3 BROAD CATEGORIES

Common Water Treatment Acronyms

AA - Activated alumina
AC - Activated carbon
ASR - Annual Status Report
As(III) - Trivalent arsenic, common inorganic form in water is arsenite, H_3AsO_3
As(V) - Pentavalent arsenic, common inorganic form in water is arsenate, H_2AsO_4
BDAT - Best demonstrated available technology
BTEX - Benzene, toluene, ethylbenzene, and xylene
CCA - Chromated copper arsenate
CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act
CERCLIS 3 - CERCLA Information System
CLU-IN - EPA's CLeanUp INformation system
CRAO- Compliance and Regulatory Affairs Office
CWS - Community Water System
cy - Cubic yard
DDT - Dichloro-diphenyl-trichloroethane
DI - De-ionized
DOC - Dissolved organic carbon
DoD - Department of Defense
DOE - Department of Energy
EDTA - Ethylenediaminetetraacetic acid
EPA - U.S. Environmental Protection Agency
EPT - Extraction Procedure Toxicity Test
FRTR - Federal Remediation Technologies Roundtable
ft - feet
gpd - gallons per day
gpm - gallons per minute
HTMR - High temperature metals recovery
MCL - Maximum Contaminant Level (enforceable drinking water standard)
MF - Microfiltration
MHO - Metallurgie-Hoboken-Overpelt
mgd - million gallons per day
mg/kg - milligrams per kilogram
mg/L - milligrams per Liter
NF - Nanofiltration
NPL - National Priorities List
OCLC - Online Computer Library Center
ORD - EPA Office of Research and Development
OU - Operable Unit
PAH - Polycyclic aromatic hydrocarbons
PCB - Polychlorinated biphenyls
P.L. – Public Laws
POTW - Publicly owned treatment works
PRB - Permeable reactive barrier
RCRA - Resource Conservation and Recovery Act
Redox - Reduction/oxidation
RO - Reverse osmosis
ROD - Record of Decision
SDWA - Safe Drinking Water Act

SMZ - Surfactant modified zeolite
SNAP - Superfund NPL Assessment Program
S/S - Solidification/Stabilization
SVOC - Semi-volatile organic compounds
TCLP - Toxicity Characteristic Leaching Procedure
TNT - 2,3,6-trinitrotoluene
TWA - Total Waste Analysis
UF - Ultrafiltration
VOC - Volatile organic compounds
WET - Waste Extraction Test
ZVI - Zero valent iron

Hyperlink to the Glossary and Appendix

http://www.abctlc.com/downloads/PDF/WTGlossary.pdf

Common Water Quality Terms

Community Water System (CWS). A public water system that serves at least 15 service connections used by year-round residents of the area served by the system or regularly serves at least 25 year-round residents.

Class V Underground Injection Control (UIC). Rule A rule under development covering wells not included in Class I, II, III or IV in which nonhazardous fluids are injected into or above underground sources of drinking water.

Contamination Source Inventory. The process of identifying and inventorying contaminant sources within delineated source water protection areas through recording existing data, describing sources within the source water protection area, targeting likely sources for further investigation, collecting and interpreting new information on existing or potential sources through surveys, and verifying accuracy and reliability of the information gathered.

Cryptosporidium. A protozoan associated with the disease cryptosporidiosis in humans. The disease can be transmitted through ingestion of drinking water, person-to-person contact, or other exposure routes. Cryptosporidiosis may cause acute diarrhea, abdominal pain, vomiting, and fever that last 1-2 weeks in healthy adults, but may be chronic or fatal in immuno-compromised people.

Drinking Water State Revolving Fund (DWSRF). Under section 1452 of the SDWA, the EPA awards capitalization grants to states to develop drinking water revolving loan funds to help finance drinking water system infrastructure improvements, source water protection, to enhance operations and management of drinking water systems, and other activities to encourage public water system compliance and protection of public health.

Exposure. Contact between a person and a chemical. Exposures are calculated as the amount of chemical available for absorption by a person.

Giardia lamblia. A protozoan, which can survive in water for 1 to 3 months, associated with the disease giardiasis. Ingestion of this protozoan in contaminated drinking water, exposure from person-to-person contact, and other exposure routes may cause giardiasis. The symptoms of this gastrointestinal disease may persist for weeks or months and include diarrhea, fatigue, and cramps.

Ground Water Disinfection Rule (GWDR). Under section 107 of the SDWA Amendments of 1996, the statute reads, ". . . the Administrator shall also promulgate national primary drinking water regulations requiring disinfection as a treatment technique for all public water systems, including surface water systems, and as necessary, ground water systems."

Maximum Contaminant Level (MCL). In the SDWA, an MCL is defined as "*the maximum permissible level of a contaminant in water which is delivered to any user of a public water system*." MCLs are enforceable standards.

Maximum Contaminant Level Goal (MCLG). The maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health effect of persons would occur, and which allows for an adequate margin of safety. MCLGs are non-enforceable public health goals.

Nepheloletric Turbidity Units (NTU). A unit of measure used to describe the turbidity of water. Turbidity is the cloudiness in water.

Nitrates. Inorganic compounds that can enter water supplies from fertilizer runoff and sanitary wastewater discharges. Nitrates in drinking water are associated with methemoglobanemia, or blue baby syndrome, which results from interferences in the blood's ability to carry oxygen.

Non-Community Water System (NCWS). A public water system that is not a community water system. There are two types of NCWSs: transient and non-transient.

Organics. Chemical molecules contain carbon and other elements such as hydrogen. Organic contaminants of concern to drinking water include chlorohydrocarbons, pesticides, and others.

Phase I Contaminants. The Phase I Rule became effective on January 9, 1989. This rule, also called the Volatile Organic Chemical Rule, or VOC Rule, set water quality standards for 8 VOCs and required all community and Non-Transient, Non-Community water systems to monitor for, and if necessary, treat their supplies for these chemicals. The 8 VOCs regulated under this rule are: Benzene, Carbon Tetrachloride, para-dichlorobenzene, trichloroethylene, vinyl chloride, 1,1,2-trichlorethane, 1,1-dichloroethylene, and 1,2-dichlorothane.

Per capita. Per person; generally used in expressions of water use, gallons per capita per day (gpcd).

Point-of-Use Water Treatment. Refers to devices used in the home or office on a specific tap to provide additional drinking water treatment.

Point-of-Entry Water Treatment. Refers to devices used in the home where water pipes enter to provide additional treatment of drinking water used throughout the home.

Primacy State – A State that has the responsibility for ensuring a law is implemented, and has the authority to enforce the law and related regulations. This State has adopted rules at least as stringent as federal regulations and has been granted primary enforcement responsibility.

Radionuclides. Elements that undergo a process of natural decay. As radionuclides decay, they emit radiation in the form of alpha or beta particles and gamma photons. Radiation can cause adverse health effects, such as cancer, so limits are placed on radionuclide concentrations in drinking water.

Risk. The potential for harm to people exposed to chemicals. In order for there to be risk, there must be hazard and there must be exposure.

SDWA - The Safe Drinking Water Act. The Safe Drinking Water Act was first passed in 1974 and established the basic requirements under which the nation's public water supplies were regulated. The US Environmental Protection Agency (EPA) is responsible for setting the national drinking water regulations, while individual states are responsible for ensuring that public water systems under their jurisdiction are complying with the regulations. The SDWA was amended in 1986 and again in 1996.

Significant Potential Source of Contamination. A facility or activity that stores, uses, or produces chemicals or elements, and that has the potential to release contaminants identified in a state program (contaminants with MCLs plus any others a state considers a health threat)

within a source water protection area in an amount which could contribute significantly to the concentration of the contaminants in the source waters of the public water supply.

Sole Source Aquifer (SSA) Designation. The surface area above a sole source aquifer and its recharge area.

Source Water Protection Area (SWPA). The area delineated by the state for a PWS or including numerous PWSs, whether the source is ground water or surface water or both, as part of the state SWAP approved by the EPA under section 1453 of the SDWA.

Sub-watershed. A topographic boundary that is the perimeter of the catchment area of a tributary of a stream.

State Source Water Petition Program. A state program implemented in accordance with the statutory language at section 1454 of the SDWA to establish local voluntary incentive-based partnerships for SWP and remediation.

State Management Plan (SMP) Program. A state management plan under FIFRA required by the EPA to allow states (i.e. states, tribes and U.S. territories) the flexibility to design and implement approaches to manage the use of certain pesticides to protect ground water.

Surface Water Treatment Rule (SWTR). The rule specifies maximum contaminant level goals for *Giardia lamblia*, viruses and *Legionella,* and promulgated filtration and disinfection requirements for public water systems using surface water sources, or by ground water sources under the direct influence of surface water. The regulations also specify water quality, treatment, and watershed protection criteria under which filtration may be avoided.

Susceptibility Analysis. An analysis to determine, with a clear understanding of where the significant potential sources of contamination are located, the susceptibility of the public water systems in the source water protection area to contamination from these sources. This analysis will assist the state in determining which potential sources of contamination are "significant."

To the Extent Practical. States must inventory sources of contamination to the extent they have the technology and resources to complete an inventory for a Source Water Protection Area delineated as described in the guidance. All information sources may be used, particularly previous Federal and state inventories of sources.

Transient/Non-Transient, Non-Community Water Systems (T/NT, NCWS). Water systems that are non-community systems: transient systems serve 25 non-resident persons per day for 6 months or less per year. Transient non-community systems typically are restaurants, hotels, large stores, etc. Non-transient systems regularly serve at least 25 of the same non-resident persons per day for more than 6 months per year. These systems typically are schools, offices, churches, factories, etc.

Treatment Technique. A specific treatment method required by the EPA to be used to control the level of a contaminant in drinking water. In specific cases where the EPA has determined it is not technically or economically feasible to establish an MCL, the EPA can instead specify a treatment technique. A treatment technique is an enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

Total Coliform. Bacteria that are used as indicators of fecal contaminants in drinking water.

Toxicity. The property of a chemical to harm people who come into contact with it.

Underground Injection Control (UIC) Program. The program is designed to prevent underground injection which endangers drinking water sources. The program applies to injection well owners and operators on Federal facilities, Native American lands, and on all U.S. land and territories.

Watershed. A topographic boundary area that is the perimeter of the catchment area of a stream.

Watershed Approach. A watershed approach is a coordinating framework for environmental management that focuses public and private sector efforts to address the highest priority problems within hydrologically-defined geographic areas, taking into consideration both ground and surface water flow.

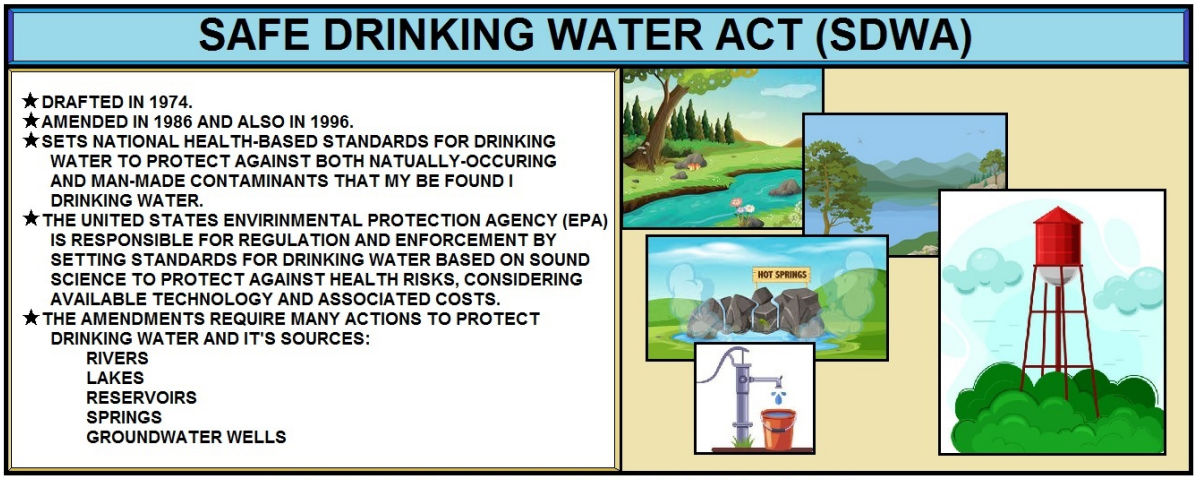
Watershed Area. A topographic area that is within a line drawn connecting the highest points uphill of a drinking water intake, from which overland flow drains to the intake.

Wellhead Protection Area (WHPA). The surface and subsurface area surrounding a well or well field, supplying a PWS, through which contaminants are reasonably likely to move toward and reach such water well or well field.

Hyperlink to the Glossary and Appendix

http://www.abctlc.com/downloads/PDF/WTGlossary.pdf

Preface



SAFE DRINKING WATER ACT FACTS

Technical Learning College

Safe Drinking Water Act of 1974 Introduction

(Public Law 93-523) as amended by:

- The Safe Drinking Water Act Amendments of 1986
- National Primary Drinking Water Regulations, 40 CFR 141
- National Interim Primary Drinking Water Regulations Implementation, 40 CFR142
- National Secondary Drinking Water Regulations, 40 CFR 143

This is the primary Federal legislation protecting drinking water supplied by public water systems (those serving more than 25 people). The Environmental Protection Agency (**EPA**) is the lead agency and is mandated to set standards for drinking water. The EPA establishes national standards of which the states are responsible for enforcing.

The act provides for the establishment of primary regulations for the protection of the public health and secondary regulations relating to the taste, odor, and appearance of drinking water. Primary drinking water regulations, by definition, include either a maximum contaminant level (**MCL**) or, when a MCL is not economically or technologically feasible, a prescribed treatment technique which would prevent adverse health effects to humans.

An MCL is the permissible level of a contaminant in water that is delivered to any user of a public water system. Primary and secondary drinking water regulations are stated in 40 CFR 141 and 143, respectively. As amended in 1986, the EPA is required to set maximum contaminant levels for 83 contaminants deemed harmful to humans (with specific deadlines). It also has authority over groundwater. Water agencies are required to monitor water to ensure it meets standards.

National Drinking Water Regulations

The Act instructs the EPA on how to select contaminants for regulation and specifies how the EPA must establish national primary drinking water regulations once a contaminant has been selected (Section 1412). As of late 1996, the EPA had promulgated 84 drinking water regulations.

Contaminant Selection
Public law 104-182 establishes a new process for the EPA to select contaminants for regulatory consideration based on occurrence, health effects, and meaningful opportunity for health risk reduction. By February 1998 and every 5 years thereafter, the EPA must publish a list of contaminants that may warrant regulation. Every 5 years thereafter, the EPA must determine whether or not to regulate at least 5 of the listed contaminants.

The Act directs the EPA to evaluate contaminants that present the greatest health concern and to regulate contaminants that occur at concentration levels and frequencies of public health concern. The law also includes a schedule for the EPA to complete regulations for disinfectants and disinfection byproducts (**D/DBPs**) and ***Cryptosporidium*** (a waterborne pathogen).

Standard Setting
Developing national drinking water regulations is a two-part process. For each contaminant that the EPA has determined merits regulation, the EPA must set a non-enforceable maximum contaminant level goal (**MCLG**) at a level at which no known or anticipated adverse health effects occur, and which allows an adequate margin of safety.

The EPA must then set an enforceable standard, a maximum contaminant level (**MCL**), as close to the MCLG as is "***feasible***" using the best technology, treatment techniques, or other means available (taking costs into consideration).

Standards are generally based on technologies that are affordable for large communities; however, under P.L. 104-182, each regulation establishing an MCL must list any technologies, treatment techniques, or other means that comply with the MCL and that are affordable for three categories of small public water systems.

The 1996 Amendments authorize the EPA to set a standard at other than the feasible level if the feasible level would lead to an increase in health risks by increasing the concentration of other contaminants or by interfering with the treatment processes used to comply with other SDWA regulations. In such cases, the standard or treatment techniques must minimize the overall health risk.

Also, when proposing a regulation, the EPA must now publish a determination as to whether or not the benefits of the standard justify the costs. If the EPA determines that the benefits do not justify the costs, the EPA may, with certain exceptions, promulgate a standard that maximizes health risk reduction benefits at a cost that is justified by the benefits.

More on these concerns in the Water Quality Section of the course.

Topic 1 - Water Quality Section

Section Focus: You will learn the basics of the EPA's Safe Water Drinking Act and the reasons why we need to ensure the water means federal standards. At the end of this section, you will be able to describe EPA's Primary and Secondary standards. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: EPA identifies contaminants to regulate in drinking water to protect public health. The Agency sets regulatory limits for the amounts of certain contaminants in water provided by public water systems. These contaminant standards are required by the Safe Drinking Water Act (SDWA). Drinking water standards may apply differently based on type and size of public water systems.

FACTOR	TYPE	SOURCE(S)	PROBLEM
FECAL COLIFORM BACTERIA	BIOLOGICAL	HUMAN SEWAGE; LIVESTOCK WASTE	POSSIBLE PRESENCE OF PATHOGENIC (DISEASE-CAUSING) ORGANISMS
DISSOLVED OXYGEN (DO)	CHEMICAL	AIR; AQUATIC PLANTS	LOW LEVELS CAN KILL AQUATIC ORGANISMS
NITROGEN AND PHOSPHORUS	CHEMICAL	FERTILIZERS AND DETERGENTS FROM LAWNS AND RUNOFF	EXCESSIVE ALGAE GROWTH CAN LEAD TO LOW DO
ZINC, ARSENIC, LEAD, MERCURY, CADMIUM, NICKEL	CHEMICAL	LANDFILLS; INDUSTRIAL DISCHARGES; RUNOFF	GENETIC MUTATIONS OR DEATH IN FISH & WILDLIFE (HUMAN HEALTH THREATS AS WELL)
SALT	CHEMICAL	SALTWATER INTRUSION (IF NEAR OCEAN)	KILLS FRESHWATER SPECIES OF PLANTS AND ANIMALS
MUD, SAND, OTHER SOLID PARTICLES (TURBIDITY)	PHYSICAL	EROSION AND RUNOFF FROM DEVELOPMENT; AGRICULTURE	REDUCES PHOTOSYNTHESIS IN AQUATIC VEGETATION; INTERFERES WITH RESPIRATION IN AQUATIC ANIMALS

IMPORTANT WATER QUALITY CONCERNS

Common Water Quality Units of Measurement

mg/l = Milligrams per liter. One milligram per liter equals one packet of artificial sweetener sprinkled into 250 gallons of iced tea.

µg/l = Micrograms per liter. One microgram per liter is equal to one packet of artificial sweetener sprinkled into an Olympic-size swimming pool.

NTU = Nephelometric Turbidity Units. A measurement on the cloudiness of the water.

pCi/l = Picocuries per liter. A measure of radioactivity.

Acronyms

Maximum Contaminant Level (MCL) - The highest level of a contaminant that is allowed in drinking water.

Maximum Contaminant Level Goal (MCLG) - The level of a contaminant in drinking water below which there is no known or expected risk to health.

Treatment Technique (TT) - A required process intended to reduce the level of a contaminant in drinking water.

Action Level (AL) - The concentration of a contaminant that, if exceeded, triggers treatment or other requirements which a water system must follow.

Federal Water Drinking Water Quality Regulations Timeline

National Interim Primary Drinking Water Regulations (NIPDWR) Promulgated 1975-1981 Contained 7 contaminants, Targeted: Trihalomethanes, Arsenic, and Radionuclides Established 22 drinking water standards.

Phase 1 Standards Promulgated 1987 Contained 8 contaminants, Targeted: VOCs.

Phase 2 Standards Promulgated 1991 Contained 36 contaminants, Targeted: VOCs, SOCs, and IOCs.

Phase 5 Standards Promulgated 1992 Contained 23 contaminants, Targeted: VOCs, SOCs, and IOCs.

Surface Water Treatment Rule (SWTR) Promulgated 1989 Contained 5 contaminants, Targeted: Microbiological and Turbidity.

Stage 1 Disinfectant/Disinfection By-product (D/DBP) Rule Promulgated 1998 Contained 14 contaminants, Targeted: DBPs and precursors.

Interim Enhanced Surface Water Treatment Rule (IESWTR) Promulgated 1998 Contained 2 contaminants, Targeted: Microbiological and Turbidity.

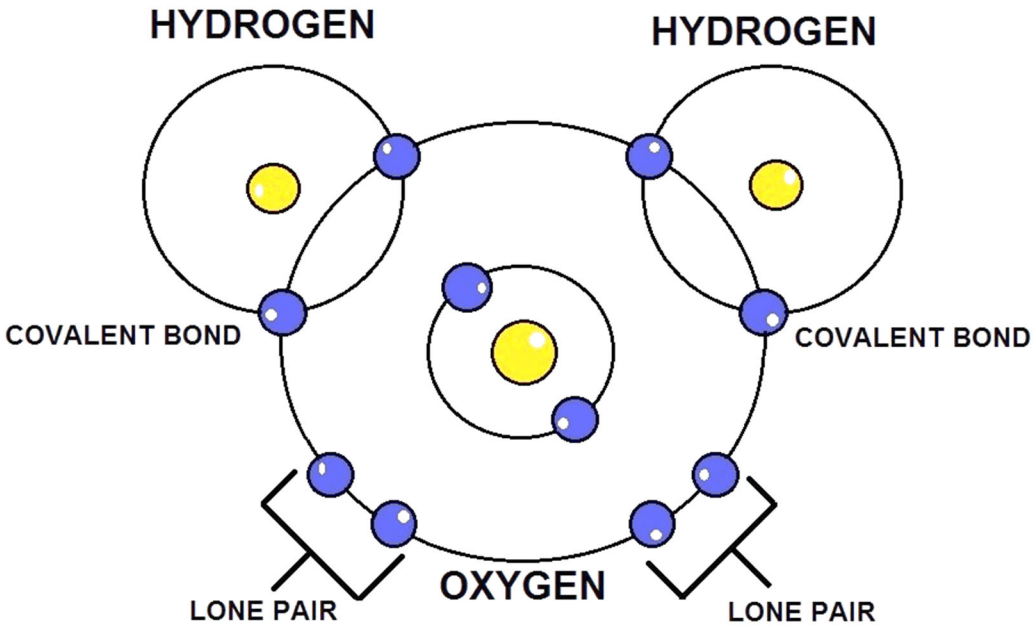
Radionuclide Rule Promulgated 2000 Contained 4 contaminants, Targeted: Radionuclides.

Arsenic Rule Promulgated 2001 Contained 1 contaminant, Targeted: Arsenic.

Filter Backwash Recycling Rule Promulgated 2001 Contained 2 contaminants, Targeted: Microbiological and Turbidity.

What is Water?

Water is the chemical substance with chemical formula H_2O: one molecule of water has two hydrogen atoms covalently bonded to a single oxygen atom. Water is a tasteless, odorless liquid at ambient temperature and pressure, and appears colorless in small quantities, although it has its own intrinsic very light blue hue. Ice also appears colorless, and water vapor is essentially invisible as a gas.

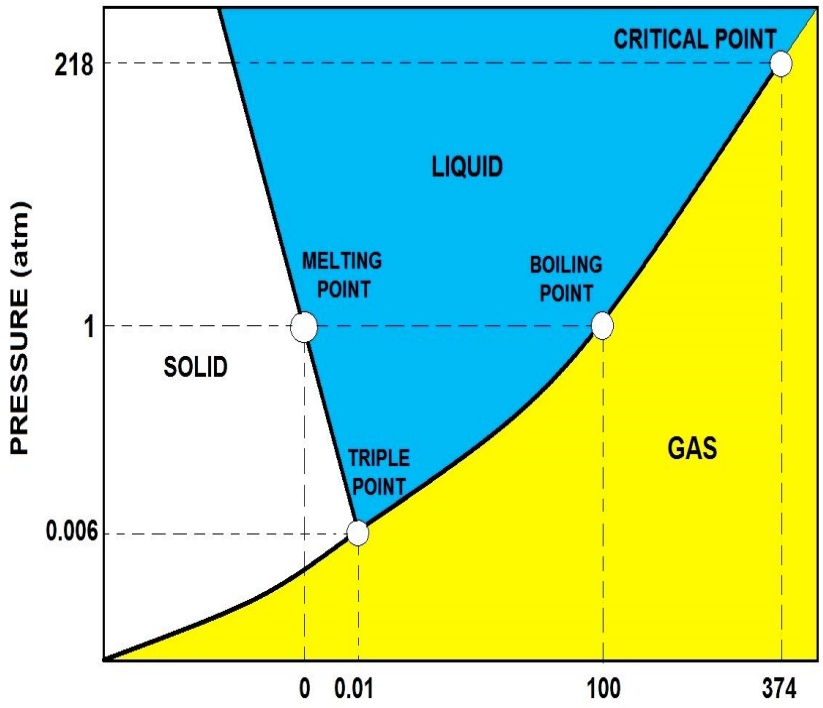


WATER MOLECULE DIAGRAM

Water is primarily a liquid under standard conditions on earth, to other analogous hydrides of the oxygen family in the periodic table, which are gases, such as hydrogen sulfide. The elements surrounding oxygen in the periodic table, nitrogen, fluorine, phosphorus, sulfur and chlorine, all combine with hydrogen to produce gases under standard conditions. The reason that water forms a liquid is that oxygen is more electronegative than all of these elements with the exception of fluorine.

Oxygen attracts electrons much more strongly than hydrogen, resulting in a net positive charge on the hydrogen atoms, and a net negative charge on the oxygen atom. The presence of a charge on each of these atoms gives each water molecule a net dipole moment.

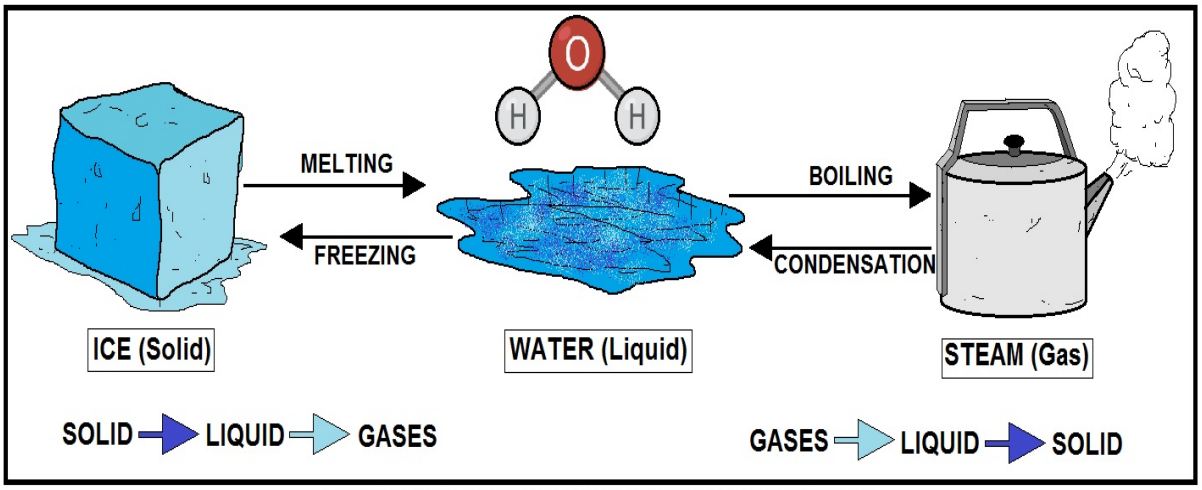
Electrical attraction between water molecules due to this dipole pulls individual molecules closer together, making it more difficult to separate the molecules and therefore raising the boiling point.



TEMPERATURE (^{O}C) WATER PHASE DIAGRAM

Boiling Phase

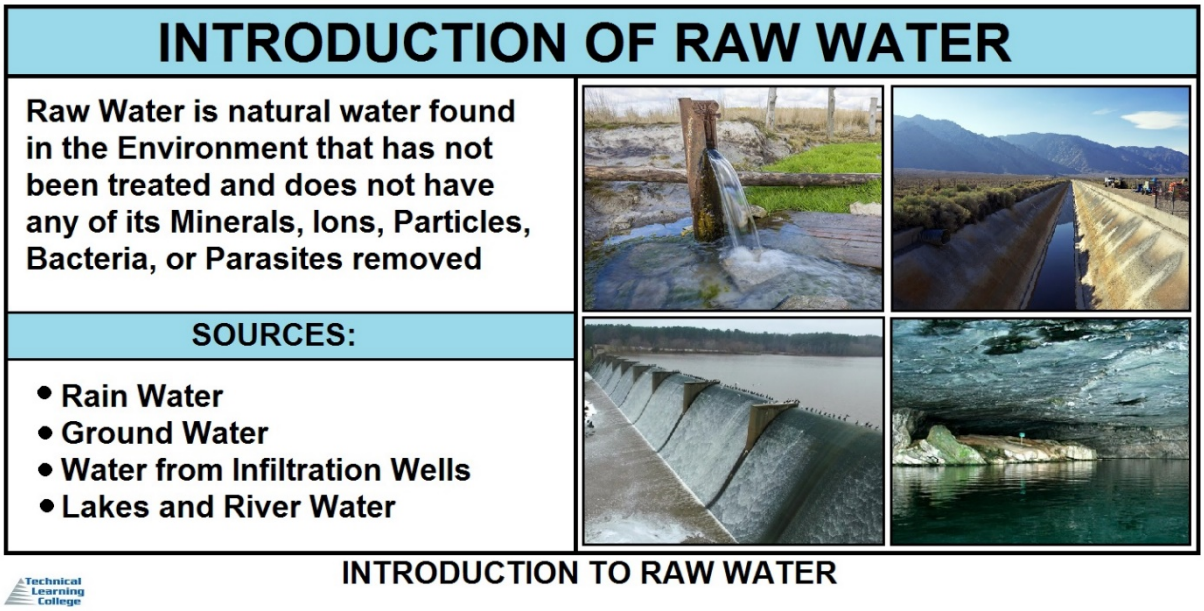
Once liquid water is heated to 212^{o}F (100^{o}C) it takes a significant amount of energy to change the "phase" of water from a liquid state to a gas state. That is one reason it's easier to heat a pot of water to boiling rather than to evaporate all of it.



PHYSICAL CHARACTERISTICS OF WATER

Surface (Raw) Water Introduction

We will go into greater detail on these concerns in the Water Analysis section.



INTRODUCTION TO RAW WATER

Because raw water (surface water) is never pure of pollution, we need to properly treat it. Most of the earth's water sources obtain their water supplies through precipitation (rain). During precipitation, water passes over (runoff) and through the ground (infiltration), acquiring a wide variety of dissolved or suspended impurities that intensely alters its usefulness. Water has unique physical, chemical and biological properties.

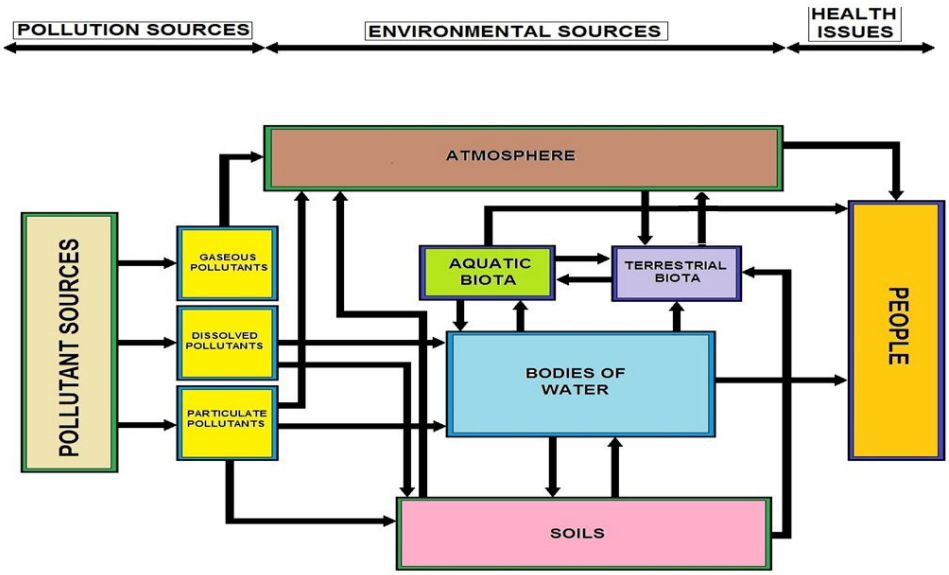
These characteristics have a direct influence on the most effective types of treatment methods and/or chemicals. The improvement of water quality and formation of policy measures (administrative and engineering) revolves around these characteristics.

It is important to remember that raw water will normally contains varying amounts of dissolved minerals including calcium, magnesium, sodium, chlorides, sulfates and bicarbonates, depending on its source.

It is also not uncommon to find traces of iron, manganese, copper, aluminum, nitrates, insecticides and herbicides.

Currently, we also need to deal with contaminants of emerging concern including Pharmaceuticals and Personal Care Products. EPA defines emerging contaminants as: An emerging contaminant (EC) is a chemical or material characterized by a perceived, potential, or real threat to human health or the environment or by a lack of published health standards.

The maximum allowable amounts of all these substances are strictly limited by the regulations (MCLS). These are usually referred to as contaminants and/or pollutants.



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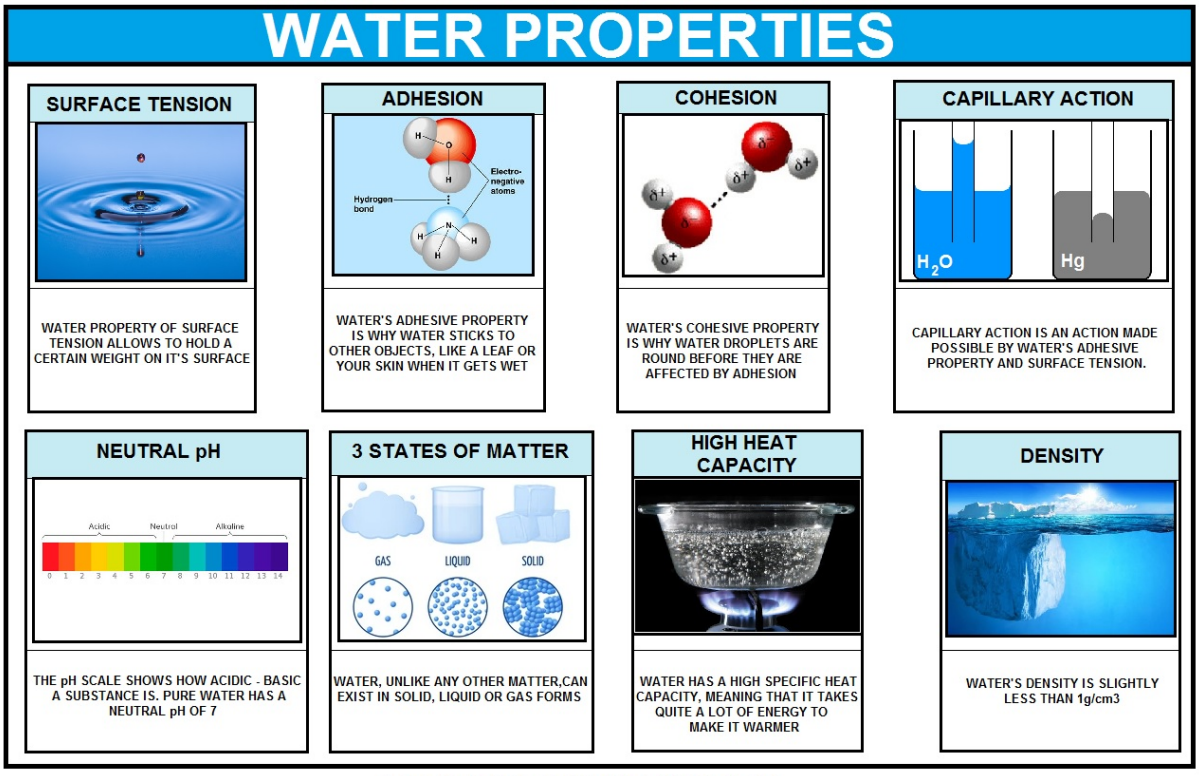
WATER QUALITY INDICATORS



PROPERTIES OF WATER

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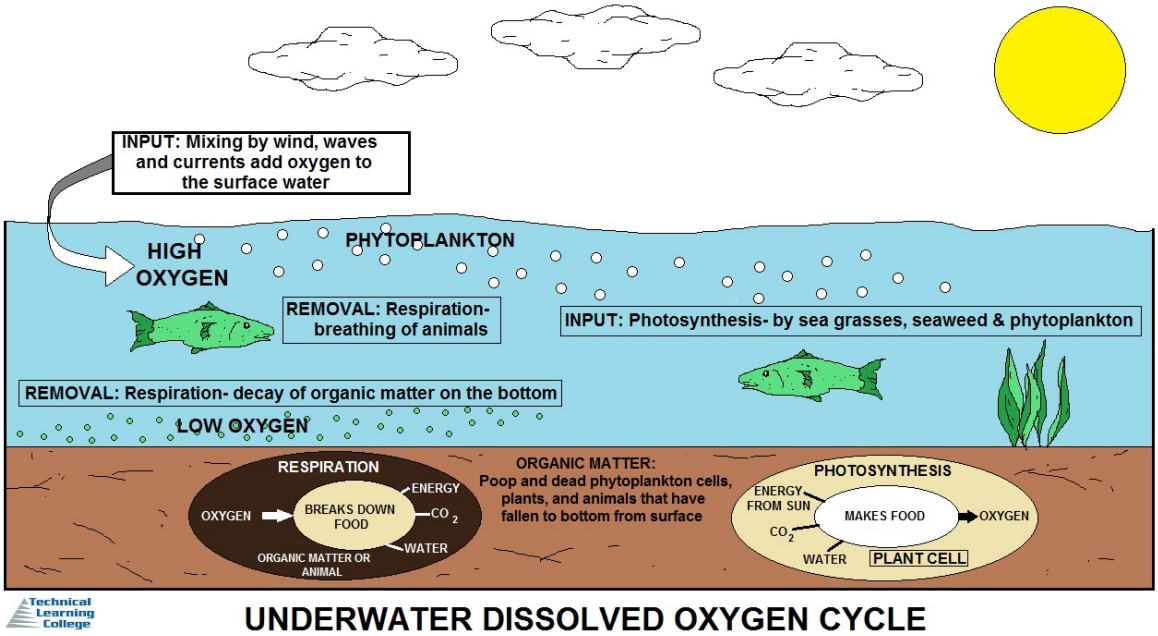
Surface Water Properties

Some of the water will be immediately impounded in lakes and reservoirs, and some will collect as runoff to form streams and rivers that will then flow into the ocean. Water is known as the universal solvent because most substances that come in contact with it will dissolve. What's the difference between lakes and reservoirs?

Reservoirs are lakes with man-made dams. Surface water is usually contaminated and unsafe to drink.

Depending on the region, some lakes and rivers receive discharge from sewer facilities or defective septic tanks. Runoff could produce mud, leaves, decayed vegetation, and human and animal refuse. The discharge from industry could increase volatile organic compounds. Some lakes and reservoirs may experience seasonal turnover.

Changes in the dissolved oxygen, algae, temperature, suspended solids, turbidity, and carbon dioxide will change because of biological activities.



UNDERWATER DISSOLVED OXYGEN CYCLE

Managing Water Quality at the Source

Depending on the region, source water may have several restrictions of use as part of a Water Shed Management Plan. In some areas, it may be restricted from recreational use, discharge or runoff from agriculture, or industrial and wastewater discharge. Another aspect of quality control is aquatic plants.

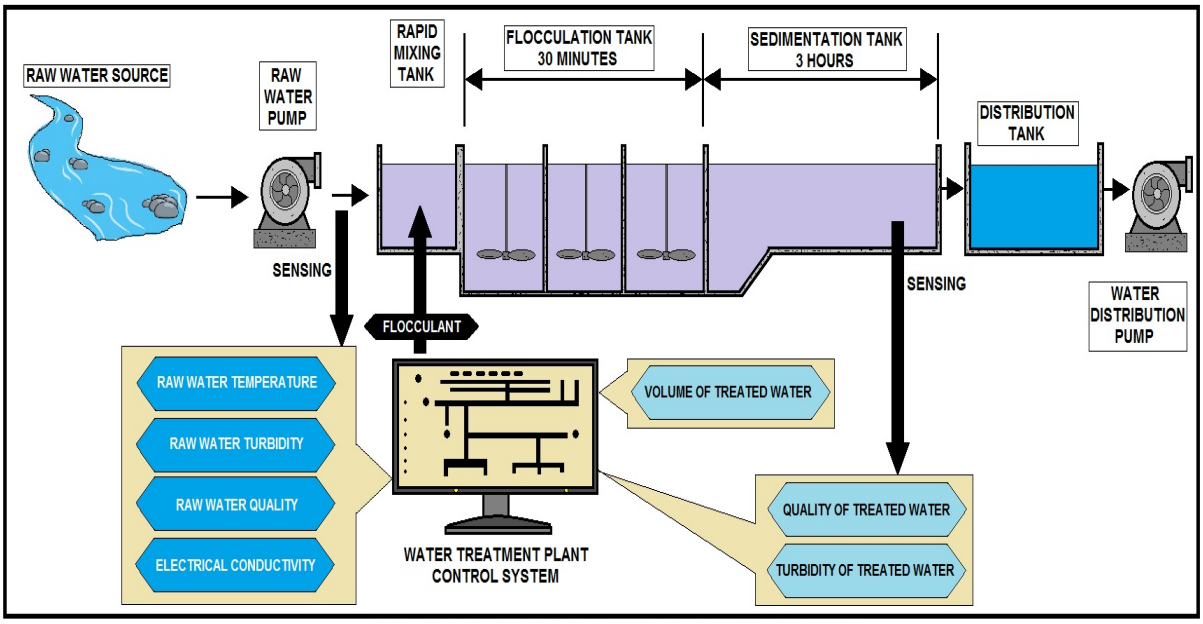
The ecological balance in lakes and reservoirs plays a natural part in purifying and sustaining the life of the lake. For example, algae and rooted aquatic plants are essential in the food chain of fish and birds. Algae growth is the result of photosynthesis. Algae growth is supplied by the energy of the sun. As algae absorbs this energy, it converts carbon dioxide to oxygen.

This creates **aerobic** conditions that supply fish with oxygen. Without sun light, the algae would consume oxygen and release carbon dioxide. The lack of dissolved oxygen in water is known as **anaerobic** conditions. Certain vegetation removes the excess nutrients that would promote the growth of algae. Too much algae will imbalance the lake and kill fish.

Most treatment plant upsets such as taste and odor, color, and filter clogging is due to algae. The type of algae determines the problem it will cause, for instance slime, corrosion, color, and toxicity. Algae can be controlled in the water supply by using chemicals such as copper sulfate.

Depending on federal regulations and the amount of copper found natural in water, operators have used potassium permanganate, powdered activated carbon and chlorine to control algae blooms. The pH and alkalinity of the water will determine how these chemicals will react.

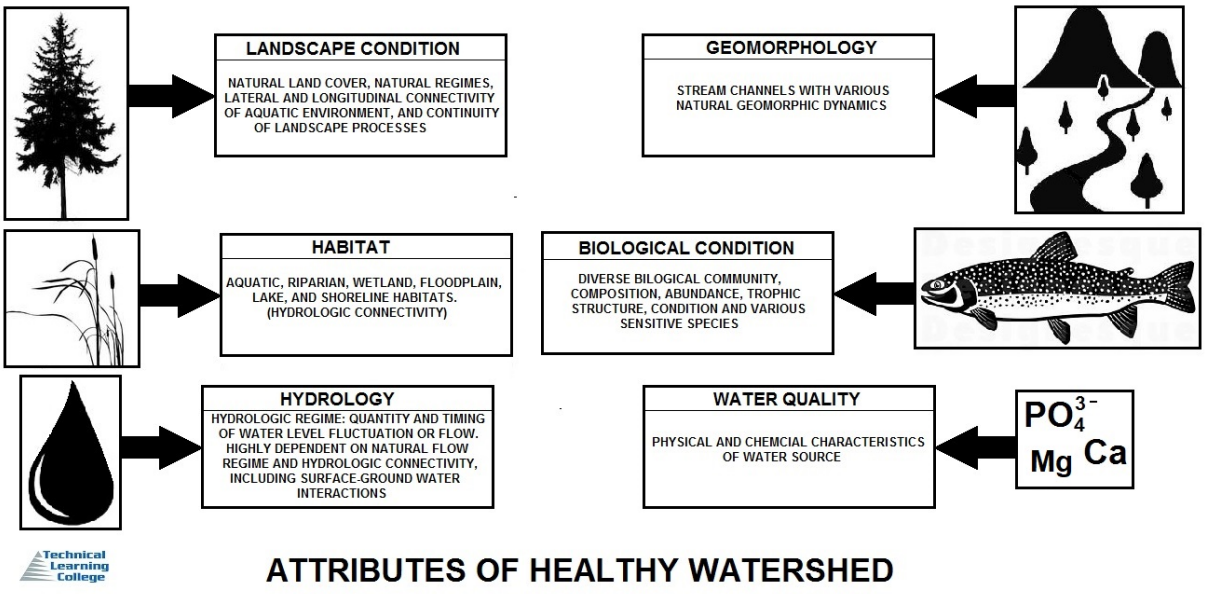
Many water systems are limiting their chlorine usage because it reacts with the organics in the water to form trihalomethanes. Most treatment plants that do not use chlorine in the disinfection process will still add chlorine for a *residual* in the distribution system.



RAW WATER TURBIDITY MONITORING

Physical Characteristics of Water

Physical characteristics such as taste, odor, temperature, pH, TDS, and turbidity; are mostly how the consumer judges how well the provider is treating the water.



ATTRIBUTES OF HEALTHY WATERSHED

Physical characteristics are the elements found that are considered alkali, metals, and non-metals such as carbonates, fluoride, sulfides or acids. The consumer relates it to scaling of faucets or staining. Particles and rust come from the distribution system, the gradual breakdown of the lining of concrete or iron water pipes (mains) or from sediment that has accumulated over the years and is disturbed in some way.

SOLIDS

Solid material in wastewater may be dissolved, suspended, or settled.

Total dissolved solids or TDS (sometimes called filterable residue) is measured as the mass of residue remaining when a measured volume of filtered water is evaporated. The mass of dried solids remaining on the filter is called **total suspended solids** (TSS) or non-filterable residue.

Settleable solids are measured as the visible volume accumulated at the bottom of an Imhoff cone after water has settled for one hour.

Turbidity is a measure of the light scattering ability of suspended matter in the water.

Salinity measures water density or conductivity changes caused by dissolved materials.

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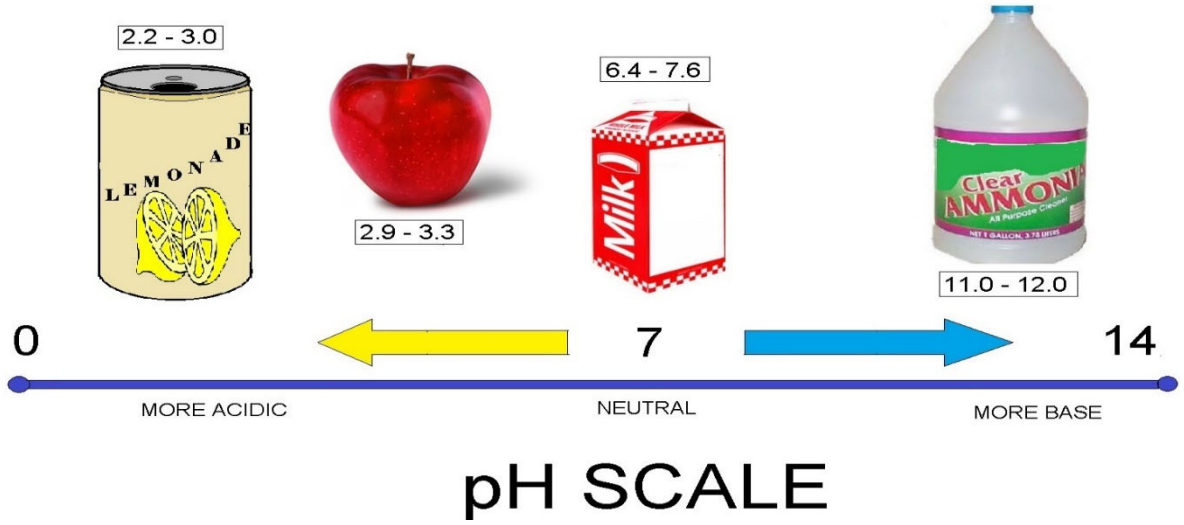
Total Dissolved Solids (TDS) is not a primary pollutant; it is an indicator of aesthetic water characteristics such as hardness and an indication of an assortment of chemical contaminants which might be present, such as Arsenic. We will cover this in a few more pages.

pH is the negative logarithm of the hydrogen ion concentration, $[H^+]$, a measure of the degree to which a solution is acidic or alkaline. An acid is a substance that can give up a hydrogen ion (H^+); a base is a substance that can accept H^+.

The more acidic a solution the greater the hydrogen ion concentration and the lower the pH; a pH of 7.0 indicates neutrality, a pH of less than 7 indicates acidity, and a pH of more than 7 indicates alkalinity. We will cover this subject further in the Water Analysis/Laboratory Section.



pH SCALE

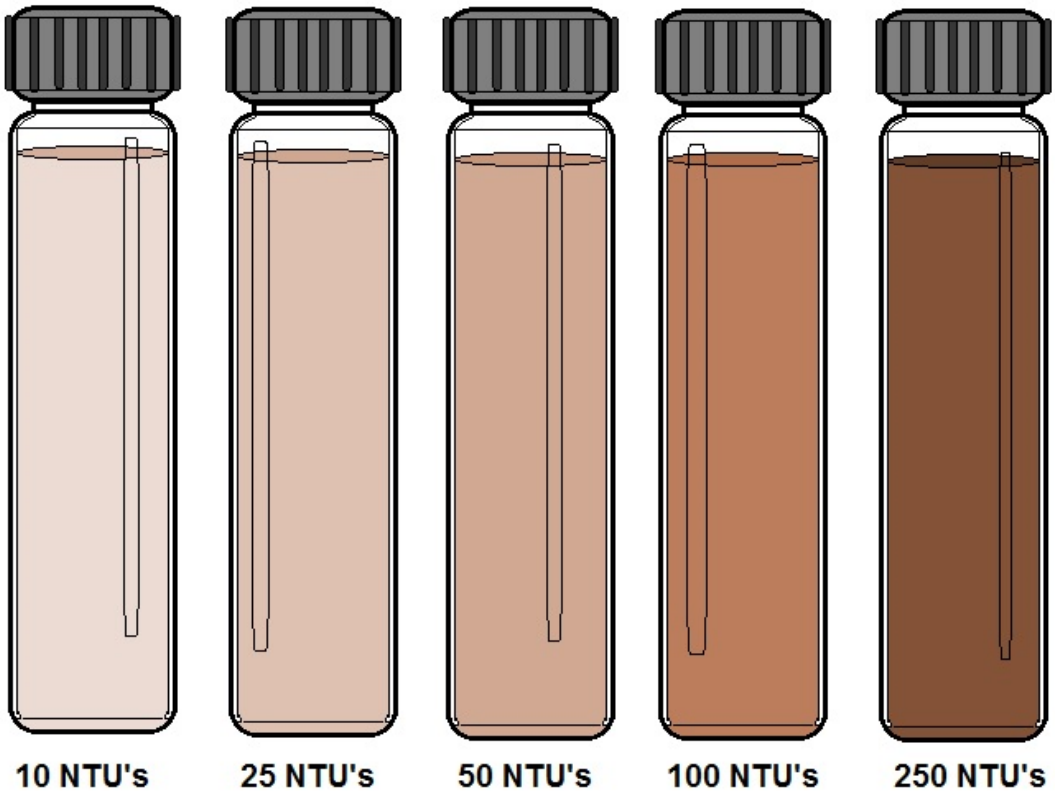
Alkalinity

Alkalinity of water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates or other bases if these are present.

Alkalinity Measurements

Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes



TURBIDITY SAMPLES IN NTU's (Neophelometric Turbidity Unit)

Turbidity Introduction

One physical characteristic of water is turbidity. A measure of the cloudiness of water caused by suspended particles. The cloudy appearance of water caused by the presence of tiny particles. High levels of turbidity may interfere with proper water treatment and monitoring. If high quality raw water is low in turbidity, there will be a reduction in water treatment costs. Turbidity is undesirable because it causes health hazards.

The turbidity in natural surface waters is composed of a large number of sizes of particles. The sizes of particles can be changing constantly, depending on precipitation and manmade factors.

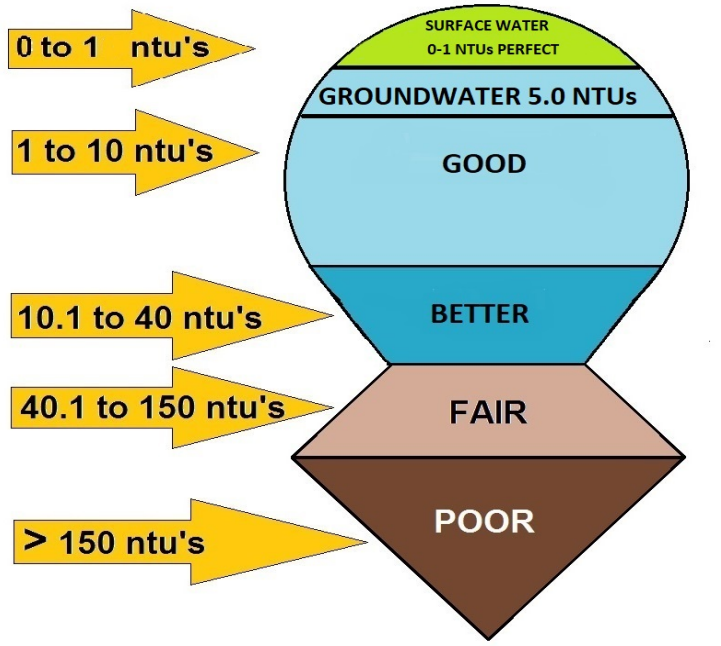
When heavy rains occur, runoff into streams, rivers, and reservoirs occurs, causing turbidity levels to increase. In most cases, the particle sizes are relatively large and settle relatively quickly in both the water treatment plant and the source of supply. However, in some instances, fine, colloidal material may be present in the supply, which may cause some difficulty in the coagulation process.

Generally, higher turbidity levels require higher coagulant dosages. However, seldom is the relationship between turbidity level and coagulant dosage linear. Usually, the additional coagulant required is relatively small when turbidities are much higher than normal due to higher collision probabilities of the colloids during high turbidities.

Conversely, low turbidity waters can be very difficult to coagulate due to the difficulty in inducing collision between the colloids.

In this instance, floc formation is poor, and much of the turbidity is carried directly to the filters. Organic colloids may be present in a water supply due to pollution, and these colloids can be difficult to remove in the coagulation process. In this situation, higher coagulant dosages are generally required.

Turbidity MCL
An MCL for turbidity established by the EPA because turbidity interferes with disinfection. This characteristic of water changes the most rapidly after a heavy rainfall. The following conditions may cause an inaccurate measure of turbidity; the temperature variation of a sample, a scratched or unclean sample tube in the nephelometer and selecting an incorrect wavelength of a light path.



TURBIDITY PARAMETERS (NTU) FOR WATER QUALITY

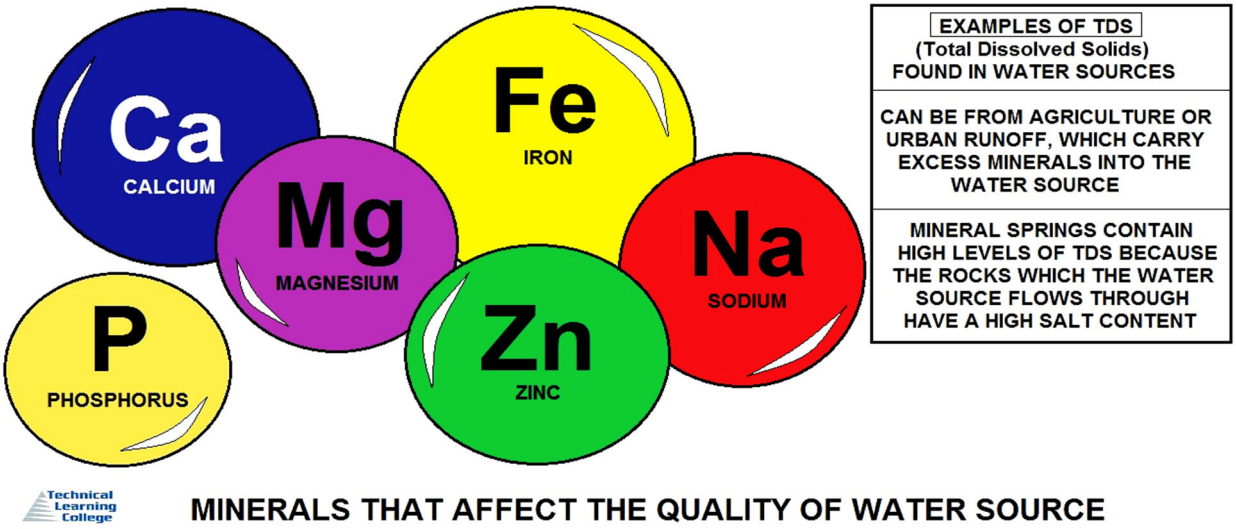
Surface Water System Compliance Information
(Depends on Systems and Rule)

- 0.34 NTU in 95% of samples, never to exceed 1.0 NTU spike
- Sample turbidity at each individual filter effluent
- Sample the combined filter turbidity at the clear well
- (Groundwater turbidity ≤ 5.0 NTU allowed)

Turbidity Key

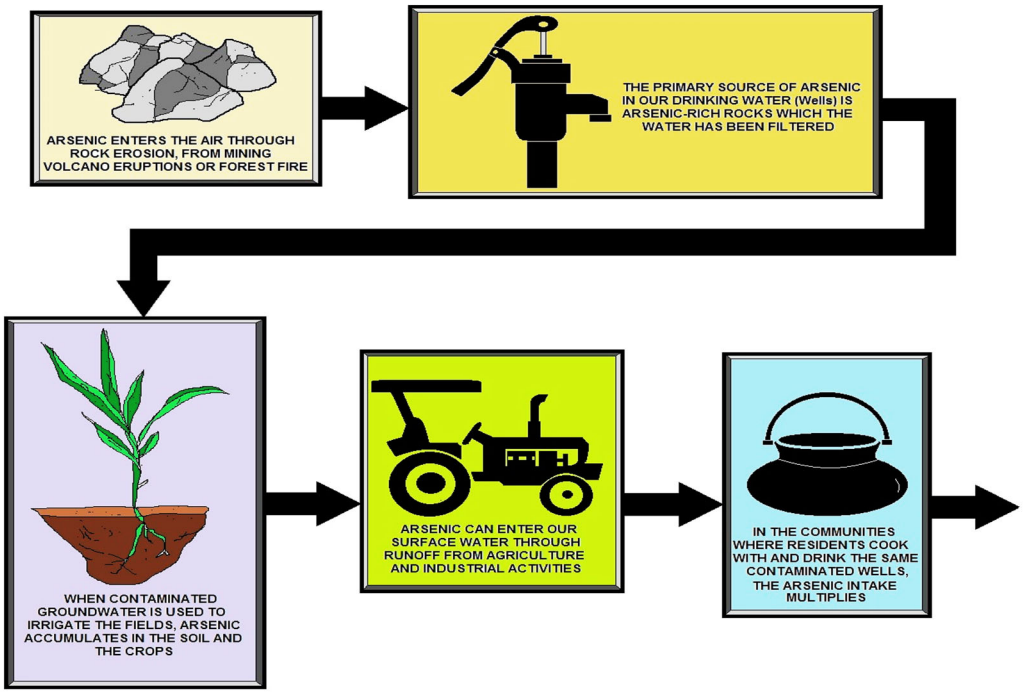
- Turbidity can also be measured in ppm (parts per million) and its size is measured in microns. Turbidity can be particles in the water consisting of finely divided solids, larger than molecules, but not visible by the naked eye; ranging in size from .001 to .150mm (1 to 150 microns).

- 0.34 NTU in 95% of surface water samples, never to exceed 1.0 NTU spike

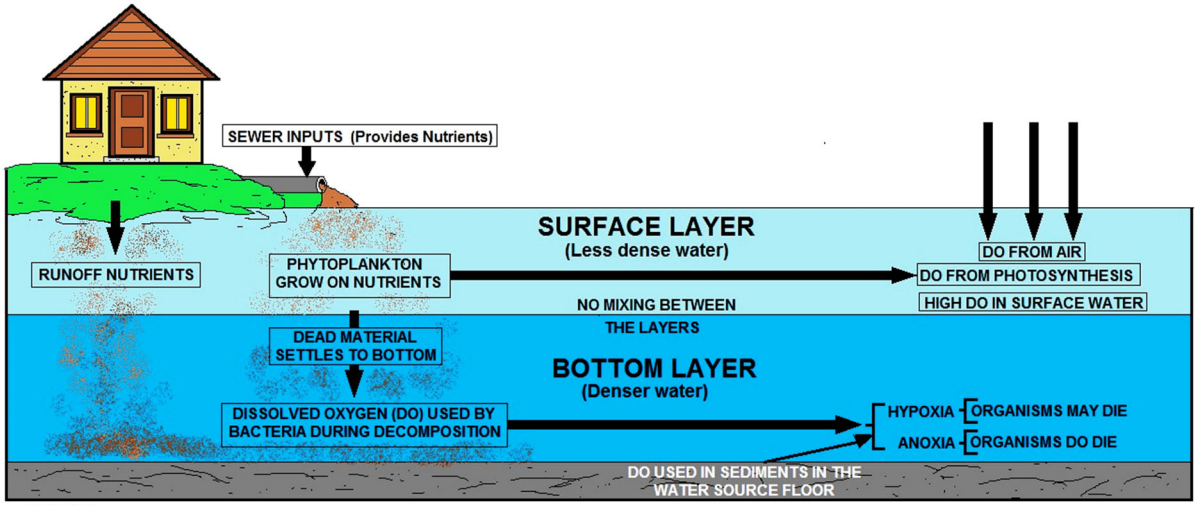


MINERALS THAT AFFECT THE QUALITY OF WATER SOURCE

Lead does not usually occur naturally in water supplies but is derived from lead distribution and domestic pipework and fittings. Water suppliers (distribution systems) have removed most of the original lead piping from the mains distribution system, however many older properties still have lead service pipes and internal lead pipework. The pipework (including the service pipe) within the boundary of the property is the responsibility of the owner of the property, not the water supplier.



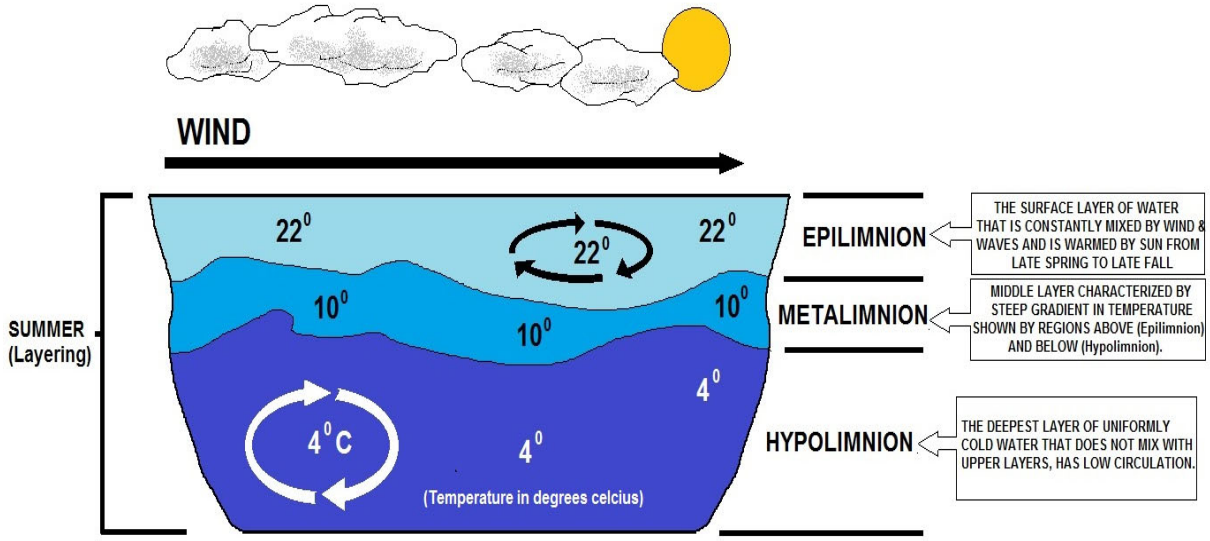
ARSENIC IN DRINKING WATER



DISSOLVED OXYGEN AFFECT IN WATER SOURCES

Dissolved Oxygen

The level of dissolved oxygen in natural waters is often a direct indication of quality, since aquatic plants produce oxygen, while microorganisms generally consume it as they feed on pollutants. At low temperatures the solubility of oxygen is increased, so that in winter, concentrations as high as 20 ppm may be found in natural waters; during summer, saturation levels can be as low as 4 or 5 ppm. Dissolved oxygen is essential for the support of fish and other aquatic life and aids in the natural decomposition of organic matter.



THERMAL STRATIFICATION (Temperature Transition Zones)

Thermal stratification is possible as **water becomes less dense when heated**, meaning water weighs less per unit volume. Therefore, warmer water will be lighter and colder water will be heavier. Due to this, there will always be a level of “self-induced” thermal stratification in a water storage.

Hardness Introduction

Temporary and Permanent

There are two types of hardness: temporary and permanent. Temporary hardness comes out of the water when it is heated and is deposited as scale and "fur" on kettles, coffee makers and taps and appears as a scum or film on tea and coffee. Permanent hardness is unaffected by heating. We will cover this in the advanced water treatment section

WATER HARDNESS (Salt Types)	
CARBONATE HARDNESS COMPOUNDS	NON-CARBONATE HARDNESS COMPOUNDS
CALCIUM CARBONATE ($CaCO_3$)	CALCIUM SULPHATE ($CaSO_4$)
MAGNESIUM CARBONATE ($MgCO_3$)	MAGNESIUM SULPHATE ($MgSO_4$)
CALCIUM BiCARBONATE ($Ca(HCO_3)_2$)	CALCIUM CHLORIDE ($CaCl_2$)
MAGNESIUM BiCARBONATE ($Mg(HCO_3)_2$)	MAGNESIUM CHLORIDE ($MgCl_2$)
CALCIUM HYDROXIDE ($Ca(OH)_2$)	
MAGNESIUM HYDROXIDE ($Mg(OH)_2$)	

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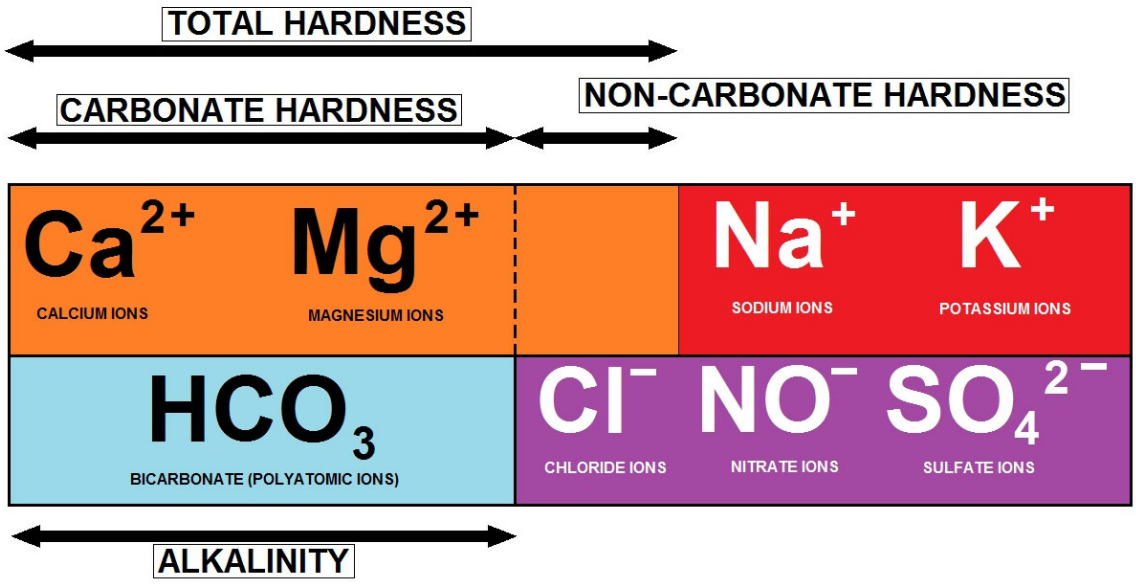
CAUSES OF HARDNESS THAT AFFECTS WATER QUALITY



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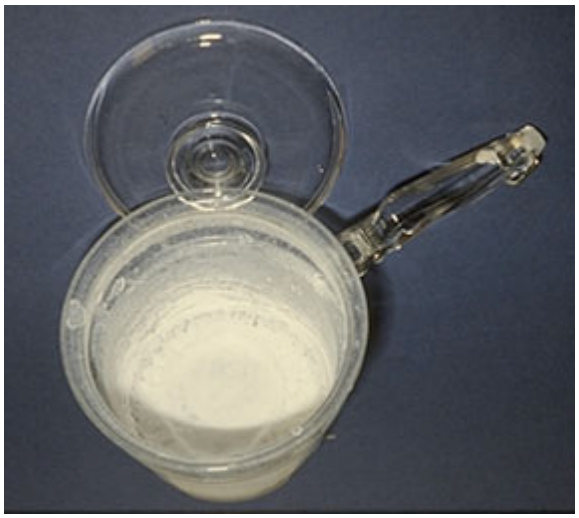
CARBONATE HARDNESS CHART

Objections to Hard Water

Scale Formation
Hard water forms scale, usually calcium carbonate, which causes a variety of problems. Left to dry on the surface of glassware and plumbing fixtures, including showers doors, faucets, and sink tops; hard water leaves unsightly white scale known as water spots. Scale that forms on the inside of water pipes will eventually reduce the flow capacity or possibly block it entirely. Scale that forms within appliances and water meters causes wear on moving parts.



When hard water is heated, scale forms much faster. In particular, when the magnesium hardness is more than about 40 mg/l (as $CaCO_3$), magnesium hydroxide scale will deposit in hot water heaters that are operated at normal temperatures of 140-150°F (60-66°C).

A coating of only 0.04 in. (1 mm) of scale on the heating surfaces of a hot water heater creates an insulation effect that will increase heating costs by about 10 percent.

Effect on Soap
The historical objection to hardness has been its effect on soap. Hardness ions form precipitates with soap, causing unsightly "**curd**," such as the familiar bathtub ring, as well as reduced efficiency in washing and laundering. To counteract these problems, synthetic detergents have been developed and are now used almost exclusively for washing clothes and dishes.

These detergents have additives known as sequestering agents that "**tie up**" the hardness ions so that they cannot form the troublesome precipitates. Although modern detergents counteract many of the problems of hard water, many customers prefer softer water. These customers can install individual softening units or use water from another source, such as a cistern, for washing.

Total Dissolved Solids (TDS)

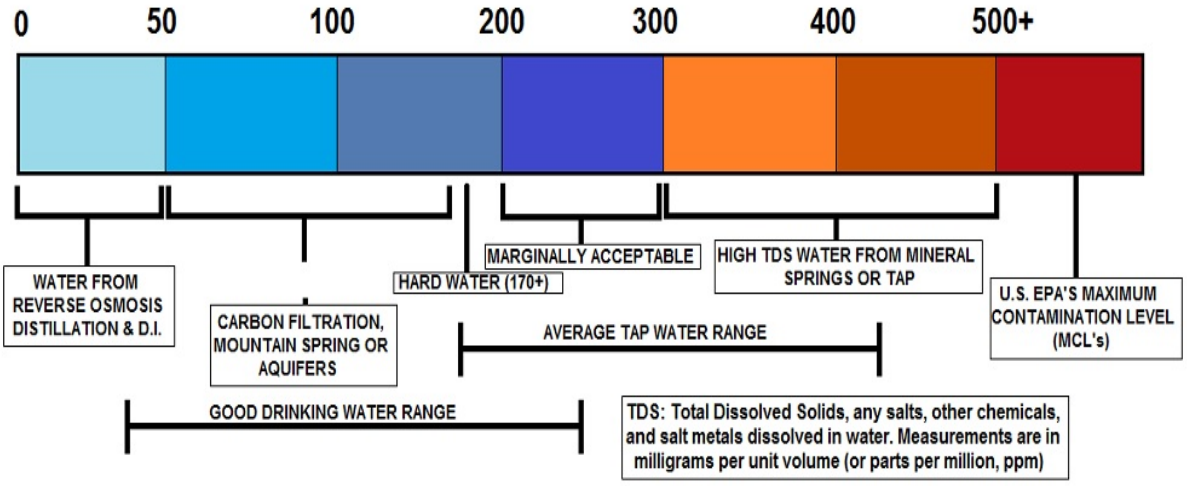
Total dissolved solids (TDS) represents the combined total of all organic and inorganic substances found in drinking water. The total dissolved solids present in water is one of the leading causes of particles and sediments in drinking water, which give water its color, odor, and flavor, and can be a general indicator of water quality.

Organic substances found in drinking water may include:

- Algae
- Bacteria
- Fungi
- Hair
- Pesticides
- Herbicides
- Fertilizers
- Disinfectants
- Pharmaceuticals

Inorganic substances found in drinking water may include:

- Arsenic
- Lead
- Mercury
- Chlorine
- Sodium
- Calcium
- Potassium
- Magnesium
- Fluoride



Secondary Standard

TDS is most often measured in parts per million (ppm) or milligrams per liter of water (mg/L). The normal TDS level ranges from 50 ppm to 1,000 ppm. The Environmental Protection Agency (EPA), which is responsible for drinking water regulations in the United States, has identified TDS as a secondary standard, meaning that it is a voluntary guideline. While the United States set legal standards for many harmful substances, TDS, along with other contaminants that cause aesthetic, cosmetic, and technical effects, has only a guideline.

Levels of TDS (milligrams per litre)	Rating
Less than 300	Excellent
300 - 600	Good
600 - 900	Fair
900 - 1,200	Poor
Above 1,200	Unacceptable

Increased concentrations of dissolved solids can also have technical effects. Dissolved solids can produce hard water, which leaves deposits and films on fixtures and can corrode the insides of hot water pipes and boilers.

PARAMETERS	CLARITY	TURBIDITY	TOTAL SUSPENDED SOLIDS (TSS)
DEFINITION	HOW FAR LIGHT CAN PASS THROUGH THE WATER COLUMN	MEASURES THE DEGREE TO WHICH WATER LOSES IT'S TRANPARENCY	PARTICLES THAT ARE LARGER THAN 2 MICRONS FOUND IN THE WATER COLUMN
COMMENTS	TURBIDITY AND CLARITY ARE DIRECTLY RELATED. TURBIDITY IS A MEASURE OF THE WATER'S CLARITY High Turbidity = Low Clarity Low Turbidity = High Clarity		WHILE TSS DIRECTLY AFFECTS TURBIDITY, TURBIDITY IS NOT A DIRECT MEASUREMENT OF TOTAL SUSPENDED SOLIDS
WHAT IS MEASURED?	ORGANIC AND INORGANIC SUSPENDED SOLIDS, LIKE CLAY, SILT, SEDIMENT, ALGAE AND BATERIA + DISSOLVED COLORED MATERIALS (Smaller than 2 Microns)		ORGANIC AND INORGANIC SUSPENDED SOLIDS SETTLEABLE SOLIDS (Solids that are moved along the bottom of water by strong flow)
HOW IS IT MEASURED?	DIRECT MEASUREMENT: TURBIDIMETER or TURBIDITY SENSOR INDIRECT MEASUREMENT: SECCHI DISK or TURBIDITY TUBE Indirect Methods are Quick and Expensive but are Dependent on the Visual Acuity of the Observer		TO MEASURE TSS, A WATER SAMPLE IS FILTERED, DRIED AND WEIGHED

TOTAL DISSOLVED SOLIDS / WATER TREATMENT

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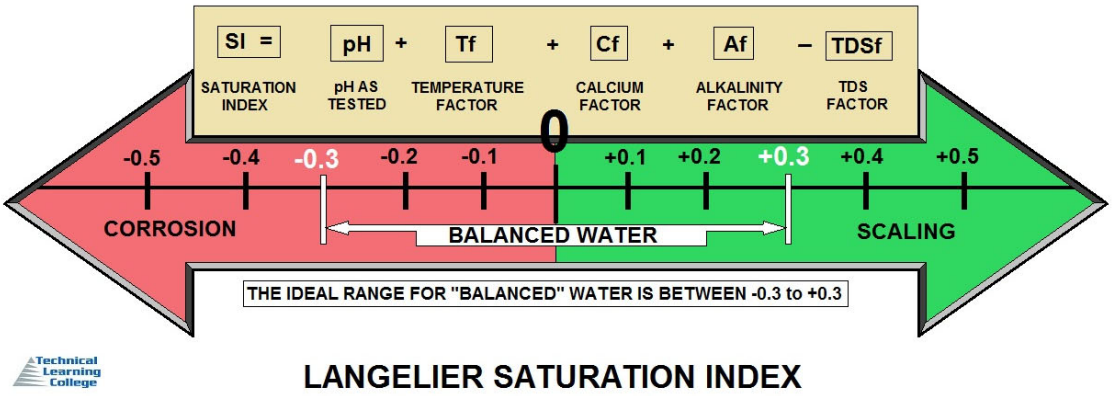
Langelier Saturation Index

The Langelier Saturation index (LSI) is an equilibrium model derived from the theoretical concept of saturation and provides an indicator of the degree of saturation of water with respect to calcium carbonate. It can be shown that the Langelier saturation index (LSI) approximates the base 10 logarithm of the calcite saturation level. The Langelier saturation level approaches the concept of saturation using pH as a main variable. The LSI can be interpreted as the pH change required to bring water to equilibrium.



LANGELIER SATURATION INDEX

Water with a Langelier saturation index of 1.0 is one pH unit above saturation. Reducing the pH by 1 unit will bring the water into equilibrium. This occurs because the portion of total alkalinity present as CO_3^{2-} decreases as the pH decreases, according to the equilibria describing the dissociation of carbonic acid:

$$H_2CO_3 \rightleftarrows HCO_3^- + H^+$$
$$HCO_3^- \rightleftarrows CO_3^{2-} + H^+$$

- If LSI is negative: No potential to scale, the water will dissolve $CaCO_3$
- If LSI is positive: Scale can form and $CaCO_3$ precipitation may occur
- If LSI is close to zero: Borderline scale potential.
- Water quality or changes in temperature, or evaporation could change the index.

The LSI is probably the most widely used indicator of cooling water scale potential. It is purely an equilibrium index and deals only with the thermodynamic driving force for calcium carbonate scale formation and growth. It provides no indication of how much scale or calcium carbonate will actually precipitate to bring water to equilibrium.

It simply indicates the driving force for scale formation and growth in terms of pH as a master variable. In order to calculate the LSI, it is necessary to know the alkalinity (mg/l as $CaCO_3$), the calcium hardness (mg/l Ca^{2+} as $CaCO_3$), the total dissolved solids (mg/l TDS), the actual pH, and the temperature of the water (^oC).

If TDS is unknown, but conductivity is, one can estimate mg/L TDS using a conversion table. LSI is defined as:

$$\mathbf{LSI = pH - pH_s}$$

Where:

pH is the measured water pH

pH_s is the pH at saturation in calcite or calcium carbonate and is defined as:

$$\mathbf{pH_s = (9.3 + A + B) - (C + D)}$$

Where:

$A = (Log_{10}\ [TDS] - 1) / 10$

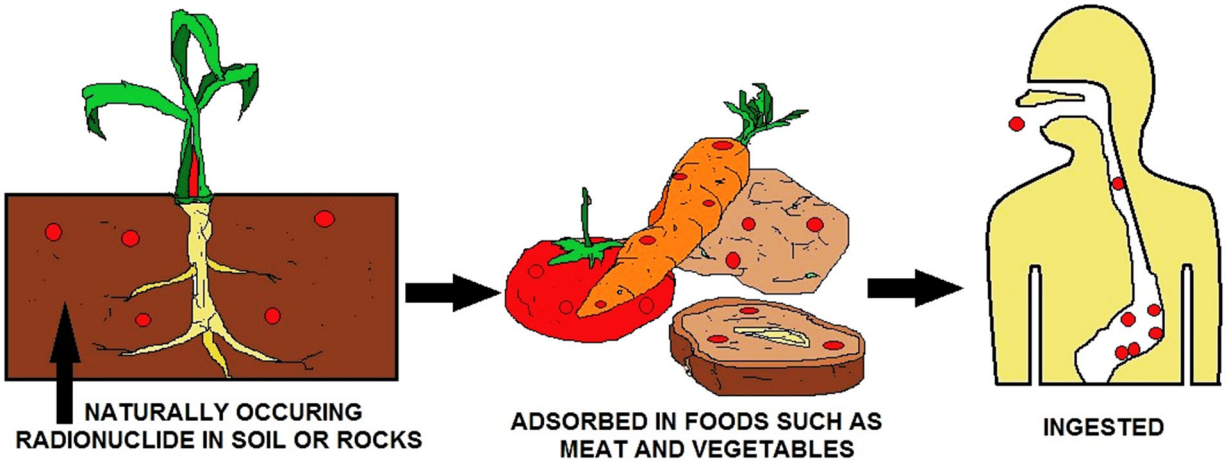
$B = -13.12 \times Log_{10}\ (^oC + 273) + 34.55$

$C = Log_{10}\ [Ca^{2+}\ as\ CaCO_3] - 0.4$

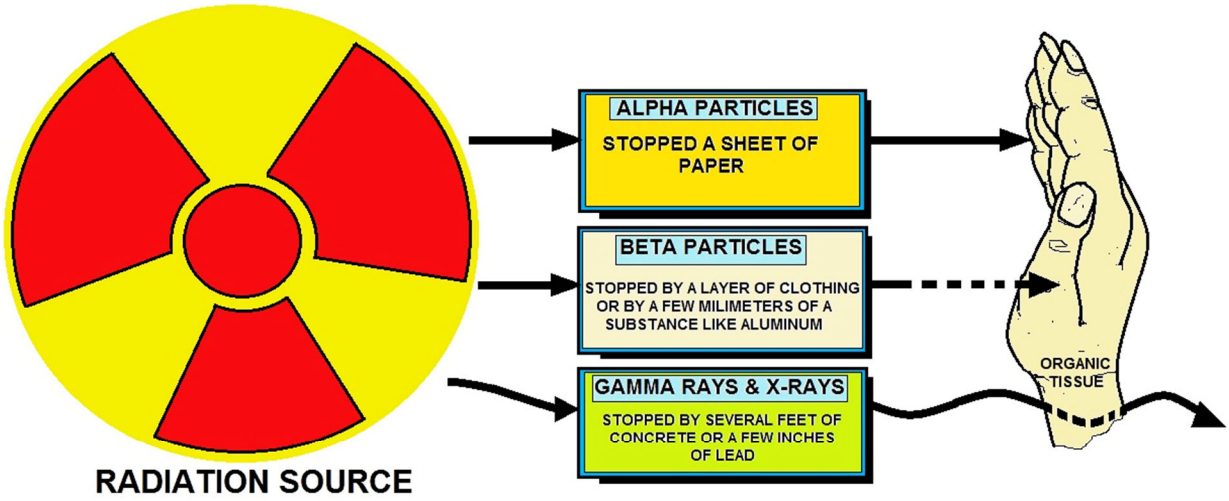
$D = Log_{10}\ [alkalinity\ as\ CaCO_3]$

Radiological Characteristics

Radiological characteristics are the result of water coming in contact with radioactive materials. This could be associated with atomic energy.

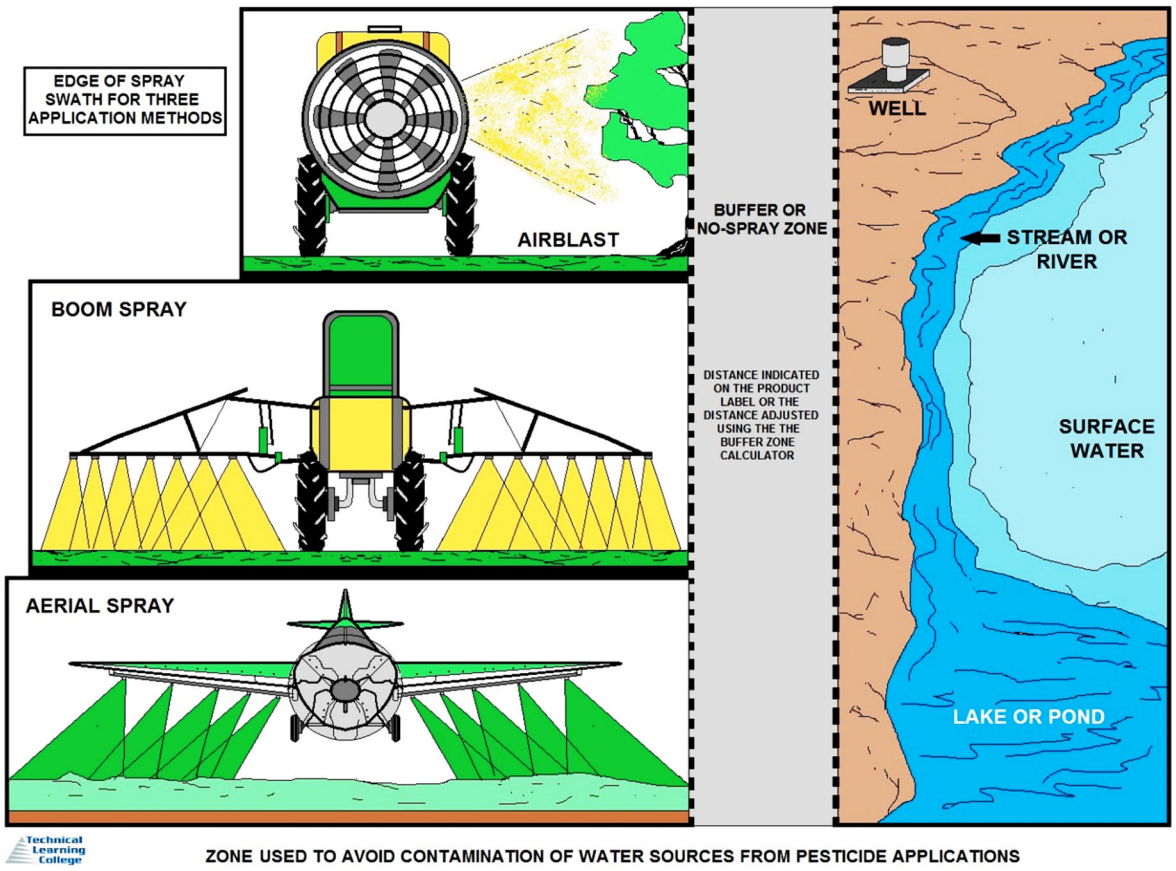


RADIONUCLIDES



PENETRATING POWER OF ALPHA / BETA PARTICLES AND GAMMA RAYS AND X-RAYS

Most of these substances are of natural origin and are picked up as water passes around the water cycle. Some are present due to the treatment processes that are used make the water suitable for drinking and cooking.



Insecticides and Herbicides

Insecticides and herbicides (sometimes referred to as pesticides) are widely used in agriculture, industry, leisure facilities and gardens to control weeds and insect pests and may enter the water cycle in many ways. Aluminum salts are usually added during water treatment to remove color and suspended solids and may reduce any residual insecticides in the water.

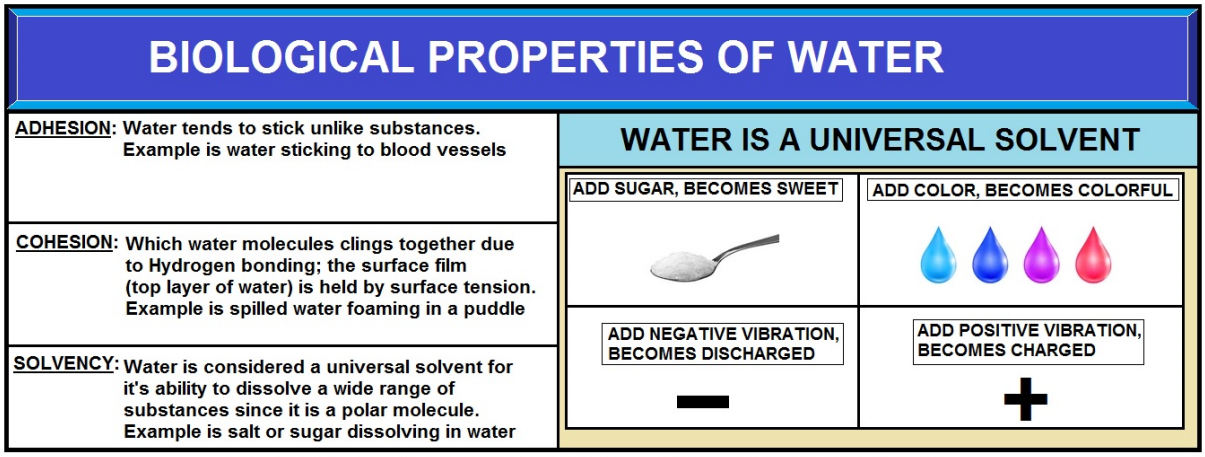
Biological Characteristics of Water

Biological characteristics are the presence of living or dead organisms. Biological characteristics will also interact with the chemical composition of the water. The consumer will become sick or complain about hydrogen sulfide odors, the rotten egg smell. We will cover the Total Coliform Rule in detail in the Water Monitoring Section - Microbiological section and again in the Appendix.

Pathogen Definition
A pathogen is an organism capable of causing disease. Pathogens include parasites, bacteria and viruses.

Biological Parameters

- Biological parameters are important factor that determine quality of drinking water. It is more important than physical and chemical parameters in term of direct effect on human health.
- Some important biological characteristics affecting quality of drinking water includes bacteria, protozoa, virus and algae.



BIOLOGICAL PROPERTIES OF WATER EXAMPLE

Bacteriological Aspects of Water Pollution

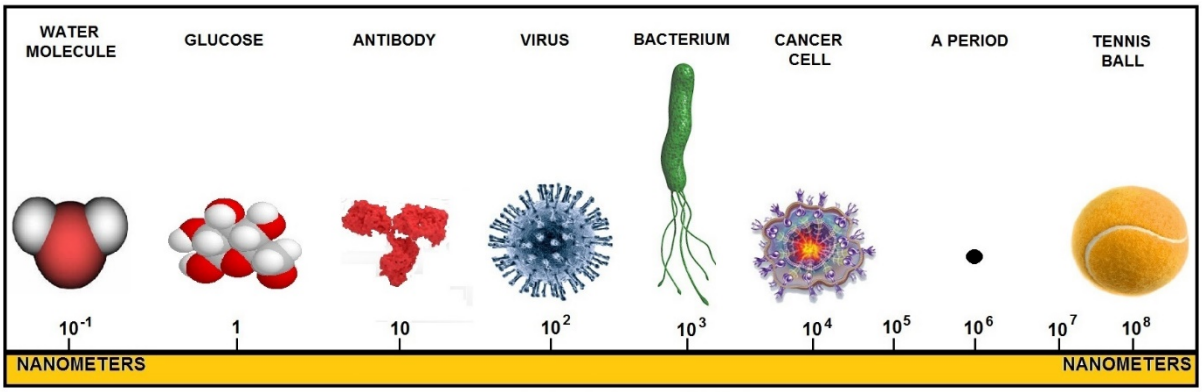
- Human beings and other animals discharge large number of intestinal bacteria into stool and urine. Therefore, bacteria appears in drinking water when water source is contaminated with feces.
- Some intestinal bacteria which are normal flora of intestine are not pathogenic while other bacteria causes serious disease when they are present in drinking water.
- Some pathogenic bacteria includes- *Salmonella, Shigella, Vibrio cholera, Yersinia enterocolitica.*
- These bacteria are only present in drinking water if source of water is contaminated with feces.
- Drinking water must be regularly check to detect intestinal pathogens. However all intestinal pathogens are difficult to cultivate and identify in routine examination. Therefore, presence of pathogenic intestinal bacteria is indirectly checked by detecting

intestinal normal flora. Such organism which are routinely checked for quality of water is known as indicator organism for fecal contamination.

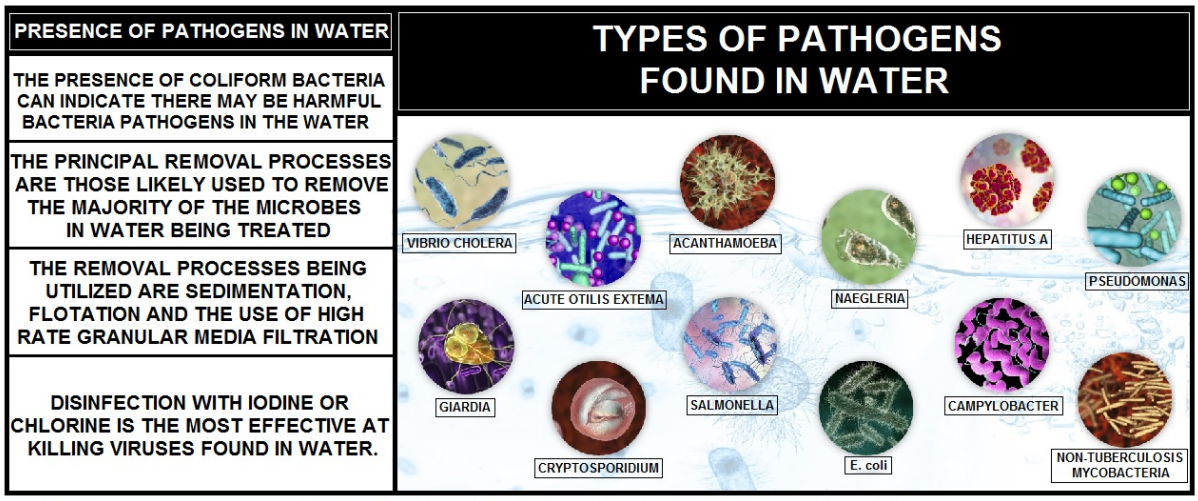
- Some indicator organism are fecal coliform (E. *coli*), fecal Streptococci (*Enterococcus*), *Clostridium perfringens*

Cysts

Cysts are associated with the reproductive stages of parasitic microorganisms (protozoans) which can cause acute diarrhea type illnesses; they come from farm animals, wild animals and people. Cysts are very resistant to normal disinfection processes but can be removed by advanced filtration processes installed in water treatment works. Cysts are rarely present in the public water supply. We will cover this area in the water monitoring section.



SIZE COMPARISON
HOW SMALL IS SMALL ?

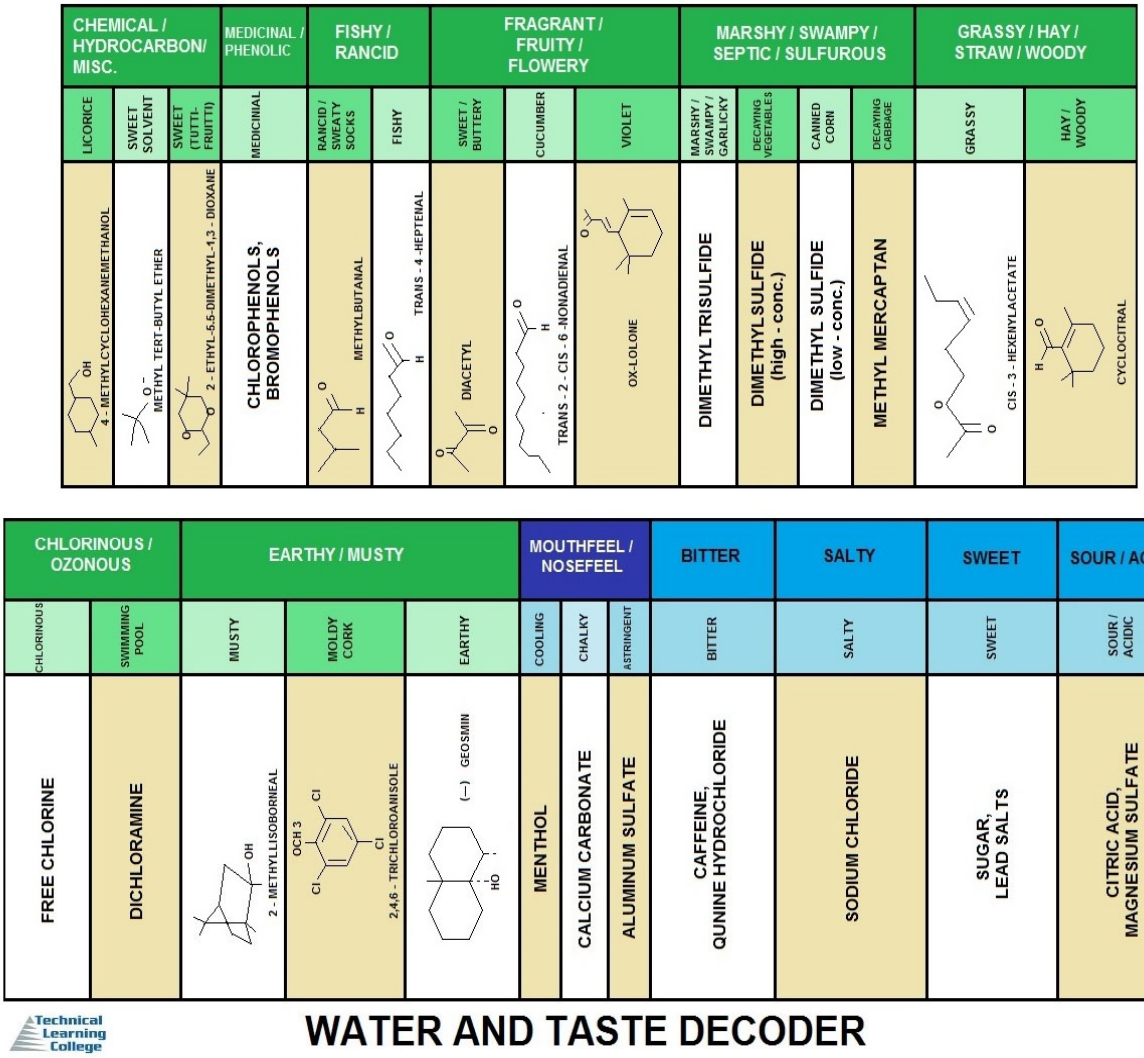


Technical Learning College

PATHOGENS FOUND IN WATER SUPPLIES

Drinking Water Tastes and Odors

Health concerns are not the only criteria that we use to judge our drinking water. In fact, often the most noticeable qualities that determine whether water is acceptable to consumers are unpleasant taste or odor, staining, poor reaction with soap, or mineral buildup in pipes and plumbing. These problems result from elevated concentrations of "nuisance" constituents.



Most nuisance constituents occur naturally. These constituents are more likely to occur at nuisance concentrations in groundwater than surface water, because they result from the reaction of groundwater with aquifer rocks and sediments as the water moves underground.

Yellow Water Complaints

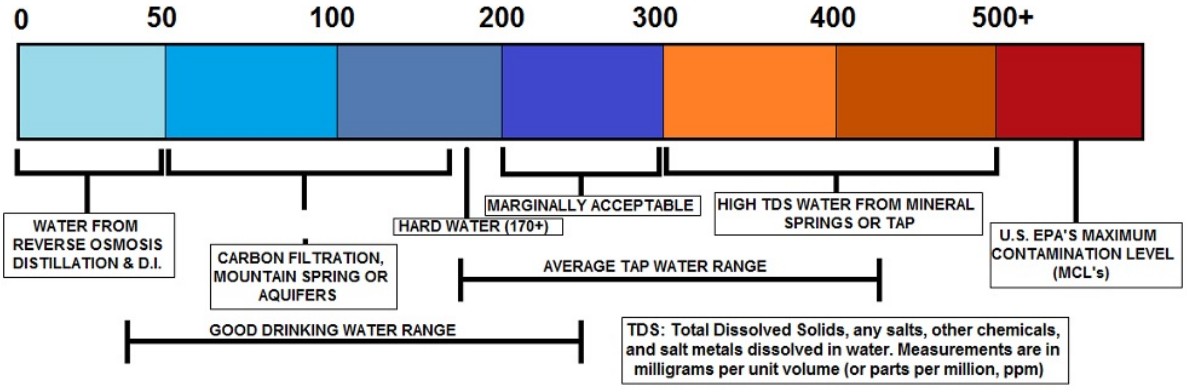
Dissolved iron in groundwater can stain laundry, sinks, bathtubs, and toilets a brownish red, and can degrade plumbing and heating systems. Iron also gives drinking water an unpleasant taste, making it undrinkable for many well owners. Manganese often co-occurs with iron and causes many of the same problems.

Hard Water
Hard water—defined by high concentrations of calcium and magnesium—causes water pipes and fixtures to become coated with scale, limits the ability of soaps and detergents to form suds, and can cause premature failure of plumbing and heating fixtures. pH outside of acceptable ranges can give water a metallic taste and can cause corrosion of pipes. A high dissolved solids concentration—a measure of all dissolved substances in water, also referred to as salinity—makes water taste disagreeably salty.

EPA Guidelines for Nuisance Constituents
The EPA recommends limits, called Secondary Maximum Contaminant Levels (SMCLs), for nuisance constituents in public water supplies. The SMCLs are non-health-based, non-enforceable guidelines for concentrations of 15 constituents in drinking water. These guidelines are designed to assist public water systems in managing their drinking water for aesthetic considerations, such as taste, color, and odor. These contaminants are not considered to present a risk to human health at the SMCL.

Because they can be smelled, tasted, or seen, nuisance constituents may be more likely to be noticed by consumers than contaminants that actually are a health risk. However, some constituents that have an SMCL also have a higher human-health benchmark. Manganese is one example—the black staining caused by manganese might be just a nuisance or might signal a concentration high enough to be a health risk.

Dissolved Solids
In other situations, the presence of nuisance constituents can signal geochemical conditions that promote high concentrations of other, more harmful contaminants. For example, high concentrations of dissolved solids are considered a nuisance because they cause water to taste salty, but high dissolved solids is not in itself a health concern. However, high dissolved solids can be an indication that there are elevated concentrations of arsenic, uranium, or other trace elements in the groundwater as well. The occurrence of nuisance constituents in drinking water therefore can indicate that testing for a broader range of constituents could be warranted to assess possible risks and to determine options for reducing those risks.



Fluoride Introduction

Some water providers will add fluoride to the water to help prevent cavities in children. Too much fluoride will mottle the teeth.

Chemical Feed
The equipment used for feeding the fluoride to water shall be accurately calibrated before being placed in operation, and at all times shall be capable of maintaining a rate of feed within 5% of the rate at which the machine is set.

The following chemical feed practices apply:
1. Where a dry feeder of the volumetric or gravimetric type is used, a suitable weighing mechanism shall be provided to check the daily amount of chemical feed.
2. Hoppers should be designed to hold a 24 hour supply of the fluoride compound and designed such that the dust hazard to operators is minimized.
3. Vacuum dust filters shall be installed with the hoppers to prevent dust from rising into the room when the hopper is filled.
4. Dissolving chambers are required for use with dry feeders, and the dissolving chambers shall be designed such that at the required rate of feed of the chemical the solution strength will not be greater than 1/4 of that of a saturated solution at the temperature of the dissolving water. The construction material of the dissolving chamber and associated piping shall be compatible with the fluoride solution to be fed.
5. Solution feeders shall be of the positive displacement type and constructed of material compatible with the fluoride solution being fed.
6. The weight of the daily amount of fluoride fed to water shall be accurately determined.
7. Feeders shall be provided with anti-siphon valves on the discharge side. Wherever possible, positive anti-siphon breakers other than valves shall be provided.
8. A "*day tank*" capable of holding a 24 hour supply of solution should be provided.
9. All equipment shall be sized such that it will be operated within the 20 to 80 percent range of their scale, and be capable of feeding over the entire pumpage range of the plant.
10. Alarm signals are recommended to detect faulty operation of equipment; and,
11. The fluoride solution should be added to the water supply at a point where the fluoride will not be removed by any following treatment processes and where it will be mixed with the water. It is undesirable to inject the fluoride compound or solution directly on-line unless there are provisions for adequate mixing.

Metering
Metering of the total water to be fluoridated shall be provided, and the operation of the feeding equipment is to be controlled. Control of the feed rate shall be automatic/ proportional controlled, whereby the fluoride feed rate is automatically adjusted in accordance with the flow changes to provide a constant pre-established dosage for all rates of flow, or (2) automatic/ residual controlled, whereby a continuous automatic fluoride analyzer determines the residual fluoride level and adjusts the rate of feed accordingly, or compound loop controlled, whereby the feed rate is controlled by a flow proportional signal and residual analyzer signal to maintain a constant residual.

Alternate Compounds
Any one of the following fluoride compounds may be used:
1. Hydrofluosilicic acid,
2. Sodium fluoride or,

3. Sodium silicofluoride*.* Other fluoride compounds may be used, if approved by the EPA.

Chemical Storage and Ventilation
The fluoride chemicals shall be stored separately from other chemicals, and the storage area shall be marked "***FLUORIDE CHEMICALS ONLY***". The storage area should be in close proximity to the feeder, kept relatively dry, and provided with pallets (if using bagged chemical) to allow circulation of air and to keep the containers off the floor.

Record of Performance
Accurate daily records shall be kept. These records shall include:
1. The daily reading of the water meter which controls the fluoridation equipment or that which determines the amount of water to which the fluoride is added.
2. The daily volume of water fluoridated.
3. The daily weight of fluoride compound in the feeder.
4. The daily weight of fluoride compound in stock.
5. The daily weight of the fluoride compound fed to the water; and,
6. The fluoride content of the raw and fluoridated water determined by laboratory analysis, with the frequency of measurement as follows:
    (i) treated water being analyzed continuously or once daily, and
    (ii) raw water being analyzed at least once a week.

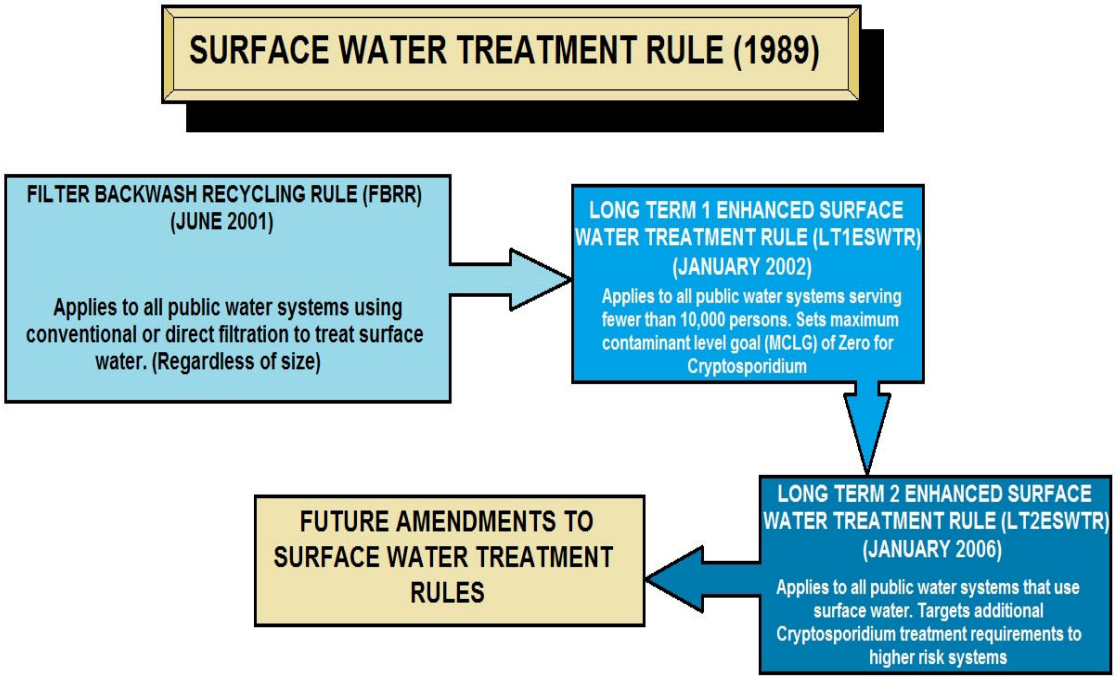
Sampling
In keeping the fluoride records, the following sampling procedures are required:
1. A sample of raw water and a sample of treated water shall be forwarded to an approved independent laboratory for fluoride analysis once a month.
2. On new installations or during start-ups of existing installations, weekly samples of raw and treated water for a period of not less than four consecutive weeks.
3. In addition to the reports required, the EPA may require other information that is deemed necessary.

Fluoride Safety
The following safety procedures shall be maintained:
1. All equipment shall be maintained at a high standard of efficiency, and all areas and appliances shall be kept clean and free of dust. Wet or damp cleaning methods shall be employed wherever practicable.
2. Personal protective equipment shall be used during the clean-up, and appropriate covers shall be maintained over all fluoride solutions.
3. At all installations, safety features are to be considered and the necessary controls built into the installation to prevent an overdose of fluoride in the water. This shall be done either by use of day tanks or containers, anti-siphon devices, over-riding flow switches, sizing of pump and feeders, determining the length and duration of impulses, or other similar safety devices.
4. Safety features shall also be provided to prevent spills and overflows.
5. Individual dust respirators, chemical safety face shields, rubber gloves, and protective clothing shall be worn by all personnel when handling or being exposed to the fluoride dust.
6. Chemical respirators, rubber gloves, boots, chemical safety goggles and acid proof aprons shall be worn where acids are handled.
7. After use, all equipment shall be thoroughly cleaned and stored in an area free of fluoride dusts. Rubber articles shall be washed in water, and hands shall be washed after the equipment is stored; and, all protective devices, whether for routine or emergency use, shall be inspected periodically and maintained in good operating condition.

Safe Drinking Water Act (SDWA) Introduction

On August 6, 1996, President Clinton signed the Reauthorization of the Safe Drinking Water Act, bringing a successful conclusion to years of work on the part of water professionals and a broad range of public interest groups throughout the nation.

This law strikes a balance among federal, state, local, urban, rural, large and small water systems in a manner that improves the protection of public health and brings reason and good science to the regulatory process.



The major elements of this law include:

- The law updates the standard-setting process by focusing regulations on contaminants known to pose greater public health risks.
- It replaces the current law's demand for 25 new standards every three years with a new process based on occurrence, relative risk and cost-benefit considerations.
- It also requires the EPA to select at least five new candidate contaminants to consider for regulation every five years.
- The EPA is directed to require public water systems to provide customers with annual "**Consumer Confidence Reports**" in newspapers and by direct mail.
- The reports must list levels of regulated contaminants along with Maximum Contaminant Levels (**MCLs**) and Maximum Contaminant Level Goals (**MCLGs**), along with plainly worded definitions of both.
- The reports must also include a plainly worded statement of the health concerns for any contaminants for which there has been a violation, describe the utility's sources of

drinking water and provide data on unregulated contaminants for which monitoring is required, including Cryptosporidium and radon.

- The EPA must establish a toll-free hot line customers can call to get additional information.
- The EPA is required to publish guidelines for states to develop water source assessment programs that delineate protection areas and assess contamination risks.
- The EPA is required to identify technologies that are affordable for small systems to comply with drinking water regulations.
- Technical assistance funds and Small System Technical Assistance Centers are authorized to meet the training and technical needs of small systems.
- States are authorized to grant variances for compliance with drinking water regulations for systems serving 3,300 or fewer persons.
- The EPA is required to publish certification guidelines for operators of community and nontransient noncommunity public water systems.
- States that do not have operator certification programs that meet the requirements of the guidelines will lose 20 percent of their SRLF grant.
- A source water petition program for voluntary, incentive-based partnerships among public water systems and others to reduce contamination in source water is authorized.
- The law establishes a new State Revolving Loan Fund (**SRLF**) of $1 billion per year to provide loans to public water systems to comply with the new SDWA.
- It also requires states to allocate 15 percent of the SRLF to systems serving 10,000 or fewer people unless no eligible projects are available for loans.
- It also allows states to jointly administer SDWA and Clean Water Act loan programs and transfer up to 33 percent between the two accounts.
- States must ensure that all new systems have compliance capacity and that all current systems maintain capacity, or lose 20 percent of their SRLF grant.

Although the EPA will continue to provide policy, regulations and guidance, state governments will now have more regulatory flexibility allowing for improved communication between water providers and their local regulators. Increased collaboration will result in solutions that work better and are more fully supported by the regulated community. States that have a source water assessment program may adopt alternative monitoring requirements to provide permanent monitoring relief for public water systems in accordance with EPA guidance.

Risk Assessment

P.L. 104-182 adds risk assessment and communication provisions to SDWA. When developing regulations, the EPA is now required to: (1) use the best available, peer-reviewed science and supporting studies and data; and (2) make publicly available a risk assessment document that discusses estimated risks, uncertainties, and studies used in the assessment. When proposing drinking water regulations, the EPA must publish a health risk reduction and cost analysis. The law permits the EPA to promulgate an interim standard without first preparing a benefit-cost analysis or making a determination as to whether the benefits of a regulation would justify the costs if the EPA determines that a contaminant presents an urgent threat to public health.

New regulations generally become effective 3 years after promulgation. Up to 2 additional years may be allowed if the EPA (or a state in the case of an individual system) determines the time is needed for capital improvements. Section 1412 includes specific provisions for arsenic, sulfate, and radon. The law authorizes states to grant Systems variances from a regulation if raw water quality prevents meeting the standards despite application of the best technology (Section 1415). A new provision authorizes small system variances based on best affordable technology.

States may grant these variances to systems serving 3,300 or fewer persons if the system cannot afford to comply (through treatment, an alternative water source, or restructuring) and the variance ensures adequate protection of public health; states may grant variances to systems serving between 3,300 and 10,000 persons with EPA approval. To receive a small system variance, the system must install a variance technology identified by the EPA. The variance technology need not meet the MCL, but must protect public health. The EPA must identify variance technologies for existing regulations. Variances are not available for microbial contaminants. The Act also provides for exemptions if a regulation cannot be met for other compelling reasons (including costs) and if the system was in operation before the effective date of a standard or treatment requirement (Section 1416). An exemption is intended to give a public water system more time to comply with a regulation and can be issued only if it will not result in an unreasonable health risk. Small systems may receive exemptions for up to 9 years.

State Primacy
The primary enforcement responsibility for public water systems lies with the states, provided they adopt regulations as stringent as the national requirements, adopt authority for administrative penalties, develop adequate procedures for enforcement, maintain records, and create a plan for providing emergency water supplies (Section 1413). Currently, 55 of 57 states and territories have primacy authority. P.L. 104-182 authorizes $100 million annually for EPA to make grants to states to carry out the public water system supervision program. States may also use a portion of their SRF grant for this purpose (Section 1443).

Whenever the EPA finds that a public water system in a state with primary enforcement authority does not comply with regulations, the Agency must notify the state and the system and provide assistance to bring the system into compliance. If the state fails to commence enforcement action within 30 days after the notification, the EPA is authorized to issue an administrative order or commence a civil action.

Nonprimacy State
In a non-primacy state, the EPA must notify an elected local official (if any has jurisdiction over the water system) before commencing an enforcement action against the system (Section 1414). Primacy states may establish alternative monitoring requirements to provide interim monitoring relief for systems serving 10,000 or fewer persons for most contaminants, if a contaminant is not detected in the first quarterly sample. States with approved source water protection programs may adopt alternative monitoring requirements to provide permanent monitoring relief to qualified systems for chemical contaminants (Section 1418).

P.L. 104-182 requires states to adopt programs for training and certifying operators of community and nontransient noncommunity systems. The EPA must publish guidelines specifying minimum standards for operator certification by February 1999. Two years thereafter, the EPA must withhold 20% of a state's SRF grant unless the state has an operator certification program (Section 1419). States are also required to establish capacity development programs based on EPA guidance.

State programs must include: 1) legal authority to ensure that new systems have the technical, financial, and managerial capacity to meet SDWA requirements; and 2) a strategy to assist existing systems that are experiencing difficulties to come into compliance. Beginning in 2001, the EPA is required to withhold a portion of SRF grants from states that do not have compliance development strategies (Section 1420).

Underground Injection Control
Another provision of the Act requires the EPA to promulgate regulations for state underground injection control (**UIC**) programs to protect underground sources of drinking water. These regulations contain minimum requirements for the underground injection of wastes in five well classes to protect underground sources of drinking water and to require that a state prohibit, by December 1977, any underground injection that was not authorized by state permit (Section 1421).

Ground Water Protection Grant Programs
The Act contains three additional ground water protection programs. Added in 1986, Section 1427 established procedures for demonstration programs to develop, implement, and assess critical aquifer protection areas already designated by the Administrator as sole source aquifers. Section 1428, also added in 1986, and established an elective state program for protecting wellhead areas around public water system wells.

If a state established a wellhead protection program by 1989, and the EPA approved the state's program, then the EPA may award grants covering between 50% and 90% of the costs of implementing the program. Section 1429, added by P.L. 104-182, authorizes the EPA to make 50% grants to states to develop programs to ensure coordinated and comprehensive protection of ground water within the states. Appropriations for these three programs and for LYIC state program grants are authorized starting back in FY2003.

Source Water Protection Programs
P.L. 104-182 broadens the pollution prevention focus of the Act to embrace surface water as well as ground water protection. New Section 1453 directs the EPA to publish guidance for states to implement source water assessment programs that delineate boundaries of assessment areas from which systems receive their water, and identify the origins of contaminants in delineated areas to determine systems' susceptibility to contamination. States with approved assessment programs may adopt alternative monitoring requirements to provide systems with monitoring relief under Section 1418.

New Section 1454 authorizes a source water petition program based on voluntary partnerships between state and local governments. States may establish a program under which a community water system or local government may submit a source water quality partnership petition to the state requesting assistance in developing a voluntary partnership to: (1) reduce the presence of contaminants in drinking water; (2) receive financial or technical assistance; and (3) develop a long-term source water protection strategy. This section authorizes $5 million each year for grants to states to support petition programs. Also, states may use up to 10% of their annual SRF capitalization grant for the source water assessment activities or for the petition program.

State Revolving Funds
Section 1452, added by P.L. 104-182 authorizes a State Revolving Loan Fund (**SRF**) program to help systems finance improvements needed to comply with drinking water regulations. The law authorizes the EPA to make grants to states to capitalize SDWA SRFs, which states then use to make loans to public water systems. States must match 20% of the federal grant.

Grants will be allotted to states using the formula for distributing state PWSS grants through FY1997; then, grants will be allotted based on a needs survey. Each state will receive at least 1% of funds.

Drinking water SRFs may be used to provide loan and grant assistance for expenditures that the EPA has determined will facilitate compliance or significantly further the Act's health protection objectives. States must make available 15% of their annual allotment for loan assistance to systems that serve 10,000 or fewer persons. States may use up to 30% of their SRF grant to provide grants or forgive loan principle to help economically disadvantaged communities. Also, states may use a portion of funds for technical assistance, source water protection and capacity development programs, and for operator certification.

Other Provisions

Public water systems must notify customers of violations with potential for serious health effects within 24 hours. Systems must also issue to customers' annual reports on contaminants detected in their drinking water (Section 1414). Section 1417 requires any pipe, solder, or flux used in the installation or repair of public water systems or of plumbing in residential or nonresidential facilities providing drinking water to be "**lead free**" (as defined in the Act).

As of August 1998, it will be unlawful to sell pipes, plumbing fittings or fixtures that are not "**lead free**" or to sell solder or flux that is not lead free (unless it is properly labeled); with the exception of pipes used in manufacturing or industrial processing. P.L. 104-182 sets limits on the amount of lead that may leach from new plumbing fixtures, and allows one year for a voluntary standard to be established before requiring EPA to take regulatory action.

The Administrator has emergency powers to issue orders and commence civil action if a contaminant likely to enter a public drinking water supply system poses a substantial threat to public health and state or local officials have not taken adequate action (Section 1431).

If a chemical necessary for water treatment is not reasonably available, the Administrator can issue a "***certification of need***," in which case the President can order an allocation of the chemical to those needing it (Section 1441).

EPA is provided authority to conduct research, studies, and demonstrations related to the causes, treatment, control, and prevention of diseases resulting from contaminants in water. The Agency is directed to provide technical assistance to the states and municipalities in administering their public water system regulatory responsibilities. The law authorizes annually, $15 million for technical assistance to small systems and Indian Tribes, and $25 million for health effects research (Section 1442). P.L. 104-182 authorizes additional appropriations for drinking water research, not to exceed $26.6 million annually.

The Administrator may make grants to develop and demonstrate new technologies for providing safe drinking water and to investigate health implications involved in the reclamation/reuse of waste waters (Section 1444).

Also, suppliers of water who may be subject to regulation under the Act are required to establish and maintain records, monitor, and provide any information that the Administrator requires to carry out the requirements of the Act (Section 1445).

The Administrator may also enter and inspect the property of water suppliers to enable him/her to carry out the purposes of the Act. Failure to comply with these provisions may result in criminal penalties.

The Act established a National Drinking Water Advisory Council, composed of 15 members (with at least 2 representing rural systems), to advise, consult, and make recommendations to the Administrator on activities and policies derived from the Act (Section 1446).

National Security
Any federal agency having jurisdiction over federally owned and maintained public water systems must comply with all federal, state, and local drinking water requirements, as well as any underground injection control programs (Section 1447). The Act provides for waivers in the interest of national security. Procedures for judicial review are outlined (Section 1448), and provision for citizens' civil actions is made (Section 1449).

Three Types of Public Water Systems

Community Water Systems (CWSs)

- Provide water to the same population year-round (for example: homes, apartment buildings)
- Approximately 52,000 systems serving the majority of the U.S. population

Non-Transient Non-Community Water Systems (NTNCWSs)

- Provide water to the same people at least six months a year, but not all year (for example: schools, factories, churches, office buildings that have their own water system)
- Approximately 85,000 systems

Transient Non-Community Water System (TNCWS)

- Provide water where people do not remain for long periods of time (for example: gas stations, campgrounds)
- Approximately 18,000 systems

SDWA MCLs Introduction

Radionuclides

Alpha Emitters Certain minerals are radioactive and may emit a form of radiation known as alpha radiation. Some people who drink water containing alpha emitters in excess of EPA standards over many years may have an increased risk of getting cancer.

Beta/photon Emitters Certain minerals are radioactive and may emit forms of radiation known as photons and beta radiation. Some people who drink water containing beta and photon emitters in excess of EPA standards over many years may have an increased risk of getting cancer.

Combined Radium 226/228 Some people who drink water containing radium 226 or 228 in excess of EPA standards over many years may have an increased risk of getting cancer.

Radon gas can dissolve and accumulate in underground water sources, such as wells, and concentrate in the air in your home. Breathing radon can cause lung cancer. Drinking water containing radon presents a risk of developing cancer. Radon in air is more dangerous than radon in water. Radon in water is typically released into the air while showering.

Water Sampling Bottles

These are commonly found examples of various water sampling bottles. VOC and THM bottles are in the front.

You will have to make sure there is absolutely no air inside these tiny bottles. Any air bubble can ruin the sample. There are several ways to get the air out. The best one is slowly overfill the bottle to get a reverse meniscus. Second, is to fill the cap with water before screwing it onto the bottle. The third one is to use a thin copper tube and slowly fill the bottle.

Inorganic Contaminants

Antimony	Cadmium	Cyanide	Nitrite
Asbestos	Chromium	Mercury	Selenium
Barium	Copper	Nitrate	Thallium
Beryllium			

Inorganic Contaminants

Arsenic. Some people who drink water containing arsenic in excess of EPA standards over many years could experience skin damage or problems with their circulatory system, and may have an increased risk of getting cancer.

Fluoride. Many communities add fluoride to their drinking water to promote dental health. Each community makes its own decision about whether or not to add fluoride. The EPA has set an enforceable drinking water standard for fluoride of 4 mg/L. Some people who drink water-containing fluoride in excess of this MCL level over many years could get bone disease, including pain and tenderness of the bones. The EPA has also set a secondary fluoride standard of 2 mg/L to protect against dental fluorosis.

Dental fluorosis, in its moderate or severe forms, may result in a brown staining and/or pitting of the permanent teeth. This problem occurs only in developing teeth, before they erupt from the gums. Children under nine should not drink water that has more than 2 mg/L of fluoride.

Lead. Typically leaches into water from plumbing in older buildings. Lead pipes and plumbing fittings have been banned since August 1998. Children and pregnant women are most susceptible to lead health risks. For advice on avoiding lead, see the EPA's "*Lead in Your Drinking Water"* fact sheet.

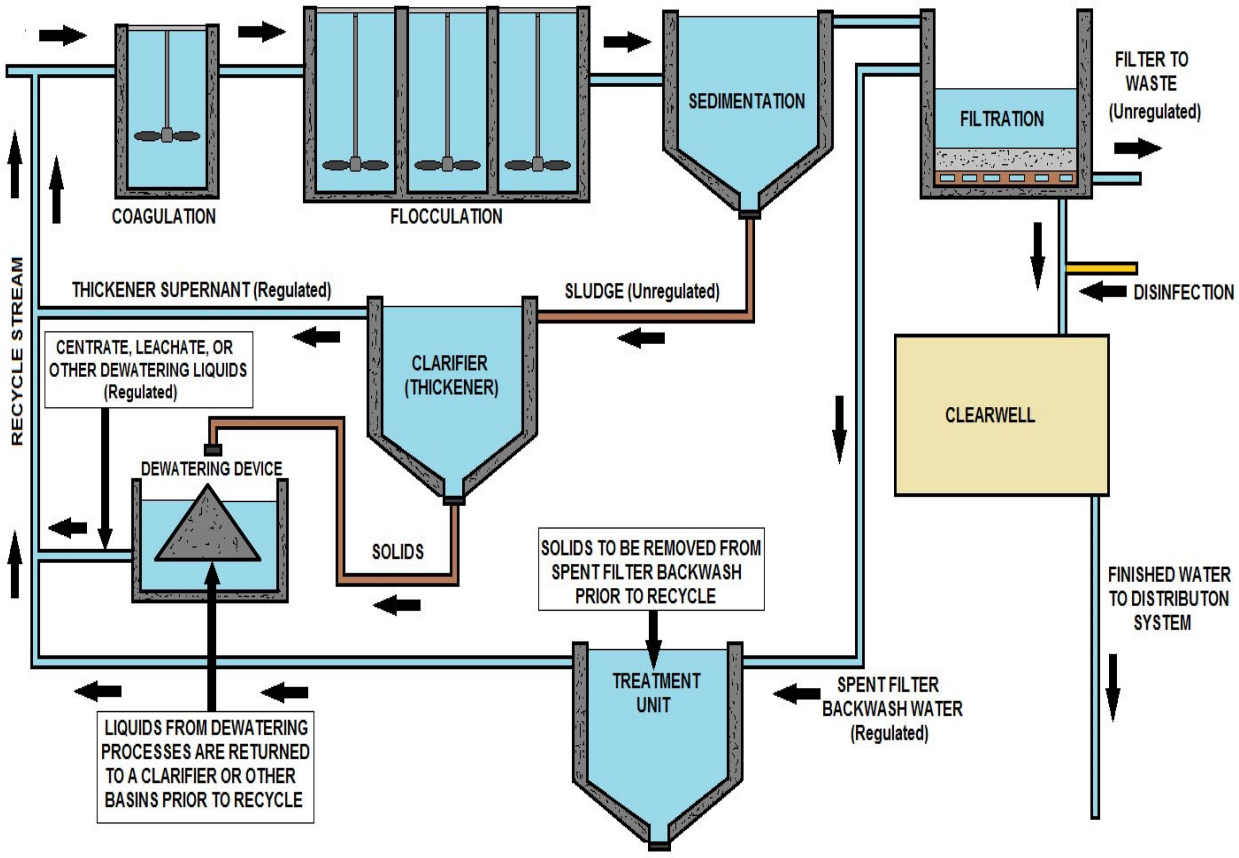
Synthetic Organic Contaminants, including Pesticides & Herbicides

2,4-D	Dibromochloropropane	Hexachlorobenzene
2,4,5-TP (Silvex)	Dinoseb	Hexachlorocyclopentadiene
Acrylamide	Dioxin (2,3,7,8-TCDD)	Lindane
Alachlor	Diquat	Methoxychlor
Atrazine	Endothall	Oxamyl [Vydate]
Benzoapyrene	Endrin	PCBs [Polychlorinated biphenyls]
Carbofuran	Epichlorohydrin	Pentachlorophenol
Chlordane	Ethylene dibromide	Picloram
Dalapon	Glyphosate	Simazine
Di 2-ethylhexyl adipate	Heptachlor	Toxaphene
Di 2-ethylhexyl phthalate	Heptachlor epoxide	

Volatile Organic Contaminants

Benzene	trans-1,2-Dicholoroethylene	1,2,4-Trichlorobenzene
Carbon Tetrachloride	Dichloromethane	1,1,1,-Trichloroethane
Chlorobenzene	1,2-Dichloroethane	1,1,2-Trichloroethane
o-Dichlorobenzene	1,2-Dichloropropane	Trichloroethylene
p-Dichlorobenzene	Ethylbenzene	Toluene
1,1-Dichloroethylene	Styrene	Vinyl Chloride
cis-1,2-Dichloroethylene	Tetrachloroethylene	Xylenes

Other Related EPA Water Treatment Rules



Filter Backwash Recycling Rule (FBRR)

The Filter Backwash Recycling Rule (FBRR) regulates the recycling of filter backwash water within the treatment process of public water systems. The FBRR requires surface water systems to review their recycle practices and to modify any recycle practices that may compromise microbial control or contribute to violations of the drinking water regulations. Recycle flows can be a source of concentrated microbial pathogens and chemical contaminants.

IESWTR

The Interim Enhanced Surface Water Treatment Rule (IESWTR) builds on the requirements of the Surface Water Treatment Rule. IESWTR specifies treatment requirements to address *Cryptosporidium* and other microbial contaminants in public water systems serving 10,000 or more persons.

The rule balances the need for treatment with potential increases in disinfection by-products. The materials found on this page are intended to assist public water systems and states in the implementation of the IESWTR.

Arsenic
Arsenic is an element that occurs naturally in the earth's crust. When certain rocks, minerals, and soil erode, they release arsenic into water supplies. When people either drink this water or eat animals and plants that drink it, they are exposed to arsenic. In the U.S., eating and drinking are the most common ways that people are exposed to arsenic, although it can also come from industrial sources. Studies have linked long-term exposure of arsenic in drinking water to a variety of cancers in humans.

To protect human health, an EPA standard limits the amount of arsenic in drinking water. Back in January 2001, the EPA revised the standard from 50 parts per billion (**ppb**), ordering that it fall to 10 ppb in 2006.

After adopting 10 ppb as the new standard for arsenic in drinking water, the EPA decided to review the decision to ensure that the final standard was based on sound science and accurate estimates of costs and benefits. In October 2001, the EPA decided to move forward with implementing the 10 ppb standard for arsenic in drinking water.

More information on the rulemaking process and the costs and benefits of setting the arsenic limit in drinking water at 10 ppb can be found at www.epa.gov/safewater/arsenic.html.

ICR Information Collection Rule
The EPA has collected data required by the Information Collection Rule (**ICR**) to support future regulation of microbial contaminants, disinfectants, and disinfection byproducts. The rule is intended to provide the EPA with information on chemical byproducts that form when disinfectants used for microbial control react with chemicals already present in source water (disinfection byproducts (DBPs)); disease-causing microorganisms (pathogens), including Cryptosporidium; and engineering data to control these contaminants.

Drinking water microbial and disinfection byproduct information collected for the ICR is now available in the EPA's *Envirofacts Warehouse*.

Gas Chromatograph
Used for micro-contaminant water analysis.

Commonly Found Distribution System Water Quality Problems

Turbidity

Turbidity is caused by particles suspended in water. These particles scatter or reflect light rays, making the water appear cloudy. Turbidity is expressed in nephelometric turbidity units (ntu) and a reading in excess of 5 ntu is generally noticeable to water system customers.

Besides the appearance being unpleasant to customers, turbidity in water is significant from a public health standpoint because suspended particles could shelter microorganisms from the disinfectant and allow them to still be viable when they reach the customer.

EPA regulations direct that, for most water systems, the turbidity of water entering the distribution system must be equal or less than 0.5 ntu in at least 95 percent of the measurements taken each month. At no time may the turbidity exceed 5 ntu.

Turbidity changes in the distribution system can indicate developing problems. Increases in turbidity may be caused by changes in velocity or inadequate flushing following main replacement or repairs.

Hardness

Hardness is a measure of the concentration of calcium and magnesium in water. Water hardness usually comes from water contacting rock formations, such as water from wells in limestone formations. Soft ground water may occur where topsoil is thin and limestone formations are sparse or absent. Most surface water is of medium hardness.

Hard and soft water are both satisfactory for human consumption, but customers may object to very hard water because of the scale it forms in plumbing fixtures and on cooking utensils. Hardness is also a problem for some industrial and commercial users because of scale buildup in boilers and other equipment.

Water generally is considered most satisfactory for household use when the hardness is between 75 and 100 mg/L as calcium carbonate ($CaCO_3$). Water with 300 mg/L of hardness usually is considered **hard**. Very soft water of 30 mg/L or less is found in some section of the United States. Soft water usually is quite corrosive, and may have to be treated to reduce the corrosivity.

Iron

Iron occurs naturally in rocks and soils and is one of the most abundant elements. It occurs in two forms. Ferrous iron (Fe^{+2}) is in a dissolved state, and water containing ferrous iron is colorless. Ferric iron (Fe^{+3}) has been oxidized, and water containing it is rust-colored.

Water from some well sources contains significant levels of dissolved iron, which is colorless, but rapidly turns brown as air reaches the water and oxidizes the iron.

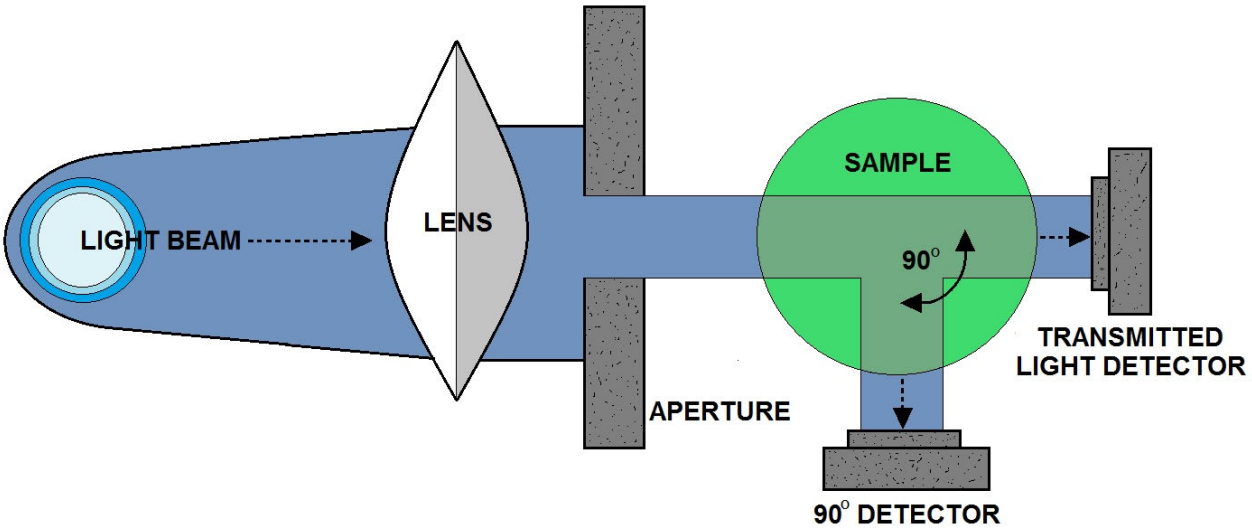
There are no known harmful effects to humans from drinking water containing iron, but NSDWR suggest a limit of 0.5 mg/L. At high levels, the staining of plumbing fixtures and clothing becomes objectionable. Iron also provides nutrient source for some bacteria that grow in distribution systems and wells. Iron bacteria, such as Gallionella, cause red water, tastes and odors, clogged pipes, and pump failure.

Whenever tests on water samples show increased iron concentrations between the point where water enters the distribution system and the consumer's tap, either corrosion, iron bacteria, or both are probably taking place. If the problem is caused by bacteria, flushing mains, shock chlorination, and carrying increased residual chlorine are alternatives to consider.

Manganese
Manganese in ground water creates problems similar to iron. It does not usually discolor the water, but will stain washed clothes and plumbing fixtures black; this is very unpopular with customers. Consumption of manganese has no known harmful effects on humans, but the NSDWR recommend a concentration not to exceed 0.05 mg/L to avoid customer complaints.

Water Quality Safeguards
The **critical** safeguard for water distribution system operations are

- continuous positive pressure in the mains; 20 pounds per square inch (psi) minimum residual pressure is recommended;
- maintenance of chlorine residual;
- cross-connection control; and
- frequent testing.



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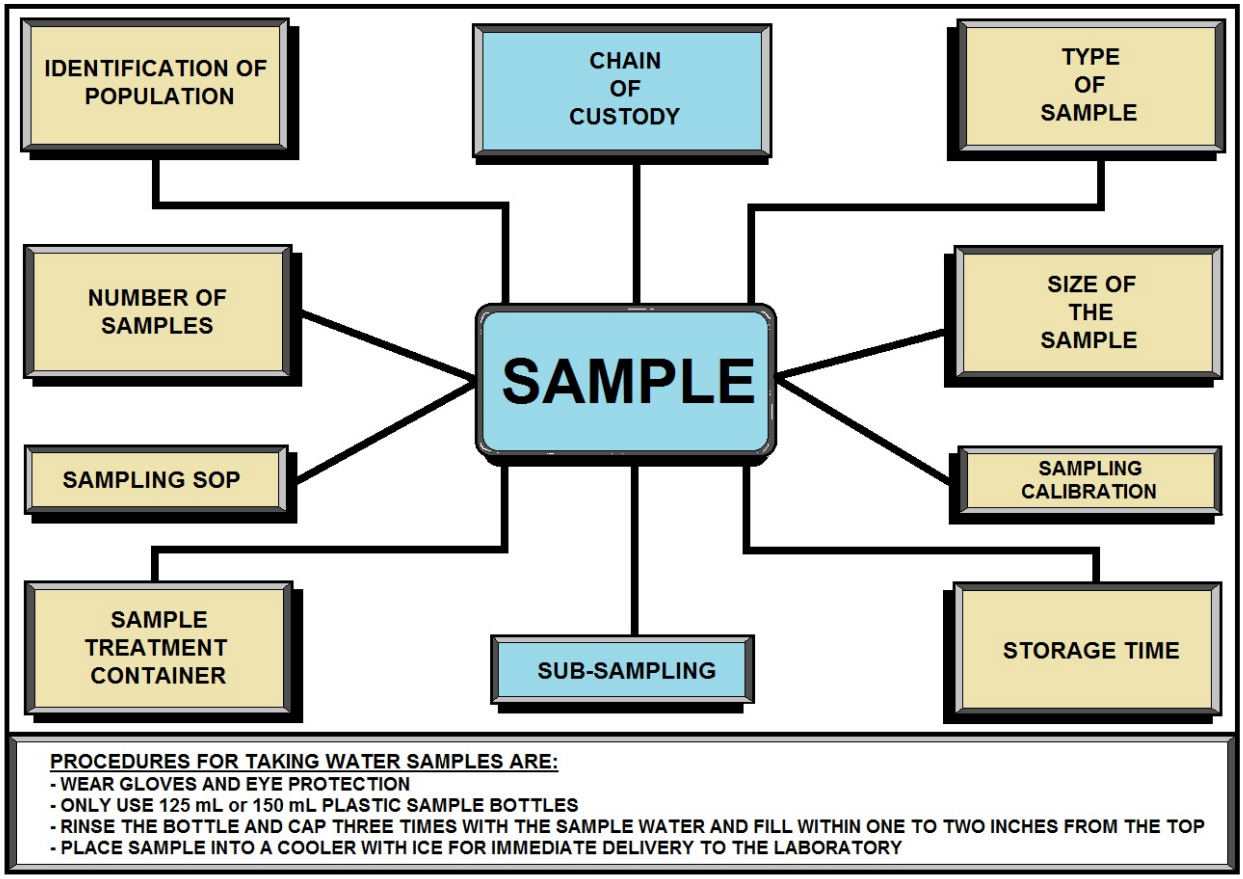
BASICS OF A TURBIDIMETER

Sampling Plan Introduction

A written sampling plan must be developed by the water system. These plans will be reviewed by the Health Department or State Drinking Water agency during routine field visits for sanitary surveys or technical assistance visits. This plan should include:

1. The location of routine sampling sites on a system distribution map. You will need to locate more routine sampling sites than the number of samples required per month or quarter. A minimum of three sites is advised and the sites should be rotated on a regular basis.
2. Map the location of repeat sampling sites for the routine sampling sites. Remember that repeat samples must be collected within five (5) connections upstream and downstream from the routine sample sites.
3. Establish a sampling frequency of the routine sites.
4. Sampling technique, establish a minimum flushing time and requirements for free chlorine residuals at the sites (if you chlorinate continuously).

The sampling sites should be representative of the distribution network and pressure zones. If someone else, e.g., the lab, collects samples for you, you should provide them with a copy of your sampling plan and make sure they have access to all sample sites.



PROPER SAMPLING PROCEDURES (WATER)

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WATER TESTING LAB
456 SOMEWHERE ST.
ANYWHERE, AZ 85002

TEST REPORT:

WATER COMPANY
123 ANYWHERE ST.
SOMEWHERE, AZ 85001

DRINKING WATER ANALYSIS RESULTS

FOR MODEL : RO103TDS

NOTE:
ND THIS CONTAMINANT WAS NOT DETECTED AT OR ABOVE OUR STATED DETECTION LEVEL
NBS NO BACTERIA SUBMITTED NBR NO BACTERIA REQUIRED
* THE MCL (Maximum Contaminant Level) OR AN ESTABLISHED GUIDELINE HAS BEEN EXCEEDED FOR THIS CONTAMINANT
** BACTERIA RESULTS MAY BE INVALID DUE TO LACK OF COLLECTION INFORMATION OR BECAUSE SAMPLE HAS EXCEEDED THE 30-HOUR HOLDING TIMES

ANALYSIS PERFORMED: P-PRESENCE A - ABSENCE EP - E.COLI PRESENCE EA - E.COLI ABSENCE NA: NOT ANALYZED

ANALYSIS	MCL (mg/l)	Det. Level	Level Detected
TOTAL COLIFORM	P	P	A
INORGANIC CHEMICALS - Metals			
Aluminum	0.2	0.1	ND
Arsenic	0.05	0.020	ND
Barium	2	0.30	ND
Cadmium	0.005	0.002	ND
Chromium	0.1	0.010	ND
Copper	1.3	0.004	ND
Iron	0.3	0.020	ND
Lead	0.015	0.002	ND
Manganese	0.05	0.004	ND
Mercury	0.002	0.001	ND
Nickel	0.1	0.02	ND
Selenium	0.05	0.020	ND
Silver	0.1	0.02	ND
Sodium	-----	1.0	ND
Zinc	5	0.004	ND
INORGANIC CHEMICALS - Other, and Physical Factors			
Alkalinity (Total as CaCO)	-----	0.1	ND
Chloride	250	5.0	ND
Fluoride	4	0.5	ND
Nitrate as N	10	0.5	ND
Nitrite as N	1	0.5	ND
Sulfate	250	5.0	ND
Hardness (suggested limit - 100)		10	ND
pH (Standard Units)	6.5 - 8.5	-----	7.7
Total Dissolved Solids	500	20	ND
Turbidity (Turbidity Units)	1.0	0.1	ND
ORGANIC CHEMICALS - Trihalomethanes:			
T THMS	0.080	0.004	ND

WATER ANALYSIS REPORT EXAMPLE

More on Evolving Disinfection Rules

In the past 40 years, the Safe Drinking Water Act (SDWA) has been highly effective in protecting public health and has also evolved to respond to new and emerging threats to safe drinking water. Disinfection of drinking water is one of the major public health advances in the 20th century. One hundred years ago, typhoid, dysentery and cholera epidemics were common through American cities; disinfection was a major factor in reducing these epidemics.

However, the disinfectants themselves can react with naturally-occurring materials in the water to form unintended byproducts which may pose health risks. In addition, in the past thirty years though, we have learned that there are specific microbial pathogens, such as *Cryptosporidium*, which can cause illness and is resistant to traditional disinfection practices.

Chlorine is the most widely used water disinfectant due to its effectiveness and cost. Most states require community water systems to use chlorination. However, research shows that chlorine has side effects. It reacts with organic matter present in water and forms a series of compounds that have been linked to cancer in animals.

These compounds are called disinfection by-products (DBPs). All disinfectants form DBPs in one of two reactions:
(1) chorine and chlorine-based compounds (halogens) react with organics in water causing the chlorine atom to substitute other atoms, resulting in halogenated by-products and
(2) oxidation reactions, where chlorine oxidizes compounds present in water. Secondary by-products are also formed when multiple disinfectants are used.

All living organisms have carbon as an essential element in their cells. When trees shed their leaves, the leaves start decomposing and are ultimately broken down by bacteria into carbon-containing compounds. Similarly, dead animals on land and fish and other aquatic life decompose and disintegrate into compounds that contain carbon as an essential element. Hence, all surface water and groundwater contain varying amounts of carbon-containing compounds called organic matter (primarily humic and fulvic acids).

The EPA Surface Water Treatment Rule (SWTR) requires systems using public water supplies from either surface water or groundwater under the direct influence of surface water to disinfect. In addition, since some disinfectants produce chemical by-products, the dual objective of disinfection is to provide the required level of organism destruction and remain within the maximum contaminant level (MCL) for the SWTR disinfection set by EPA. An MCL is set for Total Trihalomethanes and additional disinfection byproducts.

What are the microbial/disinfection byproducts (MDBP) rules and which ones apply to me?
The MDBP requirements have been in place for close to 30 years and include the following federal rules:

- Total Trihalomethanes monitoring and MCL, promulgated Nov 1979
- Surface Water Treatment Rule, promulgated June 1989
- Interim Enhanced Surface Water Treatment Rule and Stage 1 Disinfectants / Disinfection Byproducts Rule, promulgated Dec 1998
- Filter Backwash Rule, promulgated June 2001
- Long Term 1 Enhanced Surface Water Treatment Rule, promulgated Jan 2002
- Long Term 2 Enhanced Surface Water Treatment Rule and Stage 2 Disinfectants / Disinfection Byproducts Rule, promulgated Jan 2006
- Groundwater Rule, promulgated Nov 2006

The Disinfectants and Disinfection Byproducts (DBP) rules apply to all community and non-community water systems using a disinfectant such as chlorine, chloramines, ozone and chlorine dioxide.

Compliance with the Stage 1 DBP requirements began in 2000. The Stage 2 DBP requirements began in 2006 with the Initial Distribution System Evaluation (IDSE). Compliance monitoring for the Stage 2 DBP begins in April 2012. See phased compliance schedule dependent on system population below.

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2) rule applies to all water systems using surface water, groundwater under the influence of a surface water, as well as groundwater/surface water blends. The LT2 requirements began in 2006 with the characterization of raw water Cryptosporidium and E.coli levels. Systems serving <10,000 monitor for E.coli only every two weeks for one year. Compliance with the LT2 requirements begin in April 2013.

The Groundwater Rule (GWR) applies to all public water systems using groundwater. The GWR requirements begin in March 2009 with 6-months investigative monitoring (IM) for source water E.coli, for systems currently applying disinfection only. All other requirements for the GWR began back in Dec 2009.

Amendments to the SDWA in 1996 require EPA to develop rules to balance the risks between microbial pathogens and disinfection byproducts (DBPs). It is important to strengthen protection against microbial contaminants, especially *Cryptosporidium*, and at the same time, reduce potential health risks of DBPs.

The Stage 1 Disinfectants and Disinfection Byproducts Rule and Interim Enhanced Surface Water Treatment Rule, announced in December 1998, are the first of a set of rules under the 1996 SDWA Amendments. This fact sheet focuses on the Stage 1 Disinfectants and Disinfection Byproducts Rule. A separate fact sheet focuses on the Interim Enhanced Surface Water Treatment Rule (EPA 815-F-98-009).

Public Health Concerns

While disinfectants are effective in controlling many microorganisms, they react with natural organic and inorganic matter in source water and distribution systems to form DBPs. Results from toxicology studies have shown several DBPs (e.g., bromodichloromethane, bromoform, chloroform, dichloroacetic acid, and bromate) to be carcinogenic in laboratory animals.

Other DBPs (e.g., chlorite, bromodichloromethane, and certain haloacetic acids) have also been shown to cause adverse reproductive or developmental effects in laboratory animals.

Several epidemiology studies have suggested a weak association between certain cancers (e.g., bladder) or reproductive and developmental effects, and exposure to chlorinated surface water. More than 200 million people consume water that has been disinfected. Because of the large population exposed, health risks associated with DBPs, even if small, need to be taken seriously.

Disinfection Byproduct Research and Regulations Summary

Drinking water chlorination has contributed to a dramatic decline in waterborne disease rates and increased life expectancy in the United States. Largely because of this success, many Americans take it for granted that their tap water will be free of disease-causing organisms.

In recent years, regulators and the public have focused greater attention on potential health risks from chemical contaminants in drinking water. One such concern relates to disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with certain organic matter in water.

In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. Concerned that these chemicals may be carcinogenic to humans, EPA set the first regulatory limits for THMs in 1979. Since that time, a wealth of research has improved our understanding of how DBPs are formed, their potential health risks, and how they can be controlled. It is now recognized that all chemical disinfectants form some potentially harmful byproducts. The byproducts of chlorine disinfection are by far the most thoroughly studied.

While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formations are available and should be adopted where possible.

The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts.

Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic matter prior to disinfection, while ensuring that microbial protection remains the top priority.

Based largely on these animal data, EPA considers individual THMs and HAAs to be either possible or probable human carcinogens, although any risk from the low levels found in drinking water would be slight. After reviewing the full body of toxicology studies, the IPCS concluded, “None of the chlorination byproducts studied to date is a potent carcinogen at concentrations normally found in drinking water” (IPCS 2000, p. 376).

Some epidemiology studies have reported an association between human exposure to DBPs and elevated cancer risks, while other studies have found no association. EPA evaluated the existing cancer epidemiology studies and found that only for bladder cancer were associations with chlorinated water somewhat consistent.

Even in these studies, cancer risks were not strongly correlated to measured THM levels, indicating that other factors cannot be ruled out (Craun et al., 2001). EPA has concluded, “The present epidemiologic data do not support a causal relationship between exposure to chlorinated drinking water and development of cancer at this time” (EPA 1998). The IPCS reached a similar conclusion in 2000, noting that a causal relationship between DBPs and increased cancer remains an open question (IPCS 2000).

Balancing DBP and Microbial Risks
Continuing evidence of waterborne disease occurrence suggests that microbial risks should receive a much higher level of attention than disinfection byproducts. For this reason, The American Academy of Microbiology (Ford and Colwell, 1996) has recommended, the health risks posed by microbial pathogens should be placed as the highest priority in water treatment to protect public health. A report published by the International Society of Regulatory Toxicology and Pharmacology (Coulston and Kolbye, 1994) stated "The reduction in mortality due to waterborne infectious diseases, attributed largely to chlorination of potable water supplies, appears to outweigh any theoretical cancer risks (which may be as low as zero) posed by the minute quantities of chlorinated organic chemicals reported in drinking waters disinfected with chlorine."

The IPCS (IPCS 2000, p. 375) reached similar conclusions:
Disinfection is unquestionably the most important step in the treatment of water for drinking water supplies. The microbial quality of drinking water should not be compromised because of concern over the potential long-term effects of disinfectants and DBPs. The risk of illness and death resulting from exposure to pathogens in drinking water is very much greater than the risks from disinfectants and DBPs.

Controlling Disinfection Byproducts
Treatment techniques are available that provide water suppliers the opportunity to maximize potable water safety and quality while minimizing the risk of DBP risks. Generally, the best approach to reduce DBP formation is to remove natural organic matter precursors prior to disinfection. EPA has published a guidance document for water system operators entitled, Controlling Disinfection byproducts and Microbial Contaminants in Drinking Water (EPA, 2001).

The EPA guidance discusses three processes to effectively remove natural organic matter prior to disinfection:

1. Coagulation and Clarification
Most treatment plants optimize their coagulation process for turbidity (particle) removal. However, coagulation processes can also be optimized for natural organic matter removal with higher doses of inorganic coagulants (such as alum or iron salts), and optimization of pH.

2. Absorption
Activated carbon can be used to absorb soluble organics that react with disinfectants to form byproducts.

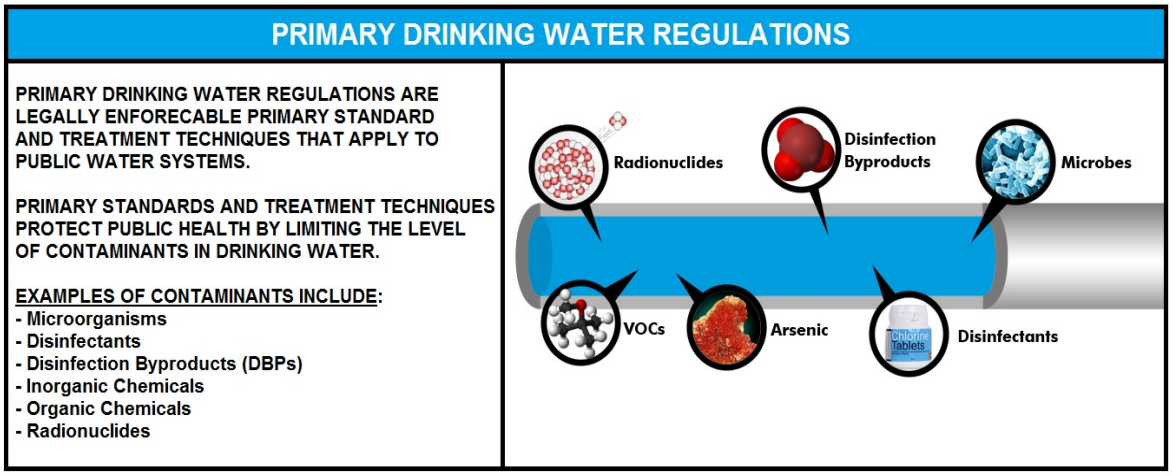
3. Membrane Technology
Membranes, used historically to desalinate brackish waters, have also demonstrated excellent removal of natural organic matter. Membrane processes use hydraulic pressure to force water through a semi-permeable membrane that rejects most contaminants. Variations of this technology include reverse osmosis (RO), nanofiltration (low pressure RO), and microfiltration (comparable to conventional sand filtration).

Other conventional methods of reducing DBP formation include changing the point of chlorination and using chloramines for residual disinfection. EPA predicted that most water systems will be able to achieve compliance with new DBP regulations through the use of one or more of these relatively low cost methods (EPA, 1998). Water system managers may also consider switching from chlorine to alternative disinfectants to reduce formation of THMs and HAAs.

National Primary Drinking Water Regulations

Inorganic Chemicals	MCLG [1] (mg/L) [4]	MCL[2] or TT[3] (mg/L) [4]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Antimony	0.006	0.006	Increase in blood cholesterol; decrease in blood glucose	Discharge from petroleum refineries; fire retardants; ceramics; electronics; solder
Arsenic	none[5]	0.010	Skin damage; circulatory system problems; increased risk of cancer	Discharge from semiconductor manufacturing; petroleum refining; wood preservatives; animal feed additives; herbicides; erosion of natural deposits
Asbestos (fiber >10 micrometers)	7 million fibers per Liter	7 MFL	Increased risk of developing benign intestinal polyps	Decay of asbestos cement in water mains; erosion of natural deposits
Barium	2	2	Increase in blood pressure	Discharge of drilling wastes; discharge from metal refineries; erosion of natural deposits
Beryllium	0.004	0.004	Intestinal lesions	Discharge from metal refineries and coal-burning factories; discharge from electrical, aerospace, and defense industries
Cadmium	0.005	0.005	Kidney damage	Corrosion of galvanized pipes; erosion of natural deposits; discharge from metal refineries; runoff from waste batteries and paints
Chromium (total)	0.1	0.1	Some people who use water containing chromium well in excess of the MCL over many years could experience allergic dermatitis	Discharge from steel and pulp mills; erosion of natural deposits
Copper	1.3	Action Level= 1.3; TT[6]	Short term exposure: Gastrointestinal distress. Long term exposure: Liver or kidney damage. Those with Wilson's Disease should consult their personal doctor if their water systems exceed the copper action level.	Corrosion of household plumbing systems; erosion of natural deposits; leaching from wood preservatives
Cyanide (as free cyanide)	0.2	0.2	Nerve damage or thyroid problems	Discharge from steel/metal factories; discharge from plastic and fertilizer factories
Fluoride	4.0	4.0	Bone disease (pain and tenderness of the bones); Children may get mottled teeth.	Water additive which promotes strong teeth; erosion of natural deposits; discharge from fertilizer and aluminum factories
Lead	zero	Action Level= 0.015; TT[6]	Infants and children: Delays in physical or mental development. Adults: Kidney problems; high blood pressure	Corrosion of household plumbing systems; erosion of natural deposits

Inorganic Mercury	0.002	0.002	Kidney damage	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills and cropland
Nitrate (measured as Nitrogen)	10	10	"Blue baby syndrome" in infants under six months - life threatening without immediate medical attention. Symptoms: Infant looks blue and has shortness of breath.	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Nitrite (measured as Nitrogen)	1	1	"Blue baby syndrome" in infants under six months - life threatening without immediate medical attention. Symptoms: Infant looks blue and has shortness of breath.	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Selenium	0.05	0.05	Hair or fingernail loss; numbness in fingers or toes; circulatory problems	Discharge from petroleum refineries; erosion of natural deposits; discharge from mines
Thallium	0.0005	0.002	Hair loss; changes in blood; kidney, intestine, or liver problems	Leaching from ore-processing sites; discharge from electronics, glass, and pharmaceutical companies



Organic Chemicals	MCLG [1] (mg/L) [4]	MCL [2] or TT [3] (mg/L) [4]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Acrylamide	zero	TT[7]	Nervous system or blood problems; increased risk of cancer	Added to water during sewage/wastewater treatment
Alachlor	zero	0.002	Eye, liver, kidney or spleen problems; anemia; increased risk of cancer	Runoff from herbicide used on row crops
Atrazine	0.003	0.003	Cardiovascular system problems; reproductive difficulties	Runoff from herbicide used on row crops
Benzene	zero	0.005	Anemia; decrease in blood platelets; increased risk of cancer	Discharge from factories; leaching from gas storage tanks and landfills
Benzo(a)pyrene	zero	0.0002	Reproductive difficulties; increased risk of cancer	Leaching from linings of water storage tanks and distribution lines
Carbofuran	0.04	0.04	Problems with blood or nervous system; reproductive difficulties.	Leaching of soil fumigant used on rice and alfalfa
Carbon tetrachloride	zero	.005	Liver problems; increased risk of cancer	Discharge from chemical plants and other industrial activities
Chlordane	zero	0.002	Liver or nervous system problems; increased risk of cancer	Residue of banned termiticide
Chlorobenzene	0.1	0.1	Liver or kidney problems	Discharger from chemical and agricultural chemical factories
2,4-D	0.07	0.07	Kidney, liver, or adrenal gland problems	Runoff from herbicide used on row crops
Dalapon	0.2	0.2	Minor kidney changes	Runoff from herbicide used on rights of way
1,2-Dibromo-3-chloropropane (DBCP)	zero	0.0002	Reproductive difficulties; increased risk of cancer	Runoff/leaching from soil fumigant used on soybeans, cotton, pineapples, and orchards
o-Dichlorobenzene	0.6	0.6	Liver, kidney, or circulatory system problems	Discharge from industrial chemical factories
p-Dichlorobenzene	0.075	0.075	Anemia; liver, kidney or spleen damage; changes in blood	Discharge from industrial chemical factories
1,2-Dichloroethane	zero	0.005	Increased risk of cancer	Discharge from industrial chemical factories
1-1-Dichloroethylene	0.007	0.007	Liver problems	Discharge from industrial chemical factories
cis-1, 2-Dichloroethylene	0.07	0.07	Liver problems	Discharge from industrial chemical factories
trans-1,2-Dichloroethylene	0.1	0.1	Liver problems	Discharge from industrial chemical factories
Dichloromethane	zero	0.005	Liver problems; increased risk of cancer	Discharge from pharmaceutical and chemical factories
1-2-Dichloropropane	zero	0.005	Increased risk of cancer	Discharge from industrial chemical factories
Di(2-ethylhexyl)adipate	0.4	0.4	General toxic effects or reproductive difficulties	Leaching from PVC plumbing systems; discharge from chemical factories
Di(2-ethylhexyl)phthalate	zero	0.006	Reproductive difficulties; liver problems; increased risk of cancer	Discharge from rubber and chemical factories

Dinoseb	0.007	0.007	Reproductive difficulties	Runoff from herbicide used on soybeans and vegetables
Dioxin (2,3,7,8-TCDD)	zero	0.00000003	Reproductive difficulties; increased risk of cancer	Emissions from waste incineration and other combustion; discharge from chemical factories
Diquat	0.02	0.02	Cataracts	Runoff from herbicide use
Endothall	0.1	0.1	Stomach and intestinal problems	Runoff from herbicide use
Endrin	0.002	0.002	Nervous system effects	Residue of banned insecticide
Epichlorohydrin	zero	TT[7]	Stomach problems; reproductive difficulties; increased risk of cancer	Discharge from industrial chemical factories; added to water during treatment process
Ethylbenzene	0.7	0.7	Liver or kidney problems	Discharge from petroleum refineries
Ethelyne dibromide	zero	0.00005	Stomach problems; reproductive difficulties; increased risk of cancer	Discharge from petroleum refineries
Glyphosate	0.7	0.7	Kidney problems; reproductive difficulties	Runoff from herbicide use
Heptachlor	zero	0.0004	Liver damage; increased risk of cancer	Residue of banned termiticide
Heptachlor epoxide	zero	0.0002	Liver damage; increased risk of cancer	Breakdown of heptachlor
Hexachlorobenzene	zero	0.001	Liver or kidney problems; reproductive difficulties; increased risk of cancer	Discharge from metal refineries and agricultural chemical factories
Hexachlorocyclopentadiene	0.05	0.05	Kidney or stomach problems	Discharge from chemical factories
Lindane	0.0002	0.0002	Liver or kidney problems	Runoff/leaching from insecticide used on cattle, lumber, gardens
Methoxychlor	0.04	0.04	Reproductive difficulties	Runoff/leaching from insecticide used on fruits, vegetables, alfalfa, livestock
Oxamyl (Vydate)	0.2	0.2	Slight nervous system effects	Runoff/leaching from insecticide used on apples, potatoes, and tomatoes
Polychlorinated biphenyls (PCBs)	zero	0.0005	Skin changes; thymus gland problems; immune deficiencies; reproductive or nervous system difficulties; increased risk of cancer	Runoff from landfills; discharge of waste chemicals
Pentachlorophenol	zero	0.001	Liver or kidney problems; increased risk of cancer	Discharge from wood preserving factories
Picloram	0.5	0.5	Liver problems	Herbicide runoff
Simazine	0.004	0.004	Problems with blood	Herbicide runoff
Styrene	0.1	0.1	Liver, kidney, and circulatory problems	Discharge from rubber and plastic factories; leaching from landfills
Tetrachloroethylene	zero	0.005	Liver problems; increased risk of cancer	Discharge from factories and dry cleaners
Toluene	1	1	Nervous system, kidney, or liver problems	Discharge from petroleum factories
Total Trihalomethanes (TTHMs)	none[5]	0.10	Liver, kidney or central nervous system problems; increased risk of cancer	Byproduct of drinking water disinfection
Toxaphene	zero	0.003	Kidney, liver, or thyroid problems; increased risk of cancer	Runoff/leaching from insecticide used on cotton and cattle
2,4,5-TP (Silvex)	0.05	0.05	Liver problems	Residue of banned herbicide

1,2,4-Trichlorobenzene	0.07	0.07	Changes in adrenal glands	Discharge from textile finishing factories
1,1,1-Trichloroethane	0.20	0.2	Liver, nervous system, or circulatory problems	Discharge from metal degreasing sites and other factories
1,1,2-Trichloroethane	0.003	0.005	Liver, kidney, or immune system problems	Discharge from industrial chemical factories
Trichloroethylene	zero	0.005	Liver problems; increased risk of cancer	Discharge from petroleum refineries
Vinyl chloride	zero	0.002	Increased risk of cancer	Leaching from PVC pipes; discharge from plastic factories
Xylenes (total)	10	10	Nervous system damage	Discharge from petroleum factories; discharge from chemical factories
Radionuclides	**MCLG [1] (mg/L) [4]**	**MCL[2] or TT[3] (mg/L) [4]**	**Potential Health Effects from Ingestion of Water**	**Sources of Contaminant in Drinking Water**
Beta particles and photon emitters	none[5]	4 millirems per year	Increased risk of cancer	Decay of natural and man-made deposits
Gross alpha particle activity	none[5]	15 picocuries per Liter (pCi/L)	Increased risk of cancer	Erosion of natural deposits
Radium 226 and Radium 228 (combined)	none[5]	5 pCi/L	Increased risk of cancer	Erosion of natural deposits
Microorganisms	**MCLG [1] (mg/L) [4]**	**MCL[2] or TT[3] (mg/L) [4]**	**Potential Health Effects from Ingestion of Water**	**Sources of Contaminant in Drinking Water**
Giardia lamblia	zero	TT[8]	Giardiasis, a gastroenteric disease	Human and animal fecal waste
Heterotrophic plate count	N/A	TT[8]	HPC has no health effects, but can indicate how effective treatment is at controlling microorganisms.	n/a
Legionella	zero	TT[8]	Legionnaire's Disease, commonly known as pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)	zero	5.0%[9]	Used as an indicator that other potentially harmful bacteria may be present[10]	Human and animal fecal waste
Turbidity	N/A	TT[8]	Turbidity has no health effects but can interfere with disinfection and provide a medium for microbial growth. It may indicate the presence of microbes.	Soil runoff
Viruses (enteric)	zero	TT[8]	Gastroenteric disease	Human and animal fecal waste

Common water sample bottles for distribution systems.

Radiochems, VOCs, (Volatile Organic Compounds), TTHMs, Total Trihalomethanes), Nitrate, Nitrite.

Most of these sample bottles will come with the preservative already inside the bottle.

Some bottles will come with a separate preservative (acid) for the field preservation.

Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.

Drinking water standards may apply differently based on type and size of public water systems.

National Secondary Drinking Water Regulations

National Secondary Drinking Water Regulations (NSDWRs or secondary standards are non-enforceable guidelines regulating contaminants that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water.

The EPA recommends secondary standards to water systems but does not require systems to comply. However, states may choose to adopt them as enforceable standards.

Contaminant	Secondary Standard
Aluminum	0.05 to 0.2 mg/L
Chloride	250 mg/L
Color	15 (color units)
Copper	1.0 mg/L
Corrosivity	noncorrosive
Fluoride	2.0 mg/L
Foaming Agents	0.5 mg/L
Iron	0.3 mg/L
Manganese	0.05 mg/L
Odor	3 threshold odor number
pH	6.5-8.5
Silver	0.10 mg/L
Sulfate	250 mg/L
Total Dissolved Solids	500 mg/L
Zinc	5 mg/L

Notes

[1] Maximum Contaminant Level Goal (**MCLG**) - The maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health effect of persons would occur, and which allows for an proper margin of safety. MCLGs are non-enforceable public health goals.

[2] Maximum Contaminant Level (**MCL**) - The maximum permissible level of a contaminant in water which is delivered to any user of a public water system. MCLs are enforceable standards. The margins of safety in MCLGs ensure that exceeding the MCL slightly does not pose significant risk to public health.

[3] Treatment Technique - An enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

[4] Units are in milligrams per Liter (mg/L) unless otherwise noted.

[5] MCLGs were not established before the 1986 Amendments to the Safe Drinking Water Act. Therefore, there is no MCLG for this contaminant.

[6] Lead and copper are regulated in a Treatment Technique which requires systems to take tap water samples at sites with lead pipes or copper pipes that have lead solder and/or are served by lead service lines. The action level, which triggers water systems into taking treatment steps, if exceeded in more than 10% of tap water samples, for copper is 1.3 mg/L, and for lead is 0.015mg/L.

[7] Each water system must certify, in writing, to the state (using third-party or manufacturer's certification) that when acrylamide and epichlorohydrin are used in drinking water systems, the combination (or product) of dose and monomer level does not exceed the levels specified, as follows:

- **Acrylamide** = 0.05% dosed at 1 mg/L (or equivalent)
- **Epichlorohydrin** = 0.01% dosed at 20 mg/L (or equivalent)

[8] The Surface Water Treatment Rule requires systems using surface water or ground water under the direct influence of surface water to (1) disinfect their water, and (2) filter their water or meet criteria for avoiding filtration so that the following contaminants are controlled at the following levels:

- ***Giardia lamblia**:* 99.9% killed/inactivated
  Viruses: 99.99% killed/inactivated
- ***Legionella**:* No limit, but EPA believes that if ***Giardia*** and viruses are inactivated, ***Legionella*** will also be controlled.
- **Turbidity**: At no time can turbidity (**cloudiness of water**) go above 5 nephelolometric turbidity units (NTU); systems that filter must ensure that the turbidity go no higher than 1 NTU (0.5 NTU for conventional or direct filtration) in at least 95% of the daily samples in any month.
- **HPC**: NO more than 500 bacterial colonies per milliliter.

[9] No more than 5.0% samples total coliform-positive in a month. (For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive). Every sample that has total coliforms must be analyzed for fecal coliforms. There cannot be any fecal coliforms.

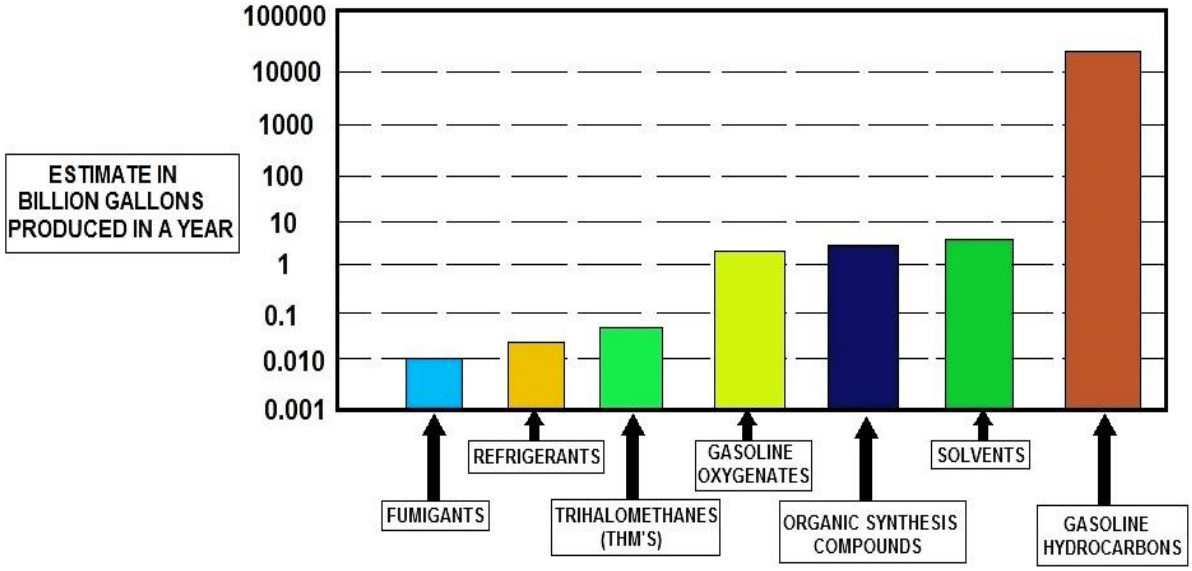
[10] Fecal coliform and *E. coli* are bacteria whose presence indicates that the water may be contaminated with human animal wastes. Microbes in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms.

Chemical Monitoring Sub- Section

The final federal rules regarding Phase II and V contaminants were promulgated by the U.S. EPA in 1992 and initial monitoring began in January 1993. This group of contaminants consists of Inorganic Chemicals (IOC), Volatile Organic Chemicals (VOC) and Synthetic Organic Chemicals (SOC) and the rule applies to all community and non-transient non-community public water systems.

The monitoring schedule for these contaminants is phased in by water system population size according to a "standardized monitoring framework" established by the U.S. EPA. This standardized monitoring framework establishes nine-year compliance cycles consisting of three 3-year compliance periods.

The first compliance cycle began back in January 1993 and ended December 31, 2001, with subsequent compliance cycles following the nine-year timeframe. The three-year compliance period of each cycle is the standard monitoring period for the water system.



VOLATILE ORGANIC COMPOUNDS FOUND IN GROUNDWATER CHART

Turbidity Monitoring
Monitoring for turbidity is applicable to all public water systems using surface water sources or ground water sources under the direct influence of surface water in whole or part. Check with your state drinking water section or health department for further instructions.

The maximum contaminant level for turbidity for systems that provide filtration treatment:
1. Conventional or direct filtration: less than or equal to 0.5 NTU in at least 95% of the measurements taken each month. Conventional filtration treatment plants should be able to achieve a level of 0.1 NTU with proper chemical addition and operation.
2. Slow sand filtration, cartridge and alternative filtration: less than or equal to 1 NTU in at least 95% of the measurements taken each month. The turbidity levels must not exceed 5 NTU at any turbidity measurements must be performed on representative samples of the filtered water every four (4) hours that the system serves water to the public.

A water system may substitute continuous turbidity monitoring for grab sample monitoring if it validates the continuous measurement for accuracy on a regular basis using a protocol approved by the Health or Drinking Water Agency, such as confirmation by a bench top turbidimeter. For systems using slow sand filtration, cartridge, or alternative filtration treatment the Health or Drinking Water Agency may reduce the sampling frequency to once per day if it determines that less frequent monitoring is sufficient to indicate effective filtration performance.

Inorganic Chemical Monitoring

All systems must monitor for inorganics. The monitoring for these contaminants is also complex with reductions, waivers and detections affecting the sampling frequency. Please refer to the monitoring schedules provided by your state health or drinking water sections for assistance in determining individual requirements. All transient non-community water systems are required to complete a one-time inorganic chemical analysis. The sample is to be collected at entry points (**POE**) to the distribution system representative of each source after any application of treatment.

Nitrates

Nitrate is an inorganic chemical that occurs naturally in some groundwater but most often is introduced into ground and surface waters by man. The most common sources are from fertilizers and treated sewage or septic systems.

At high levels (over 10 mg/l) it can cause the "**blue baby**" syndrome in young infants, which can lead to serious illness and even death. It is regarded as an "**acute health risk**" because it can quickly cause illness.

Every water system must test for ***Nitrate*** at least yearly. Systems that use ground water only must test yearly. Systems that use surface water and those that mix surface and ground water must test every quarter. A surface water system may go to yearly testing if community and nontransient noncommunity water must do quarterly monitoring whenever they exceed 5 mg/l in a test. After 4 quarters of testing and if the results show that the nitrate level has not exceeded 5 mg/L, they may go back to yearly testing.

Radiological Contaminants

All community water systems shall monitor for gross alpha activity every four years for each source. Depending on your state rules, compliance will be based on the annual composite of 4 consecutive quarters or the average of the analyses of 4 quarterly samples. If the average annual concentration is less than one half the MCL, an analysis of a single sample may be substituted for the quarterly sampling procedure.

Total Trihalomethanes (TTHM)

All community water systems serving a population of 10,000 or more and which add a disinfectant in any part of the drinking water treatment process shall monitor for total trihalomethanes (**TTHM**). The MCL is 0.08 mg/l (80 ppb) and consists of a calculation of the running average of quarterly analyses of the sum of the concentrations of bromodichloromethane, di-bromochloromethane, bromoform and chloroform.

Lead and Copper Rule
The Lead and Copper Rule was promulgated by the U.S. EPA on June 7, 1991, with monitoring to begin in January 1992 for larger water systems. This rule applies to all community and nontransient, noncommunity water systems and establishes action levels for these two contaminants at the consumer's tap. Action levels of 0.015 mg/l for lead and 1.3 mg/l for copper have been established.

This rule establishes maximum contaminant level goals (**MCLGs**) for lead and copper, treatment technique requirements for optimal corrosion control, source water treatment, public education and lead service line replacement. Whenever an action level is exceeded, the corrosion control treatment requirement is triggered. This is determined by the concentration measured in the 90th percentile highest sample from the samples collected at consumers' taps.

Sample results are assembled in ascending order (lowest to highest) with the result at the 90th percentile being the action level for the system. For example, if a water system collected 20 samples, the result of the 18th highest sample would be the action level for the system.

The rule also includes the best available technology (**BAT**) for complying with the treatment technique requirements, mandatory health effects language for public notification of violations and analytical methods and laboratory performance requirements.

Initial monitoring began in January 1992 for systems with a population of 50,000 or more, in July 1992 for medium-sized systems (3,300 to 50,000 population) and in July 1993 for small-sized systems (less than 3,300 population),

One-liter tap water samples are to be collected at high-risk locations by either water system personnel or residents.

Generally, high-risk locations are homes with lead-based solder installed after 1982 or with lead pipes or service lines. If not enough of these locations exist in the water system, the rule provides specific guidelines for selecting other sample sites.

The water must be allowed to stand motionless in the plumbing pipes for at least six (6) hours and collected from a cold water tap in the kitchen or bathroom. It is a first draw sample, which means the line is not to be flushed prior to sample collection.

The number of sampling sites is determined by the population of the system and sample collection consists of two, six-month monitoring periods; check with your state rule or drinking water section for more information.

Sampling Sites by Population

System size - No. of sites - No. of sites		
(no. of persons served)	*(standard monitoring)*	*(reduced monitoring)*
>100,000	100	50
10,001-100,000	60	30
3,301 to 10,000	40	20
501 to 3,300	20	10
101 to 500	10	5
< 100	5	5

If a system meets the lead and copper action levels or maintains optimal corrosion control treatment for two consecutive six-month monitoring periods, then reduced monitoring is allowed and sampling frequency drops to once per year.
After three consecutive years of reduced monitoring, sample frequency drops to once every three years. In addition to lead and copper testing, all large water systems and those medium- and small-sized systems that exceed the lead or copper action levels will be required to monitor for the following water quality parameters: pH, alkalinity, calcium, conductivity, orthophosphate, silica and water temperature.

These parameters are used to identify optimal corrosion control treatment and determine compliance with the rule once treatment is installed.

The sampling locations for monitoring water quality parameters are at entry points and representative taps throughout the distribution system.

Coliform sampling sites can be used for distribution system sampling. The number of sites required for monitoring water quality during each six-month period is shown below.

Number of Water Quality Parameters per Population

System size # (no. of persons served) no. of sites for water quality parameters

>100,000	25
10,001-100,000	10
3,301 to 10,000	3
501 to 3,300	2
101 to 500	1
<100	1

Water systems which maintain water quality parameters reflecting optimal corrosion control for two consecutive six-month monitoring periods qualify for reduced monitoring. After three consecutive years, the monitoring frequency can drop to once per year.

All large water systems must demonstrate that their water is minimally corrosive or install corrosion control treatment regardless of lead and copper sampling results.

Quality Assurance /Quality Control Measures - Introduction

In addition to standard samples, the field technicians collect equipment blanks (**EB**), field cleaned equipment blanks (**FB**), split samples (**SS**), and field duplicate samples (**FD**).

Overall care must be taken in regards to equipment handling, container handling/storage, decontamination, and record keeping. Sample collection equipment and non-preserved sample containers must be rinsed three times with sample water before the actual sample is taken. Exceptions to this are any pre-preserved container or bac-t type samples.

If protective gloves are used, they shall be clean, new and disposable. These should be changed upon arrival at a new sampling point. Highly contaminated samples shall never be placed in the same ice chest as environmental samples. It is good practice to enclose highly contaminated samples in a plastic bag before placing them in ice chests. The same is true for wastewater and drinking water samples.

Ice chests or shipping containers with samples suspected of being highly contaminated shall be lined with new, clean, plastic bags. If possible, one member of the field team should take all the notes, fill out labels, etc., while the other member does all of the sampling.

Preservation of Samples
Proper sample preservation is the responsibility of the sampling team, not the lab providing sample containers. The best reference for preservatives is Standard Methods or your local laboratory.

It is the responsibility of the field team to assure that all samples are appropriately preserved.

Follow the preservative solution preparation instructions.

Always use strong safety precautions when diluting any acid.

Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.

Put a new label on the dispensing bottle with the current date.

Wait 3-4 hours for the preservative to cool most samples down to 4 degrees Celsius.

Most preservatives have a shelf life of one year from the preparation date.

When samples are analyzed for TKN, TP, NH_4 and NOx 1 mL of 50% Trace Metal grade sulfuric acid is added to each discrete auto sampler bottles/bags in the field lab before sampling collection. The preservative maintains the sample at 1.5<pH<2 after collection. To meet maximum holding time for these preserved samples (28 days), pull and ship samples every 14 days.

Narrow range pH paper (test strips) can be used to test an aliquot of the preserved sample.

Place the pH paper into the container and compare the color with the manufacturer's color chart.

FINISHED WATER REPORT	UNITS OF MEASURE
FINISHED WATER TURBIDITY	NTU Neophelometric Turbidity Unit
FINSHED WATER TEMPERATURE	Deg. C Degrees Celcius
FINISHED WATER pH	SU Standard Units
FINISHED WATER ALKALINITY	mg/l Milligrams per Liter
FINISHED WATER HARDNESS	mS/cm Millisiemens per Centimeter
FINISHED WATER CONDUCTIVITY	mg/l Milligrams per Liter
FINISHED WATER TOTAL DISSOLVED SOLIDS	mg/l Milligrams per Liter
FINISHED WATER FLUORIDE	mg/l Milligrams per Liter
FINISHED WATER IRON	mg/l Milligrams per Liter
FINISHED WATER MANGANESE	mg/l Milligrams per Liter
FINISHED WATER PHOSPHATE	mg/l Milligrams per Liter
HARDNESS PER GALLON	GRAINS

WATER QUALITY REPORT INCLUDING UNITS OF MEASUREMENT

FINISHED WATER REPORTING INFO	UNITS OF MEASUREMENT
FINSIHED WATER TURBIDITY	NTU – NEOPHELOMETRIC TURBIDITY UNIT
FINISHED WATER TEMPERATURE	DEGREES CELCIUS
FINISHED WATER pH	SU – STANDARD UNITS
FINISHED WATER ALKALINITY	PPM or GRAINS PER GALLON
FINISHED WATER HARDNESS	Degrees of general hardness (dGH or °GH) Milligrams of $CaCO_3$ per Liter
FINISHED WATER CONDUCTIVITY	Millimhos per Centimeter [mmho/cm]
FINISHED WATER TOTAL DISSOLVED SOLIDS	Mg/L - Milligrams per Liter
FINISHED WATER FLUORIDE	Mg/L - Milligrams per Liter
FINISHED WATER IRON	Mg/L - Milligrams per Liter
FINISHED WATER MANGANESE	Mg/L - Milligrams per Liter
FINISHED WATER PHOSPHATE	Mg/L - Milligrams per Liter
HARDNESS PER GALLON	GRAINS PER GALLON

Water quality reports are used not only to satisfy state and federal compliance. It is a great reference tool for evaluating changes to source water due to human influence and unforeseen weather changes.

Since the Lead and Copper rule was enacted by EPA water systems analyze the water to see if it will leach the metals from the pipe, causing corrosion, or chemicals will precipitate out causing scaling in pipes and industrial processes such as boilers.

Drinking Water Sampling - Analysis Charts

ANALYSIS	METHOD	HOLDING TIME
Inorganic Compounds (IOC) Antimony, Arsenic, Barium, Beryllium, Cadmium, Chromium, Copper, Iron, Lead, Manganese, Mercury, Nickel, Selenium, Silver, Sodium, Thallium, Zinc, Hardness, Conductivity, Turbidity, Color, Chloride, Cyanide, Fluoride, Nitrate, Nitrite, Sulfate, and Total Dissolved Solids.	**(various)**	**48 hours**
Primary Pollutants (Short IOC) Antimony, Arsenic, Barium, Beryllium, Cadmium, Chromium, Lead, Mercury, Selenium, Silver, Sodium, Thallium, Turbidity, Fluoride, Cyanide, Nitrate, and Nitrite.	**(various)**	**48 hours**
Municipal Testing		
Lead and Copper	**EPA 200.9 for Pb** **EPA 200.7 for Cu**	**14 days**
Public or Individual Water Source Testing		
Nitrate	**SM-4500 NO3 D**	**48 hours**
Total Coliform & E. Coli	**SM-9223 B**	**30 Hours**
Metals Analysis on Drinking Water (per element)		
GFAA **(As, Pb, Sb, Se, Tl)**	**EPA 200.9**	**6 months**
ICP (Ag, Al, B, Ba, Be, Cd, Cr, Cu, Fe, Mn, Mo, Na, Ni, Zn)	**EPA 200.7**	**6 months**
CVAA (Hg)	**EPA 245.1**	**6 months**
Primary Pollutant Metals	**GFAA/ICP/CVAA**	**6 months**
Drinking Water Analysis		
PH	**EPA 150.1**	
Acidity	**SM-2310 B (4b)**	**14 days**
Alkalinity (Bicarbonate & Carbonate)	**SM-2320 B (4a)**	**14 days**
BOD	**SM-5210 B**	**48 hours**
Calcium	**EPA 200.7**	**6 months**
Chloride	**SM-4500 Cl**	**8 days**
Chlorine, total	**SM-4500 Cl**	**5 hours**
Color	**SM-2120 B**	**8 hours**
COD	**EPA 410.4 (7.3)**	**28 days**
Cyanide	**EPA 335.2 (8.7)**	**28 days**
Dissolved Oxygen	**SM-4500 O C**	**8 hours**
Fluoride	**SM-4500 F C**	**28 days**
Hardness	**SM-2340 B**	**6 months**

Magnesium	**EPA 200.7**	**6 months**
Nitrogen, ammonia	**SM-4500 NH3 E** **SM-4500 NH3 H**	**28 days**
Nitrogen, nitrate	**SM-4500 NO3 D**	**48 hours**
Nitrogen, nitrite	**SM-4500 NO2**	**48 hours**
Nitrate + Nitrite	**SM-4500 NO3 E**	**48 hours**
Nitrogen, TKN	**EPA 351.4**	**28 days**
Odor	**SM-2150**	**6 days**
Phosphorous, ortho	**EPA 200.7**	**48 hours**
Phosphorous, total	**SM-4500 P**	**28 days**
Solids, settle able	**SM-2540**	**7 days**
Solids, suspended	**SM-2540 D**	**7 days**
Drinking Water Analysis		
Solids, total dissolved	**SM-2540 B**	**7 days**
Solids, volatile	**SM-2540 E**	**7 days**
Specific Conductance	**SM-2510 B**	**28 days**
Sulfate	**SM-4500 SO-4 E**	**28 days**
Sulfide	**SM-4500 S-2 D**	**28 days**
Sulfite	**EPA 377.1**	**28 days**
Silica	**SM-4500 SI E**	**28 days**
Total Organic Carbon	**EPA 415.1**	**28 days**
Turbidity	**SM- 2130 B**	**48 hours**

ORGANICS		
Semi-volatile Organics in Water (SOC)*	**(various)**	**7 days**
Volatile Organics in Water*	**(various)**	**7 days**
Trihalomethanes*	**EPA 501.1**	**7 days**
Gross Alpha & Bata (Radionuclides)*	**(various)**	**7 days**
BOD	**SM-5210 B**	**48 hours**
COD	**EPA 410.4(7.3)**	**28 days**
Oil and Grease	**EPA 413.1(1.2)**	**28 days**
Hardness W/digestion	**SM-2340 B**	**6 months**
Nitrogen, TKN	**EPA 351.4**	**28 days**
Nitrogen, ammonia	**SM-4500 NH3 F**	**28 days**
Nitrogen, Total Organic	**SM-4500 NorgNH3**	**28 days**
Nitrogen, nitrate	**SM-4500 NO3 D**	**48 hours**
Nitrogen, nitrite	**SM-4500 NO2 B**	**48 hours**
Phosphorous, ortho	**SM-4500 P E**	**48 hours**
Sulfate	**SM-4500 SO4 E**	**28 days**
Solids, dissolved	**SM-2540**	**7 days**
Solids, settle able	**SM-2540 F**	**7 days**
Solids, suspended	**SM-2540 D**	**7 days**
Solids, total	**SM-2540 B**	**7 days**
Solids, volatile	**SM-2540 E**	**7 days**
Total Organic Carbon	**EPA 415.1**	**28 days**
PH	**EPA 150.1**	
Metals (per element)		

ICP **(Ag, Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Sb, V, Zn)**	**EPA 200.7**	**6 months**
GFAA **(As, Pb, Ba, Se, Tl)**	**EPA 200.9**	**6 months**
CVAA (Hg)	**EPA 245.1**	**6 months**

Definitions:

Action level - the concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.

Maximum Contaminant Level - the "Maximum Allowed" (MCL) is the highest level of a contaminant that is allowed in drinking water. MCLs are set as close to the MCLGs as feasible using the best available treatment technology.

Maximum Contaminant Level Goal - the "Goal" (MCLG) is the level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety.

Non-Detects (ND) - laboratory analysis indicates that the constituent is not present.

Parts per million (ppm) or Milligrams per liter (mg/L) - one part per million corresponds to one minute in two years or a single penny in $10,000.

Parts per billion (ppb) or Micrograms per liter (ug/L) - one part per billion corresponds to one minute in 2,000 years, or a single penny in $10,000,000.

Picocuries per liter (pCi/L) - picocuries per liter is a measure of the radioactivity in water.

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

SAMPLE CONTAINERS and PRESERVATION

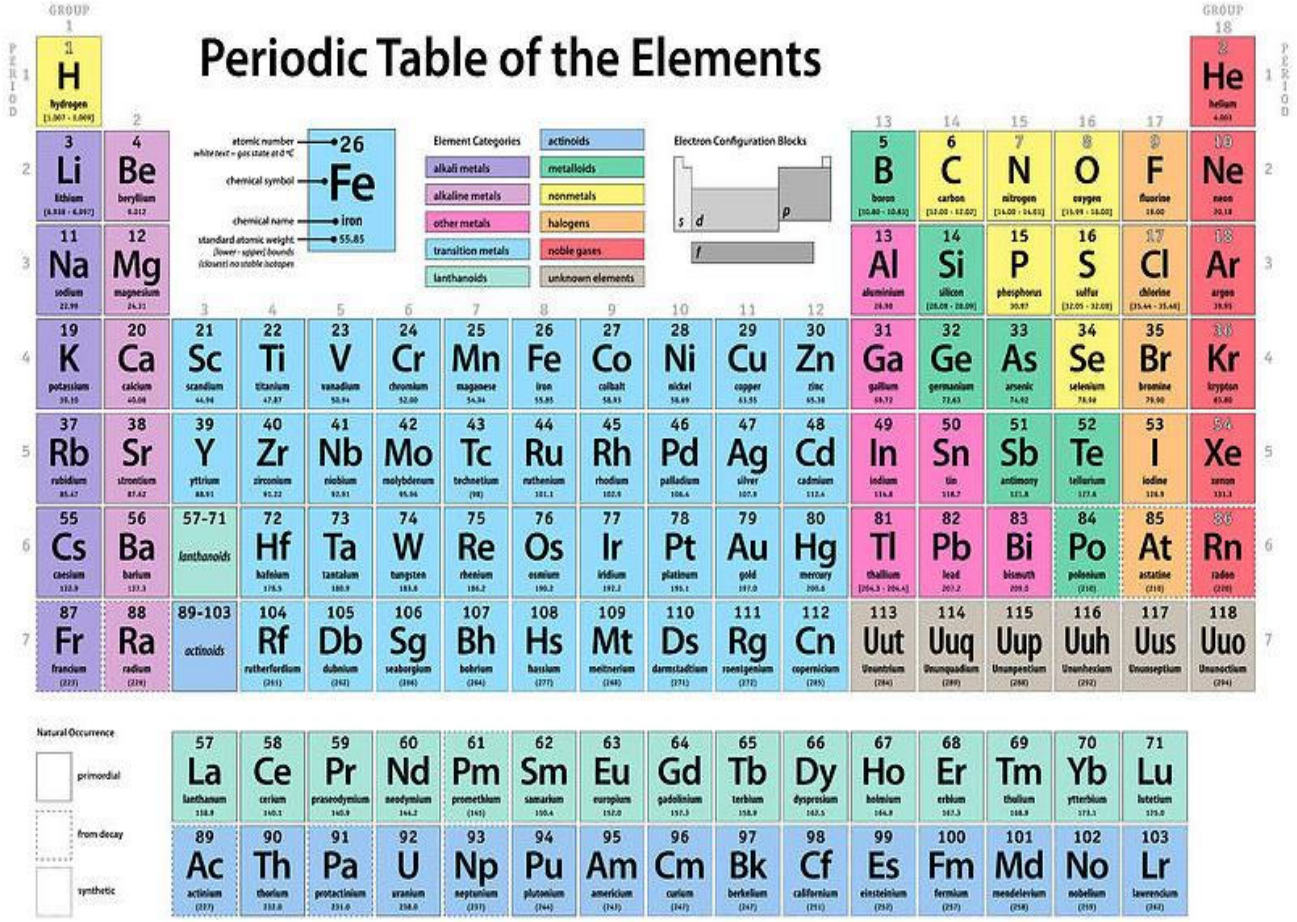
Methods used by the laboratory usually specify what type of container and how much sample is required to run an analysis. The following table provides a summary of the sample handling and preservation requirements for some of the most common tests.

Parameter	Bottle Type	Minimum Sample Size	Maximum Holding Time	Storage & Preservation
Acidity	P or G[B]	100ml	24 hrs/14 days	refrigerate
Alkalinity	P or G	200ml	24 hrs/14 days	refrigerate
BOD (5 day)	P or G	1L	6 hrs/48 hrs	refrigerate
Boron	P	100ml	28 days/6 months	
Chloride	P or G	250ml	28 days	
Chlorine, residual	P or G	500ml	0.5 hr/stat	analyze on site ASAP
COD	P or G	500ml	28 days/28 days	analyze on site ASAP
Color	P or G	500ml	48 hrs/48 hrs	refrigerate
Coliform, Total	P or G	125ml	30 hrs	refrigerate
Conductivity	P or G	500ml	48 hrs/48 hrs	refrigerate
Cyanide, Total	P or G	500ml	28 days/28 days	add NaOH to pH>12
				refrigerate in dark
Fluoride	P	300ml	28days/ 28 days	
Hardness	P or G	100ml	6 months/6 months	add HNO_3 to pH<2
Metals, general	P[A] or G[A]	250ml	6 months/6 months	add HNO_3 to pH<2
Furnace	P[A] or G[A]	250ml	6 months/6 months	
Flame	P[A] or G[A]	250ml	6 months/6 months	
Mercury	P[A] or G[A]	500ml	28 days/28 days	add HNO_3 to pH<2
Nitrogen *Ammonia*	P or G	500ml	7 days/ 28 days	ASAP or add H_2SO_4 to pH<2 & refrigerate
Nitrate	P or G	100ml	48 hrs/48 hrs	ASAP & refrigerate
Nitrate + Nitrite	P or G	200ml	48 hrs/28 days	ASAP & refrigerate
Nitrite	P or G	100ml	none/48 hrs	ASAP & refrigerate

TKN	P or G	500ml	7 days/28 days	add H_2SO_4 to pH<2
Oxygen, dissolved	G (BOD)	300ml		
Electrode			0.5 hrs/stat	ASAP on site
Winkler			8hrs/8 hrs	ASAP on site
pH	P or G	50ml	2 hrs/stat	ASAP on site
Phosphate,	G^A			
Ortho		100ml	48hrs	filter ASAP refrigerate
Total		100ml	28 days/28 days	refrigerate
Solids,	P or G			
Dissolved		250ml	7 days	refrigerate
Settleable		1L	48 hrs	refrigerate
Suspended		250ml	7 days	refrigerate
Total		250ml	7 days	refrigerate
Volatile		250ml	7 days	refrigerate
Silica	P	200ml	28 days/28 days	refrigerate
Sulfate	P or G	100ml	28 days/28 days	refrigerate
Turbidity	P or G	100ml	24 hrs/48 hrs	ASAP/refrigerate, store in dark up to 24 hrs

Refrigerate = storage at 4 degrees C, in the dark. P = plastic (polyethylene or equivalent); G = glass, G^A or P^A = rinsed with 1:1 HNO_3; G^B = glass, borosilicate, G^S = glass rinsed with organic solvents; NS = not stated in cited reference; stat = no storage allowed; analyze immediately.

Inorganic Compound (IOC) Section



Inorganic Compound

Inorganic Contaminants (IOCs) are elements or compounds found in water supplies and may be natural in the geology or caused by activities of man through mining, industry or agriculture. An inorganic compound is typically a chemical compound that lacks Carbon-Hydrogen bonds, that is, a compound that is not an organic compound, but the distinction is not defined or even of particular interest. Some simple compounds that contain carbon are often considered inorganic.

Examples include many toxic or poisonous compounds like:

carbon monoxide, carbon dioxide, carbonates, cyanides, cyanates, carbides, and thiocyanates. Many of these are normal parts of mostly organic systems, including organisms, which means that describing a chemical as inorganic does not obligately mean that it does not occur within living things.

It is common to have trace amounts of many Inorganic Contaminants in water supplies. Amounts above the Maximum Contaminant Levels may cause a variety of damaging effects to the liver, kidney, nervous system circulatory system, blood, gastrointestinal system, bones, or skin depending upon the inorganic contaminant and level of exposure.

IOC Sample Collection – Things to Remember

Sample instructions should be supplied with the sample containers from the laboratory. If the laboratory fails to include sample instructions, contact the laboratory and request sample instructions.

Some general practices to remember:

• Samples should be collected at the entry point to the distribution system after all treatment (finished water)

• Select a sampling faucet that does NOT have an aerator (sampling must be done with minimum aeration

• Run the water until the temperature is as cold as it gets (except for Pb and Cu samples.)

• Just before sample collection, adjust to a very low flow. Do not change the flow while collecting the sample

• Routine nitrate and nitrite samples should be collected on a Monday or a Tuesday

• When filling sample bottle, tip bottle slightly so that water flows down the side wall of the container. Bring bottle to an upright position as it fills

• Call the laboratory if bottles are received broken (or break while collecting samples)

• The owner or operator of a water supply must maintain chemical analysis reports (results) or a summary of those reports for at least 10 years

Inorganic Chemicals

Contaminant	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Long-Term Exposure Above the MCL (unless specified as short-term)	Sources of Contaminant in Drinking Water
Antimony	0.006	0.006	Increase in blood cholesterol; decrease in blood sugar	Discharge from petroleum refineries; fire retardants; ceramics; electronics; solder
Arsenic	0[7]	0.010 as of 01/23/06	Skin damage or problems with circulatory systems, and may have increased risk of getting cancer	Erosion of natural deposits; runoff from orchards, runoff from glass & electronics production wastes
Asbestos (fiber >10 micrometers)	7 million fibers per liter	7 MFL	Increased risk of developing benign intestinal polyps	Decay of asbestos cement in water mains; erosion of natural deposits
Barium	2	2	Increase in blood pressure	Discharge of drilling wastes; discharge from metal refineries; erosion of natural deposits
Beryllium	0.004	0.004	Intestinal lesions	Discharge from metal refineries and coal-burning factories; discharge from electrical, aerospace, and defense industries
Cadmium	0.005	0.005	Kidney damage	Corrosion of galvanized pipes; erosion of natural deposits; discharge from metal refineries; runoff from waste batteries and paints
Chromium (total)	0.1	0.1	Allergic dermatitis	Discharge from steel and pulp mills; erosion of natural deposits
Copper	1.3	TT[7]; Action Level=1.3	Short term exposure: Gastrointestinal distress Long term exposure: Liver or kidney damage People with Wilson's Disease should consult their personal doctor if the amount of copper in their water exceeds the action level	Corrosion of household plumbing systems; erosion of natural deposits

Inorganic Chemicals

Contaminant	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Long-Term Exposure Above the MCL (unless specified as short-term)	Sources of Contaminant in Drinking Water
Cyanide (as free cyanide)	0.2	0.2	Nerve damage or thyroid problems	Discharge from steel/metal factories; discharge from plastic and fertilizer factories
Fluoride	4.0	4.0	Bone disease (pain and tenderness of the bones); Children may get mottled teeth	Water additive which promotes strong teeth; erosion of natural deposits; discharge from fertilizer and aluminum factories
Lead	zero	TT[7]; Action Level=0.015	Infants and children: Delays in physical or mental development; children could show slight deficits in attention span and learning abilities Adults: Kidney problems; high blood pressure	Corrosion of household plumbing systems; erosion of natural deposits
Mercury (inorganic)	0.002	0.002	Kidney damage	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills and croplands
Nitrate (measured as Nitrogen)	10	10	Infants below the age of six months who drink water containing nitrate in excess of the MCL could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue-baby syndrome.	Runoff from fertilizer use; leaking from septic tanks, sewage; erosion of natural deposits
Nitrite (measured as Nitrogen)	1	1	Infants below the age of six months who drink water containing nitrite in excess of the MCL could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue-baby syndrome.	Runoff from fertilizer use; leaking from septic tanks, sewage; erosion of natural deposits
Selenium	0.05	0.05	Hair or fingernail loss; numbness in fingers or toes; circulatory problems	Discharge from petroleum refineries; erosion of natural deposits; discharge from mines

Synthetic Organic Chemicals (SOCs) Section

SOC/VOC bottles are the smaller, thin bottles with the septum tops. Be careful not to get any air bubbles in the SOC/VOC bottles. It may take a few weeks to learn to collect a proper sample.

SOC

Synthetic Organic Chemicals (SOCs) are organic (carbon based) chemicals that are less volatile than Volatile Organic Compounds (VOCs). SOCs are used as pesticides, defoliants, fuel additives and as ingredients for other organic compounds. They are all man made and do not naturally occur in the environment. Some of the more well-known SOCs are Atrazine, 2,4-D, Dioxin and Polychlorinated Biphenyls (PCBs).

SOCs most often enter the natural environment through application of pesticide (including runoff from areas where they are applied), as part of a legally discharged waste stream, improper or illegal waste disposal, accidental releases or as a byproduct of incineration. Some SOCs are very persistent in the environment, whether in soil or water.

SOCs are generally toxic and can have substantial health impacts from both acute (short-term) and chronic (long-term) exposure. Many are known carcinogens (cancer causing). EPA has set Maximum Contaminant Levels (MCL) for 30 SOCs under the Safe Drinking Water Act.

The Safe Drinking Water Act requires that all water sources of all public water systems be periodically monitored for regulated SOCs. The monitoring frequency can be adjusted through a waiver if SOCs are not detected.

EPA established Maximum Contaminant Levels (MCL), Maximum Contaminant Level Goals (MCLG), monitoring requirements and best available technologies for removal for 65 chemical contaminants over a five-year period as EPA gathered and analyzed occurrence and health effects data. This series of rules are known as the Chemical Phase Rules and they define regulations for three contaminant groups:

- ✓ Inorganic Chemicals (IOC),
- ✓ Synthetic Organic Chemicals (SOC), and
- ✓ Volatile Organic Chemicals (VOC).

The Chemical Phase rules provide public health protection through the reduction of chronic risks from:

- ✓ cancer;
- ✓ organ damage; and
- ✓ circulatory,
- ✓ nervous, and
- ✓ reproductive system disorders.

They also help to reduce the occurrence of Methemoglobinemia or "blue baby syndrome" from ingestion of elevated levels of nitrate or nitrite. All public water systems must monitor for Nitrate and Nitrite.

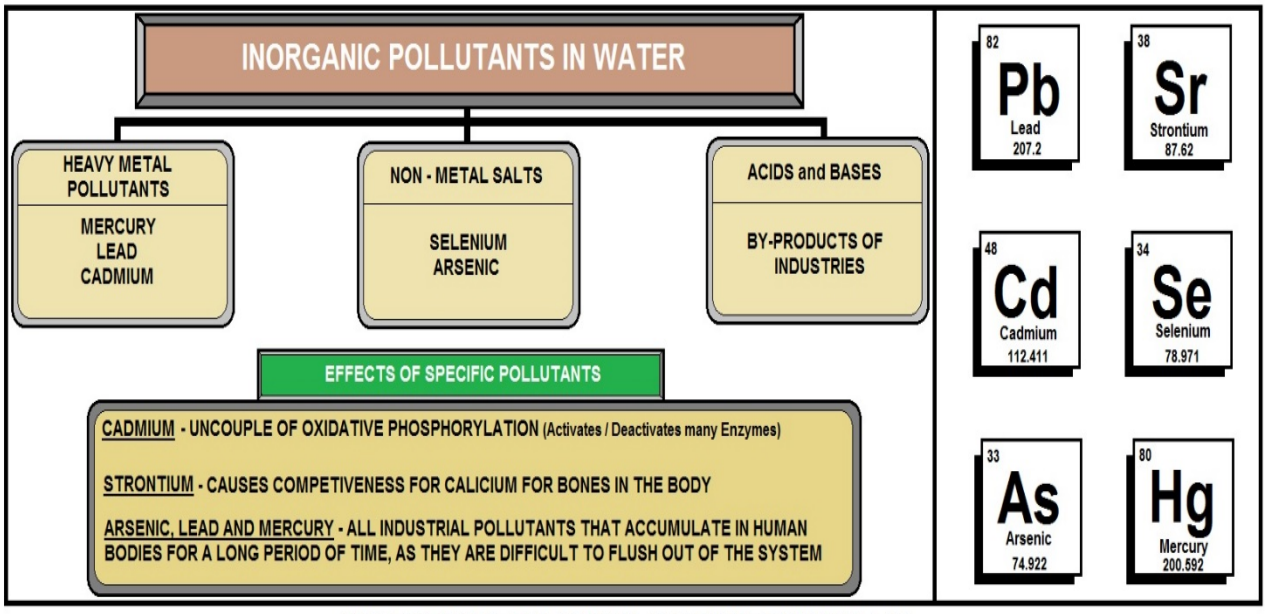
Community water systems and Non-transient non-community water systems must also monitor for IOCs, SOCs, and VOCs.

These lists of the organic chemicals—which include pesticides, industrial chemicals, and disinfection by-products—that are tested for in public water systems (those that provide water to the public), along with the maximum standard for the contaminant, and a brief description of the potential health effects associated with long-term consumption of elevated levels of the contaminants.

The federal standard for most contaminants is listed as a Maximum Contaminant Level (MCL), the lowest concentration at which that particular contaminant is believed to represent a potential health concern. Unless otherwise noted, the MCL is expressed as parts per billion (ppb).

Also, because of technological limitations or other factors, it is not possible to test for some contaminants in a reliable fashion. Instead, public water systems are required to use specific Treatment Techniques (TT) that are designed to remove these particular contaminants from the water.

Unregulated Chemicals
In addition to the chemicals listed, monitoring is done for approximately 60 organic chemicals for which MCLs have not been established. If unacceptable levels are found of these “unregulated” contaminants—based on established state health standards and an assessment of the risks they pose—the response is the same as if an MCL has been exceeded: the public water system must notify those served by the system.



INORGANIC CHEMICALS FOUND IN WATER

Technical Learning College

Synthetic Organic Chemicals	MCL (ppb)	Potential Health Effects
Acrylamide	TT	Cancer, nervous system effects
Alachlor	2	Cancer
Aldicarb	3	Nervous system effects
Aldicarb sulfoxide	4	Nervous system effects
Aldicarb sulfone	2	Nervous system effects
Atrazine	3	Liver, kidney, lung, cardiovascular effects; possible carcinogen
Benzo(a)pyrene (PAHs)	0.2	Liver, kidney effects, possible carcinogen
Carbofuran	40	Nervous system, reproductive system effects
Chlordane	2	Cancer
2,4-D	70	Liver, kidney effects
Di(2-ethylhexyl) adipate	400	Reproductive effects
Di(2-ethylhexyl) phthalate	6	Cancer
Dibromochloro-propane (DBCP)	0.2	Cancer
Dinoseb	7	Thyroid, reproductive effects
Diquat	20	Ocular, liver, kidney effects
Endothall	100	Liver, kidney, gastrointestinal effects
Endrin	2	Liver, kidney effects
Epichlorohydrin	TT	Cancer
Ethylene dibromide (EDB)	0.05	Cancer
Glyphosate	700	Liver, kidney effects
Heptachlor	0.4	Cancer
Heptachlor epoxide	0.2	Cancer
Hexachlorobenzene	1	Cancer
Hexachlorocyclopentadiene (HEX)	50	Kidney, stomach effects

Lindane	0.2	Liver, kidney, nervous system, immune system, circulatory system effects
Methoxychlor	40	Developmental, liver, kidney, nervous system effects
Oxamyl (Vydate)	200	Kidney effects
Pentachlorophenol	1	Cancer
Picloram	500	Kidney, liver effects
Polychlorinated biphenyls (PCBs)	0.5	Cancer
Simazine	4	Body weight and blood effects, possible carcinogen
2,3,7,8-TCDD (Dioxin)	0.00003	Cancer
Toxaphene	3	Cancer
2,4,5-TP (Silvex)	50	Liver, kidney effects

Volatile Organic Compounds (VOCs)

Definitions

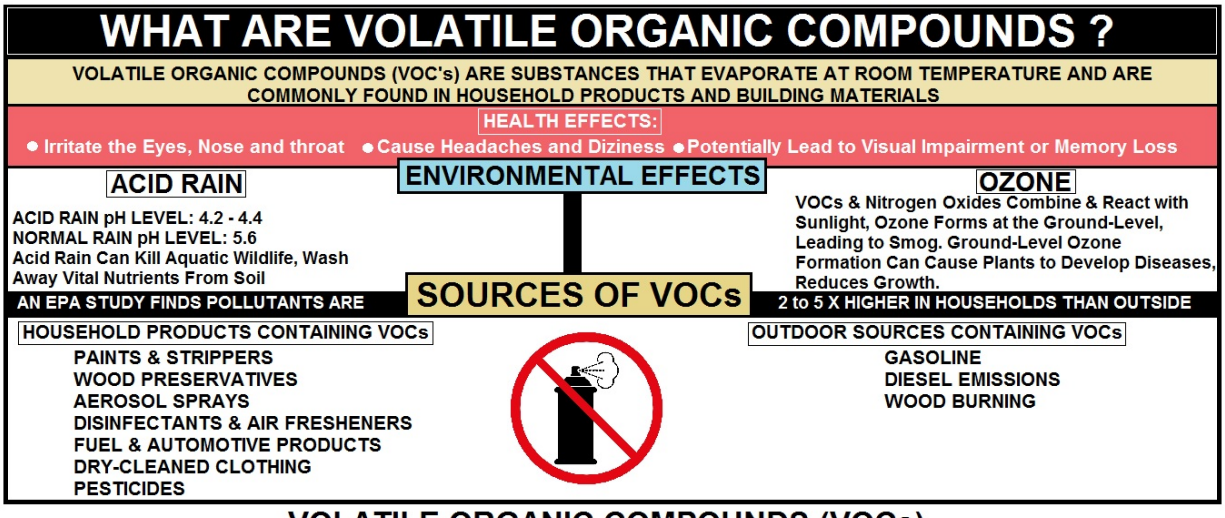
Volatile Organic Compounds (VOCs) – "VOCs are ground-water contaminants of concern because of very large environmental releases, human toxicity, and a tendency for some compounds to persist in and migrate with ground-water to drinking-water supply well … In general, VOCs have high vapor pressures, low-to-medium water solubilities, and low molecular weights. Some VOCs may occur naturally in the environment, other compounds occur only as a result of manmade activities, and some compounds have both origins." - Zogorski and others, 2006

Volatile Organic Compounds (VOCs) – "Volatile organic compounds released into the atmosphere by anthropogenic and natural emissions which are important because of their involvement in photochemical pollution." - Lincoln and others, 1998

Volatile Organic Compounds (VOCs) – "Hydrocarbon compounds that have low boiling points, usually less than 100ºC, and therefore evaporate readily. Some are gases at room temperature. Propane, benzene, and other components of gasoline are all volatile organic compounds." - Art, 1993

Volatile Organic Compounds (VOCs) – "VOCs are organic compounds that can be isolated from the water phase of a sample by purging the water sample with inert gas, such as helium, and, subsequently, analyzed by gas chromatography.

Many VOCs are human-made chemicals that are used and produced in the manufacture of paints, adhesives, petroleum products, pharmaceuticals, and refrigerants. They often are compounds of fuels, solvents, hydraulic fluids, paint thinners, and dry-cleaning agents commonly used in urban settings. VOC contamination of drinking water supplies is a human-health concern because many are toxic and are known or suspected human carcinogens." - U.S. Geological Survey, 2005



VOLATILE ORGANIC COMPOUNDS (VOCs)

VOCs *Explained*

Volatile organic compounds (VOCs) are organic chemicals that have a high vapor pressure at ordinary, room-temperature conditions. Their high vapor pressure results from a low boiling point, which causes large numbers of molecules to evaporate or sublimate from the liquid or solid form of the compound and enter the surrounding air. An example is formaldehyde, with a boiling point of –19 °C (–2 °F), slowly exiting paint and getting into the air.

VOCs are numerous, varied, and ubiquitous. They include both human-made and naturally occurring chemical compounds. Most scents or odors are of VOCs. VOCs play an important role in communication between plants. Some VOCs are dangerous to human health or cause harm to the environment.

Anthropogenic VOCs are regulated by law, especially indoors, where concentrations are the highest. Harmful VOCs are typically not acutely toxic, but instead have compounding long-term health effects. Because the concentrations are usually low and the symptoms slow to develop, research into VOCs and their effects is difficult.

Specific Components

Paints and Coatings

A major source of man-made VOCs are coatings, especially paints and protective coatings. Solvents are required to spread a protective or decorative film. Approximately 12 billion liters of paints are produced annually. Typical solvents are aliphatic hydrocarbons, ethyl acetate, glycol ethers, and acetone. Motivated by cost, environmental concerns, and regulation, the paint and coating industries are increasingly shifting toward aqueous (water-based) solvents.

Chlorofluorocarbons and Chlorocarbons

Chlorofluorocarbons, which are banned or highly regulated, were widely used cleaning products and refrigerants. Tetrachloroethene is used widely in dry cleaning and by industry. Industrial use of fossil fuels produces VOCs either directly as products (e.g., gasoline) or indirectly as byproducts (e.g., automobile exhaust).

Benzene

One VOC that is a known human carcinogen is benzene, which is a chemical found in environmental tobacco smoke, stored fuels, and exhaust from cars in an attached garage. Benzene also has natural sources such as volcanoes and forest fires. It is frequently used to make other chemicals in the production of plastics, resins, and synthetic fibers. Benzene evaporates into the air quickly and the vapor of benzene is heavier than air allowing the compound to sink into low-lying areas. Benzene has also been known to contaminate food and water and if digested can lead to vomiting, dizziness, sleepiness, rapid heartbeat, and at high levels, even death may occur.

Methylene Chloride

Methylene chloride is another VOC that is highly dangerous to human health. It can be found in adhesive removers and aerosol spray paints and the chemical has been proven to cause cancer in animals. In the human body, methylene chloride is converted to carbon monoxide and a person will suffer the same symptoms as exposure to carbon monoxide. If a product that contains methylene chloride needs to be used the best way to protect human health is to use the product outdoors. If it must be used indoors, proper ventilation is essential to keeping exposure levels down.

Perchloroethylene
Perchloroethylene is a volatile organic compound that has been linked to causing cancer in animals. It is also suspected to cause many of the breathing related symptoms of exposure to VOC's. Perchloroethylene is used mostly in dry cleaning.

Studies show that people breathe in low levels of this VOC in homes where dry-cleaned clothes are stored and while wearing dry-cleaned clothing. While dry cleaners attempt to recapture perchlorothylene in the dry cleaning process to reuse it in an effort to save money, they can't recapture it all. To avoid exposure to perchlorothylene, if a strong chemical odor is coming from clothing when picked up from the dry cleaner, do not accept them and request that less of the chemical be used as well as a complete drying of the garments

MTBE
MTBE was banned in the US around 2004 in order to limit further contamination of drinking water aquifers primarily from leaking underground gasoline storage tanks where MTBE was used as an octane booster and oxygenated-additive.

Formaldehyde
Many building materials such as paints, adhesives, wall boards, and ceiling tiles slowly emit formaldehyde, which irritates the mucous membranes and can make a person irritated and uncomfortable. Formaldehyde emissions from treated wood are in the range of 0.02 – 0.04 ppm. Relative humidity within an indoor environment can also affect the emissions of formaldehyde. High relative humidity and high temperatures allow more vaporization of formaldehyde from wood materials.

Health Risks
Respiratory, allergic, or immune effects in infants or children are associated with man-made VOCs and other indoor or outdoor air pollutants. Some VOCs, such as styrene and limonene, can react with nitrogen oxides or with ozone to produce new oxidation products and secondary aerosols, which can cause sensory irritation symptoms. Unspecified VOCs are important in the creation of smog.

Health Effects Include:
Eye, nose, and throat irritation; headaches, loss of coordination, nausea; damage to liver, kidney, and central nervous system. Some organics can cause cancer in animals; some are suspected or known to cause cancer in humans. Key signs or symptoms associated with exposure to VOCs include conjunctival irritation, nose and throat discomfort, headache, allergic skin reaction, dyspnea, declines in serum cholinesterase levels, nausea, emesis, epistaxis, fatigue, dizziness.

The ability of organic chemicals to cause health effects varies greatly from those that are highly toxic, to those with no known health effects. As with other pollutants, the extent and nature of the health effect will depend on many factors including level of exposure and length of time exposed.

Routes of Entry
Eye and respiratory tract irritation, headaches, dizziness, visual disorders, and memory impairment are among the immediate symptoms that some people have experienced soon after exposure to some organics. At present, not much is known about what health effects occur from the levels of organics usually found in homes. Many organic compounds are known to cause cancer in animals; some are suspected of causing, or are known to cause, cancer in humans.

Reducing Exposure
To reduce exposure to these toxins, one should buy products that contain Low-VOC's or No VOC's. Only the quantity which will soon be needed should be purchased, eliminating stockpiling of these chemicals. Use products with VOC's in well ventilated areas. When designing homes and buildings, design teams can implement the best possible ventilation plans, call for the best mechanical systems available, and design assemblies to reduce the amount of infiltration into the building.

These methods will help improve indoor air quality, but by themselves they cannot keep a building from becoming an unhealthy place to breathe. While proper building ventilation is a key component to improving indoor air quality, it cannot do the job on its own. As stated earlier, awareness is the key component to improving air quality, when choosing building materials, furnishings, and decorations. When architects and engineers implement best practices in ventilation and mechanical systems, the owner must maintain good air quality levels thereafter.

Chemical Fingerprinting
The exhaled human breath contains a few hundred volatile organic compounds and is used in breath analysis to serve as a VOC biomarker to test for diseases such as lung cancer. One study has shown that "volatile organic compounds ... are mainly blood borne and therefore enable monitoring of different processes in the body." And it appears that VOC compounds in the body "may be either produced by metabolic processes or inhaled/absorbed from exogenous sources" such as environmental tobacco smoke. Research is still in the process to determine whether VOCs in the body are contributed by cellular processes or by the cancerous tumors in the lung or other organs.

Volatile Organic Chemicals	MCL (ppb)	Potential Health Effects
Benzene	5	Cancer
Carbon tetrachloride	5	Liver effects, cancer
Chlorobenzene	100	Liver, kidney, nervous system effects
o-Dichlorobenzene	600	Liver, kidney, blood cell effects
para-Dichlorobenzene	175	Kidney effects, possible carcinogen
1,2-Dichloroethane	5	Cancer
1,1-Dichloroethylene	7	Liver, kidney effects, possible carcinogen
cis-1,2-Dichloroethylene	70	Liver, kidney, nervous system, circulatory system effects
trans-1,2-Dichloroethylene	100	Liver, kidney, nervous system, circulatory system effects
1,2-Dichloropropane	5	Cancer
Ethylbenzene	700	Liver, kidney, nervous system effects
Methylene chloride	5	Cancer
Styrene	100	Liver, nervous systems effects, possible carcinogen
Tetrachloroethylene (PCE)	5	Cancer
Toluene	1,000	Liver, kidney, nervous system, circulatory system effects
Total trihalomethanes Chloroform Bromoform Bromodichloromethane Chlorodibromomethane	100	Cancer
1,2,4-Trichlorobenzene	70	Liver, kidney effects
1,1,1-Trichloroethane	200	Liver, nervous system effects
1,1,2-Trichloroethane	5	Kidney, liver effects, possible carcinogen
Trichloroethylene (TCE)	5	Cancer
Vinyl chloride	2	Nervous system, liver effects, cancer
Xylenes (total)	10,000	Liver, kidney, nervous system effects

Disinfection By-products	MCL (ppb)	Potential Health Effects
Bromate	10	Cancer
Chlorate	1,000	Anemia, nervous system effects
Haloacetic Acids (HAA5)*	60	Cancer
Total trihalomethanes (TTHMs)**	80	Cancer

*Haloacetic acids consist of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid.

**Total trihalomethanes consist of chloroform, bromoform, bromodichloromethane, and chlorodibromomethane.

Safe Drinking Water Act (SDWA) Summary

In 1974, Congress passed the Safe Drinking Water Act (SDWA) setting up a regulatory program among local, state, and federal agencies to help ensure the provision of safe drinking water in the U.S. The states are expected to administer and enforce these regulations for public water systems (systems that either have 15 or more service connections or regularly serve an average of 25 or more people daily for at least 60 days each year). Public water systems must provide water treatment, ensure proper drinking water quality through monitoring, and provide public notification of contamination problems.

Relating to prevention of waterborne disease, the SDWA required EPA to:
1) set numerical standards, referred to as Maximum Contaminant Levels (MCLs — the highest allowable contaminant concentrations in drinking water) or treatment technique requirements for contaminants in public water supplies;
2) issue regulations requiring monitoring of all regulated and certain unregulated contaminants, depending on the number of people served by the system, the source of the water supply, and the contaminants likely to be found;
3) set criteria under which systems are obligated to filter water from surface water sources; it must also develop procedures for states to determine which systems have to filter;
4) develop disinfection rules for all public water supplies; and
5) require all states to develop Wellhead Protection Programs designed to protect from sources of contamination areas around wells that supply public drinking water systems.

Through the Surface Water Treatment Rule (SWTR), EPA has set treatment requirements to control microbiological contaminants in public water systems using surface water sources (and ground-water sources under the direct influence of surface water). These requirements include the following:
1) treatment must remove or inactivate at least 99.9% of *Giardia lamblia* cysts and 99.99% of viruses;
2) all systems must disinfect, and are required to filter if certain source water quality criteria and site-specific criteria are not met;
3) the regulations set criteria for determining if treatment, including turbidity (suspended particulate matter) removal and disinfection requirements, is adequate for filtered systems; and
4) all systems must be operated by qualified operators as determined by the states.

Current EPA Research –Barriers to Contamination
Although water treatment and disinfection techniques are quite effective at microbe reduction, finished drinking water is not sterile. Survival and regrowth of microorganisms in drinking water distribution systems can lead to the deterioration of water quality and even noncompliance of a supply.

Regrowth has largely been associated with heterotrophic bacteria (i.e., those bacteria – including pathogens – that require preformed organic compounds as carbon and energy sources).

Bacterial growth occurs on the walls of the distribution system (referred to as “biofilms”) and in the water either as free living cells or cells attached to suspended solids. A multi-faceted phenomenon, bacterial regrowth is influenced primarily by temperature, residence time in mains and storage units, the efficacy of disinfection, and nutrients.

Assimilable Organic Carbon (AOC)
Assimilable organic carbon (AOC) is the portion of the total organic carbon (TOC) dissolved in water that is easily used by microorganisms as a carbon source (i.e., nutrients). Researchers are currently investigating treatment processes to control AOC.

One promising process is biologically active filtration wherein bacterial communities are intentionally established in the filters to use up, or biodegrade, the AOC as it passes through. This treatment process must be employed before final disinfection so that bacteria escaping from the filter can be properly controlled.

Most water utilities do not disinfect with chlorine until late in the treatment train. This limits the formation of disinfection by-products (i.e., those compounds like chloroform produced when chlorine reacts with naturally occurring organic carbon).

To accomplish disinfection earlier in treatment, some water utilities employ ozonation. While ozone is a very strong disinfectant, it also converts a portion of the TOC into AOC. Researchers are examining the advantages (e.g., disinfection of bacteria, viruses and protozoan cysts, control of color, control of taste and odor, enhancement of coagulation, and partial oxidation of the naturally occurring organic carbon that reacts with chlorine) and disadvantages of ozone (e.g., enhancement of AOC, conversion of bromide to bromate, and formation of its own disinfection byproducts like formaldehyde).

EPANET
The project entitled “EPANET” involves the development and testing of a water quality model for drinking water distribution systems. The EPANET model is a computer program that performs extended period simulation of hydraulic and water quality behavior within water distribution networks. It tracks the flow of water in each pipe, the pressure at each pipe junction, the height of water in each tank, and the concentration of a contaminant throughout the network during a multiple time period simulation. Water age and source tracing can also be simulated.

EPANET can be useful for analyzing the loss of disinfectant residual, designing water quality sampling programs, performing drinking water exposure risk assessments, and calibrating network hydraulic models. It can provide insight into how changes in water source utilization, pumping water storage levels, use of satellite treatment and targeted pipe cleaning and replacement would affect drinking water quality. In support of small community and non-community (less than 3,300 people) drinking water treatment systems, researchers are designing, modifying and testing “Hybrid Drinking Water Treatment Package Plants.”

These package plants are factory-built, skid-mounted, and ready to be operated in the field with minimal site preparation. They exhibit lower capital cost than custom designed facilities built onsite and can incorporate any drinking water treatment process. Promising technologies being considered for incorporation include membranes, advanced oxidation, bag filters, and photocatalytic oxidation.

By merging, modifying, and adapting conventional treatment trains with innovative treatment technologies, a broader variety of contaminants (including pathogens) can be removed and SDWA compliance can be facilitated. Concern has recently mounted over the ability of certain pathogenic protozoan (*Cryptosporidium)* cysts to survive treatment processes and enter the distribution system.

Chapter 1 - Water Quality Post Quiz

Internet Link to Assignment…
http://www.abctlc.com/downloads/PDF/LabAnalystAss.pdf

The answers for the post quiz are located in the rear before the References.

1. What is the substance or compound manufactured from aluminum hydroxide by dehydroxylating it in a way that produces a highly porous material?

2. Define TDS?

3. What is the substance or compound forms especially strong complexes with Mn(II), Cu(II), Fe(III), Pb (II) and Cr(III)?

4. Which compound/element can dissolve and accumulate in underground water sources, such as wells, and in the air in your home?

5. The EPA set a standard limit or the amount of what element in drinking water to 10 ppb?

6. Which compound/element/substance is a chemical that occurs naturally in the earth's crust. When rocks, minerals, and soil erode, they release this compound/element/substance into water supplies?

ICR

7. The EPA has collected data required by the Information Collection Rule (ICR) to support future regulation of *Microbial contaminants*, disinfectants, and disinfection byproducts. True or False

8. The rule is intended to provide EPA with information on chemical byproducts that form when disinfectants used for microbial control react with chemicals already present in source water (disinfection byproducts (DBPs)); *Disease-causing microorganisms (pathogens)*, including Cryptosporidium; and engineering data to control these contaminants. True or False

Stage 2 DBP Rule Federal Register Notices

9. Which rule is one part of the Microbial and Disinfection Byproducts Rules, which are a set of interrelated regulations that address risks from microbial pathogens and disinfectants/disinfection byproducts?

10. Which rule focuses on public health protection by limiting exposure to DBPs, specifically total trihalomethanes and five haloacetic acids, which can form in water through disinfectants used to control microbial pathogens?

11. There are specific microbial pathogens, such as_____, which can cause illness, and are highly resistant to traditional disinfection practices.

12. Which rule and the Long Term 2 Enhanced Surface Water Treatment Rule are the second phase of rules required by Congress?

13. Which rule is being promulgated simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule to address concerns about risk tradeoffs between pathogens and DBPs?

14. Which term requires systems to conduct an evaluation of their distribution systems, known as an Initial Distribution System Evaluation?

Filter Backwash Recycling Rule (FBRR)

15. The Filter Backwash Recycling Rule (FBRR) regulates the chlorination within the treatment process of public water systems. True or False

16. The FBRR requires surface water systems to review their recycle practices and to modify any recycle practices that may compromise microbial control or contribute to violations of the drinking water regulations. Recycle flows can be a source of concentrated microbial pathogens and chemical contaminants.
True or False

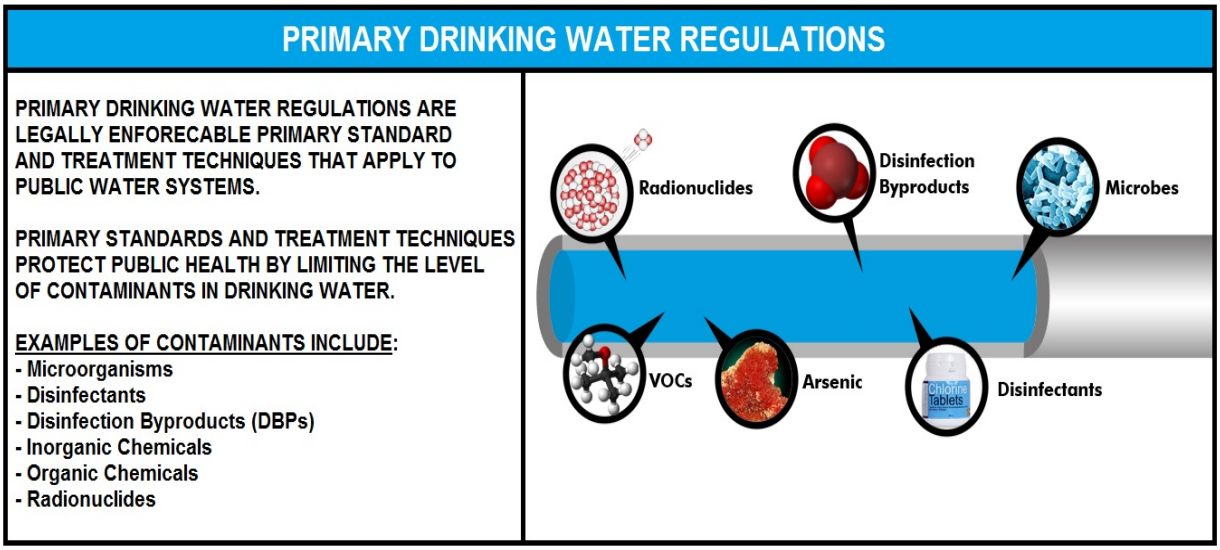
Water Quality Post Quiz Answers

1. Activated alumina, 2. Total Dissolved Solids, 3. Ethylenediaminetetraacetic acid (EDTA), 4. Radon gas, 5. Arsenic, 6. Arsenic,7. True, 8. True, 9. The Stage 2 DBP rule, 10. The Stage 2 DBP rule, 11. Cryptosporidium, 12. The Stage 2 DBPR, 13. The Stage 2 DBPR, 14. Stage 2 DBPR, 15. False, 16. True,

Chapter 2- Water Sampling and Laboratory Procedures

Section Focus: You will learn the basics of the EPA's Safe Water Drinking Act water sampling and basic laboratory procedures. At the end of this section, you will be able to describe EPA's Primary and Secondary standards. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: EPA identifies contaminants to regulate in drinking water to protect public health. The Agency sets regulatory limits for the amounts of certain contaminants in water provided by public water systems. These contaminant standards are required by the Safe Drinking Water Act (SDWA). Drinking water standards may apply differently based on type and size of public water systems.



PRIMARY DRINKING WATER REGULATIONS / STANDARDS

New EPA Rules

Arsenic

Arsenic is a chemical that occurs naturally in the earth's crust. When rocks, minerals, and soil erode, they release arsenic into water supplies. When people either drink this water or eat animals and plants that drink it, they are exposed to arsenic. For most people in the U.S., eating and drinking are the most common ways that people are exposed to arsenic, although it can also come from industrial sources. Studies have linked long-term exposure of arsenic in drinking water to a variety of cancers in humans.

To protect human health, an EPA standard limits the amount of arsenic in drinking water. In January 2001, the EPA revised the standard from 50 parts per billion (ppb), ordered that it fall to 10 ppb by 2006. After adopting 10ppb as the new standard for arsenic in drinking water, the EPA decided to review the decision to ensure that the final standard was based on sound science and accurate estimates of costs and benefits. In October 2001, the EPA decided to move forward with implementing the 10 ppb standard for arsenic in drinking water.

More information on the rulemaking process and the costs and benefits of setting the arsenic limit in drinking water at 10 ppb can be found at www.epa.gov/safewater/arsenic.html.

DISINFECTANTS AND DISINFECTION BYPRODUCTS RULE			
DISINFECTION RESIDUAL	**MRDLG (mg/L)**	**MRDL (mg/L)**	**COMPLIANCE BASED ON:**
CHLORINE	4 (as Cl_2)	4.0 (as Cl_2)	ANNUAL AVERAGE
CHLORAMINE	4 (as Cl_2)	4.0 (as Cl_2)	ANNUAL AVERAGE
CHLORINE DIOXIDE	0.8 (as ClO_2)	0.8 (as ClO_2)	ANNUAL AVERAGE
DISINFECTION BYPRODUCTS	**MCLG (mg/L)**	**MCL (mg/L)**	**COMPLIANCE BASED ON:**
TOTAL TRIHALOMETHANES (TTHM)[1] - CHLOROFORM - BROMODICHLOROMETHANE - DIBROMOCHLOROMETHANE - BROMOFORM	N/A *** 0 0.06 0	0.080	ANNUAL AVERAGE
HALOACETIC ACIDS (five) (HAA5)[2] - DICHLOROACETIC ACID - TRICHLOROACETIC ACID	N/A 0 0.3	0.60	ANNUAL AVERAGE
CHLORITE	0.8	1.0	MONTHLY AVERAGE
BROMATE	0	0.010	ANNUAL AVERAGE

DISINFECTION BYPRODUCTS RULE PARAMETERS EXAMPLE

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ICR

The EPA has collected data required by the Information Collection Rule (ICR) to support future regulation of microbial contaminants, disinfectants, and disinfection byproducts. The rule is intended to provide EPA with information on chemical byproducts that form when disinfectants used for microbial control react with chemicals already present in source water (disinfection byproducts (DBPs)); disease-causing microorganisms (pathogens), including Cryptosporidium; and engineering data to control these contaminants.

Drinking water microbial and disinfection byproduct information collected for the ICR is now available in the EPA's Envirofacts Warehouse website.

Disinfection Rules Stages 1 & 2 DBPR

The following are EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

Stage 1 and 2 Rules Introduction

Chlorine is the most widely used water disinfectant due to its effectiveness and cost. Using chlorine as a drinking water disinfectant has prevented millions of water borne diseases, such as typhoid, cholera, dysentery, and diarrhea. Most states require community water systems to use chlorination.

All disinfectants form DBPs in one of two reactions: Chorine and chlorine-based compounds (halogens) react with organics in water causing the chlorine atom to substitute other atoms resulting in halogenated by-products.

Oxidation reactions, where chlorine oxidizes compounds present in water. Secondary by-products are also formed when multiple disinfectants are used.

The EPA's Surface Water Treatment Rule (SWTR) requires systems using public water supplies from either surface water or groundwater under the direct influence of surface water to disinfect.

Public Health Concerns

While disinfectants are effective in controlling many microorganisms, they react with natural organic and inorganic matter in source water and distribution systems to form DBPs. Results from toxicology studies have shown several DBPs (e.g., bromodichloromethane, bromoform, chloroform, dichloroacetic acid, and bromate) to be carcinogenic in laboratory animals.

Other DBPs (e.g., chlorite, bromodichloromethane, and certain haloacetic acids) have also been shown to cause adverse reproductive or developmental effects in laboratory animals.

Several epidemiology studies have suggested a weak association between certain cancers (e.g., bladder) or reproductive and developmental effects, and exposure to chlorinated surface water. More than 200 million people consume water that has been disinfected. Because of the large population exposed, health risks associated with DBPs, even if small, need to be taken seriously.

Stage 2 DBPR

EPA finalized the Stage 2 Disinfectants and Disinfection Byproduct Rule (DBPR) to reduce potential health risks from DBPs. The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) is being finalized and implemented at the same time as the Stage 2 DBPR to ensure that drinking water is safe from both microbial pathogens and DBPs.

General Requirements

To comply with the Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR), published on January 4, 2006 (71 FR 388) systems must do the following:

• **Conduct an Initial Distribution System Evaluation (IDSE)** to find locations in the distribution system that have high levels of TTHM and HAA5 and that can be used as compliance monitoring sites for the Stage 2 DBPR.

• **Use a locational running annual average (LRAA) calculation to determine compliance** with the Stage 2 DBPR maximum contaminant levels (MCLs) of:

– 0.080 mg/L for total trihalomethanes (TTHM), and

– 0.060 mg/L for five haloacetic acids (HAA5).

Note: The MCL values are the same as the Stage 1 MCLs; only the calculation method changes.

• **Monitor for Stage 2 compliance** at the required number of locations for each system's retail population

• **Identify when TTHM or HAA5 levels exceed the operational evaluation level** and, when this happens, look at source water, operational practices, and treatment to find ways to reduce TTHM and HAA5 concentrations in the distribution system. Each of these general requirements are covered in more detail in the rest of this guidance manual. The Stage 2 DBPR is an extension of the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR). Systems must also continue to comply with the other requirements of the Stage 1 DBPR in addition to meeting the requirements of the Stage 2 DBPR. This includes compliance with the MCLs for bromate (for systems using ozone) and chlorite (for systems using chlorine dioxide), the MRDLs for chlorine or chloramine (depending on the residual disinfectant used), as well as TOC removal requirements.

Compliance Timeline
Your compliance schedule for the Stage 2 DBPR are based on whether your system is part of a *combined distribution system*:

• If your system **is** part of a combined distribution system, you must comply with the revised MCLs by the same date as required for the largest system in your combined distribution system.

Example: if your system serves 8,000 people, but you purchase water from a system that serves 250,000 people, you must comply by the dates shown in Schedule 1.

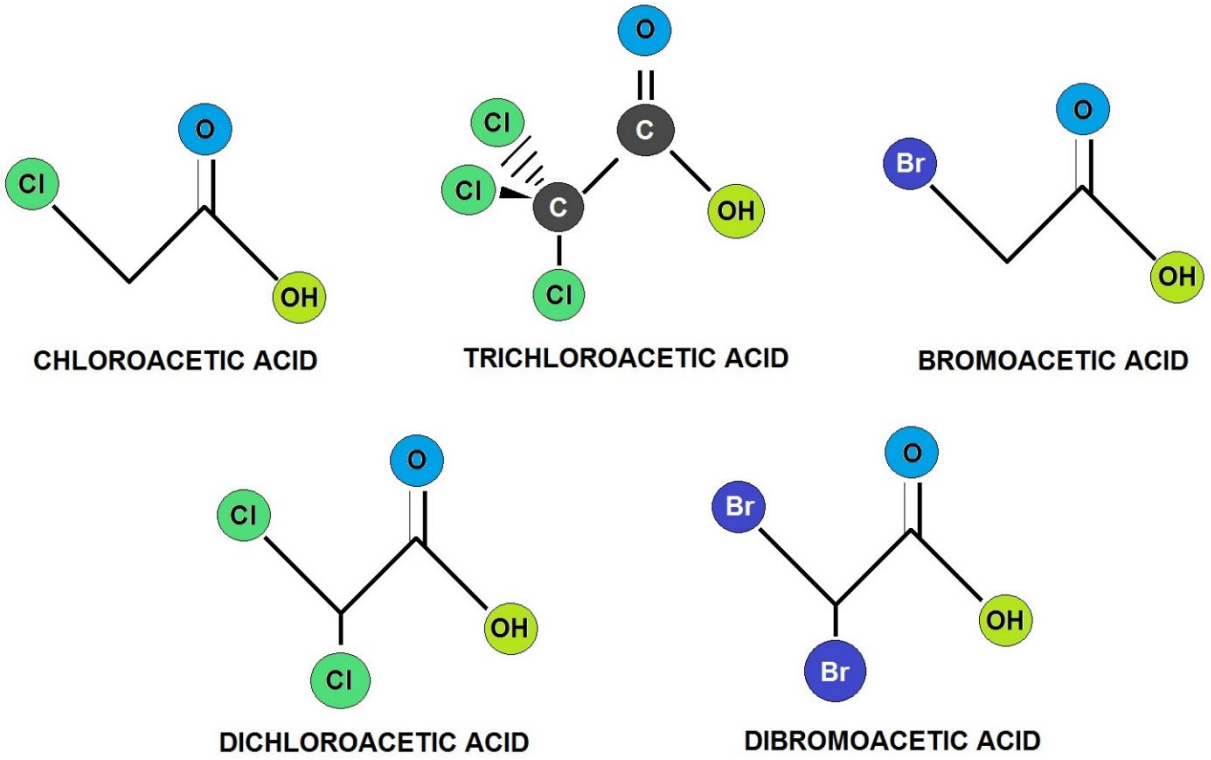
• If your system **is not** part of a combined distribution system, compliance dates are based on the population served by your system.

If you are using this guidance manual, you likely serve fewer than 10,000 people and you must comply by the dates shown in Schedule 4.

Your State (or EPA) should have sent you a letter telling you what schedule you are on. If you did not receive this letter or you have questions about your schedule, contact your State (contact information is listed in Appendix C).

Note: You are on the same schedule for Stage 2 DBPR compliance as you were on for the IDSE.The timeline on the next page shows important dates for the Stage 2 DBPR as well as periods for *Cryptosporidium* and *E. coli* required under the LT2ESWTR.

Note: The figure shows the 2-year period after systems must begin compliance as a "possible extension." States may give you up to an additional 2 years to comply if you need time to install capital improvements.



HALOACETIC ACIDS (HAA5)

As noted earlier, the Stage 2 DBPR is an extension of the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR). The Stage 2 DBPR and the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) were published together to address the balance between protection from microbial pathogens and the potential health effects from disinfectants and their byproducts.

You are still required to continue to meet all existing federal requirements. You may call the Safe Drinking Water Hotline at (800) 426-4791 (e-mail: hotline-sdwa@epa.gov) for more information on other drinking water rules.

Where do DBPs come from?
Chlorine and other chemical disinfectants have been widely used by public water systems (along with filtration) to protect the public from microbial pathogens in drinking water. DBPs are formed when certain disinfectants react with DBP precursors (organic and inorganic materials) in source waters. In most cases, natural organic matter (NOM) is an important factor that affects the levels of DBPs that form (NOM is usually measured as TOC).

The levels of DBPs in drinking water can vary significantly from one point in a distribution system to another, as many continue to form in the distribution system. DBP levels are generally higher in surface water systems because surface water usually contains higher DBP precursor levels and requires stronger disinfection.

Ensuring Safe Drinking Water
All drinking water systems want to provide water that is safe. One aspect of providing safe drinking water is limiting the levels of DBPs in it. Long-term exposure to DBPs has been linked to bladder cancer, and possibly colon and rectal cancers. More recent studies have shown that shorter-term exposure to high levels of DBPs may be associated with adverse reproductive and developmental health effects.

Limiting the levels of DBPs in your drinking water may require you to make some adjustments to your current operations, such as:

- Making operational improvements at the plant or in the distribution system
- Modifying current treatment operations to remove more DBP precursors or form lower levels of DBPs
- Upgrading or installing a new treatment technology

What Does Compliance Monitoring Involve?
Monitoring requirements for TTHM and HAA5 are based on your source water type and the population your system serves. Note that this is different than the Stage 1 DBPR monitoring requirements that were based on the number of treatment plants in your system.

With population-based monitoring, there are five categories of small systems under the Stage 2 DBPR:

- Subpart H systems that serve fewer than 500 people.
- Subpart H systems that serve 500 to 3,300 people.
- Subpart H systems that serve 3,301 to 9,999 people.
- Ground water systems that serve fewer than 500 people.
- Ground water systems that serve 500 to 9,999 people.

If you do not know what type of system you are, you should contact your State to confirm this information.

Older Stage 1 DBPR Information

Disinfection Byproduct Regulations
In December 1998, the EPA established the Stage 1 Disinfectants/Disinfection Byproducts Rule that requires public water systems to use treatment measures to reduce the formation of disinfection byproducts and to meet the following specific standards:

Total Trihalomethanes (TTHM)	80 parts per billion (ppb)
Haloacetic Acids (HAA5)	60 ppb
Bromate	10 ppb
Chlorite	1.0 parts per million (ppm)

Trihalomethanes were regulated at a maximum allowable annual average level of 100 parts per billion for water systems serving over 10,000 people under the Total Trihalomethane Rule finalized by the EPA in 1979. The Stage 1 Disinfectant/Disinfection Byproduct Rule standards became effective for trihalomethanes and other disinfection byproducts listed above in December 2001 for large surface water public water systems. Those standards became effective in December 2003 for small surface water and all ground water public water systems.

Disinfection byproducts are formed when disinfectants used in water treatment plants react with bromide and/or natural organic matter (i.e., decaying vegetation) present in the source water. Different disinfectants produce different types or amounts of disinfection byproducts. Disinfection byproducts for which regulations have been established have been identified in drinking water, including trihalomethanes, haloacetic acids, bromate, and chlorite.

Trihalomethanes (**THM**) are a group of four chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The trihalomethanes are chloroform, bromodichloromethane, dibromochloromethane, and bromoform.

The EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate total trihalomethanes (TTHM) at a maximum allowable annual average level of 80 parts per billion. This new standard replaced the old standard of a maximum allowable annual average level of 100 parts per billion back in December 2001 for large surface water public water systems. The standard became effective for the first time back in December 2003 for small surface water and all ground water systems.

Haloacetic Acids (**HAA5**) are a group of chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The regulated haloacetic acids, known as HAA5, are: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate HAA5 at 60 parts per billion annual average.

This standard became effective for large surface water public water systems back in December 2001 and for small surface water and all ground water public water systems back in December 2003.

Summary
In the past 30 years, the Safe Drinking Water Act (SDWA) has been highly effective in protecting public health and has also evolved to respond to new and emerging threats to safe drinking water.

There are specific microbial pathogens, such as Cryptosporidium, which can cause illness, and are highly resistant to traditional disinfection practices.

Stage 1 and 2 DBP Rule Federal Register Notices
Chlorine and its halogen are neutrally charged and therefore easily penetrate the negatively charged surface of pathogens.

Interim Enhanced Surface Water Treatment Rule was established to maintain control of pathogens while systems lower disinfection byproduct levels to comply with the Stage 1 Disinfectants/Disinfection Byproducts Rule and to control Cryptosporidium.

The EPA established a MCL of o for all public water systems and a TT3 MCLG (mg/L) removal requirement for Cryptosporidium in filtered public water systems that serve at least 100,000 people.

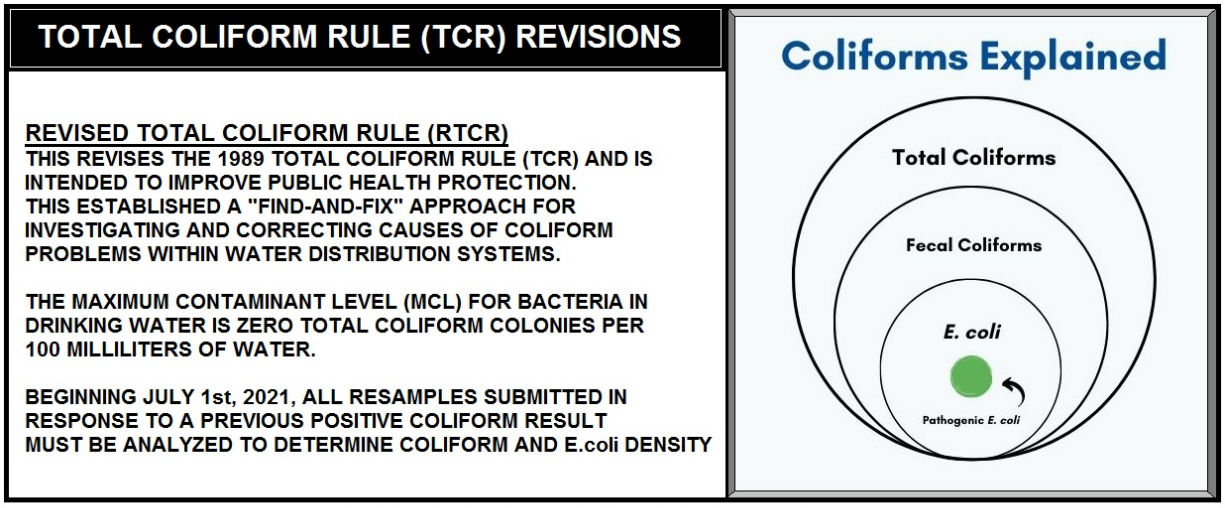
Turbidly measurement is an indicator of the physical removal of particulates, including pathogens.

Stage 1 Disinfectants/Disinfection Byproducts Rule improves physical removal of Cryptosporidium, and to maintain control of pathogens.

Revised Total Coliform Rule (RTCR)

The following are EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

EPA published the Revised Total Coliform Rule (RTCR) in the Federal Register (FR) on February 13, 2013 (78 FR 10269). It is the revision to the 1989 Total Coliform Rule (TCR).



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TOTAL COLIFORM RULE (TCR) REVISIONS

Why revise the 1989 TCR?
The 1996 amendments to the Safe Drinking Water Act [Section 1412(b) (9)] require the Administrator to review and revise, as appropriate, each national primary drinking water regulation not less often that every six years. EPA published its decision to revise the TCR in July 2003 as part of its National Primary Drinking Water Regulation (NPDWR) review.

The RTCR:

- Upholds the purpose of the 1989 TCR to protect public health by ensuring the integrity of the drinking water distribution system and monitoring for the presence of microbial contamination.
- Requires public water systems (PWSs) to meet a legal limit for E. coli, as demonstrated by required monitoring.
- Specifies the frequency and timing of required microbial testing based on population served, public water system type and source water type: ground water or surface water.

When must PWSs comply with the RTCR requirements?
Unless a State determines an earlier effective date, all PWSs must comply with the RTCR requirements starting April 1, 2016. All PWSs include:

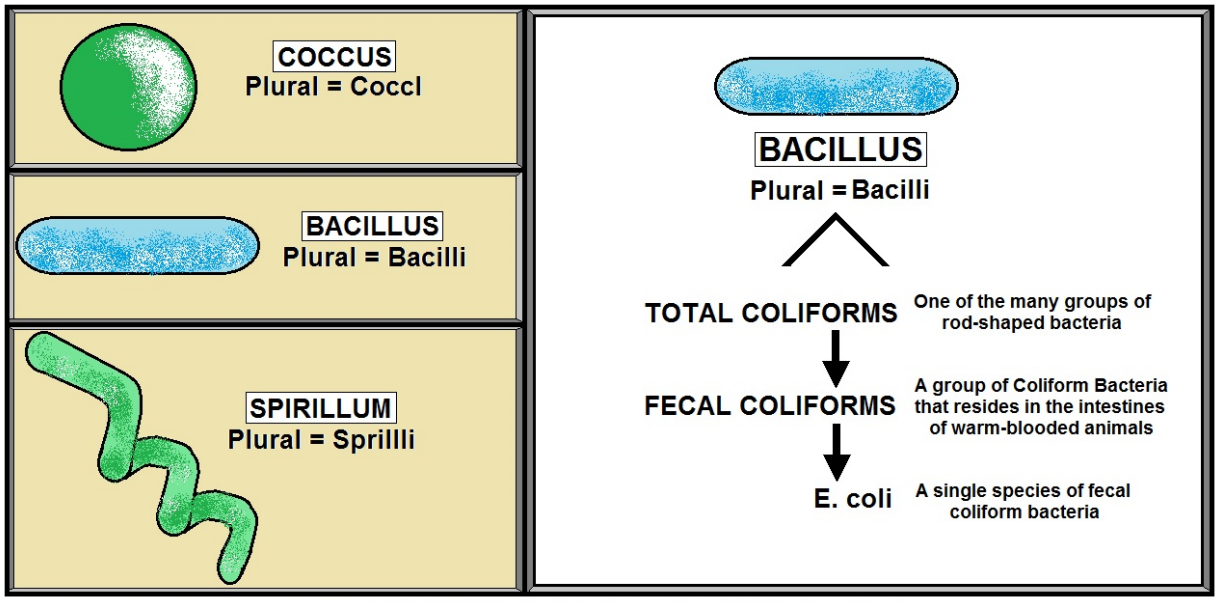
- Community Water Systems (CWSs),
- Non-Transient Non-Community Water Systems (NTNCWSs), and
- Transient Non-Community Water Systems (TNCWSs).

Minor Corrections to the Revised Total Coliform Rule (RTCR)
Minor corrections to the final RTCR became effective on April 28, 2014. No comments were received on the Direct Final Rule published on February 26, 2014 and the corrections therefore became effective without further notice. See the **Direct Final Rule** Federal Register Notice.

Revised Total Coliform Rule (RTCR) – Final Rule
On February 13, 2013, EPA published in the Federal Register the revisions to the 1989 TCR. EPA anticipates greater public health protection under the Revised Total Coliform Rule (RTCR) requirements. The RTCR:

- Requires public water systems that are vulnerable to microbial contamination to identify and fix problems; and
- Establishes criteria for systems to qualify for and stay on reduced monitoring, which could reduce water system burden and provide incentives for better system operation.

Public water systems (PWSs) and primacy agencies must comply with the revised requirements by April, 2016. Until then, PWSs and primacy agencies must continue complying with the 1989 TCR.



COLIFORM BACTERIA EXAMPLE

What are the key provisions PWSs must comply with under the RTCR?

Provision Category	Key Provisions
Contaminant Level	• Addresses the presence of total coliforms and E. coli in drinking water. • For E. coli (EC), the Maximum Contaminant Level Goal (MCLG) is set at zero and the Maximum Contaminant Level (MCL) is based on the occurrence of a condition that includes routine and repeat samples. • For total coliforms (TC), PWSs must conduct a Level 1 or Level 2 assessment of their system when they exceed a specified frequency of total coliform occurrence. Other events such as an MCL violation or failure to take repeat samples following a routine total coliform-positive sample will also trigger an assessment. Any sanitary defects identified during an assessment must be corrected by the PWS. These are the treatment technique requirements of the RTCR.
Monitoring	• Develop and follow a sample siting plan that designates the PWS's collection schedule and location of routine and repeat water samples. • Collect routine water samples on a regular basis (monthly, quarterly, annually) and have them tested for the presence of total coliforms by a state certified laboratory. • Analyze all routine or repeat samples that are total coliform positive (TC+) for E. coli. • Collect repeat samples (at least 3) for each TC+ positive routine sample. • For PWSs on quarterly or annual routine sampling, collect additional routine samples (at least 3) in the month after a TC+ routine or repeat sample. • Seasonal systems must monitor and certify the completion of a state-approved start-up procedures
Level 1 and Level 2 Assessments and Corrective Actions	• PWSs are required to conduct a Level 1 or Level 2 assessment if certain conditions indicate that they might be vulnerable to contamination, and fix any sanitary defects within a required timeframe.
Reporting and Recordkeeping	• PWSs are required to report certain items to their states. These reporting and recordkeeping requirements are essentially the same as under TCR with the addition of Level 1 and Level 2 requirements.
Violations, Public Notification (PN) and Consumer Confidence Report (CCR)	• PWSs incur violations if they do not comply with the requirements of the RTCR. The violation types are essentially the same as under the TCR with few changes. The biggest change is no acute or monthly MCL violation for total coliform positive samples only. • PN is required for violations incurred. Within required timeframes, the PWS must use the required health effects language and notify the public if they did not comply with

	certain requirements of the RTCR. The type of PN depends on the severity of the violation. • Community water systems (CWSs) must use specific language in their CCRs when they must conduct an assessment or if they incur an E. coli MCL violation.

CHAIN OF CUSTODY RECORD

- A CHAIN OF CUSTODY IS THE PROPER FORM USED TO ACCOMPANY SAMPLES WHEN THEY ARE DELIVERED TO A LABORATORY.
- THIS FORM GIVES THE LABORATORY YOUR CLIENT INFORMATION AS WELL AS IDENTIFICATION OF THE SAMPLE TYPE, DESCRIPTION, SAMPLING DATE and TIME, AND WHAT TESTS YOU WANT RUN ON SAMPLE

CHAIN OF CUSTODY PROCEDURES:

- COLLECTION TECHNIQUES
- PRESERVATION
- PACKAGING
- TRANSPORTATION
- STORAGE
- CREATION OF INVENTORY LIST

CHAIN OF CUSTODY EXAMPLE:

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LABORATORIES TESTING & INSPECTION SERVICES, INC.
123 SAMPLE AVENUE STE# 1
WAYTOHOT, ARIZONA, 85938

CHAIN OF CUSTODY RECORD

PROJECT NUMBER | PROJECT NAME & ADDRESS | NUMBER OF CONTAINERS | ANALYSES / CONTAINERS AND PRESERVATIVES

SAMPLERS (Signature)

LAB USE ONLY LAB NUMBER	DATE	TIME	TOTAL RESIDUAL CHLORINE	COLL. METHOD	SAMPLE MATRIX	STATION LOCATION / DESCRIPTION	NUMBER OF CONTAINERS	COMMENTS

ΔSampling Methods abbreviations: Grab (G), Composite (C), Equipment Blank (EQ)
ΔSample Matrix abbreviations: Groundwater (GW), Surface Water (SW), Marine Water (MW), Drinking Water (DW), Sludge (Slg), Soil, Waste (Wt), Domestic Effluent (Dom Eff), Industrial Effluent (Ind Eff), Influent (Inf)

Kit prepared and relinquished by:	Date/Time:	Received by: Company Name:	Date/Time:	Relinquished by: Company Name:	Date/Time:	Common Carrier:	
Received by: Company Name:	Date/Time:	Relinquished by: Company Name:	Date/Time:	Received by: Company Name:	Date/Time:		
Relinquished by: Company Name:	Date/Time:	Received in the Laboratory by:	Date/Time:				

NOTE: PLEASE RETURN THIS CHAIN OF CUSTODY WITH SAMPLES!

CHAIN OF CUSTODY EXAMPLE

More on the Current Stage 2 DBP Rule

The following are EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

The Stage 2 DBP rule is one part of the Microbial and Disinfection Byproducts Rules (MDBPs), which are a set of interrelated regulations that address risks from microbial pathogens and disinfectants/disinfection byproducts. The Stage 2 DBP rule focuses on public health protection by limiting exposure to DBPs, specifically total trihalomethanes (TTHM) and five haloacetic acids (HAA5), which can form in water through disinfectants used to control microbial pathogens. This rule will apply to all community water systems and nontransient noncommunity water systems that add a primary or residual disinfectant other than ultraviolet (UV) light or deliver water that has been disinfected by a primary or residual disinfectant other than UV.

Amendments to the SDWA in 1996 require EPA to develop rules to balance the risks between microbial pathogens and disinfection byproducts (DBPs). The Stage 1 Disinfectants and Disinfection Byproducts Rule and Interim Enhanced Surface Water Treatment Rule, promulgated in December 1998, were the first phase in a rulemaking strategy required by Congress as part of the 1996 Amendments to the Safe Drinking Water Act.

The Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) builds upon the Stage 1 DBPR to address higher risk public water systems for protection measures beyond those required for existing regulations. The Stage 2 DBPR and the Long Term 2 Enhanced Surface Water Treatment Rule are the second phase of rules required by Congress. These rules strengthen protection against microbial contaminants, especially *Cryptosporidium*, and at the same time, reduce potential health risks of DBPs.

What is the Stage 2 DBPR?

The Stage 2 Disinfection Byproducts Rule will reduce potential cancer and reproductive and developmental health risks from disinfection byproducts (DBPs) in drinking water, which form when disinfectants are used to control microbial pathogens. Over 260 million individuals are exposed to DBPs.

This final rule strengthens public health protection for customers by tightening compliance monitoring requirements for two groups of DBPs, trihalomethanes (TTHM) and haloacetic acids (HAA5). The rule targets systems with the greatest risk and builds incrementally on existing rules. This regulation will reduce DBP exposure and related potential health risks and provide more equitable public health protection. The Stage 2 DBPR is being promulgated simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule to address concerns about risk tradeoffs between pathogens and DBPs.

What does the rule require?

Under the Stage 2 DBPR, systems will conduct an evaluation of their distribution systems, known as an Initial Distribution System Evaluation (IDSE), to identify the locations with high disinfection byproduct concentrations.

These locations will then be used by the systems as the sampling sites for Stage 2 DBPR compliance monitoring. Compliance with the maximum contaminant levels for two groups of disinfection byproducts (TTHM and HAA5) will be calculated for each monitoring location in the distribution system. This approach, referred to as the locational running annual average (LRAA), differs from current requirements, which determine compliance by calculating the running annual average of samples from all monitoring locations across the system.

The Stage 2 DBPR also requires each system to determine if they have exceeded an operational evaluation level, which is identified using their compliance monitoring results. The operational evaluation level provides an early warning of possible future MCL violations, which allows the system to take proactive steps to remain in compliance.

A system that exceeds an operational evaluation level is required to review their operational practices and submit a report to their state that identifies actions that may be taken to mitigate future high DBP levels, particularly those that may jeopardize their compliance with the DBP MCLs.

Who must comply with the rule?
Entities potentially regulated by the Stage 2 DBPR are community and nontransient noncommunity water systems that produce and/or deliver water that is treated with a primary or residual disinfectant other than ultraviolet light.

A community water system (CWS) is a public water system that serves year-round residents of a community, subdivision, or mobile home park that has at least 15 service connections or an average of at least 25 residents.

A nontransient noncommunity water system (NTNCWS) is a water system that serves at least 25 of the same people more than six months of the year, but not as primary residence, such as schools, businesses, and day care facilities.

What are disinfection byproducts (DBPs)?
Disinfectants are an essential element of drinking water treatment because of the barrier they provide against waterborne disease-causing microorganisms. Disinfection byproducts (DBPs) form when disinfectants used to treat drinking water react with naturally occurring materials in the water (e.g., decomposing plant material).

Total trihalomethanes (TTHM - chloroform, bromoform, bromodichloromethane, and dibromochloromethane) and haloacetic acids (HAA5 - monochloro-, dichloro-, trichloro-, monobromo-, dibromo-) are widely occurring classes of DBPs formed during disinfection with chlorine and chloramine. The amount of trihalomethanes and haloacetic acids in drinking water can change from day to day, depending on the season, water temperature, amount of disinfectant added, the amount of plant material in the water, and a variety of other factors.

Are THMs and HAAs the only disinfection byproducts?
No. The four THMs (TTHM) and five HAAs (HAA5) measured and regulated in the Stage 2 DBPR act as indicators for DBP occurrence. There are many other known DBPs, in addition to the possibility of unidentified DBPs present in disinfected water. THMs and HAAs typically occur at higher levels than other known and unknown DBPs. The presence of TTHM and HAA5 is representative of the occurrence of many other chlorination DBPs; thus, a reduction in the TTHM and HAA5 generally indicates a reduction of DBPs from chlorination.

Microbial Regulations

One of the key regulations developed and implemented by the United States Environmental Protection Agency (USEPA) to counter pathogens in drinking water is the Surface Water Treatment Rule.

Among its provisions, the rule requires that a public water system, using surface water (or ground water under the direct influence of surface water) as its source, have sufficient treatment to reduce the source water concentration of *Giardia* and viruses by at least 99.9% and 99.99%, respectively. The Surface Water Treatment Rule specifies treatment criteria to assure that these performance requirements are met; they include turbidity limits, disinfectant residual and disinfectant contact time conditions.

Disinfectant Review Statements:

Disinfectant residual: The CT values for disinfection are used to determine the disinfection efficiency based upon time and what other parameter?

Bacteria, Virus and Intestinal parasites: What types of organisms may transmit waterborne diseases?

Disinfection By-Products (DBPs): The products created due to the reaction of chlorine with organic materials (e.g. leaves, soil) present in raw water during the water treatment process. The EPA has determined that these DBPs can cause cancer.

How is the effectiveness of disinfection determined? *From the results of coliform testing.*

The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

What types of source water are required by law to treat water using filtration and disinfection? *Groundwater under the direct influence of surface water, and related surface water sources.*

E. Coli, *Escherichia coli*: A bacterium commonly found in the human intestine. For water quality analyses purposes, it is considered an indicator organism. These are considered evidence of water contamination. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

pH Strips

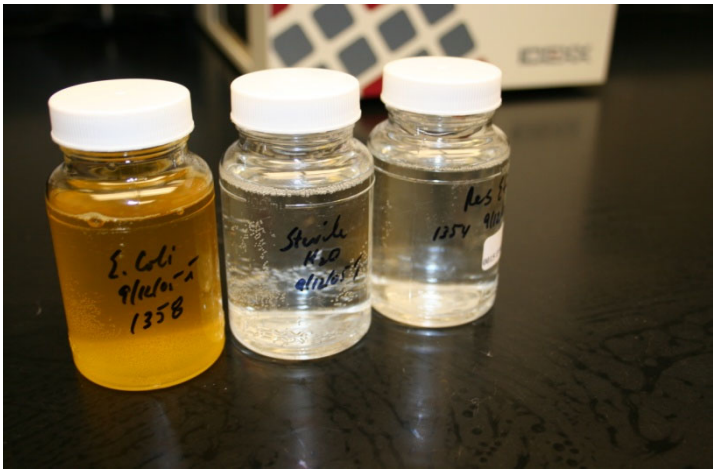
pH is on a scale from 0-14. 7 is considered neutral and acid is on the 0 to 7 side and the base is 7-14. pH is known as the Power of Hydroxyl Ion activity.

Common water distribution sample bottles, Radiochems, VOCs, (Volatile Organic Compounds), TTHMs, Total Trihalomethanes), Nitrate, Nitrite.

Most of these sample bottles will come with the preservative already inside the bottle. Some bottles will come with a separate preservative (acid) for the field preservation. Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.

Bac-T Sample Bottle, often referred to as a Standard Sample, 100 mls. Notice the white powder inside the bottle. That is Sodium Thiosulfate, a de-chlorination agent. Be careful not to wash-out this chemical while sampling. Notice the custody seal on the bottle.

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.



The bottle with the yellow color on the left indicates coliform bacteria is present. If the bottle fluoresces under a black light, fecal bacteria is present.

Common Water Quality Definitions

Units of Measurement

mg/l = Milligrams per liter. One milligram per liter equals one packet of artificial sweetener sprinkled into 250 gallons of iced tea.
µg/l = Micrograms per liter. One microgram per liter is equal to one packet of artificial sweetener sprinkled into an Olympic-size swimming pool.
NTU = Nephelometric Turbidity Units. A measurement on the cloudiness of the water.
pCi/l = Picocuries per liter. A measure of radioactivity.

Acronyms

Maximum Contaminant Level (MCL) - The highest level of a contaminant that is allowed in drinking water.

Maximum Contaminant Level Goal (MCLG) - The level of a contaminant in drinking water below which there is no known or expected risk to health.

Treatment Technique (TT) - A required process intended to reduce the level of a contaminant in drinking water.

Action Level (AL) - The concentration of a contaminant that, if exceeded, triggers treatment or other requirements which a water system must follow.

National Interim Primary Drinking Water Regulations (NIPDWR) Promulgated 1975-1981 Contained 7 contaminants Targeted: Trihalomethanes, Arsenic, and Radionuclides Established 22 drinking water standards.

Phase 1 Standards Promulgated 1987 Contained 8 contaminants Targeted: VOCs.

Phase 2 Standards Promulgated 1991 Contained 36 contaminants Targeted: VOCs, SOCs, and IOCs.

Phase 5 Standards Promulgated 1992 Contained 23 contaminants Targeted: VOCs, SOCs, and IOCs.

Surface Water Treatment Rule (SWTR) Promulgated 1989 Contained 5 contaminants Targeted: Microbiological and Turbidity.

Stage 1 Disinfectant/Disinfection By-product (D/DBP) Rule Promulgated 1998 Contained 14 contaminants Targeted: DBPs and precursors.

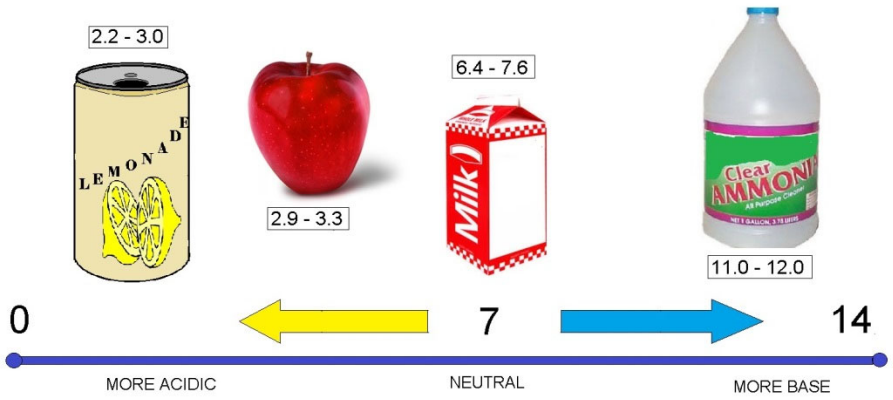
Interim Enhanced Surface Water Treatment Rule (IESWTR) Promulgated 1998 Contained 2 contaminants Targeted: Microbiological and Turbidity.

Radionuclide Rule Promulgated 2000 Contained 4 contaminants Targeted: Radionuclides.

Arsenic Rule Promulgated 2001 Contained 1 contaminant Targeted: Arsenic.

Filter Backwash Recycling Rule Promulgated 2001 Contained - Targeted: Microbiological and Turbidity.

pH Sub-Section – Detailed



pH SCALE

In water and wastewater processes, **pH** is a measure of the acidity or basicity of an aqueous solution. Solutions with a pH greater than 7 are basic or alkaline and solution or samples with a pH less than 7 are said to be acidic. Pure water has a pH very close to 7.

The Arrhenius theory states than an acid is a substance that produces Hydronium ions when it is dissolved in water, and a base is one that produces hydroxide ions when dissolved in water.

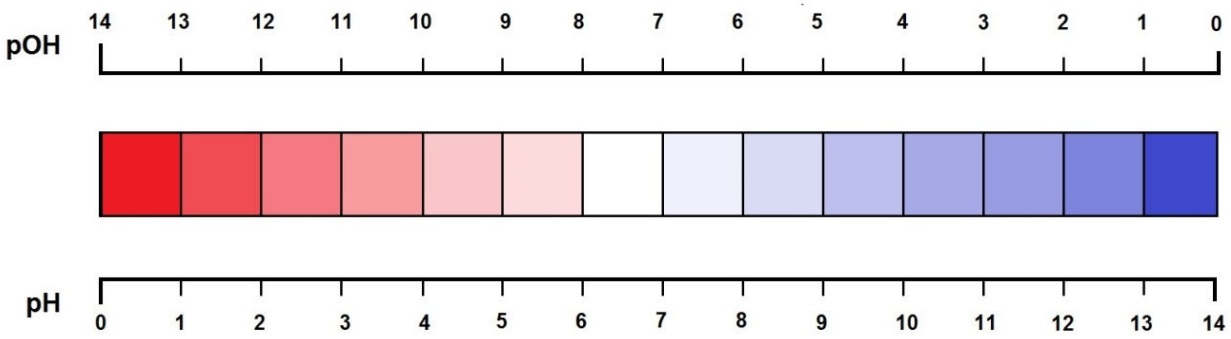
Primary pH standard values are determined using a concentration cell with transference, by measuring the potential difference between a hydrogen electrode and a standard electrode such as the silver chloride electrode. The pH scale is traceable to a set of standard solutions whose pH is established by international agreement.

Measurement of pH for aqueous solutions can be done with a glass electrode and a pH meter, or using indicators like strip test paper. pH measurements are important in water and wastewater processes (sampling) but also in medicine, biology, chemistry, agriculture, forestry, food science, environmental science, oceanography, civil engineering, chemical engineering, nutrition, water treatment & water purification, and many other applications.

Mathematically, pH is the measurement of hydroxyl ion activity and expressed as the negative logarithm of the activity of the (solvated) hydronium ion, more often expressed as the measure of the hydronium ion concentration.

Ions and Salts

An *ion* is a charged species, an atom or a molecule, that has lost or gained one or more electrons. When an atom loses an electron and thus has more protons than electrons, the atom is a positively-charged ion or cation. When an atom gains an electron and thus has more electrons than protons, the atom is a negatively-charged ion or anion. Cations and anions can form a crystalline lattice of neutral salts, such as the Na^+ and Cl^- ions forming sodium chloride, or NaCl.



IN RELATION BETWEEN p(OH) AND p(H) (red= ACIDIC / blue= BASIC)

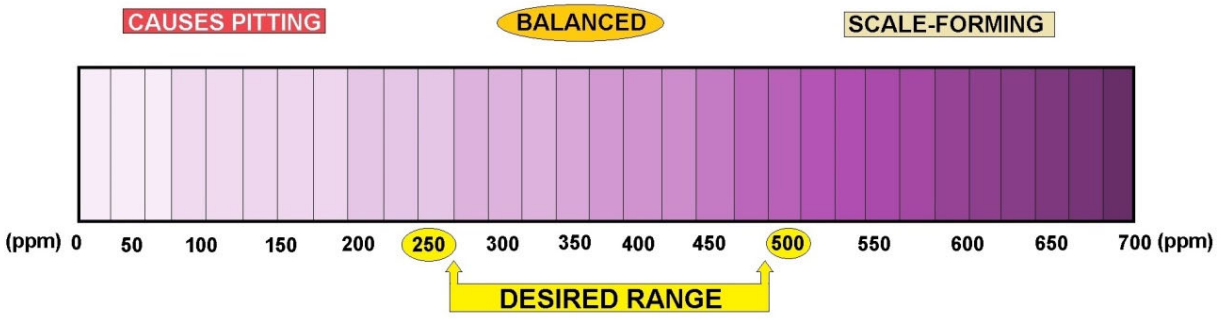
Contents

History

The scientific discovery of the p[H] concept of was first introduced by Danish chemist Søren Peder Lauritz Sørensen at the Carlsberg Laboratory back in 1909 and revised to the modern pH in 1924 to accommodate definitions and measurements in terms of electrochemical cells. In the first papers, the notation had the "H" as a subscript to the lowercase "p", as so: pH.

Alkalinity

Alkalinity is the quantitative capacity of an aqueous solution to neutralize an acid. Measuring alkalinity is important in determining a stream's ability to neutralize acidic pollution from rainfall or wastewater. It is one of the best measures of the sensitivity of the stream to acid inputs. There can be long-term changes in the alkalinity of rivers and streams in response to human disturbances.



CALCIUM HARDNESS MEASUREMENT

Reference. Bates, Roger G. Determination of pH: theory and practice. Wiley, 1973.

pH Definition and Measurement

CONCENTRATION OF HYDROGEN IONS COMPARED TO DISTILLED H2O				EXAMPLES OF SOLUTIONS AND THEIR RESPECTIVE pH
	1/10,000,000	14	LIQUID DRAIN CLEANER CAUSTIC SODA	
	1/1,000,000	13	BLEACHES OVEN CLEANERS	
	1/100,000	12	SOAPY WATER	
	1/10,000	11	HOUSEHOLD AMMONIA (11.9)	
	1/1,000	10	MILK OF MAGNESIUM (10.5)	
	1/100	9	TOOTHPASTE (9.9)	
	1/10	8	BAKING SODA (8.4) / SEA WATER EGGS	
	0	7	"PURE" WATER (7)	
	10	6	URINE (6) / MILK (6.6)	
	100	5	ACID RAIN (5.6) BLACK COFFEE (5)	
	1000	4	TOMATO JUICE (4.1)	
	10,000	3	GRAPEFRUIT & ORANGE JUICE SOFT DRINK	
	100,000	2	LEMON JUICE (2.3) VINEGAR (2.9)	
	1,000,000	1	HYDROCHLORIC ACID SECRETED FROM STOMACH LINING (1)	
	10,000,000	0	BATTERY ACID	

pH Scale

Technical Definition of pH

In technical terms, pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, *a*H+, in a solution.

$$pH = -\log_{10}(a_{H^+}) = \log_{10}\left(\frac{1}{aH+}\right)$$

Ion-selective electrodes are often used to measure pH, respond to activity.

In this calculation of electrode potential, *E*, follows the Nernst equation, which, for the hydrogen ion can be written as

$$E = E^0 + \frac{RT}{F} In\,(a_H+) = E^0 - \frac{2.303RT}{F} pH$$

where E is a measured potential, E^0 is the standard electrode potential, R is the gas constant, T is the temperature in kelvin, F is the Faraday constant. For H^+ number of electrons transferred is one. It follows that electrode potential is proportional to pH when pH is defined in terms of activity.

International Standard ISO 31-8 is the standard for the precise measurement of pH as follows: A galvanic cell is set up to measure the electromotive force (EMF) between a reference electrode and an electrode sensitive to the hydrogen ion activity when they are both immersed in the same aqueous solution.

The reference electrode may be a silver chloride electrode or a calomel electrode. The hydrogen-ion selective electrode is a standard hydrogen electrode.

Reference electrode | concentrated solution of KCl || test solution | H_2 | Pt

Firstly, the cell is filled with a solution of known hydrogen ion activity and the emf, E_S, is measured. Then the emf, E_X, of the same cell containing the solution of unknown pH is measured.

$$pH(X) = pH(S) + \frac{E_S - E_x}{Z}$$

The difference between the two measured emf values is proportional to pH. This method of calibration avoids the need to know the standard electrode potential. The proportionality

constant, *1/z* is ideally equal to $\frac{1}{2.303RT/F}$ the "Nernstian slope".

If you were to apply this practice the above calculation, a glass electrode is used rather than the cumbersome hydrogen electrode. A combined glass electrode has an in-built reference electrode. It is calibrated against buffer solutions of known hydrogen ion activity. IUPAC has proposed the use of a set of buffer solutions of known H^+ activity.

Two or more buffer solutions should be used in order to accommodate the fact that the "slope" may differ slightly from ideal.

The electrode is first immersed in a standard solution and the reading on a pH meter is adjusted to be equal to the standard buffer's value, to implement the proper calibration. The reading from a second standard buffer solution is then adjusted, using the "slope" control, to be equal to the pH for that solution. Further details, are given in the IUPAC recommendations.

When more than two buffer solutions are used the electrode is calibrated by fitting observed pH values to a straight line with respect to standard buffer values. Commercial standard buffer solutions usually come with information on the value at 25 °C and a correction factor to be applied for other temperatures. The pH scale is logarithmic and pH is a dimensionless quantity.

pH Indicators
Visual comparison of the color of a test solution with a standard color chart provides a means to measure pH accurate to the nearest whole number. Indicators may be used to measure pH, by making use of the fact that their color changes with pH. More precise measurements are possible if the color is measured spectrophotometrically, using a colorimeter of spectrophotometer. Universal indicator consists of a mixture of indicators such that there is a continuous color change from about pH 2 to pH 10. Universal indicator paper is made from absorbent paper that has been impregnated with universal indicator.

pOH
pOH is sometimes used as a measure of the concentration of hydroxide ions, OH^-, or alkalinity. pOH values are derived from pH measurements. The concentration of hydroxide ions in water is related to the concentration of hydrogen ions by

$$[\mathrm{OH^-}] = \frac{K_W}{[\mathrm{H^+}]}$$

where K_W is the self-ionization constant of water. Taking logarithms

$$\mathrm{pOH} = \mathrm{pK_W} - \mathrm{pH}$$

So, at room temperature pOH ≈ 14 − pH. However this relationship is not strictly valid in other circumstances, such as in measurements of soil alkalinity.

Extremes of pH
Measurement of pH below about 2.5 (ca. 0.003 mol dm^{-3} acid) and above about 10.5 (ca. 0.0003 mol dm^{-3} alkali) requires special procedures because, when using the glass electrode, the Nernst law breaks down under those conditions.

Extreme pH measurements imply that the solution may be concentrated, so electrode potentials are affected by ionic strength variation. At high pH the glass electrode may be affected by "alkaline error", because the electrode becomes sensitive to the concentration of cations such as Na^+ and K^+ in the solution. Specially constructed electrodes are available which partly overcome these problems. Runoff from industrial outfalls, restaurant grease, mines or mine tailings can produce some very low pH values.

Non-aqueous Solutions
Hydrogen ion concentrations (activities) can be measured in non-aqueous solvents. pH values based on these measurements belong to a different scale from aqueous pH values, because activities relate to different standard states. Hydrogen ion activity, a_{H^+}, can be defined as:

$$a_{H^+} = \exp\left(\frac{\mu_{H^+} - \mu^{\ominus}_{H^+}}{RT}\right)$$

where μ_{H^+} is the chemical potential of the hydrogen ion, $\mu^{\ominus}_{H^+}$ is its chemical potential in the chosen standard state, R is the gas constant and T is the thermodynamic temperature. Therefore pH values on the different scales cannot be compared directly, requiring an intersolvent scale which involves the transfer activity coefficient of hydrolyonium ion.

pH is an example of an acidity function. Other acidity functions can be defined. For example, the Hammett acidity function, H_0, has been developed in connection with superacids.

The concept of "Unified pH scale" has been developed on the basis of the absolute chemical potential of the proton. This scale applies to liquids, gases and even solids.

Applications
Water has a pH of $pK_w/2$, so the pH of pure water is about 7 at 25 °C; this value varies with temperature. When an acid is dissolved in water, the pH will be less than that of pure water. When a base, or alkali, is dissolved in water, the pH will be greater than that of pure water.

A solution of a strong acid, such as hydrochloric acid, at concentration 1 mol dm^{-3} has a pH of 0. A solution of a strong alkali, such as sodium hydroxide, at concentration 1 mol dm^{-3}, has a pH of 14. Thus, measured pH values will lie mostly in the range 0 to 14, though negative pH values and values above 14 are entirely possible.

Since pH is a logarithmic scale, a difference of one pH unit is equivalent to a tenfold difference in hydrogen ion concentration.

The pH of an aqueous solution of pure water is slightly different from that of, a salt such as sodium chloride even though the salt is neither acidic nor basic. In this case, the hydrogen and hydroxide ions' activity is dependent on ionic strength, so K_w varies with ionic strength.

The pH of pure water decreases with increasing temperatures. One example is the pH of pure water at 50 °C is 6.55.

Seawater
The pH of seawater plays an important role in the ocean's carbon cycle, and there is evidence of ongoing ocean acidification caused by carbon dioxide emissions. pH measurement can be complicated by the chemical properties of seawater, and several distinct pH scales exist in chemical oceanography.

As part of its operational definition of the pH scale, the IUPAC defines a series of buffer solutions across a range of pH values (often denoted with NBS or NIST designation).

These solutions have a relatively low ionic strength (~0.1) compared to that of seawater (~0.7), and, as a consequence, are not recommended for use in characterizing the pH of seawater, since the ionic strength differences cause changes in electrode potential.

To resolve this problem, an alternative series of buffers based on artificial seawater was developed. This new series resolves the problem of ionic strength differences between samples and the buffers. The newest pH scale is referred to as the **total scale**, often denoted as $\mathbf{pH_T}$.

Calculations of pH

The calculation of the pH of a solution containing acids and/or bases is an example of a chemical speciation calculation, that is, a mathematical procedure for calculating the concentrations of all chemical species that are present in the solution.

The complexity of the procedure depends on the nature of the solution.

If the pH of a solution contains a weak acid requires the solution of a quadratic equation.

If the pH of a solution contains a weak base may require the solution of a cubic equation.

For strong acids and bases no calculations are necessary except in extreme situations.

The general case requires the solution of a set of non-linear simultaneous equations.

A complicating factor is that water itself is a weak acid and a weak base. It dissociates according to the equilibrium

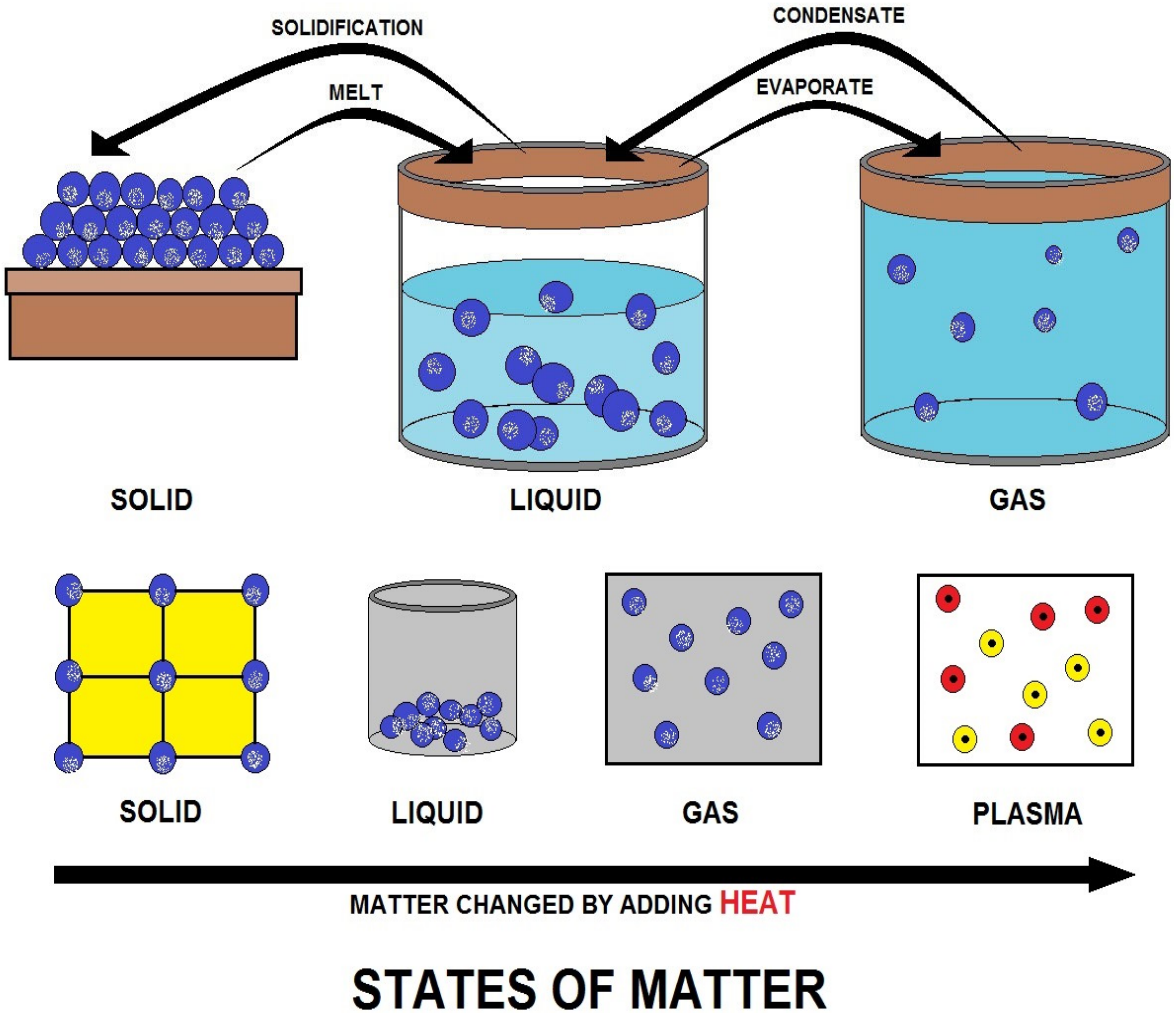
$$2H_2O \rightleftharpoons H_3O^+(aq) + OH^-(aq)$$

with a dissociation constant, K_w defined as

$$K_w = [H^+][OH^-]$$

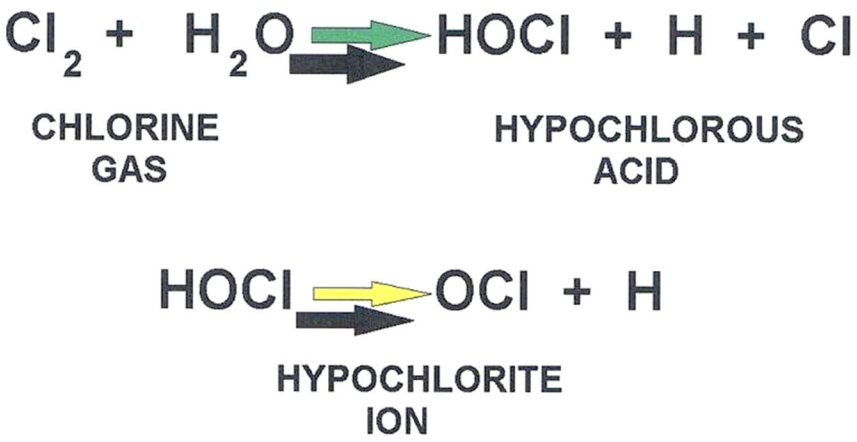
where $[H^+]$ represents for the concentration of the aquated hydronium ion and $[OH^-]$ stands for the concentration of the hydroxide ion. K_w has a value of about 10^{-14} at 25 °C, so pure water has a pH of approximately 7.

This equilibrium needs to be considered at high pH and when the solute concentration is extremely low.



STATES OF MATTER

Strong Acids and Bases



Strong Acids and Bases
Strong acids and bases are compounds that, for practical purposes, are completely dissociated in water. Under normal circumstances this means that the concentration of hydrogen ions in acidic solution can be taken to be equal to the concentration of the acid. The pH is then equal to minus the logarithm of the concentration value.

Hydrochloric acid (HCl) is an example of a strong acid. The pH of a 0.01M solution of HCl is equal to $-\log_{10}(0.01)$, that is, pH = 2.

Sodium hydroxide, NaOH, is an example of a strong base. The p[OH] value of a 0.01M solution of NaOH is equal to $-\log_{10}(0.01)$, that is, p[OH] = 2.

From the definition of p[OH] above, this means that the pH is equal to about 12. For solutions of sodium hydroxide at higher concentrations the self-ionization equilibrium must be taken into account.

Weak Acids and Bases
A weak acid or the conjugate acid of a weak base can be treated using the same formalism.

Acid: $HA \rightleftharpoons H^+ + A^-$
Base: $HA^+ \rightleftharpoons H^+ + A$

First, an acid dissociation constant is defined as follows. Electrical charges are omitted from subsequent equations for the sake of generality

$$K_a = \frac{[H][A]}{[HA]}$$

and its value is assumed to have been determined by experiment. This being so, there are three unknown concentrations, [HA], $[H^+]$ and $[A^-]$ to determine by calculation. Two additional equations are needed.

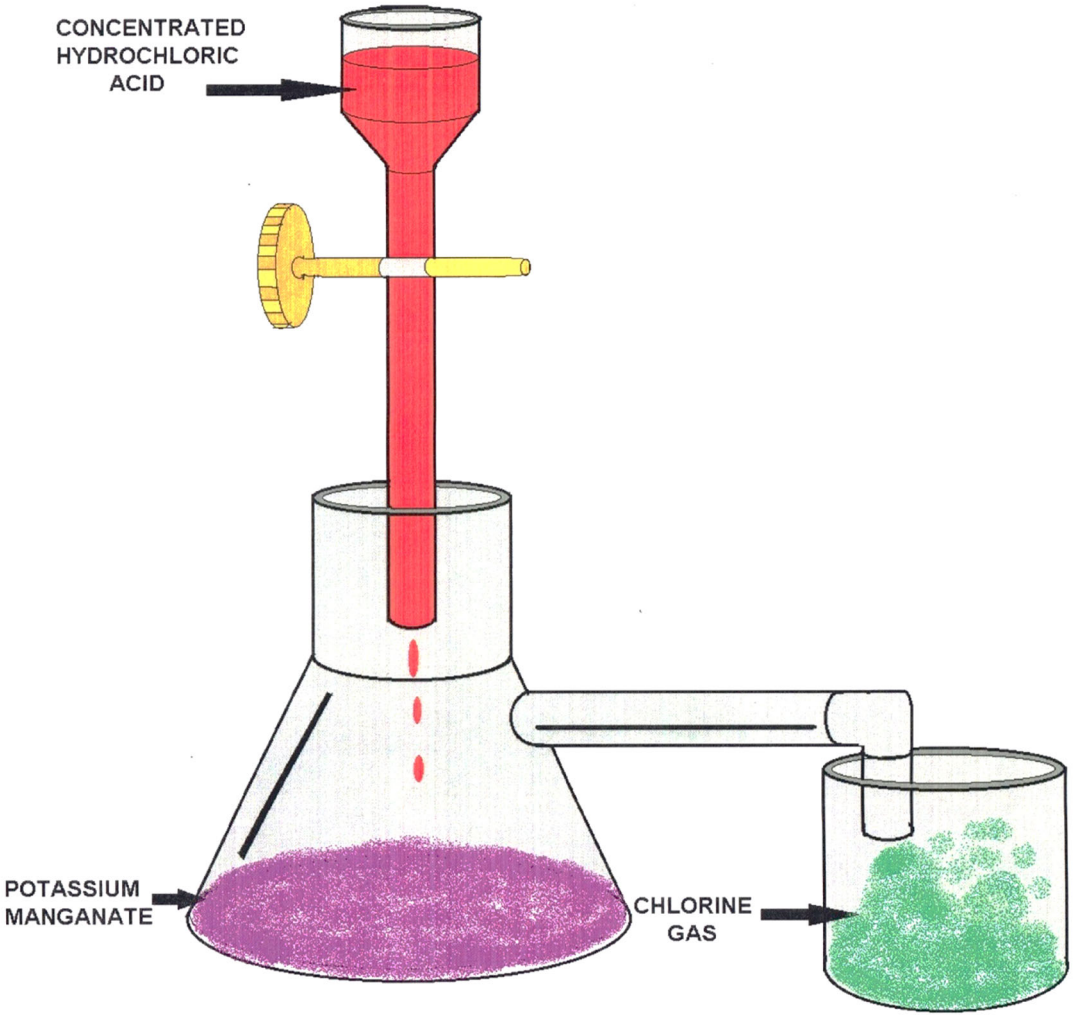
One way to provide them is to apply the law of mass conservation in terms of the two "reagents" H and A.

$$C_A = [A] + [HA]$$
$$C_H = [H] + [HA]$$

C stands for analytical concentration. In some texts one mass balance equation is replaced by an equation of charge balance. This is satisfactory for simple cases like this one, but is more difficult to apply to more complicated cases as those below.

Together with the equation defining K_a, there are now three equations in three unknowns. When an acid is dissolved in water $C_A = C_H = C_a$, the concentration of the acid, so [A] = [H]. After some further algebraic manipulation an equation in the hydrogen ion concentration may be obtained.

$$[H]^2 + K_a[H] - K_aC_a = 0$$



Alkalinity Sub-Section

Introduction
Alkalinity of water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates or other bases if these are present. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes.

Titration Method
a. Principle
Hydroxyl ions present in a sample, as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used.

b. Reagents
i) Standard Hydrochloric Acid – 0.02 N.
ii) Methyl Orange Indicator – Dissolve 0.1 g of methyl orange in distilled water and dilute to 1 liter.
iii) Sodium carbonate solution, 0.02 N : Dry 3 to 5 g primary standard Na_2CO_3 at 250°C for 4 h and cool in a desiccator. Weigh 1.03 gm. (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, dissolve and mix reagent. Do no keep longer than 1 week.

c. Procedure
Titrate over a white surface 100 ml of the sample contained in a 250-ml conical flask with standard hydrochloric acid using two or three drops of methyl orange Indicator. (**NOTE** – If more than 30 ml of acid is required for the titration, a smaller suitable aliquot of the sample shall be taken.)

d. Calculation
Total alkalinity (as $CaCO_3$), mg/l = 10 V or NxVx50x1000

$$\text{T.A. (as } CaCO_3) = \frac{\text{NxVx50x1000}}{\text{Sample Amount}}$$

Where N = Normality of HCl used
V = volume in ml of standard hydrochloric acid used in the titration.

Alkalinity to Phenolphthalein
The sample is titrated against standard acid using phenolphthalein indicator.

a. Reagents

i) Phenolphthalein Indicator Solution :

Dissolve 0.1 g of phenolphthalein in 60 ml of ETHANOL and dilute with Distilled water to 100 ml.

ii) Standard hydrochloric Acid – 0.02 N.

b. Procedure

Add 2 drops of phenolphthalein indicator solution to a sample of suitable size, 50 or 100 ml, in a conical flask and titrate over a while surface with standard hydrochloric acid.

c. Calculation

$$\textbf{Alkalinity to phenolphthalein (as } CaCO_3\textbf{)}, \text{ mg/l} = \frac{1000\ V_1}{V_2}$$

Where

V_1 = volume in ml of standard hydrochloric acid used in the titration , and

V_2 = Volume in ml of the sample taken for the test.

Caustic Alkalinity

a. General

Caustic alkalinity is the alkalinity corresponding to the hydroxides present in water and is calculated from total alkalinity (T) and alkalinity to phenolphthalein (P).

b. Procedure Determine total alkalinity and alkalinity to phenolphthalein and calculate caustic alkalinity as shown in Table below. Result of Titration Caustic Alkalinity or Hydroxide Alkalinity as $CaCO_3$ Carbonate Alkalinity as $CaCO_3$ Bicarbonate Concentration as $CaCO_3$ **Result of Titration**	**Caustic Alkalinity or Hydroxide Alkalinity as $CaCO_3$**	**Carbonate Alkalinity as $CaCO_3$**	**Bicarbonate Concentration as $CaCO_3$**
P=0	0	0	0
P<1/2T	0	2P	T-2P
P=1/2T	0	2P	0
P>1/2T	2P-T	2(T-P)	0
P=T	T	0	0

The alkalinity of water is a measure of its capacity to neutralize acids. The alkalinity of natural water is due to the salts of carbonate, bicarbonate, borates, silicates and phosphates along with the hydroxyl ions in free state.

However, the major portion of the alkalinity in natural waters is caused by hydroxide, carbonate, and bicarbonates which may be ranked in order of their association with high pH values. Alkalinity values provide guidance in applying proper doses of chemicals in water and waste water treatment processes, particularly in coagulation and softening.

Water Sampling Terms and Definitions

Microbes

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.

Fecal Coliform and E. coli are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms.

Turbidity has no health effects. However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems. The EPA and the CDC have prepared advice for those with severely compromised immune systems who are concerned about *Cryptosporidium*.

Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhea, vomiting, and cramps).

Radionuclides

Alpha emitters. Certain minerals are radioactive and may emit a form of radiation known as alpha radiation. Some people who drink water containing alpha emitters in excess of the EPA standard over many years may have an increased risk of getting cancer.

Beta/photon emitters. Certain minerals are radioactive and may emit forms of radiation known as photons and beta radiation. Some people who drink water containing beta and photon emitters in excess of the EPA standard over many years may have an increased risk of getting cancer.

Combined Radium 226/228. Some people who drink water containing radium 226 or 228 in excess of EPA standard over many years may have an increased risk of getting cancer.

Radon gas can dissolve and accumulate in underground water sources, such as wells, and in the air in your home. Breathing radon can cause lung cancer. Drinking water containing radon presents a risk of developing cancer. Radon in air is more dangerous than radon in water.

Inorganic Contaminants

Antimony	Cadmium	Cyanide	Nitrite
Asbestos	Chromium	Mercury	Selenium
Barium	Copper	Nitrate	Thallium
Beryllium			

Arsenic. Some people who drink water containing arsenic in excess of the EPA standard over many years could experience skin damage or problems with their circulatory system, and may have an increased risk of getting cancer.

Fluoride. Many communities add fluoride to their drinking water to promote dental health. Each community makes its own decision about whether or not to add fluoride. The EPA has set an enforceable drinking water standard for fluoride of 4 mg/L (some people who drink water containing fluoride in excess of this level over many years could get bone disease, including pain and tenderness of the bones). The EPA has also set a secondary fluoride standard of 2 mg/L to protect against dental fluorosis. Dental fluorosis, in its moderate or severe forms, may result in a brown staining and/or pitting of the permanent teeth. This problem occurs only in developing teeth, before they erupt from the gums. Children under nine should not drink water that has more than 2 mg/L of fluoride.

Lead typically leaches into water from plumbing in older buildings. Lead pipes and plumbing fittings have been banned since August 1998. Children and pregnant women are most susceptible to lead health risks. For advice on avoiding lead, see the EPA's "*Lead in Your Drinking Water*" fact sheet.

Synthetic Organic Contaminants, including pesticides & herbicides

2,4-D	Dibromochloropropane	Hexachlorobenzene
2,4,5-TP (Silvex)	Dinoseb	Hexachlorocyclopentadiene
Acrylamide	Dioxin (2,3,7,8-TCDD)	Lindane
Alachlor	Diquat	Methoxychlor
Atrazine	Endothall	Oxamyl [Vydate]
Benzoapyrene	Endrin	PCBs [Polychlorinated biphenyls]
Carbofuran	Epichlorohydrin	Pentachlorophenol
Chlordane	Ethylene dibromide	Picloram
Dalapon	Glyphosate	Simazine
Di 2-ethylhexyl adipate	Heptachlor	Toxaphene
Di 2-ethylhexyl phthalate	Heptachlor epoxide	

Volatile Organic Contaminants

Benzene	trans-1,2-Dicholoroethylene	1,2,4-Trichlorobenzene
Carbon Tetrachloride	Dichloromethane	1,1,1,-Trichloroethane
Chlorobenzene	1,2-Dichloroethane	1,1,2-Trichloroethane
o-Dichlorobenzene	1,2-Dichloropropane	Trichloroethylene
p-Dichlorobenzene	Ethylbenzene	Toluene
1,1-Dichloroethylene	Styrene	Vinyl Chloride
cis-1,2-Dichloroethylene	Tetrachloroethylene	Xylenes

Point-of-Entry P.O.E. will usually be a designated sampling point on a water treatment or distribution system.

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

Water Sampling and Laboratory Procedures Sub-Section

Before we can identify our waterborne disease, we first must sample the water. Most of you are very familiar with water sampling and may skip this section and proceed to the other chapters. Proper collection and handling of a water sample is critical for obtaining a valid water test. Sample containers should always be obtained from the testing laboratory because containers may be specially prepared for a specific contaminant. Sampling and handling procedures will depend on the specific water quality concern and should be followed carefully. If the water is being treated, it may be necessary to sample both before and after the water goes through the treatment equipment.

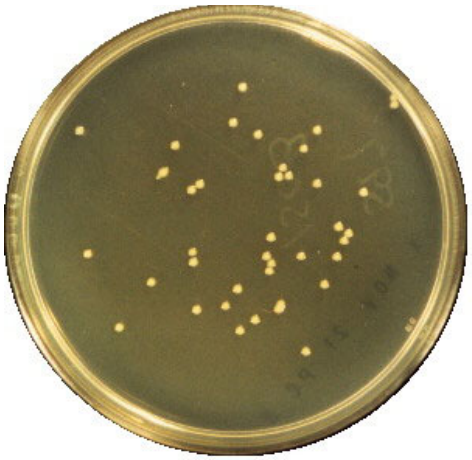
Clean sample containers, preservatives and coolers are generally provided by most laboratories. Contact the laboratory about a month before the sampling date to schedule analyses and container shipment or pickup. Collecting water-quality samples involves not only the process of physically acquiring the best possible sample for the intended analysis, but also characterizing the environment from which the sample was drawn, and handling the sample so as to protect its value for its intended purpose. The goal of sample collection and field measurements is to accurately represent the water resource being sampled at that time. This means obtaining a series of measurements (field parameters or in situ measurements) in a prescribed manner, preserving and maintaining water-quality and QA/QC samples according to established guidelines, and observing chain of custody requirements.

Obtaining a representative sample means being careful in your choice of equipment. If you are sampling for the presence of heavy metals, do not use samplers with metal components. When sampling for organics, avoid using samplers with plastic components, as the plastic may adsorb and contaminate the samples. Most importantly, always decontaminate equipment before use. Once the equipment is decontaminated, wrap inorganic equipment in plastic and organic equipment in aluminum foil for transport to the site.

Colilert tests simultaneously detects and confirms coliforms and E. coli in water samples in 24 hours or less.

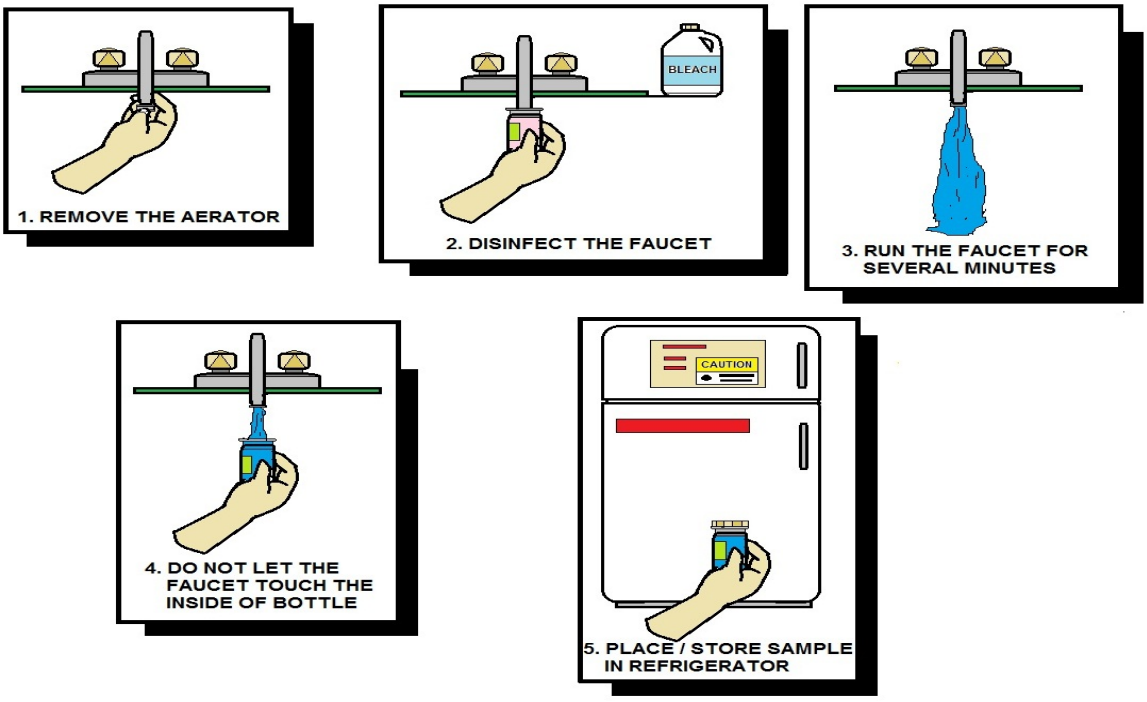
Simply add the Colilert reagent to the sample, incubate for 24 hours, and read results.

Colilert is easy to read, as positive coliform samples turn yellow or blue, and when E. coli is present, samples fluoresce under UV light.



Top photograph, HPC plate. Bottom, Bac-T or Colilert samples, the yellow indicates coliform bacteria, if this sample fluoresces under a black light that means that fecal or e. coli is present.

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.



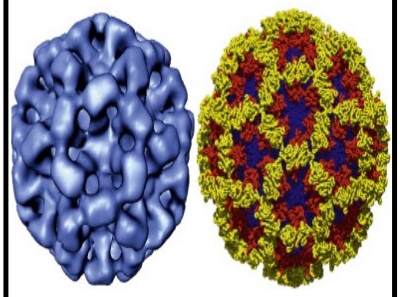
HOW TO TAKE A WATER SAMPLE

MICROBIOLOGICAL CONTAMINANTS FOUND IN WATER

THESE ARE OFTEN OF FECAL NATURE RELATED TO HUMANS, DOMESTIC ANIMALS OR WILDLIFE

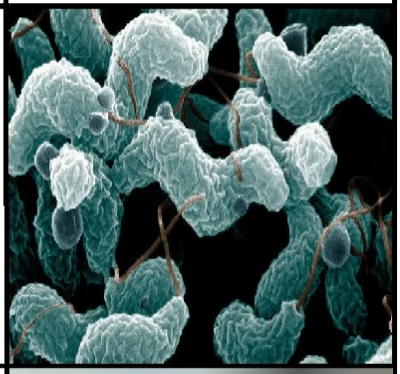
CALIVIVIRUS:

SIGNS OF THIS VIRUS INCLUDE SNEEZING, NASAL DISCHARGE, OCULAR DISCHARGE, CONJUNCTIVITUS, ULCERATION OF THE TONGUE, LETHARGY, INAPPENTENCE (Lack of Appetite) AND FEVER



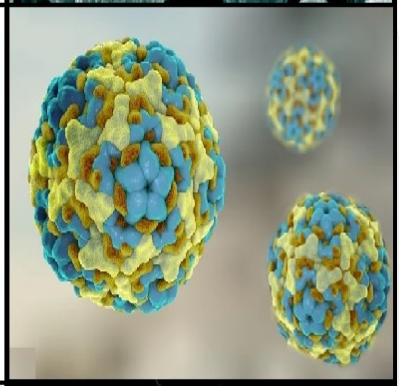
CAMPYLOBACTOR JEJUNI:

THIS IS ONE OF THE MOST COMMON CAUSES OF FOOD POISIONING. IT IS CHARACTERIZED BY DIARRHEA, ABDOMINAL PAIN, FEVER, NAUSEA AND SOMETIMES VOMITING



ENTEROVIRUS:

SYMPTOMS OF THIS VIRUS INFECTION MAY INCLUDE FEVER, RUNNY NOSE, SNEEZING, COUGH, SKIN RASH, MOUTH BLISTERS, AND BODY AND MUSCLE ACHES

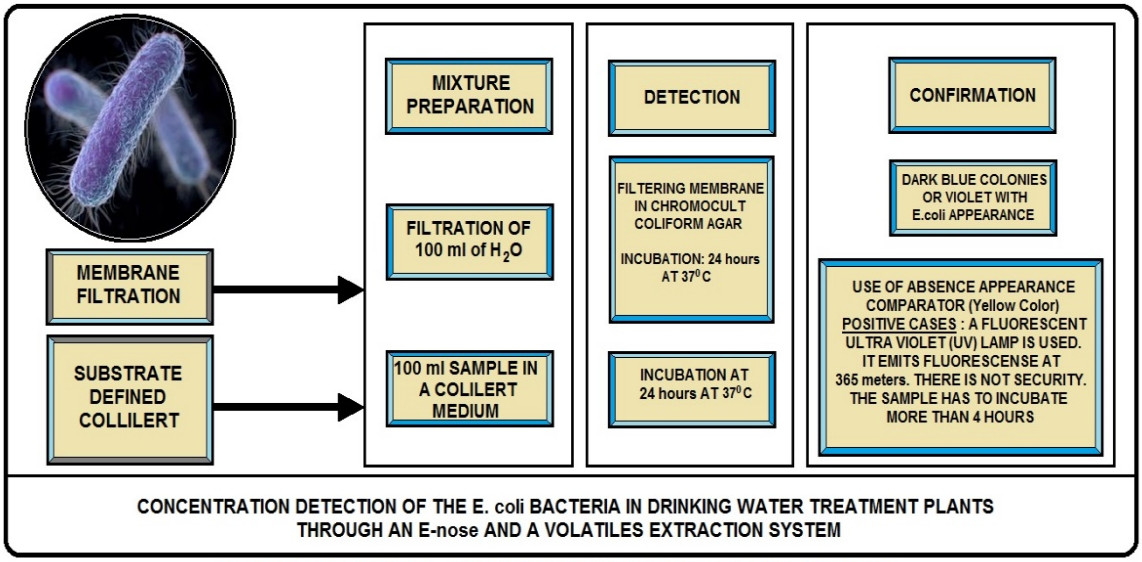


ESCHERICHIA COLI:

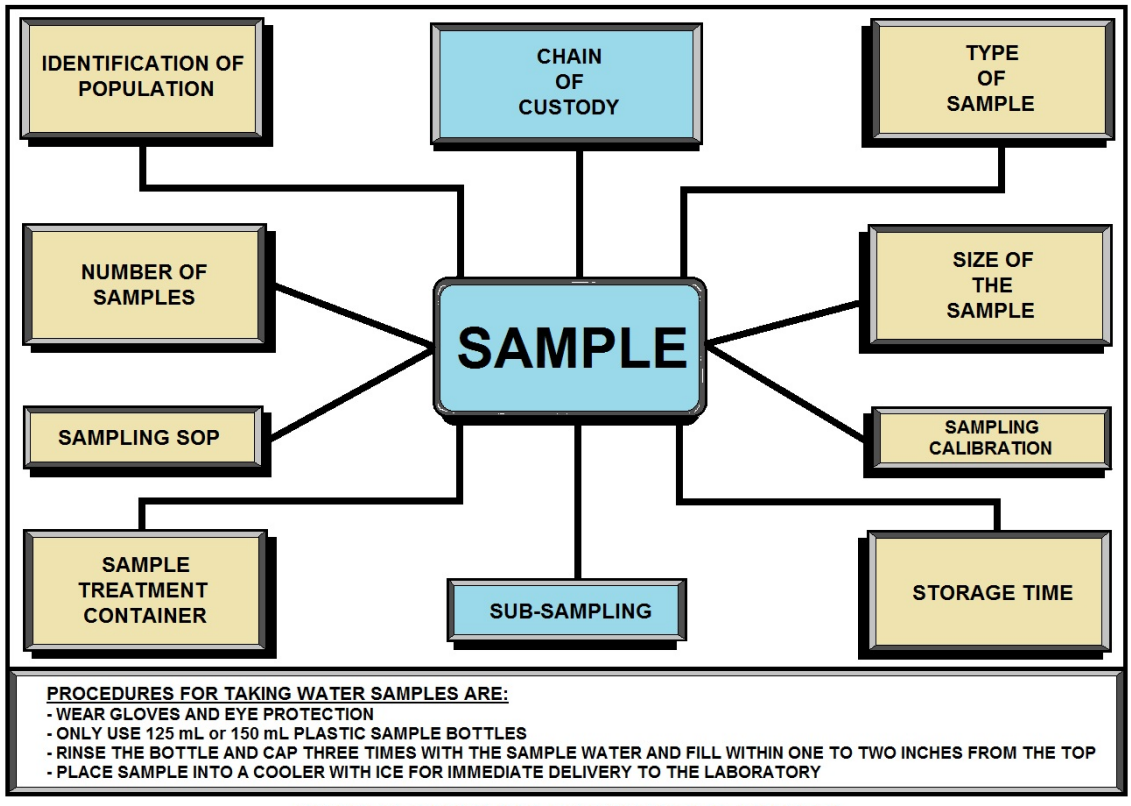
NORMALLY LIVES IN THE INTESTINES OF HEALTHY PEOPLE AND ANIMALS. MOST E.Coli ARE HARMLESS OR RELATIVELY BRIEF DIARRHEA. SOME E.Coli CAN CAUSE SEVERE STOMACH CRAMPS, BLOODY DIARRHEA AND VOMITING

MICROBIOLOGICAL CONTAMINANTS

Technical Learning College



CONVENTIONAL BACTERIOLOGICAL MONITORING



PROPER SAMPLING PROCEDURES (WATER)

Bacteriological Monitoring

Most of us have gathered samples and the primary reason is that most waterborne diseases and illnesses have been related to the microbiological quality of drinking water. The routine microbiological analysis of your water is for coliform bacteria. The coliform bacteria group is used as an indicator organism to determine the biological quality of your water.

The presence of an indicator or pathogenic bacteria in your drinking water is an important health concern. Indicator bacteria signal possible fecal contamination, and therefore, the potential presence of pathogens. They are used to monitor for pathogens because of the difficulties in determining the presence of specific disease-causing microorganisms.

Indicator bacteria are usually harmless, occur in high densities in their natural environment, and are easily cultured in relatively simple bacteriological media. Indicators in common use today for routine monitoring of drinking water include total coliforms, fecal coliforms, and *Escherichia coli (E. coli).*

Bacteria Sampling
Water samples for bacteria tests must always be collected in a sterile container. Take the sample from an inside faucet with the aerator removed. Sterilize by spraying a 5% household bleach or alcohol solution or flaming the end of the tap with disposable butane lighter or propane torch.

Run the water for five minutes to clear the water lines and bring in fresh water. Do not touch or contaminate the inside of the bottle or cap. Carefully open the sample container and hold the outside of the cap. Fill the container and replace the top.

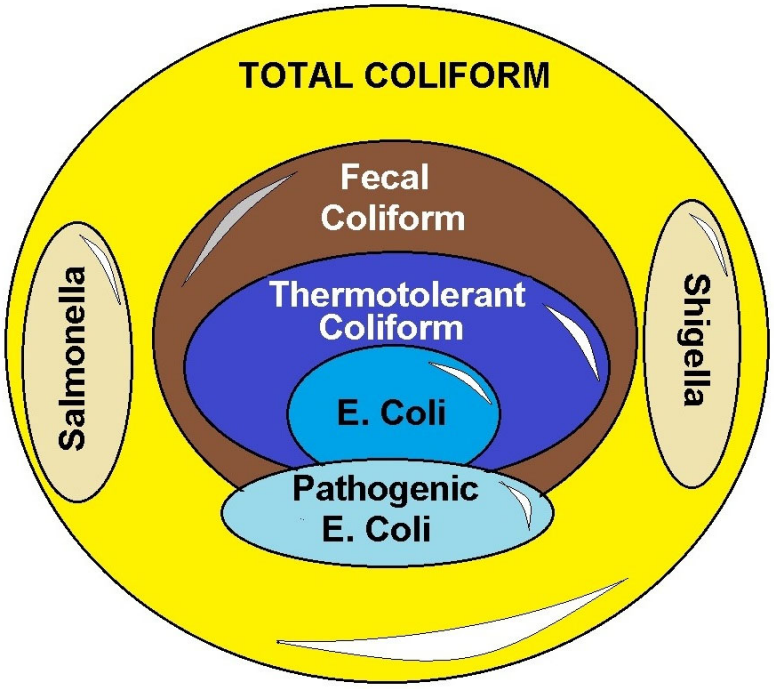
Refrigerate the sample and transport it to the testing laboratory within six hours (in an ice chest). Many labs will not accept bacteria samples on Friday so check the lab's schedule. Mailing bacteria samples is not recommended because laboratory analysis results are not as reliable.

Iron bacteria forms an obvious slime on the inside of pipes and fixtures. A water test is not needed for identification. Check for a reddish-brown slime inside a toilet tank or where water stands for several days.

Standard Sample Coliform Bacteria Bac-T.

Bac-T Sample Bottle, often referred to as a Standard Sample, 100 mls, Notice the white powder inside the bottle. That is Sodium Thiosulfate, a de-chlorination agent. Be careful not to wash-out this chemical while sampling. Notice the custody seal on the bottle.

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.



COLIFORM BACTERIA DIAGRAM

Laboratory Procedures, more detailed information in the next section

The laboratory may perform the total coliform analysis in one of four methods approved by the U.S. EPA and your local environmental or health division.

Methods
The MMO-MUG test, a product marketed as Colilert is the most common. The sample results will be reported by the laboratories as simply coliforms present or absent. If coliforms are present, the laboratory will analyze the sample further to determine if these are fecal coliforms or E. coli and report their presence or absence.

Types of Water Samples
It is important to properly identify the type of sample you are collecting. Please indicate in the space provided on the laboratory form the type of sample.

The three (3) primary types of samples are:
1. **Routine:** Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.
2. **Repeat:** Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect.
3. **Special:** Samples collected for other reasons.
Examples would be a sample collected after repairs to the system and before it is placed back into operation, or a sample collected at a wellhead prior to a disinfection injection point.

Routine Coliform Sampling (*Check with your governmental environmental or health agency for more information*)
The number of routine samples and frequency of collection for community public water systems is shown in **Table 3-1** next page.

Noncommunity and nontransient noncommunity public water systems will sample at the same frequency as a like sized community public water system if:
1. It has more than 1,000 daily population and has ground water as a source, or

2. It serves 25 or more daily population and utilizes surface water as a source or ground water under the direct influence of surface water as its source.

Noncommunity and nontransient, noncommunity water systems with less than 1,000 daily population and groundwater as a source will sample on a quarterly basis.

Table 3 Number of Samples per System Population

Persons served - Samples per month

up to 1,000	1
1,001-2,500	2
2,501-3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 12,900	10
12,901 to 17,200	15
17,201 to 21,500	20
21,501 to 25,000	25
25,001 to 33,000	30
33,001 to 41,000	40
41,001 to 50,000	50
50,001 to 59,000	60
59,001 to 70,000	70
70,001 to 83,000	80
83,001 to 96,000	90
96,001 to 130,000	100
130,001 to 220,000	120
220,001 to 320,000	150
320,001 to 450,000	180
450,001 to 600,000	210
600,001 to 780,000	240

Repeat Sampling

Repeat sampling replaces the old check sampling with a more comprehensive procedure to try to identify problem areas in the system. Whenever a routine sample is positive for total coliform or fecal coliform present, a set of repeat samples must be collected within 24 hours after being notified by the laboratory.

Before I start out to re-sample, I always call the Distribution Section and have their personnel flush the area and obtain a good Chlorine residual.

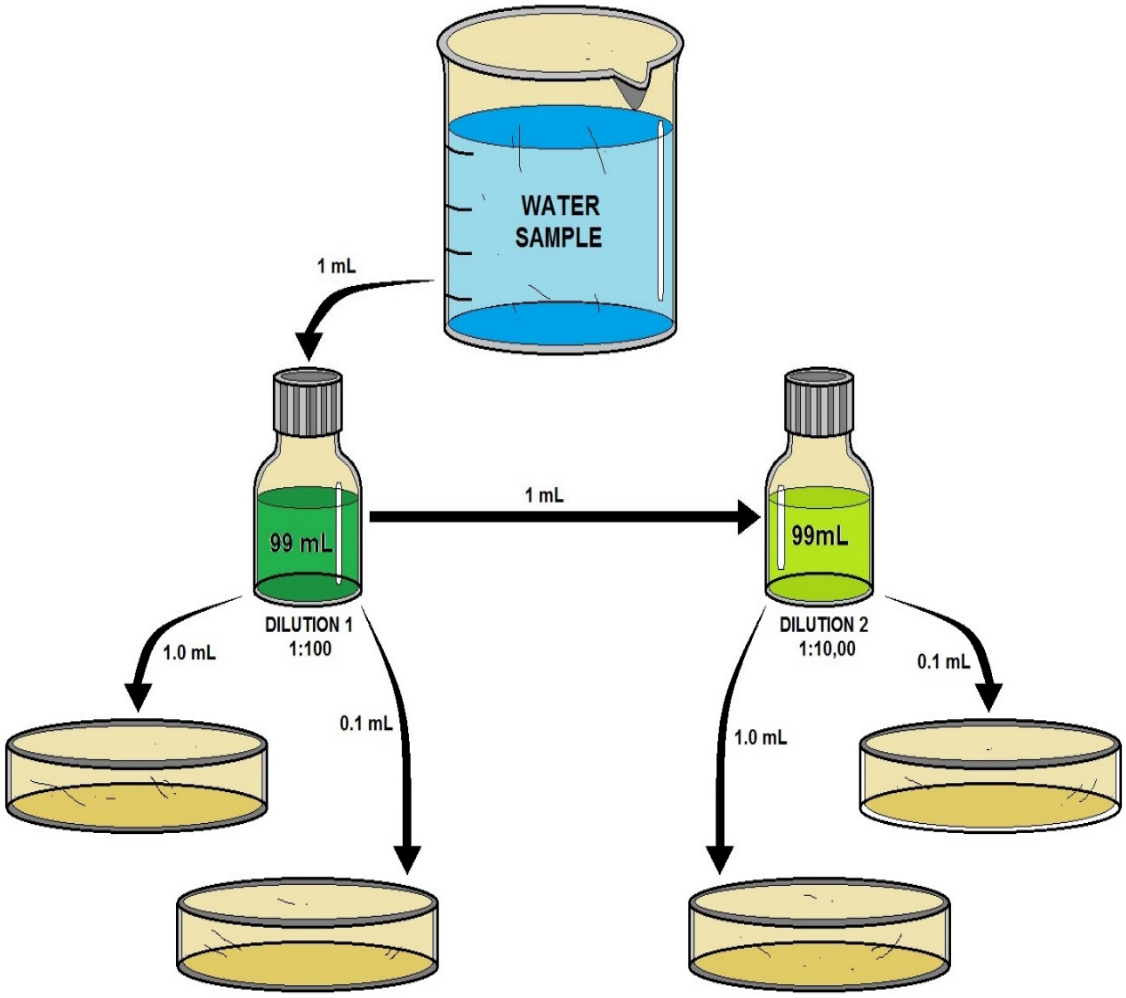
The follow-up for repeat sampling is:

1. If only one routine sample per month or quarter is required, four (4) repeat samples must be collected.
2. For systems collecting two (2) or more routine samples per month, three (3) repeat samples must be collected.
3. Repeat samples must be collected from:

a. The original sampling location of the coliform present sample.
b. Within five (5) service connections upstream from the original sampling location.
c. Within five (5) service connections downstream from the original sampling location.
d. Elsewhere in the distribution system or at the wellhead, if necessary.

4. If the system has only one service connection, the repeat samples must be collected from the same sampling location over a four-day period or on the same day.

5. All repeat samples are included in the MCL compliance calculation.
6. If a system which normally collects fewer than five (5) routine samples per month has a coliform present sample, it must collect five (5) routine samples the following month or quarter regardless of whether an MCL violation occurred or if repeat sampling was coliform absent. **(*Check with your governmental environmental or health agency for more information*).**



STANDARD PLATE COUNT PROCEDURE
(Basic test used to detect general bacteria in samples)

Specific Types of Water Samples

It is important to properly identify the type of sample you are collecting. Please indicate in the space provided on the laboratory form the type of sample.

The three (3) primary types of samples are:

1. **Routine:** Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.

2. **Repeat:** Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect.

3. **Special:** Samples collected for other reasons.
Examples would be a sample collected after repairs to the system and before it is placed back into operation or a sample collected at a wellhead prior to a disinfection injection point.

a. **Trigger: Level 1 Assessment** is triggered if any one of the following occurs:
   ► A PWS collecting fewer than 40 samples per month has 2 or more TC+ routine/ repeat samples in the same month.
   ► A PWS collecting at least 40 samples per month has greater than 5.0 percent of the routine/repeat samples in the same month that are TC+.
   ► A PWS fails to take every required repeat sample after any single TC+ sample

b. **Trigger: Level 2 Assessment** is triggered if any one of the following occurs:
   ► A PWS incurs an E. coli MCL violation.
   ► A PWS has a second Level 1 Assessment within a rolling 12-month period.
   ► A PWS on state-approved annual monitoring has a Level 1 Assessment trigger in 2 consecutive years.

Routine Coliform Sampling
The number of routine samples and frequency of collection for community public water systems is shown in Table 3-1 below.

Noncommunity and nontransient noncommunity public water systems will sample at the same frequency as a like sized community public water system if:
1. It has more than 1,000 daily population and has ground water as a source, or
2. It serves 25 or more daily population and utilizes surface water as a source or ground water under the direct influence of surface water as its source.

Noncommunity and nontransient, noncommunity water systems with less than 1,000 daily population and groundwater as a source will sample on a quarterly basis.

Positive or Coliform Present Results (1 Example)

What do you do when your sample is positive or coliform present?

When you are notified of a positive test result you need to contact either the Drinking Water Program or your local county health department within 24 hours, or by the next business day after the results are reported to you. The Drinking Water Program contracts with many of the local health departments to provide assistance to water systems. Most of us have had this problem. It can be stressful, and people have lost their jobs over these problems. The best option is to have a SOP in place because positive samples or false positives will come to your section.

Assistance

After you have contacted an agency for assistance, you will be instructed as to the proper repeat sampling procedures and possible corrective measures for solving the problem. It is very important to initiate the repeat sampling immediately as the corrective measures will be based on those results.

Some examples of typical corrective measures to coliform problems are:

1. Shock chlorination of a ground water well. The recommended dose of 5% household bleach is 2 cups per 100 gallons of water in the well. This should be done anytime the bell is opened for repair (pump replacement, etc.). If you plan to shock the entire system, calculate the total gallonage of storage and distribution.
2. Conduct routine distribution line flushing. Install blowoffs on all dead end lines.
3. Conduct a cross connection program to identify all connections with non-potable water sources. Eliminate all of these connections or provide approved backflow prevention devices.
4. Upgrade the wellhead area to meet current construction standards as set by your state environmental or health agency.
5. If you continuously chlorinate, review your operation and be sure to maintain a detectable residual (**0.2 mg/l free chlorine**) at all times in the distribution system.
6. Perform routine cleaning of the storage system.

This list provides some basic operation and maintenance procedures that could help eliminate potential bacteriological problems, check with your state drinking water section or health department for further instructions.

Maximum Contaminant Levels (MCLs)

State and federal laws establish standards for drinking water quality. Under normal circumstances when these standards are being met, the water is safe to drink with no threat to human health. These standards are known as maximum contaminant levels (MCL). When a particular contaminant exceeds its MCL a potential health threat may occur.

The MCLs are based on extensive research of the toxicological properties of the contaminants, risk assessments and factors, short term (acute) exposure, and long term (chronic) exposure. You conduct the monitoring to make sure your water is in compliance with the MCL. There are two types of MCL violations for coliform bacteria. The first is for total coliform; the second is an acute risk to health violation characterized by the confirmed presence of fecal coliform or E. coli.

(Check with your governmental environmental or health agency for more information.)

Common Sample bottles, *Radiochems, VOCs, (Volatile Organic Compounds), TTHMs, Total Trihalomethanes), Nitrate, Nitrite.*

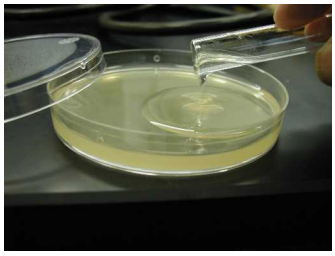
Most of these sample bottles will come with the preservative already inside the bottle. Some bottles will come with a separate preservative (acid) for the field preservation. Slowly add the acid or other preservative to the water sample; not water to the acid or preservative. Put a new label on the preservative dispensing bottle with the current date.

Heterotrophic Plate Count, *more detailed information in the next section (Check with your governmental environmental or health agency for more information.)*

Heterotrophic Plate Count (HPC) --- formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria and measuring changes during water treatment and distribution in water or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term "*colony-forming units*" (CFU).

Method:

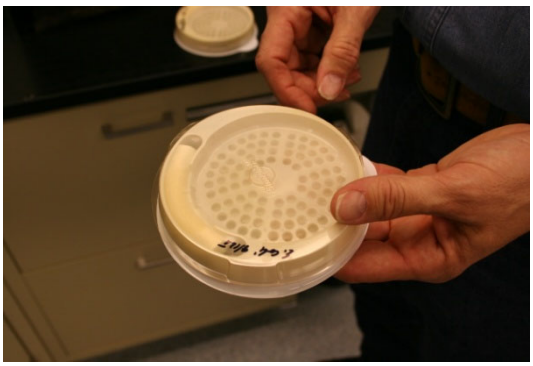
There are three methods for standard plate count:



1. **Pour Plate Method**
The colonies produced are relatively small and compact, showing less tendency to encroach on each other than those produced by surface growth. On the other hand, submerged colonies often are slower growing and are difficult to transfer.

2. Spread Plate Method
All colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Colonies can be transferred quickly, and colony morphology easily can be discerned and compared to published descriptions.

3. Membrane Filter Method
This method permits testing large volumes of low-turbidity water and is the method of choice for low-count waters.



Material:

i) Apparatus
- Glass rod
- Erlenmeyer flask
- Graduated Cylinder
- Pipette
- Petri dish
- Incubator

ii) Reagent and sample
- Reagent-grade water
- Nutrient agar
- Sample

Procedure*

1. Boil mixture of nutrient agar and nutrient broth for 15 minutes, then cool for about 20 minutes.
2. Pour approximately 15 ml of medium in each Petri dish, let medium solidify.
3. Pipet 0.1 ml of each dilution onto surface of pre-dried plate, starting with the highest dilution.
4. Distribute inoculum over surface of the medium using a sterile bent glass rod.
5. Incubate plates at 35^{o}C for 48h.

6.Count all colonies on selected plates promptly after incubation, consider only plates having 30 to 300 colonies in determining the plate count.
*Duplicate samples

Computing and Reporting: Compute bacterial count per milliliter by the following equation:
CFU/ml = colonies counted / actual volume of sample in dish
a) If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. (*Check with your governmental environmental or health agency for more information*)
b) If plates from all dilutions of any sample have no colony, report the count as less than 1/actual volume of sample in dish estimated CFU/ml.
c) Avoid creating fictitious precision and accuracy when computing CFU by recording only the first two left-hand digits.

Heterotrophic Plate Count

(Spread Plate Method) ***(Check with your governmental environmental or health agency for more information.)***
Heterotrophic organisms utilize organic compounds as their carbon source (food or substrate). In contrast, autotrophic organisms use inorganic carbon sources. The Heterotrophic Plate Count provides a technique to quantify the bacteriological activity of a sample. The R2A agar provides a medium that will support a large variety of heterotrophic bacteria. After an incubation period, a bacteriological colony count provides an estimate of the concentration of heterotrophs in the sample of interest.

Required Laboratory Equipment

100 x 15 Petri Dishes
Turntable
Glass Rods: Bend fire polished glass rod 45 degrees about 40 mm from one end. Sterilize before using.
Pipette: Glass, 1.1 mL. Sterilize before using.
Quebec Colony Counter
Hand Tally Counter

Reagents

1) R2A Agar: Dissolve and dilute 0.5 g of yeast extract, 0.5 g of proteose peptone No. 3, 0.5 g of casamino acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of dipotassium hydrogen phosphate, 0.05 g of magnesium sulfate heptahydrate, 0.3 g of sodium pyruvate, 15.0 g of agar to 1 L. Adjust pH to 7.2 with dipotassium hydrogen phosphate before adding agar**.** Heat to dissolve agar and sterilize at 121 C for 15 minutes.
2) Ethanol: As needed for flame sterilization.

Preparation of Spread Plates
Immediately after agar sterilization, pour 15 mL of R2A agar into sterile 100 x 15 Petri dishes; let agar solidify. Pre-dry plates inverted so that there is a 2 to 3 g water loss overnight with the lids on. Use pre-dried plates immediately or store up to two weeks in sealed plastic bags at 4 degrees C.

Sample Preparation
Mark each plate with sample type, dilution, date, and any other information before sample application. Prepare at least duplicate plates for each volume of sample or dilution examined. Thoroughly mix all samples by rapidly making about 25 complete up-and-down movements.

Sample Application
Uncover pre-dried agar plate. Minimize time plate remains uncovered. Pipette 0.1 or 0.5 mL sample onto surface of pre-dried agar plate.

Record volume of sample used. Using a sterile bent glass rod, distribute the sample over the surface of the medium by rotating the dish by hand on a turntable. Let the sample be absorbed completely into the medium before incubating. Put cover back on Petri dish and invert for duration of incubation time. Incubate at 28°C for 7 days. Remove Petri dishes from incubator for counting.

Counting and Recording
After incubation period, promptly count all colonies on the plates. To count, uncover plate and place on Quebec colony counter. Use hand tally counter to maintain count. Count all colonies on the plate, regardless of size. Compute bacterial count per milliliter by the following equation:

$$\text{CFU}/\text{mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish, mL}}$$

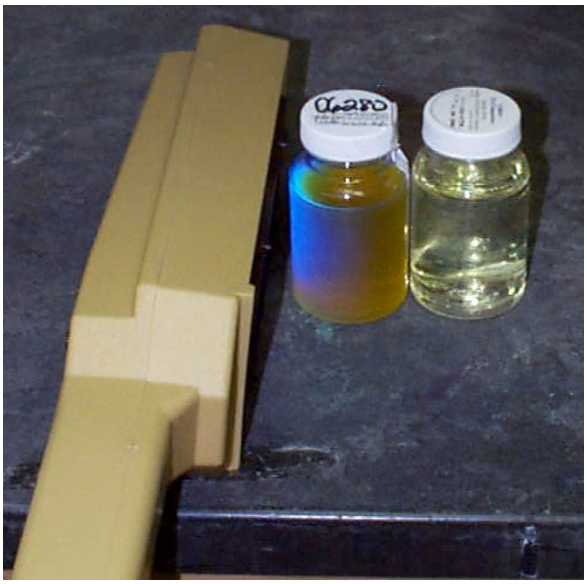
To report counts on a plate with no colonies, report the count as less than one (<1) divided by the sample volume put on that plate (remember to account for any dilution of that sample.)

If plates of all dilutions for a sample have no colonies, report the count as less than one (<1) divided by the largest sample volume used. Example: if 0.1 mL of a 100:1 and 10000:1 dilution of a sample both turned up with no colonies formed, the reported result would be <1 divided by the largest sample volume 0.001 mL (0.1 mL divided by 100). The final reported result for the sample is <1000 CFU per mL.

Assignment
1. Report the number of colony forming units (**CFU**) found on each plate.
2. Calculate the **CFU** per mL for each plate.
3. The aim of diluting samples is to produce a plate having 30 to 300 colonies, which plates meet these criteria. If no sample produces a plate with a count in this range, use the plate(s) with a count closest to 300. Based on these criteria, use your calculated results to report the **CFU** per mL for each sample. In the conclusion of your lab report, comment on your final results for each sample type as well as the quality of your application of this analysis technique. Feel free to justify your comments using statistical analysis. Also, comment on the general accuracy of this analytical technique and the factors that affect its accuracy and or applicability.

Data Table for Samples

Sample ID	Volume of Sample, mL	Colonies Counted per plate



E. coli or fecal bacteria will fluoresce.

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.

Total Coliforms, more detailed information in the next section

This MCL is based on the presence of total coliforms, and compliance is on a monthly or quarterly basis, depending on your water system type and state rule. For systems which collect *fewer* than 40 samples per month, no more than one sample per month may be positive. In other words, the second positive result (repeat or routine) in a month or quarter results in an MCL violation.

For systems which collect 40 or more samples per month, no more than five (5) percent may be positive. Check with your state drinking water section or health department for further instructions.

Acute Risk to Health (Fecal coliforms and E. coli)
An acute risk to human health violation occurs if either one of the following happen:
1. A routine analysis shows total coliform present and is followed by a repeat analysis which indicates fecal coliform or E. coli present.
2. A routine analysis shows total and fecal coliform or E. coli present and is followed by a repeat analysis which indicates total coliform present. An acute health risk violation requires the water system to provide public notice via radio and television stations in the area. This type of contamination can pose an immediate threat to human health and notice must be given as soon as possible, but no later than 24 hours after notification from your laboratory of the test results.

Certain language may be mandatory for both these violations and is included in your state drinking water rule.

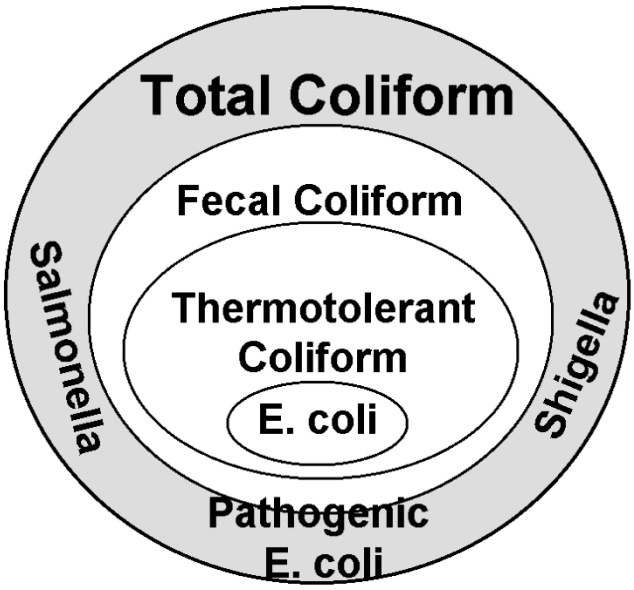
Public Notice
A public notice is required to be issued by a water system whenever it fails to comply with an applicable MCL or treatment technique, or fails to comply with the requirements of any scheduled variance or permit. This will inform users when there is a problem with the system and give them information.

A public notice is also required whenever a water system fails to comply with its monitoring and/or reporting requirements or testing procedure. Each public notice must contain certain information, be issued properly and in a timely manner, and contain certain mandatory language.

The timing and place of posting of the public notice depends on whether an acute risk is present to users. Check with your state drinking water section or health department for further instructions.

The following are acute violations:
1. Violation of the MCL for nitrate.
2. Any violation of the MCL for total coliforms, when fecal coliforms or E. coli are present in the distribution system.
3. Any outbreak of waterborne disease, as defined by the rules.

(Check with your governmental environmental or health agency for more information.)



General Contaminant Information
The sources of Drinking water include rivers, lakes, streams, ponds, reservoirs, springs, and wells.

TCR Section

Background
Coliform bacteria and chlorine residual are the only routine sampling and monitoring requirements for small ground water systems with chlorination.

The Total Coliform Rule or (TCR) requires all Public Water Systems (PWS) to monitor their distribution system for coliform bacteria according to the written sample siting plan for that system.

Coliform contamination can occur anywhere in the system, possibly due to problems such as; low pressure conditions, line breaks, or well contamination, and therefore routine monitoring is required.

Number of Monthly Samples
The number of Samples to be collected monthly depends on the size of the system.

This is especially true if the system consists of POEs, pressure zones, booster pumps, long transmission lines, or extensive distribution system piping.

The Sample siting plan should be updated as changes are made in the water system, especially the distribution system.

In order to properly implement the sample siting plan, staff must be aware of how often sampling must be done, the proper procedures and sampling containers to be used for collecting the samples, and the proper procedures for identification, storage and transport of the Samples to an approved laboratory.

General Questions about Coliform

Always check with your State to ensure this information is correct, for many States have stricter laws than what is in this section.

What is the Total Coliform Rule (TCR)?

The Total Coliform Rule (TCR) requires all public water supplies to monitor for the presence of total coliforms in the distribution system. For drinking water, total coliforms are used to monitor the sanitary quality of the water, adequacy of water treatment, and the integrity of the distribution system. The absence of total coliforms in the distribution system minimizes the likelihood that fecal pathogens are present. Thus, total coliforms are used to determine the vulnerability of a system to fecal contamination**.**

What is total coliform bacteria?

Total coliforms are a group of closely related bacteria that are (with few exceptions) not harmful to humans. They are an indicator of other pathogens that can be present in water. Coliform bacteria are present in the intestinal tract of warm-blooded animals.

They are shed from the body in the feces. Because these organisms are shed from the body in large numbers and are relatively easy to detect in the laboratory, they have been accepted as an indicator of contamination. All bacteriological samples are analyzed for the coliform group; however, a positive reaction to these coliform analyses may be from sources other than fecal. In order to differentiate between these sources, all samples that are total coliform positive must be analyzed again to determine if fecal coliform or *E. coli* are present.

What are fecal coliform and *E. coli*?

Fecal coliform is bacteria whose presence indicates that the water may be contaminated with human or animal wastes. *E. coli* is a member of the fecal coliform group. Microbes in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, and people with severely compromised immune systems. The presence of fecal coliform or *E. coli* in drinking water sample indicate recent contamination and signals a greater possibility that pathogenic organisms are also in the water.

Why must a water system monitor for total coliform, fecal coliform, and *E. coli*?

Total coliforms serve as indicators of the efficiency of water treatment, of the integrity of the pipes in the distribution system, and as a screen for the presence of fecal contamination. Usually, coliforms are a sign that there could be a problem with the system's treatment or distribution system. Fecal and/or *E. coli* indicates that pathogenic organisms may be present that can cause short-term health effects, such as diarrhea, cramps, nausea, headaches, fatigue and jaundice. They may pose a special health risk for infants, young children, and people with severely compromised immune systems.

Monitoring Requirements

How many coliform samples must a water system collect?

Every Public Water System (WS or PWS) must collect a given number of "routine" coliform samples from the distribution system per month generally based on the population served.

Additional raw, finished, and distribution water samples may also be required depending on your source of water, water treatment facilities, service area of the distribution system,

and any related sanitary survey deficiencies. After a WS or PWS sanitary survey is conducted by the official State water or health agency inspector, the total number of coliform samples required each month is listed on the follow-up inspection report that is mailed to the WS or PWS. If you are not sure how many samples you are required to collect, please contact your **Drinking Water Agency.**

Introduction.
If a WS or PWS has a positive coliform sample, confirmation (repeat) samples must be collected immediately. The number of repeats will vary.

Does the number of monthly routine distribution coliform samples ever change?
Yes. Two events may change your monthly distribution monitoring requirements:
1) A change in population may cause a change in the number of samples. If a change occurs, your official State Drinking Water Agency will notify the WS or PWS.
2) One coliform positive routine finished or routine distribution system sample result during any month requires a minimum of **FIVE** routine distribution samples be collected the following month. If your system is already required to collect a minimum of five distribution samples each month, this does not affect you.

Do I have to collect my monthly routine coliform samples from specific locations?
Yes. Failure to do so may result in a monitoring violation. Every WS or PWS must have on file an approved coliform sample site plan at the appropriate **Drinking Water Agency**. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What is a coliform sample site plan?
A coliform sample site plan is a list of sites by street address, lot number, or other permanent description, that identifies all the approved locations where your routine (monthly) coliform samples may be collected. The list of sites must be plotted on a map of your service area. Larger water systems will divide their distribution system into specific sample areas.

Does the site plan have to be approved by the official State water or health agency?
Yes. The list of sites and the map is reviewed by the official State water or health agency Drinking Water Agency serving your facility to insure representative sites have been selected. A site number is then assigned to each sample location.

All reporting forms must be completed using the sample site number rather than the street address. Your approved site plan MUST be followed each time you collect routine samples. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How can I find out the water system's sampling locations?
A copy of the WS or PWS's coliform site plan (and sample site numbers) is on file at your official State water or health agency

How often and when must I collect my routine samples?
Routine compliance samples must be collected every month. Each month is a sampling period.

Samples Should be Collected as Early as Possible

Samples should be collected as early each month as possible, but not before the first day of each month. Prompt collection allows WS or PWS sufficient time for replacement sampling if samples are discarded. Reasons for discarding samples include: failure to record date or time of collection, sample(s) more than 30 hours old upon arrival at the certified laboratory, and sample(s) broken or frozen in transit. If the sample(s) and/or any necessary replacement samples are not collected within the monthly monitoring period, the WS or PWS will be in violation of their monitoring requirements and must make public notice. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Collecting Samples (1 Example)
How do I choose a proper sample faucet?
Since coliform samples must be representative of the water quality in the distribution system, it is important to select proper sampling locations. Visit the site before selecting it for your coliform sample site plan. See if a smooth-nosed cold-water faucet is available that will allow the collector to run the faucet at a constant flushing rate for 30 to 60 seconds without flooding the sink. You may choose to install a smooth nosed faucet at your designated sample locations.

The sampling faucet should be conveniently located and readily accessible to the collector. Avoid faucets that are connected to private water treatment equipment such as water softeners or filters. Faucets that are subject to exterior contamination because they are too close to a sink bottom or to the ground must be avoided. It is difficult to place a bottle beneath a low faucet without touching the interior of the bottle's neck against the outside of the faucet. Threaded faucets that might harbor bacteria around the threads should not be used. Leaking faucets that allow water to flow around the stem and over the outside of the faucet should be avoided. If an even stream of water cannot be sustained, a more suitable tap should be found. Failure to follow these precautions could result in a contaminated sample.

Keep in mind that in the event of a positive coliform sample, two (or three) additional distribution sites meeting the above criteria, one within five (5) service connections upstream and one within five (5) service connections downstream, will be needed for the collection of repeat samples. These additional sites should be included in your coliform sample site plan. You may wish to install dedicated sampling faucets or sampling stations to assure access and satisfactory sampling conditions at all times. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How should a coliform sample be collected?
Use great care when collecting coliform samples. Only bottles received from the certified laboratory should be used to collect samples. It is extremely important that the sample collector uses only the approved sampling locations and follow proper sampling techniques.

Contamination from the sampling faucet can easily occur if extreme caution is not used. Should an incident occur during sample collection that may result in contamination, the sample should be discarded and a new bottle requested.
It is assumed that all samples submitted for testing are properly collected. Sample error will not be accepted as an excuse to avoid repeat sampling.

Generally, the following protocol should be followed:
1. Sample bottles should be examined when received. If for any reason (loose caps, caps off, etc.) the sterility of the bottle is in question, the bottles should not be used.
2. Open an approved sampling faucet so that a smooth flow of water at moderate pressure is obtained. Be sure that there is no splashing. Allow the water to flow for sufficient time to clear the service line. Depending on time of year and water source you may notice a water temperature change when the line has been cleared.
3. **If your WS or PWS is chlorinated,** check for residual chlorine. These results MUST be included on the reporting form. Indicate whether the residual measured is free (F) or total (T) chlorine.
4. The bottle cap should not be removed until you are ready to collect the sample. Do not lay the bottle cap down or put it in a pocket. Hold the bottle in one hand and the cap in the other, keeping the bottle cap right side up (threads down) and taking care not to touch the inside of the cap. Avoid touching the inside of the sterile bottle(s) with your fingers or the faucet nose.
5. Once you start filling the bottles do not adjust the stream flow. Do not allow splashing drops of water from the ground or sink to enter the bottle. Fill the bottle to the 100 ml mark on the side of the bottle. Cap the bottle immediately. Then turn off the faucet.

Can I use any container to collect the sample?
No. You must use sterile 100 ml plastic (or glass) bottles obtained from a certified laboratory. These bottles contain a small amount of sodium thiosulfate to neutralize the residual chlorine in the sample. The sodium thiosulfate may appear as a tablet or as a white residue in the bottle. Do NOT rinse this material out of the bottle.

Do we need to use any special reporting forms when submitting samples to the laboratory?
Yes. These forms can be obtained by calling your **certified laboratory**. The reporting forms should be included with the bottles. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How do I complete the reporting/collection form?
For the most part, the form is self-explanatory. A few reminders:
1. Always use your official State water or health agency assigned sample site numbers (not sample address). If a site number does not exist for a location, please add it to your site plan by calling your **official Drinking Water Agency**. Simply record the address if it's a "one-time" sample location.
2. Be-sure you include all "Contact Person" information including telephone number and cellular number. This information is very important in case there is a positive sample.
3. Clearly mark sample purpose.
4. If the WS or PWS is chlorinated, you must include the chlorine residual on the form. Failure to do so will result in a violation.

After I collect the sample, how long does it have to reach the laboratory?
Always check with your Agency to ensure this rule is correct, for this rule is different in some States. In order for the laboratory to analyze the sample(s), it must be received within 24 or 30 hours of collection. If not, a replacement must be collected. It is strongly recommended that the monthly routine samples be collected within the first few days of each month. This will allow ample time for the collection of replacement sample(s) if they are required.

What days of the week should I collect my samples?
It is preferred that routine samples be collected and shipped to the laboratory on Mondays or Tuesdays. If routine samples are hand delivered to the laboratory, they should be delivered no later than Thursday. However, emergency or repeat samples may be submitted at any time. If you anticipate the samples will arrive on a weekend or holiday, you should contact your certified laboratory and make the necessary arrangements. It is recommended that you call your certified laboratory for a contact person, business hours, and any special delivery instructions. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Do all coliform samples collected during the month count towards meeting our compliance monitoring requirements?
No. Special purpose samples, such as those taken to lift a boil order, or new construction samples to determine whether disinfection practices are sufficient following pipe placement, replacement or repair, will not be used to determine compliance with the Maximum Contaminant Level (MCL) or towards the routine number of samples required each month.

Routine raw and/or finished water entry point samples do not count towards meeting the total number of distribution samples required each month. However, finished water entry point samples will be used to determine compliance with the MCL. Thus, if you have a positive finished water entry point sample, you must follow-up with the collection of repeat samples.

Our water system is chlorine exempt. Are there any special monitoring requirements?
Yes. In past years, some very small systems have been granted an exemption from chlorination by State water or health agencies. New exemptions are no longer granted. Official State water or health agency may still honors past chlorine exemptions, but recognizes that the water lacks the protection of the residual chlorine.

Consequently, it is especially important that the bacterial quality of the water be monitored at frequent intervals. One of the conditions of the exemption is that samples be collected and analyzed at twice the frequency required of a chlorinated WS or PWS (two times a month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Interpretation of Results
How do I know if the sample is satisfactory?
The laboratory will determine if any bacteria are present in your sample(s). If no bacteria are present the sample is considered satisfactory with a (S) recorded on the reporting form.

If bacteria are detected in the sample, the laboratory will record the number of colonies (if using the membrane filter technique) and analyze the sample to see if the bacteria are coliform. If the bacteria are determined to be coliform the laboratory will use the designation of “P” (positive).If no coliform is detected, the designation will be given as “N” (negative). *Any sample with a non-coliform bacteria count of 200 colonies or less is considered a satisfactory (S) sample.*

If any coliform bacteria are found, the sample is positive and requires collection of repeat samples as described in **– Repeat Samples / Follow-up to Coliform Positive Samples**. Additional tests are performed on the original total coliform positive sample to determine if fecal coliform or *E. coli* is present. This result is also recorded on the reporting form with a P or N.

A sample analyzed by the membrane filter technique is deemed invalid (I) in the following scenarios:
1. Samples are negative for coliform but exhibit confluent growth. Confluent growth is continuous bacterial growth covering all or part of a membrane filter.
2. Samples are negative for coliform but bacteria colonies are too numerous to count (reported as G- or TNTC by the laboratory).

Submission of replacement sample(s) is required. When using the multiple fermentation tube procedure or the presence-absence procedure, if the media is turbid with no production of gas or acid, the sample also is invalidated with replacement sample(s) required.

How long must I keep coliform results and other related paperwork?
5 years. Records of total coliform analyses must be kept for no less than five (5) years. The actual laboratory reports may be kept or the data may be transferred to tabular summaries.

Local procedures may contain requirements concerning the retention of records. The most stringent requirement should be followed. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

A WS or PWS with only a **groundwater source** (not under the direct influence of surface water) that **serves 4,900 persons or fewer**, may collect all required samples on a single day if they are taken from each area of the approved sample site plan. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Surface water supplies, supplies using groundwater under the direct influence of surface water and groundwater supplies that serve more than 4,900 people must collect samples throughout the month. WS or PWS that collect weekly or more frequently should continue this practice.

Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Laboratory Related Questions

Can we have our coliform samples analyzed anywhere?
No. A laboratory certified by your State Drinking Water Agency or Health Department must analyze samples. A sample analyzed at a laboratory that is not certified cannot be used for compliance.

What laboratory methods are used for analyzing coliform samples?
WS or PWS must conduct total coliform analyses in accordance with one of the analytical methods in the following table:

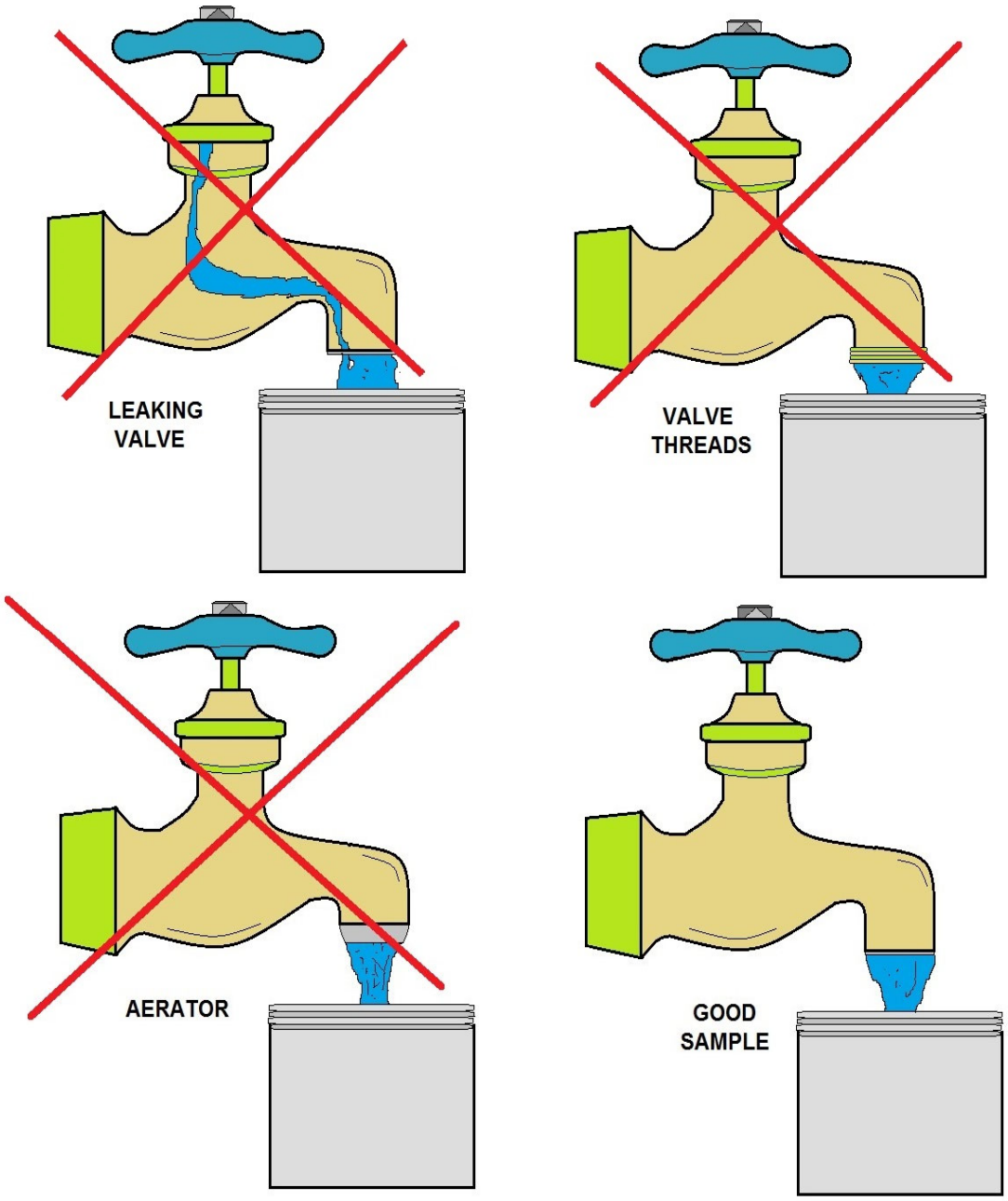
Organism Methodology
Total Coliforms Total Coliform Fermentation Technique
Total Coliform Membrane Filter Technique
Presence-Absence (P-A) Coliform Test
ONPG-MUG Test (Colilert)
Colisure Test
E*Colite® Test
m-ColiBlue24® Test
Readycult coliform 100 Presence/Absence
Colitag® Test

Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Do we need to use any special reporting forms when submitting samples to the laboratory?
Yes. These forms can be obtained by calling your **certified laboratory**. The reporting forms should be included with the bottles.

Is the water system or the laboratory responsible for getting results to the State water or health agency?
The water system. Regardless of whether a State of private laboratory is used, the WS or PWS is ultimately held accountable. It is very important that the WS or PWS is in frequent contact with the laboratory to confirm: the samples reach the laboratory, the status of results (positive vs. negative), and the results are sent to the official State water or health agency in a timely manner (within 10 days of the sampling period).

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water regulations that may be more stringent than EPA's regulations. Check with your state environmental agency for more information.



PROPER COLIFORM SAMPLING SITE

Repeat Samples / Follow-up to Coliform Positive Samples

How am I notified of positive coliform samples?
If a routine or replacement sample is total coliform positive, the certified laboratory and the Drinking Water Agency will try to contact the WS or PWS by telephone using the contact name and number provided on the reporting form. All Water Systems should keep a small number of extra coliform bottles/reporting forms in case of required repeat sampling. If for some unforeseen reason, the WS or PWS does not have extra coliform bottles on hand, the laboratory will overnight coliform bottles to the facility. However, this is not a preferred situation. Remember, responsibility for timely sampling ultimately falls on the WS or PWS. Therefore, waiting for bottles to arrive in the mail incurs risk on the WS or PWS. Obviously, to receive “repeat” sample bottles in the mail is an indication that there is a positive sample and immediate action is needed.

What is a repeat sample?
Any sample that is analyzed as a follow-up to an initial positive result is referred to as “repeat” sample. After a routine coliform sample is found to be total coliform positive, repeat samples are required to confirm the initial positive result(s), to determine if the contamination is ongoing, and to evaluate the extent of the contamination within the distribution system.

How many repeat samples must I collect?
The number of repeats samples is dependent on the number of routine samples collected for the month. **Three** repeat samples are required for each distribution sample that is coliform positive; **however**, if the WS or PWS sends in only one routine distribution sample per month, **four** repeat samples are required for each sample that is coliform positive. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Where must repeat samples be collected?
If **three** repeat samples are required, one repeat sample must be collected from a tap within five (5) service connections upstream from the original sample, another repeat sample must be collected within five (5) service connections downstream from the original sampling site, and the last must be collected at the original site.

If **four** repeat samples are required, one repeat sample must be collected from a tap within five (5) service connections upstream from the original sample, another repeat sample must be collected within five (5) service connections downstream from the original sampling site, another must be collected at the original site, and the fourth may be collected anywhere within the distribution system (this may aid in identifying the possible source of contamination).

All repeat samples (or sample set) must be collected on the same day. Exceptions to this rule are WS or PWS with a single service connection. In this case the official State water or health agency may allow the WS or PWS to collect the repeat samples over a four-day period or to collect a single sample of at least 400 ml (300 ml if the system collects more than one sample per month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Where should the repeat samples be collected if the positive sample was collected at the end of the distribution system?
The WS or PWS is still required to collect three (or four when applicable) repeat samples. If the original sampling site is at the end of the distribution system (or one tap away from the end) the State Drinking Water agency may waive the requirement to collect one of the repeat samples downstream. An additional sample will be required upstream or from the same building. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How many repeats are required if a finished water entry point sample or raw well sample is positive?
One. Only one repeat sample should be collected from the positive finished water entry point location or raw positive location. Raw or finished entry point samples (or raw/entry point repeat samples) are not used when determining compliance. Prior to August 2007, a routine coliform positive finished water (entry point) sample required three or four repeats. This has changed. Only one repeat is now required and it is to be collected from the same entry point location as the positive (downstream repeat samples are no longer required). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How much time do I have to collect repeat sample(s)?
24 Hours. If a routine or replacement sample is total coliform positive, the WS or PWS must collect a set of repeat samples within 24 hours of being notified of the positive result. Again, **all WS or PWS should keep a small number of extra coliform bottles/reporting forms in case of required repeat sampling.** If the WS or PWS is waiting for bottles to be shipped from the laboratory, repeat samples must be collected on the day of bottle receipt.

All repeat samples (or sample set) must be collected on the same day. Exceptions to this rule are WS or PWS with a single service connection. In this case the official State water or health agency may allow the WS or PWS to collect the repeat samples over a four-day period or to collect a single sample of at least 400 ml (300 ml if the system collects more than one sample per month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

When does the repeat sample collection “start clock” begin?
The 24-hour clock starts when the laboratory (or State) notifies the water system of the initial positive coliform result. You have 24 hours from the time of notification to collect your repeat samples and return them to a laboratory for analysis. If you fail to meet this window, a violation will be issued, provided that no extension had been granted.

If the WS or PWS cannot be reached via phone, the official State water or health agency has defined “notified” as the date that the laboratory initiates shipment of repeat sample bottles. The laboratory records this date on the coliform reporting form.

The repeat samples must be collected on the on the day that the bottles are received at the WS or PWS. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What if I cannot meet the 24-hour repeat collection requirement?
your official State water or health agency acknowledges that some circumstances may arise, totally beyond the control of the WS or PWS, which prevent repeat samples from being collected within the 24-hour period. Therefore, extensions may be granted by your **Drinking Water Agency**. A valid reason for the extension must be provided. Extensions cannot be granted after the fact. Therefore, requests for extensions MUST be made prior to or at the same time the repeat samples are being collected. Extensions will always specify exactly how much time the WS or PWS has to collect and return the repeat samples. Failure to obtain the extension or failure to meet the terms of the extension will result in a monitoring violation. Please remember that an extension on the 24-hour repeat collection requirement is NOT a waiver for actually collecting the repeat samples. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What happens if I am notified on a Friday of positive routine results (or receive repeat bottles on a Friday or Holiday)?
The 24-hour collection requirement must still be met. You should contact your **certified laboratory** to arrange a time on Saturday to collect the repeat samples and drive them to the laboratory. If this is not possible, the WS or PWS must request an extension the first business day following the weekend or holiday. Please call the official State water or health agency at the earliest possible time to request an extension on the 24-hour requirement.

It is strongly recommended that all routine coliform samples be collected and mailed on a Monday or Tuesday to avoid this situation.

What happens if any of the repeats are positive or invalid?
If one or more repeat samples in the set are total coliform positive or invalid, the whole repeat monitoring process must start over. A new “set” of three or four (if only one routine sample is collected per month) repeats must be collected within 24 hours of being notified of the positive or invalid repeat.

If a repeat sample location is positive and is not from the same location as the original positive sample (and/or the original site is negative), the next repeat collection should be based on the original positive site location and NOT the positive repeat location. Every consecutive set of repeat samples must be collected at the same locations as the 1st set of repeat samples.

The WS or PWS must repeat this process until either total coliform are not detected in one complete “set” of repeat samples or the WS or PWS determines that the total coliform Maximum Contaminant Level(MCL) has been exceeded and notifies your official State water or health agency. It is highly recommended that sampling be repeated until a “set” is satisfactory. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Does one (or more) positive routine or repeat sample change the following month’s monitoring requirements?
Yes. If you collect less than 5 routine distribution samples per month and have at least one positive routine, repeat or replacement sample, 5 routine distribution samples MUST be collected

the following month. The samples can be collected from other approved coliform sites or from other locations in the distribution system. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Who is responsible for notifying the official State water or health agency if results are positive?
The WS or PWS is responsible for notifying your official State water or health agency when a total coliform positive sample is found and for having the appropriate repeat samples analyzed. In most cases, an agreement has been made between the certified laboratory and the WS or PWS that the laboratory will notify the State Agency; however, even in this case, the WS or PWS is held accountable for this notification requirement.

Invalidating Sample Results
Can a sample result be invalidated?
A coliform positive can be invalidated when there is a significant reason to believe the test results are not accurate or not representative of the water quality. These samples are not used in compliance calculations and a replacement must be collected within the same monitoring period (same month) at the same location to avoid a possible monitoring violation.

There are three conditions in which a total coliform positive sample result may be invalidated:
1. The laboratory establishes that an error in its analytical procedure caused the total coliform positive result.
2. The State water or health agency, on the basis of the results of repeat samples collected determines that the total coliform positive sample resulted from a domestic or other non-distribution system-plumbing problem.
3. The State water or health agency determines that there are substantial grounds to believe that a total coliform positive result is due to a circumstance or condition that does not reflect water quality in the distribution system.

The laboratory will invalidate the results if they are unable to obtain a true result according to the test method used to analyze the sample. If a laboratory invalidates a routine sample due to interference, the WS or PWS must collect another sample from the same location as the original sample within 24 hours of being notified of the interference problem.

How can I have a positive coliform result invalidated?
Always check with your Agency to ensure this rule is correct, for this rule is different in some States. The invalidation process involves 3 steps:
1. All repeat samples must have been collected in accordance with the repeat sampling requirements.
2. As soon as you feel a sample should be invalidated, the **Drinking Water Agency** should be contacted by telephone and the situation discussed. If the Drinking Water Agency verbally agrees that the sample is not representative of the water quality, they will direct you as to what certain steps need to be taken (e.g., proof, additional samples, etc.). Ultimately, the Drinking Water Agency will recommend to the Compliance or Regulatory division whether or not a sample should be invalidated. Failure to get the Drinking Water Agency's concurrence will result in your request being rejected by the Compliance or Regulatory division.

3. A formal written requested must be mailed to the **Drinking Water Agency** and Compliance or Regulatory division within four weeks of the original routine sample collection date. The written documentation must state the specific cause of the total coliform positive sample and what action the supplier has taken, or will take, to correct this problem. The official State water or health agency will provide a written notification to the WS or PWS as to whether or not the request for invalidation was granted.

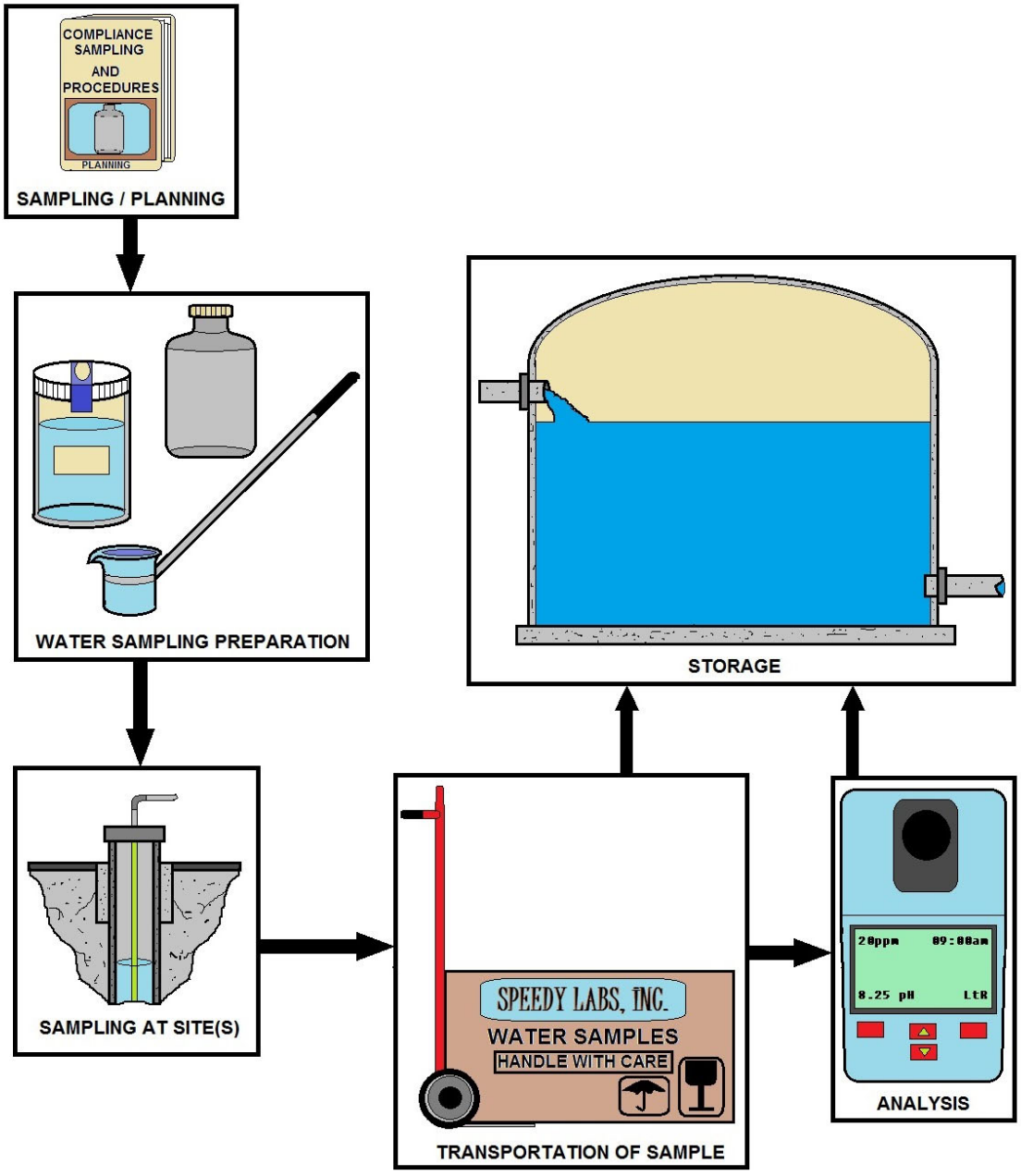
The State water or health agency will not invalidate a total coliform positive sample solely on the grounds that all repeat samples are total coliform negative.

How soon do I have to initiate the invalidation process?
At the time of receiving notice of positive results, the WS or PWS should immediately begin an investigation and collect repeat samples. At that time, any reason to question validity of a result should be acted on promptly while the situation is fresh at hand.

If a sample is invalidated by the State water or health agency or the certified laboratory does it still count towards meeting the monthly monitoring requirements?
No. You MUST collect another routine sample to replace any invalidated routine sample. To confirm the sample purpose look at the reporting form. Remember that all routine samples are marked with a sample purpose of “Routine” on the reporting form.

The replacement sample(s) MUST be collected within the same monitoring period (same month). Repeat samples cannot be used to meet this requirement. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

My repeat sample is invalid. Now what?
If one or more repeat samples in a set are invalid, the whole repeat monitoring process must begin over starting with the collection of a new “set” (3 or 4) of repeat samples within 24 hours. Every consecutive set of repeat samples should be collected at the same locations as the 1st set of repeats.



WATER SAMPLING FLOW CHART

Compliance, Violations, and Follow-up Actions

What is an MCL?
Maximum Contaminant Level. State and federal regulations set maximum contaminant levels (MCLs) on contaminants that have been determined to cause possible health effects. When MCLs are exceeded, the WS or PWS must accomplish all the required actions that generally include public notice within a specified time period. There are two types of coliform MCL violations, monthly and acute.

What is a MONTHLY coliform MCL exceedance and how is it determined?
A monthly coliform MCL violation occurs when a WS or PWS exceeds the number of allowed coliform positive samples. The number allowed depends on the number of routine distribution samples collected during the monthly sample period.

What is an ACUTE coliform MCL exceedance and how is it determined?
Acute MCLs are extremely serious and require immediate action. Coliform sample results have indicated an immediate threat to the WS or PWS and serious health effects can result. Therefore, water customers must be notified without delay. All samples that are total coliform positive must be examined for fecal coliform or *E. coli.* Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What is a monitoring violation and how can I avoid one?
A monitoring violation occurs when the coliform monitoring requirements for a month are not met. All WS or PWS are required to submit samples for bacteriological analysis during each calendar month. Do NOT sample before the beginning of the month.

The WS or PWS must meet several criteria to avoid a monitoring violation:
1. At a minimum, collect the required number of distribution samples each month. The number of distribution samples required is based on population served. It is crucial that every WS or PWS operator know the coliform monitoring requirements for his or her WS or PWS.

2. Sampling locations used must be in accordance with the approved written coliform sample site plan. All sampling areas must be represented during each monthly sampling period.

3. All replacement and repeat samples must be returned promptly to the laboratory for analysis. These samples will be included in determining compliance.

4. Repeat samples and replacement samples for invalid (negative coliform growth) samples must be collected within 24 hours of notification. If these samples cannot be collected within 24 hours, you must contact the official State water or health agency for an extension. The time extension will be given on a case-by-case basis and will always specify exactly how much time the WS or PWS has to collect and return the repeat samples. Failure to obtain the extension or failure to meet the terms of the extension will result in a monitoring violation.

5. If a private certified laboratory analyzes samples, it is the responsibility of the WS or PWS to make sure that copies of the reports are sent to the official State water or health agency within 10 days of the end of the sampling period (month).

6. Collect at least five coliform distribution system samples the month following a coliform positive finished or distribution system sample.

How are repeat samples used in determining compliance?
Repeat samples are not counted towards determining monthly distribution monitoring compliance. However, failure to collect repeats within 24-hours (following notification of a positive routine sample) will result in a monitoring violation (and possible MCL violation) unless the WS or PWS obtained a waiver from the 24-hour collection requirement. In addition, if a WS or PWS fails to collect any of the repeat samples for a positive routine sample, the missing repeats will be “assumed” positive and included when totaling the number of positive samples for the month. In most cases, this will result in an MCL violation and require public notification.

Do I need to notify the water customers of a violation?
Yes. Each violation and situation requiring notice has been assigned to one of three categories, or tiers, based on the risk of adverse health effects. Public notice is required for any of the following:

Tier 1 Violations or other Situations
Tier 1 violations or problems may result in an immediate adverse health problem for some consumers. Violation of the MCL for total coliform, when ***fecal coliform or E. coli*** are present in the water distribution system, or ***failure to test*** for fecal coliform or *E. coli* when any repeat sample tests positive for coliform.

Tier 2 Violations
All violations of the ***MCL***, ***MRDL***, and ***treatment technique*** requirements ***except*** where ***Tier 1 notice*** is required Violations of the ***monitoring*** requirements **when** required by the official State water or health agency (see Tier 3).

Tier 3 Violations or other Situations
Monitoring violations, except where Tier 1 or Tier 2 notice is required as determined by your official State water or health agency.

Common Mistakes to Avoid
Below describes some common errors that may result in a violation.
1. WS or PWS does not collect the correct number of repeat samples following a routine positive sample.
2. Failure of small WS or PWS (that serve a population 4,100 or less) to collect a minimum of 5 routine distribution coliform samples the month following a coliform positive sample.
3. Marking the wrong sample purpose box on the reporting form. Most often, this occurs when collecting a repeat (due to positive sample) sample and marking it as an invalid replacement or replacement (due to invalid, TNTC, or broken) sample and vice versa.
4. Failure to use sample site numbers.
5. Failure to report the chlorine residual (if chlorine/chloramines is added) on the reporting form.
6. Failure to monitor early in the month. Waiting until the end of the month to collect the routine samples does not allow enough time for follow-up actions if required.

Proper Sampling Handling (1 Example)

The proper handling of water quality samples also includes wearing gloves. Gloves not only protect field personnel, but also prevent potential contamination to the water sample. Always wear powderless, disposable gloves. When sampling for inorganics, wear latex gloves. Nitrile gloves are appropriate for organics.

The following will provide a field reference for chain of custody procedures, sampling surface water and ground water, and further provide procedures for measuring field parameters and handling water-quality samples.

Use chain-of-custody procedures when coolers and containers are prepared, sealed and shipped. They will remain sealed until used in the field. When making arrangements with the laboratory, make sure you request enough containers, including those for blank and duplicate samples. Order extra sample bottles to allow for breakage or contamination in the field.

Some samples require low-temperature storage and/or preservation with chemicals to maintain their integrity during shipment and before analysis in the laboratory. The most common preservatives are hydrochloric, nitric, sulfuric and ascorbic acids, sodium hydroxide, sodium thiosulfate, and biocides. Many laboratories provide pre-preserved bottles filled with measured amounts of preservatives.

Although most federal and state agencies allow the use of pre-preserved sample containers, some may require either cool temperatures or added preservatives in the field.

When the containers and preservatives are received from the laboratory, check to see that none have leaked. Be aware that many preservatives can burn eyes and skin, and must be handled carefully.

Sampling bottles should be labeled with type of preservative used, type of analysis to be done and be accompanied by a Safety Data Sheet (Formerly Material Safety Data Sheet) (SDS). Make sure you can tell which containers are pre-preserved, because extra care must be taken not to overfill them when collecting samples in the field. Check with the laboratory about quality control procedures when using pre-preserved bottles.

Coolers used for sample shipment must be large enough to store containers, packing materials and ice. Obtain extra coolers, if necessary. Never store coolers and containers near solvents, fuels or other sources of contamination or combustion. In warm weather, keep coolers and samples in the shade.

Field Parameters
Measure and record the field parameters of temperature, electrical conductivity, pH and dissolved oxygen in an undisturbed section of streamflow. Other parameters may be measured, if desired.

***(Check with your governmental environmental or health agency for more information*).**

QA/QC Measures (1 Example)

In addition to standard samples, the field technicians collect equipment blanks (**EB**), field cleaned equipment blanks (**FB**), split samples (**SS**), and field duplicate samples (**FD**).

Overall care must be taken in regards to equipment handling, container handling/storage, decontamination, and record keeping. Sample collection equipment and non-preserved sample containers must be rinsed three times with sample water before the actual sample is taken. Exceptions to this are any pre-preserved container or bac-t type samples.

If protective gloves are used, they shall be clean, new and disposable. These should be changed upon arrival at a new sampling point.

Highly contaminated samples shall never be placed in the same ice chest as environmental samples. It is good practice to enclose highly contaminated samples in a plastic bag before placing them in ice chests. The same is true for wastewater and drinking water samples.

Ice chests or shipping containers with samples suspected of being highly contaminated shall be lined with new, clean, plastic bags.

If possible, one member of the field team should take all the notes, fill out labels, etc., while the other member does all of the sampling.

Preservation of Samples
Proper sample preservation is the responsibility of the sampling team, not the lab providing sample containers. The best reference for preservatives is a current edition of ***Standard Methods*** or your local sampling laboratory.

It is the responsibility of the field team to assure that all samples are appropriately preserved.

- Follow the preservative solution preparation instructions.
- Always use strong safety precautions diluting the acid.
- Put a new label on the dispensing bottle with the current date.
- Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.
- Wait 3-4 hours for the preservative to cool most samples down to 4°C.
- Most preservatives have a shelf life of one year from the preparation date.

When samples are analyzed for TKN, TP, NH_4 and NOx, 1 mL of 50% Trace Metal grade sulfuric acid is added to the each discrete auto sampler bottles/bags in the field lab before sampling collection. The preservative maintains the sample at 1.5<pH<2 after collection. To meet maximum holding time for these preserved samples (28 days), pull and ship samples every 14 days. Narrow range pH paper (test strips) can be used to test an aliquot of the preserved sample. Place the pH paper into the container and compare the color with the manufacturer's color chart.

Collection of Surface Water Samples(1 Example)

Representative samples may be collected from rivers, streams and lakes if certain rules are followed:

1. Watch out for flash floods! If a flooding event is likely and samples must be obtained, always go in two-person teams for safety. Look for an easy route of escape;

2. Select a sampling location at or near a gauging station so that stream discharge can be related to water-quality loading. If no gauging station exists, then measure the flow rate at the time of sampling, using the streamflow method described below;

3. Locate a straight and uniform channel for sampling;

4. Unless specified in the sampling plan, avoid sampling locations next to confluences or point sources of contamination;

5. Use bridges or boats for deep rivers and lakes where wading is dangerous or impractical;

6. Do not collect samples along a bank as they may not be representative of the surface water body as a whole; and

7. Use appropriate gloves when collecting the sample.

Streamflow Measurement (1 Example)

Before collecting water quality samples, record the stream's flow rate at the selected station. The flow rate measurement is important for estimating contaminant loading and other impacts. The first step in streamflow measurement is selecting a cross-section. Select a straight reach where the stream bed is uniform and relatively free of boulders and aquatic growth. Be certain that the flow is uniform and free of eddies, slack water and excessive turbulence.

After the cross-section has been selected, determine the width of the stream by stringing a measuring tape from bank-to-bank at right angles to the direction of flow. Next, determine the spacing of the verticals. Space the verticals so that no partial section has more than 5 percent of the total discharge within it.

At the first vertical, face upstream and lower the velocity meter to the channel bottom, record its depth, then raise the meter to 0.8 and 0.2 of the distance from the stream surface, measure the water velocities at each level, and average them.

Move to the next vertical and repeat the procedure until you reach the opposite bank. Once the velocity, depth and distance of the cross-section have been determined, the mid-section method can be used for determining discharge. Calculate the discharge in each increment by multiplying the averaged velocity in each increment by the increment width and averaged depth.

(Note that the first and last stations are located at the edge of the waterway and have a depth and velocity of zero.) Add up the discharges for each increment to calculate total stream discharge. Record the flow in liters (or cubic feet) per second in your field book.

River and Stream Sampling

Collection of samples from rivers and stream involves transporting all necessary items to the water-quality station and setting up field notes, instrumentation, filtration equipment (if not performed elsewhere as with microbiologicals), sample containers and decontamination washes near the channel. The first step is to measure all field parameters and then measure streamflow. After collecting and preserving the samples, equipment storage and decontamination will follow. Avoid spills when decontaminating equipment. For remote sites, extra collections equipment may be used to eliminate the need for field decontamination. Your governmental agencies have written procedures covering all aspects of surface-water characterization and sampling.

Composite Sampling
Composite sampling is intended to produce a water quality sample representative of the total stream discharge at the sampling station. If your sampling plan calls for composite sampling, use an automatic type sampler.

River or Channel Grab Sampling
Grab sampling is performed when uniform mixing in the river or stream channel makes composite sampling unnecessary, when point samples are desired, when sample degassing may occur, or when the water is too shallow for composite sampling. Record any decision to use grab sampling in the sampling plan. For streams at least 4 inches (10 cm) deep, collect grab samples in the middle of the channel using a laboratory cleaned or decontaminated glass or plastic container, and add the required preservatives.

An automatic refrigerator sampler with a Pickle Jar, this automatic sampler can also do grab type samples.

Laboratory
123 W. Main St
Sun City, Arizona 85541

LAB I.D. NUMBER

Sampler: DATE: PAGE 1 OF 1

Company:
Department:
Address:
Contact:
Telephone:

Sample Identification	Date	Time	Matrix	Lab ID	Metals* See Attached	TSS	Lead/Copper	BOD/COD	Nitrate	Nitrate + Nitrite	TKN / Ammonia	VOC / THM's	Semi Volital Organics (625)	Chloride		Cyanide	Florida	Surfactants (MBAS)	Tot. Coliform MPN	Fecal Coliform MPN-HPC	Organo-Phosphorus Pest. (8141)	Sulfate	EC Conductivity	Number/Containers

Project Name

Project Number

Field Measurements:
pH:
Temp:

Sample Receipt
No. Containers: _____
Custody Seals: Yes No
Received Intact: Yes No
Received Cold: Yes No
Temperature:
PRIORITY:

RELINQUISHED BY:
Signature: Time:
Printed Name: Date:
Company

RELINQUISHED BY:
Signature: Time:
Printed Name: Date:
Company

SAMPLED RECEIVED BY:
Signature: Time:
Printed Name: Date:
Company

SAMPLED RECEIVED BY:
Signature: Time:
Printed Name: Date:
Company

Chain-of-Custody Report Example

Chain of Custody Procedures

Because a sample is physical evidence, chain of custody procedures are used to maintain and document sample possession from the time the sample is collected until it is introduced as evidence. Chain of custody requirements will vary from agency to agency. However, these procedures are similar and the chain of custody outlined in this manual is only a guideline. Consult your project manager for specific requirements.

If you have physical possession of a sample, have it in view, or have it physically secured to prevent tampering, then it is defined as being in "***custody***." A chain of custody record, therefore, begins when the sample containers are obtained from the laboratory. From this point on, a chain of custody record will accompany the sample containers.

Handle the samples as little as possible in the field. Each custody sample requires a chain of custody record and may require a seal. If you do not seal individual samples, then seal the containers in which the samples are shipped.

When the samples transfer possession, both parties involved in the transfer must sign, date and note the time on the chain of custody record. If a shipper refuses to sign, you must seal the samples and chain of custody documents inside a box or cooler with bottle seals or evidence tape. The recipient will then attach the shipping invoices showing the transfer dates and times to the custody sheets.

If the samples are split and sent to more than one laboratory, prepare a separate chain of custody record for each sample. If the samples are delivered to after-hours night drop-off boxes, the custody record should note such a transfer and be locked with the sealed samples inside sealed boxes.

(***Check with your governmental environmental or health agency for more information.***)

Chapter 3- The Main Players- History and Biology

Section Focus: You will learn the basics of waterborne diseases and water biology. At the end of this section, you will be able to describe the history of waterborne diseases. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: By the last half of the 19th century, the microbial world was known to consist of protozoa, fungi, and bacteria, all visible with a light microscope. In the 1840s, the German scientist Jacob Henle suggested that there were infectious agents too small to be seen with a light microscope, but for the lack of direct proof, his hypothesis was not accepted. Although the French scientist Louis Pasteur was working to develop a vaccine for rabies in the 1880s, he did not understand the concept of a virus.

History of Research

LOUIS PASTEUR

Before we define the major waterborne diseases, let's first examine the germs and other creatures that cause the diseases. Most of the following information may be simple or instruction that you already know. But to be safe, let's review the basics.

During the last half of the 19th century, several key discoveries were made that set the stage for the discovery of viruses. Pasteur is usually credited for dispelling the notion of spontaneous generation and proving that organisms reproduce new organisms. The German scientist Robert Koch, a student of Jacob Henle, and the British surgeon Joseph Lister developed techniques for growing cultures of single organisms that allowed the assignment of specific bacteria to specific diseases.

First Experiment

The first experimental transmission of a viral infection was accomplished in about 1880 by the German scientist Adolf Mayer, when he demonstrated that extracts from infected tobacco leaves could transfer tobacco mosaic disease to a new plant, causing spots on the leaves.

Because Mayer was unable to isolate a bacterium or fungus from the tobacco leaf extracts, he considered the idea that tobacco mosaic disease might be caused by a soluble agent, but he concluded incorrectly that a new type of bacteria was likely to be the cause. The Russian scientist Dmitri Ivanofsky extended Mayer's observation and reported in 1892 that the tobacco mosaic agent was small enough to pass through a porcelain filter known to block the passage of bacteria. He too failed to isolate bacteria or fungi from the filtered material. But Ivanofsky, like Mayer, was bound by the dogma of his times and concluded in 1903 that the filter might be defective or that the disease agent was a toxin rather than a reproducing organism.

Unaware of Ivanofsky's results, the Dutch scientist Martinus Beijerinck, who collaborated with Mayer, repeated the filter experiment but extended this finding by demonstrating that the filtered material was not a toxin because it could grow and reproduce in the cells of the plant tissues. In his 1898 publication, Beijerinck referred to this new disease agent as a contagious living liquid—contagium vivum fluid—initiating a 20-year controversy over whether viruses were liquids or particles.

The conclusion that viruses are particles came from several important observations. In 1917 the French-Canadian scientist Félix H. d'Hérelle discovered that viruses of bacteria, which he named bacteriophage, could make holes in a culture of bacteria. Because each hole, or plaque, developed from a single bacteriophage, this experiment provided the first method for counting infectious viruses (the plaque assay). In 1935 the American biochemist Wendell Meredith Stanley crystallized tobacco mosaic virus to demonstrate that viruses had regular shapes, and in 1939 tobacco mosaic virus was first visualized using the electron microscope.

In 1898 the German bacteriologists Friedrich August Johannes Löffler and Paul F. Frosch (both trained by Robert Koch) described foot-and-mouth disease virus as the first filterable agent of animals, and in 1900, the American bacteriologist Walter Reed and colleagues recognized yellow fever virus as the first human filterable agent. For several decades' viruses were referred to as filterable agents, and gradually the term virus (Latin for "slimy liquid" or "poison") was employed strictly for this new class of infectious agents. Through the 1940s and 1950s many critical discoveries were made about viruses through the study of bacteriophages because of the ease with which the bacteria they infect could be grown in the laboratory.

Between 1948 and 1955, scientists at the National Institutes of Health (NIH) and at Johns Hopkins Medical Institutions revolutionized the study of animal viruses by developing cell culture systems that permitted the growth and study of many animal viruses in laboratory dishes.

Germ Theory of Disease History

Louis Pasteur along with Robert Koch developed the germ theory of disease which states that "a specific disease is caused by a specific type of microorganism." In 1876, Robert Koch established an experimental procedure to prove the germ theory of disease. This scientific procedure is known as Koch's postulates.

Koch's Postulates

- the causative agent must be present in every case of the disease and must not be present in healthy animals.
- the pathogen must be isolated from the diseased host animal and must be grown in pure culture.
- the same disease must be produced when microbes from the pure culture are inoculated into healthy susceptible animals.
- the same pathogen must be recoverable once again from this artificially infected animal and it must be able to be grown in pure culture.

Koch’s postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.

Circumstances under which Koch’s postulates do not easily apply

- Many healthy people carry pathogens but do not exhibit the symptoms of disease. These "carriers" may transmit the pathogens to others who then may become diseased. Example: epidemics of certain hospital acquired (nosocomial) infections, gonorrhea, typhoid, pneumonia, and AIDS.
- Some microbes are very difficult to grow under in-vitro (in the laboratory) conditions. Example: viruses, chlamydia, rickettsias, and bacteria that cause leprosy and syphilis. Some of the fastidious organisms can now be grown in cultures of human or animal cells or in small animals.
- Not all laboratory animals are susceptible to all pathogens. Many pathogens are species specific. Ethical considerations limit the use of laboratory animals and human volunteers.
- Certain diseases develop only when an opportunistic pathogen invades a susceptible host. These secondary invaders or opportunists cause disease only when a person is ill or recovering from another disease. For example, in the case of pneumonia and ear infections following influenza, isolation of bacteria causing pneumonia may mislead the isolation of influenza virus.
- Not all diseases are caused by microorganisms. Many diseases are caused by dietary deficiencies (scurvy, rickets). Some of the diseases are inherited or are caused by abnormality in chromosomes. Still others, such as cancer of the lungs and skin, are influenced by environmental factors.

Cells
Robert Hooke observed small empty chambers in the structure of cork with the help of his crude microscope. He called them cells. With the help of advanced microscopes, it is now known that a cell is composed of many different substances and contains tiny particles called organelles that have important functions.

Two German biologists Matthias Schleiden and Theodore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.

Rudolph Virchow completed the cell theory with the idea that all cells must arise from preexisting cells.

In biology, a cell is defined as the fundamental living unit of any organism and exhibits the basic characteristics of life. A cell obtains food from the environment to produce energy and nutrients for metabolism.

Metabolism
Metabolism is a term that describes all the chemical reactions by which food is transformed for use by the cells.

Through its metabolism, a cell can grow, reproduce and it can respond to changes in its environment. As a result of accidental changes in its environment, a cell can undergo changes in its genetic material. This is called mutation.

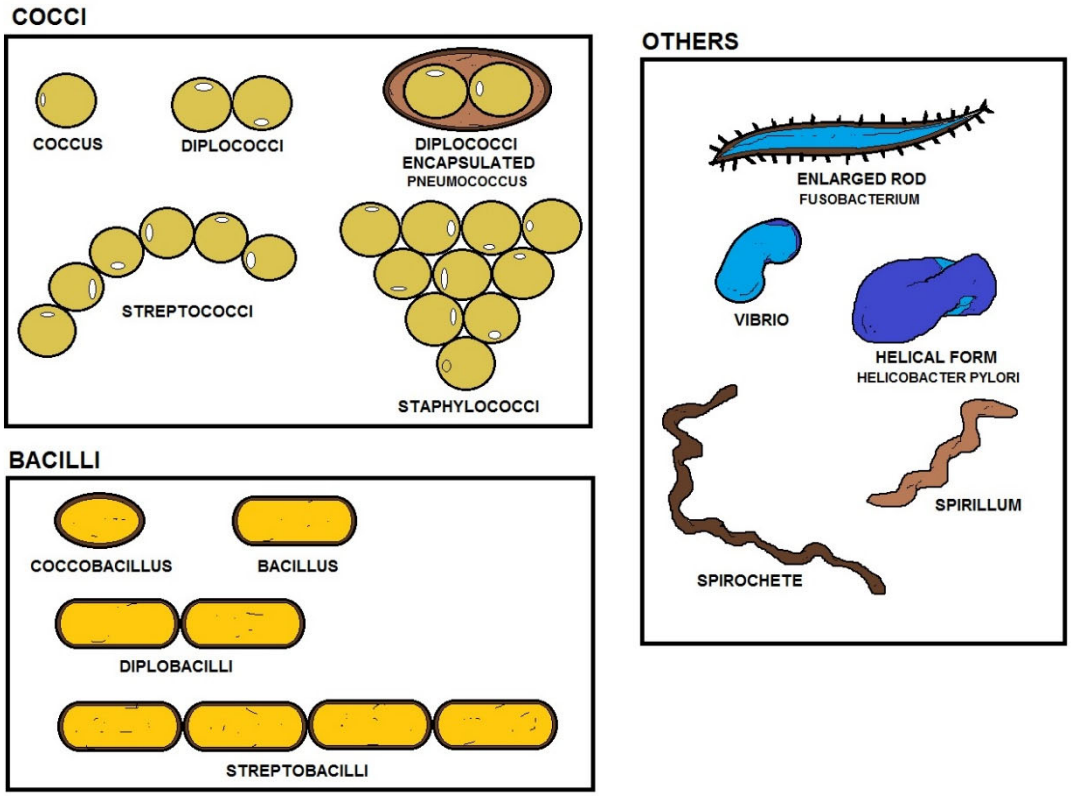
CLASSIFICATION OF LIVING THINGS

DOMAIN	BACTERIA	ARCHAEA	EUKARYA			
KINGDOM	EUBACTERIA	ARCHAEBACTERIA	PROTISTS	FUNGI	PLANTAE	ANIMALIA
CELL TYPE	PROKARYOTE	PROKARYOTE	EUKARYOTE	EUKARYOTE	EUKARYOTE	EUKARYOTE
CELL STRUCTURES	CELL WALLS WITH PEPTIDOGLYCAN	CELL WALLS WITHOUT PEPTIDOGLYCAN	CELL WALLS OF CELLULOSE IN SOME; SOME HAVE CHLOROPLASTS	CELL WALLS OF CHITIN	CELL WALLS OF CELLULOSE; CHLOROPLASTS	NO CELL WALLS OR CHLOROPLASTS
NUMBER OF CELLS	UNICELLULAR	UNICELLULAR	MOST UNICELLULAR; SOME COLONIAL; SOME MULTICELLULAR	MOST MULTICELLULAR; SOME UNICELLULAR	MULTICELLULAR	MULTICELLULAR
MODE OF NUTRITION	AUTOTROPH OR HETEROTROPH	AUTOTROPH OR HETEROTROPH	AUTOTROPH OR HETEROTROPH	HETEROTROPH	AUTOTROPH	HETEROTROPH
EXAMPLES	STREPTOCOCCUS, ESCHERICHIA COLI	METHANOGENS, HALOPHILES	AMOEBA, PARAMECIUM, SLIME MOLDS, GIANT KELP	MUSHROOMS, YEASTS	MOSSES, FERNS, FLOWERING PLANTS	SPONGES, WORMS, INSECTS, FISHES MAMMALS

Bacteria Sub-Section

Bacteria consist of only a single cell, but don't let their small size and seeming simplicity fool you. They're an amazingly complex and fascinating group of creatures. Bacteria have been found that can live in temperatures above the boiling point and in cold that would freeze your blood.

They "eat" everything from sugar and starch to sunlight, sulfur and iron. There's even a species of bacteria—*Deinococcus radiodurans*—that can withstand blasts of radiation 1,000 times greater than would kill a human being.

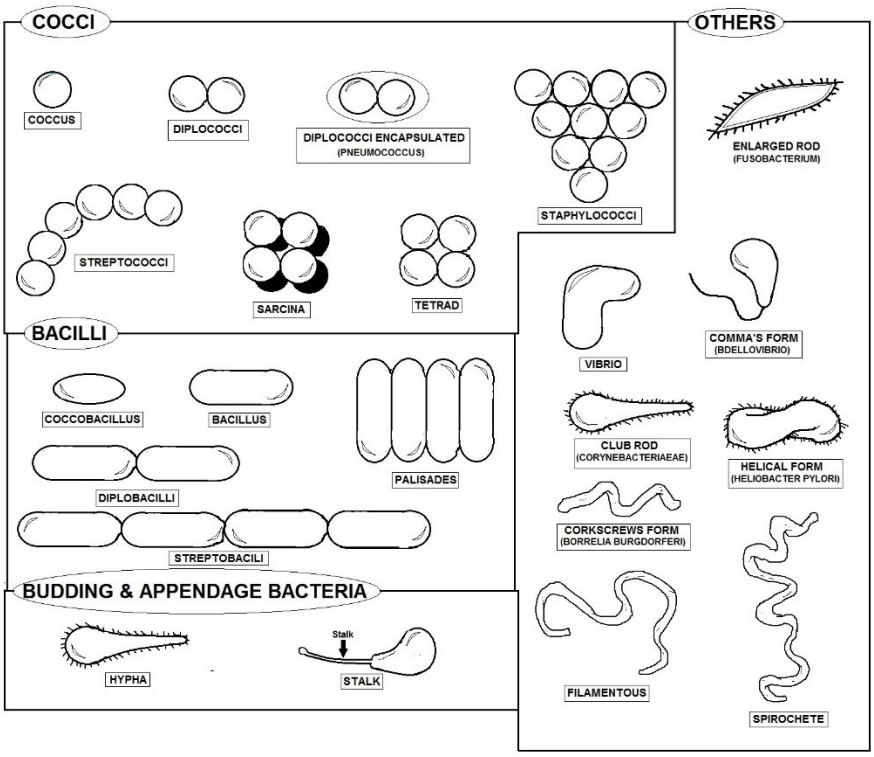


THE DIFFERENT SHAPES OF BACTERIA

“Bacteria” is a plural word. The singular for this word is “bacterium” (**bacter** = rod, staff). Bacteria are prokaryotes (Kingdom Monera), which means that they have no true nucleus. They do have one chromosome of double-stranded DNA in a ring.

They reproduce by binary fission. Most bacteria lack or have very few internal membranes, which means that they don’t have some kinds of organelles (like mitochondria or chloroplasts). Most bacteria are **benign** (**benign** = good, friendly, kind) or beneficial, and only a few are “bad guys” or **pathogens**.

Kingdom Monera is a very diverse group. There are some bacteria relatives that can do photosynthesis--they don’t have chloroplasts, but their chlorophyll and other needed chemicals are built into their cell membranes.



BACTERIA SHAPES

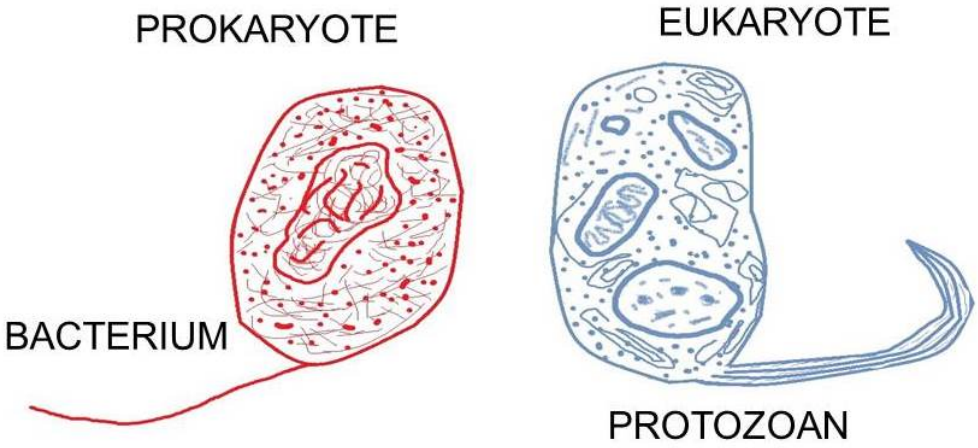
Organisms Descriptors and Meanings Chart

Description	Meaning
Aerobic	With air
Anaerobic	Without air
Auto	Self (Inorganic carbon)
Facultative	With air or without air
Hetero	Other (Organic carbon)
Troph	Feed or nourish
Photo	Light
Chemo	Chemical
Organo	Organic
Litho	Rock

These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they're not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_2, while others are poisoned or killed by it.

Prokaryotes
Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes *(you-carry-oats)*, creatures whose cells have nuclei.

(Note: viruses are not considered true cells, so they don't fit into either of these categories; this will be covered in the next few pages.)



PROKARYOTE ARE SIMPLER THAN EUKARYOTE

Early Origins
Bacteria are among the earliest forms of life that appeared on Earth thousands of years ago. Scientists think that bacteria helped shape and change the young planet's environment, eventually creating atmospheric oxygen that enabled other, more complex life forms to develop.

Many believe that more complex cells developed as once free-living bacteria took up residence in other cells, eventually becoming the organelles in modern complex cells.

The mitochondria *(mite-oh-con-dree-uh)* that make energy for your body cells is one example of such an organelle.

There are thousands of species of bacteria, but all of them are basically one of three different shapes. Some are rod - or stick-shaped and called bacilli *(buh-sill-eye)*. Others are shaped like little balls and called cocci *(cox-eye)*.

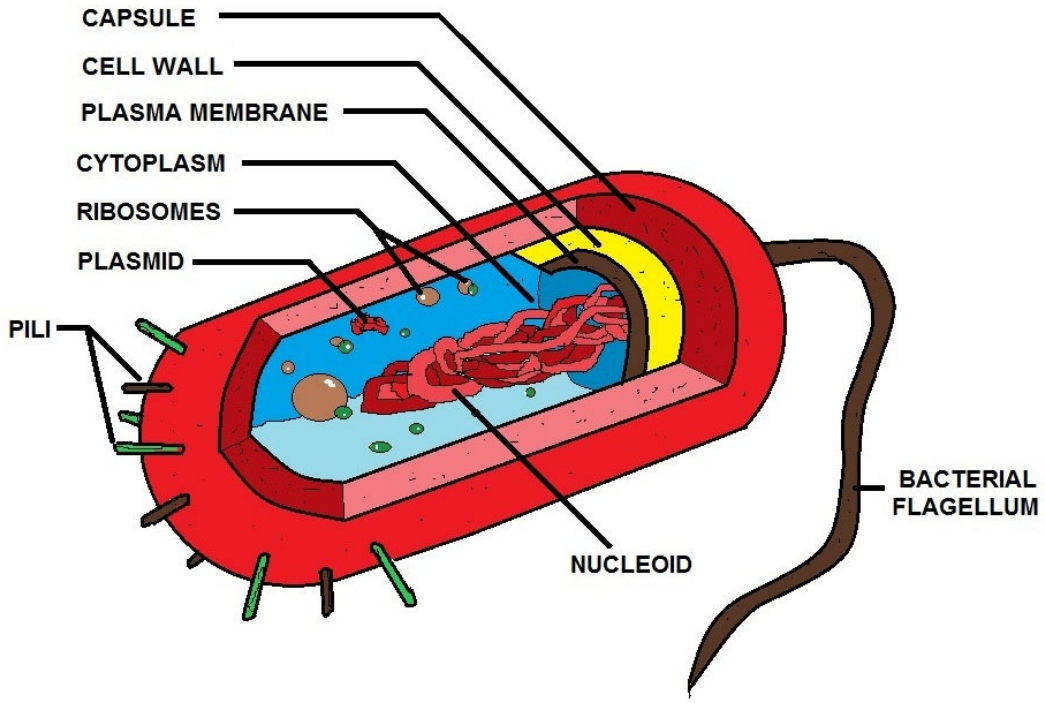
Others still are helical or spiral in shape. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other groupings. Bacteria live on or in just about every material and environment on Earth from soil to water to air, and from your house to arctic ice to volcanic vents.

Each square centimeter of your skin averages about 100,000 bacteria. A single teaspoon of topsoil contains more than a billion (1,000,000,000) bacteria.

Peptidoglycan
Most bacteria secrete a covering for themselves which we call a **cell wall**. However, bacterial cell walls are a totally different thing than the cell walls we talk about plants having.

Bacterial cell walls do **NOT** contain cellulose like plant cell walls do. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.

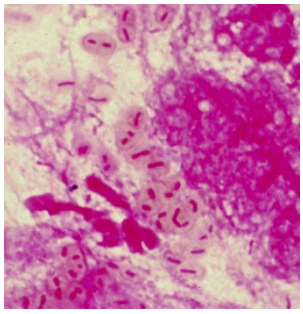
Some antibiotics, like penicillin, inhibit the formation of the chemical cross linkages needed to make peptidoglycan. These antibiotics don't kill the bacteria outright; just stop them from being able to make more cell wall so they can grow. That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.



PROKARYOTIC CELL (BACTERIA)

Gram Stain

However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. **Dr. Hans Christian Gram**, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Gram stain**. In this process, the amount of peptidoglycan in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained; thus, bacterial cells can be $Gram^+$ or $Gram^-$. $Gram^+$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color. $Gram^-$ bacteria have more complex cell walls with less peptidoglycan, thus absorb less of the purple dye used and stain a pinkish color instead. Also, $Gram^-$ bacteria often incorporate toxic chemicals into their cell walls, and thus tend to cause worse reactions in our bodies. Because $Gram^-$ bacteria have less peptidoglycan, antibiotics like penicillin are less effective against them. As we have discussed before, taking antibiotics that don't work can be bad for you, thus a good doctor should always have a culture done before prescribing antibiotics to make sure the person is getting something that will help.



Pseudomonas aeruginosa is a strictly aerobic, oxidase positive, gram-negative non-fermentative bacterium. The Gram-stain appearance is not particularly characteristic although rods are somewhat thinner than those seen for the enteric-like bacteria. Mucoid strains that produce an extracellular polysaccharide are frequently isolated from patients with cystic fibrosis and this capsular material can be seen in the photo.

Peritrichous Bacteria

Microbiologists broadly classify Bacteria according to their shape: spherical, rod-shaped, and spiral-shaped. Pleomorphic bacteria can assume a variety of shapes. Bacteria may be further classified according to whether they require oxygen (aerobic or anaerobic) and how they react to a test with Gram's stain. Bacteria in which alcohol washes away Gram's stain is called gram-negative, while bacteria in which alcohol causes the bacteria's walls to absorb the stain are called Gram-positive.

Two types of cells- Procaryotes and Eucaryotes

A Procaryotic cell exhibits all the characteristics of life but it lacks the complex system of membranes and organelles. ***Example***: Bacterial cells and cyanobacteria. A Eukaryotic cell has a complex structure. It contains a true nucleus and many membranes bound organelles. ***Example***: Protozoa, fungi, algae, all plants and animal cells.

Structure of a Eukaryotic Cell

Cell Membrane: The cell is enclosed and held intact by the cell membrane/plasma membrane/cytoplasmic membrane. It is composed of large molecules of proteins and phospholipids. These large molecules permit the passage of nutrients, waste products and secretions across the cellular membrane. The cell membrane is selectively permeable.

Nucleus
The Nucleus unifies, controls and integrates the function of the entire cell. The nucleus is enclosed in the nuclear membrane and contains chromosomes; the number and composition of chromosomes and the number of genes on each chromosome are characteristic of each species. Human cells have 46 (23 pairs) chromosomes. Each chromosome consists of many genes. A gene is a coiled unit made up of DNA and proteins that code for or determine a particular characteristic of an individual organism.

Cytoplasm
Cytoplasm is the cellular material outside the nucleus. It is composed of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, centrioles, microtubules, lysosomes and vacoules.

Cell Wall
A cell wall is found as an external structure of plant cells, algae, and fungi. It consists of cellulose, pectin, chitin, and some mineral salts. A rigid exterior cell wall defines the shape of bacterial cells. It is different from the simple cell wall of plant cells and is made up of macromolecular polymer-peptidoglycan (protein and polysaccharide chain). The thickness and its exact composition varies with the species of bacteria.

Cilia and Flagella
Some eukaryotic cells possess relatively long and thin structures called flagella. These are organs of locomotion. Cilia are also organs of locomotion but are shorter and more numerous

Structure of a Procaryotic Cell
All bacteria are procaryotes and are simple cells. They divide by binary fission.

Chromosome
The chromosome of a prokaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell. A typical bacterial chromosome contains approximately 10,000 genes.

Cytoplasm
Cytoplasm is a semi-liquid that surrounds the chromosome and is contained within the plasma membrane. Located within the cytoplasm are several ribosomes, which are the sites of protein synthesis. Cytoplasmic granules occur in certain species of bacteria which can be specifically stained and used to identify the bacteria.

Cell Membrane
The Cell Membrane is similar to that of the eukaryotic cell membrane. It is selectively permeable and controls the substances entering or leaving the cell. It is very thin and can be seen by electron microscopes only.

Capsules
Some bacteria have a layer of material outside the cell wall. When highly organized and firmly attached to the cell wall, this layer is called a capsule or if it is not highly organized and not firmly attached, a slime layer. Capsules consist of complex sugars or polysaccharides combined with lipids and proteins. The composition of the capsule is

useful in differentiating between different types of bacteria. Encapsulated bacteria produce colonies on nutrient agar that are smooth, mucoid and glistening, whereas the noncapsulated bacteria produce rough and dry colonies. Capsules enable the bacterial species to attach to mucus membranes and protect the bacteria from phagocytosis.

Flagella
Flagella are thread-like proteins that enable the bacteria to move. Flagellated bacteria are said to be motile while non-flagellated bacteria are generally non-motile. The number and arrangement of flagella are species specific and can be used to classify bacteria.
Peritrichous bacteria- possess flagella over the entire surface.
Lophotrichous bacteria-possess a tuft of flagella at one or both ends.
Amphitrichous bacteria-bacteria with one flagellum at each end.
Monotrichous bacteria-bacteria with a single polar flagellum.

Pili or Fimbriae
Pili or Fimbriae are thin hair-like structures observed on gram negative bacteria. They are not associated with motility. They enable the bacteria to attach to other bacteria or to membrane surfaces such as intestinal linings or RBC. They are also used to transfer genetic material from one bacteria cell to another.

Spores
Some bacteria are capable of forming spores (also called endospore) as a means of survival under adverse conditions. During sporulation the genetic material is enclosed in several protein coats that are resistant to heat, drying and most chemicals. Spores have been shown to survive in soil or dust. When the dried spore lands on a nutrient rich surface, it forms a new vegetative cell. Spore formation is related to the survival of bacterial cells, not reproduction.

Bacterial Nutrition

All life has the same basic nutritional requirements which include:
Energy. This may be light (the sun or lamps) or inorganic substances like sulfur, carbon monoxide or ammonia, or preformed organic matter like sugar, protein, fats etc. Without energy life cannot exist and quickly dies or becomes inactive.
Nitrogen. This may be nitrogen gas, ammonia, nitrate/nitrite, or a nitrogenous organic compound like protein or nucleic acid.
Carbon. This can be carbon dioxide, methane, carbon monoxide, or a complex organic material.
Oxygen. All cells use oxygen in a bound form and many require gaseous oxygen (air), but oxygen is lethal to many microbes.
Phosphorous, Sulfur, Magnesium, Potassium, and Sodium.
Calcium. Most cells require calcium in significant quantities, but some seem to only need it in trace amounts.
Water. All life requires liquid water in order to grow and reproduce; which is why the Mars Mission is so interested in water on Mars. Some resting stages of cells, like bacterial spores, can exist for long periods without free water, but they do not grow or metabolize.
Iron, Zinc, Cobalt. These are called trace metals that are required by some enzymes to function.

The sources of these various requirements define an organism, so a description of every organism should include this information.

Fastidious

Many bacteria can synthesize every complex molecule they need from the basic minerals, but others, said to be fastidious, require preformed organic molecules like vitamins, amino acids, nucleic acids, carbohydrates; humans are ***fastidious***. In general, bacterial pathogens need more preformed organic molecules than do non-pathogens, but that is not always true. For example, some bacteria that are found in milk hardly make any of their own basic organic molecules; that is, they let the cow (or more to the point the number of microbes that live in the cow's gut) make these things for them. A simple rule of thumb is "***if humans can use something for food, many microbes will also love it***". The reverse is not always true, as microbes can "digest" some very strange substances including cellulose, sulfur, some plastics, turkey feathers and asphalt, just to name a few.

Table of differences between Archaea, Bacteria and Eukaryotes

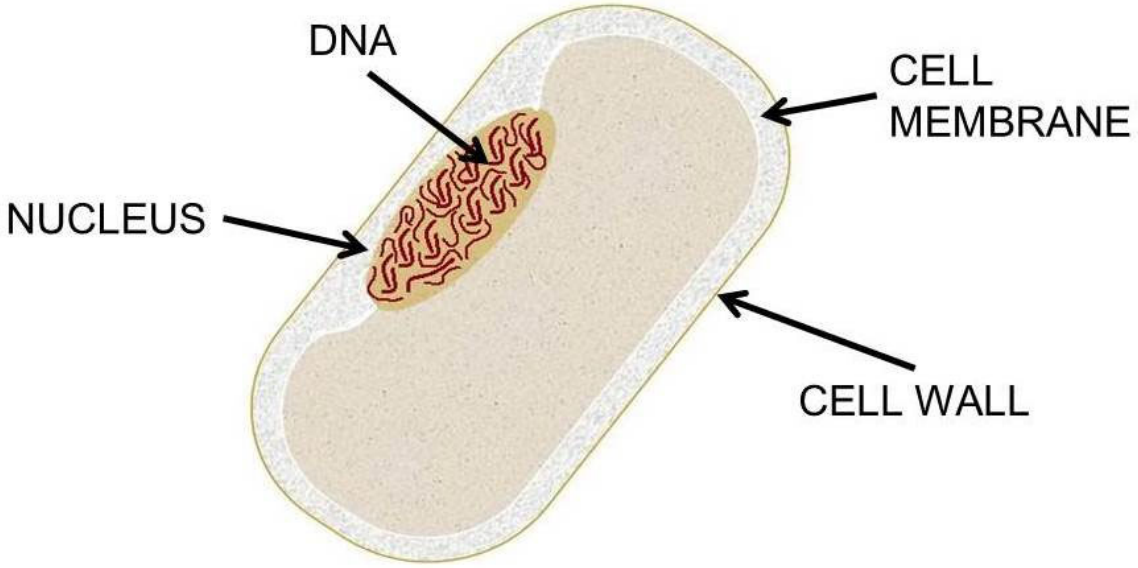
Characteristic	Archaea	Bacteria	Eukaryotes
Predominantly multicellular	No	No	Yes
Cell contains a nucleus and other membrane bound organelles	No	No	Yes
DNA occurs in a circular form*	Yes	Yes	No
Ribosome size	70s	70s	80s
Membrane lipids ester-linked**	No	Yes	Yes
Photosynthesis with chlorophyll	No	Yes	Yes
Capable of growth at temperatures greater than 80 C	Yes	Yes	No
Histone proteins present in cell	Yes	No	Yes
Methionine used as tRNA Initiator***	Yes	No	Yes
Operons present in DNA	Yes	Yes	No
Interon present in most genes	No	No	Yes
Capping and poly-A tailing of mRNA	No	No	Yes
Gas vesicles present	Yes	Yes	No
Capable of Methanogenesis	Yes	No	No
Sensitive to chloramphenicol, kanamycin and streptomycin	No	Yes	No
Transcription factors required	No	Yes	Yes
Capable of Nitrification	No	Yes	No
Capable of Denitrification	Yes	Yes	No
Capable of Nitrogen Fixation	Yes	Yes	No
Capable of Chemolithotrophy	Yes	Yes	No

***** Eukaryote DNA is linear

****** Archaea membrane lipids are ether-linked

******* Bacteria use Formylmethionine

Eukaryotes



EUKARYOTIC CELL

Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei. They include the animals, plants, and fungi, which are mostly multicellular, as well as various other groups called protists, many of which are unicellular. In contrast, other organisms such as bacteria lack nuclei and other complex cell structures, and are called prokaryotes. The eukaryotes share a common origin, and are often treated formally as a super kingdom, empire, or domain. The name comes from the Greek *eus* or true and *karyon* or nut, referring to the nucleus.

What are Protists?

- They are **eukaryotes** because they all have a **nucleus**.
- Most have **mitochondria** although some have later lost theirs. Mitochondria were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
- Many have **chloroplasts** with which they carry on photosynthesis. Chloroplasts were derived from photosynthetic **cyanobacteria** (also prokaryotes) living within their cells.

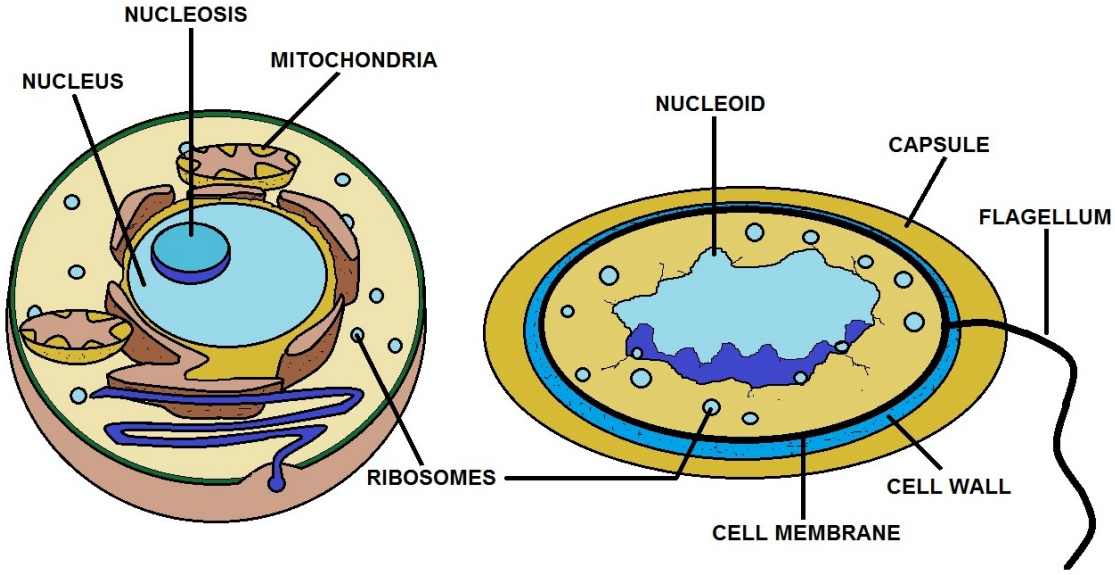
Eukaryotic Cells

Eukaryotic cells are generally much larger than prokaryotes, typically with a thousand times their volumes. They have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of microtubules and microfilaments, which plays an important role in defining the cell's organization. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among prokaryotes.

Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or vacuoles, can form by budding off of other membranes. Many cells ingest food and other materials through a process of endocytosis, where the outer membrane invaginates and then pinches off to form a vesicle. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles.

The nucleus is surrounded by a double membrane, with pores that allow material to move in and out. Various tube- and sheet-like extensions of the nuclear membrane form what is called the endoplasmic reticulum or ER, which is involved in protein transport. It includes rough sections where ribosomes are attached, and the proteins they synthesize enter the interior space or lumen. Subsequently, they generally enter vesicles, which bud off from the smooth section. In most eukaryotes, the proteins may be further modified in stacks of flattened vesicles, called Golgi bodies or dictyosomes.

Vesicles may be specialized for various purposes. For instance, lysosomes contain enzymes that break down the contents of food vacuoles, and peroxisomes are used to break down peroxide which is toxic otherwise.



Contractile Vacuoles

Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure. Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short. These are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes, and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles

Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

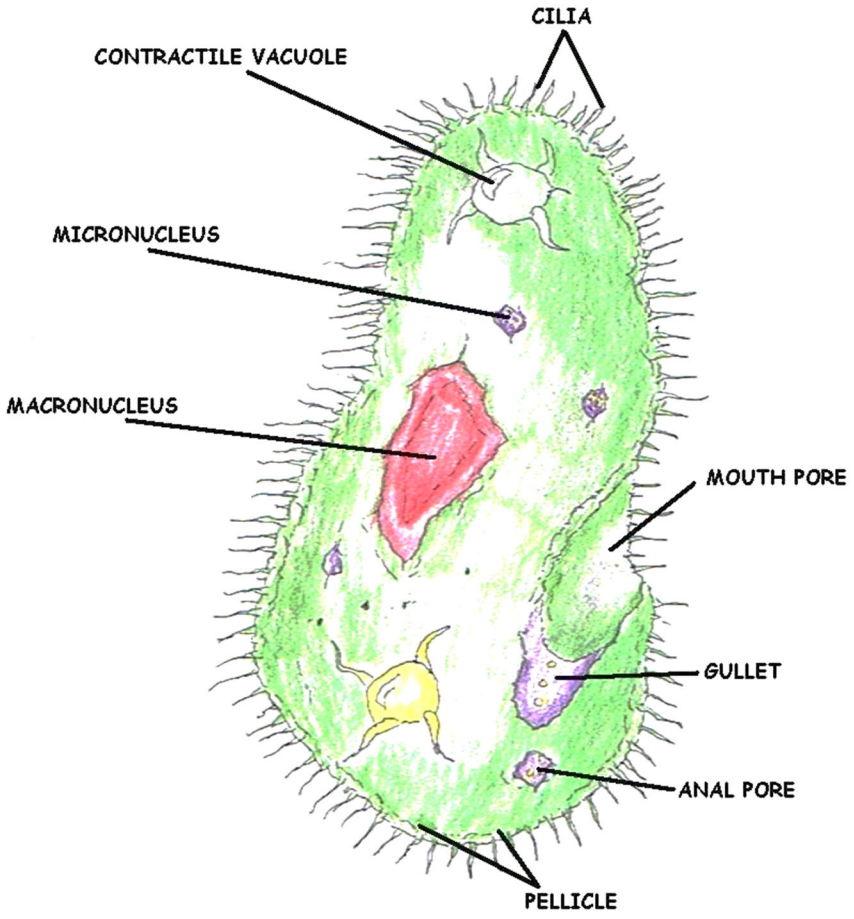
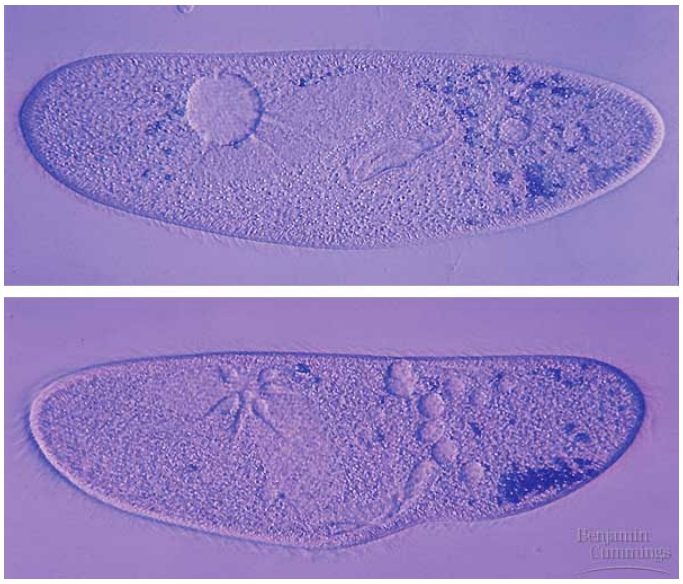


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.

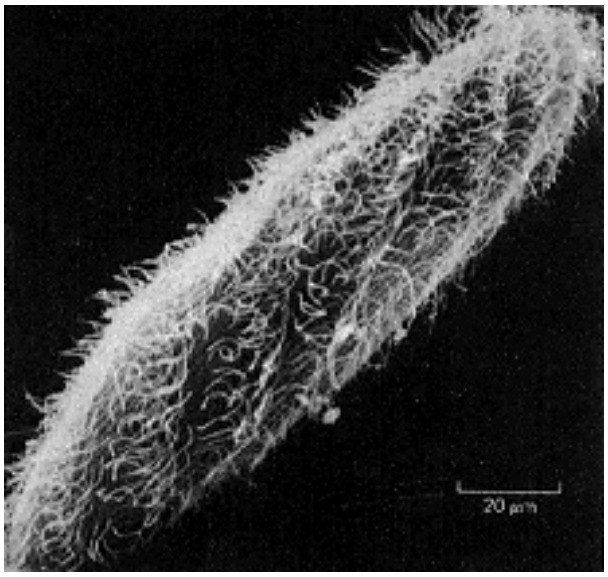


Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

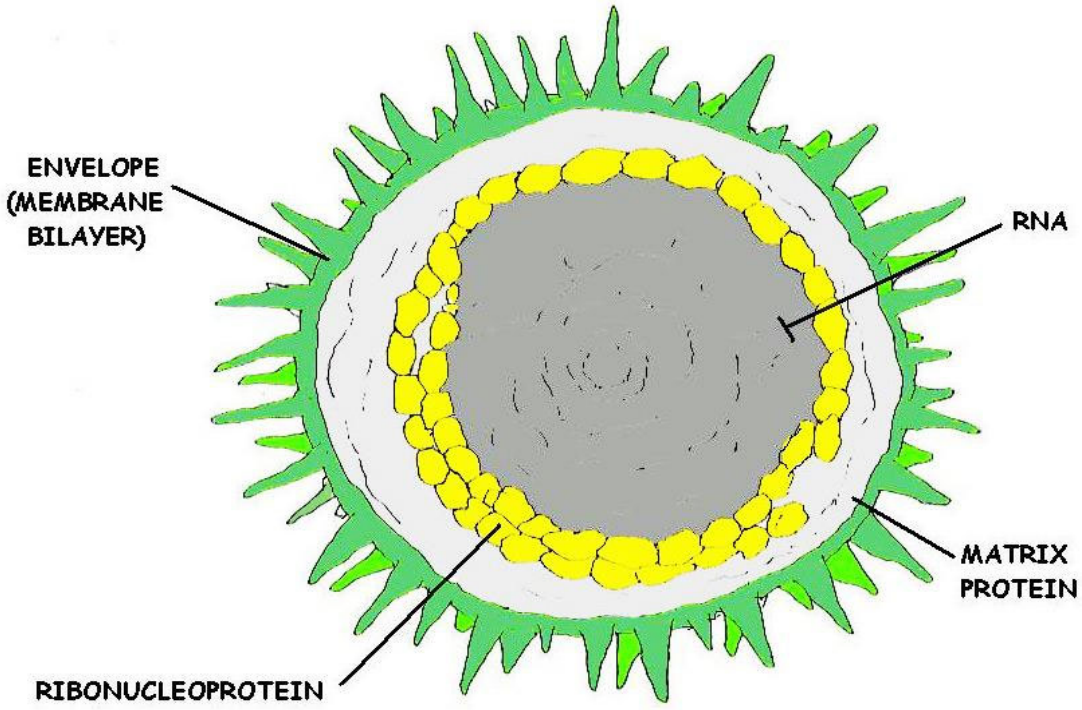
Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration. If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).



Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce. A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



CROSS SECTIONAL VIEW

Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication.

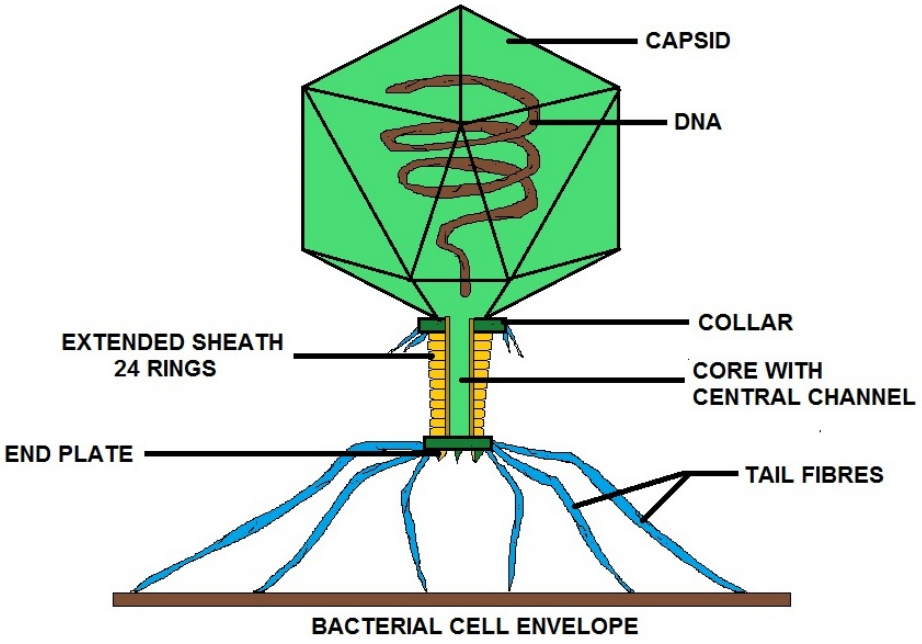
Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.

Virions
Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA. Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or nonsegmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.



VIRUS CAPSID (BACTERIOPHAGES)

Bacteriophages
The most elaborate viruses are the bacteriophages, which use bacteria as their hosts. Some bacteriophages resemble an insect with an icosahedral head attached to a tubular sheath. From the base of the sheath extend several long tail fibers that help the virus attach to the bacterium and inject its DNA to be replicated, direct capsid production, and virus particle assembly inside the cell.

Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule.

The single-stranded RNA circle collapses on itself to form a rodlike structure. The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

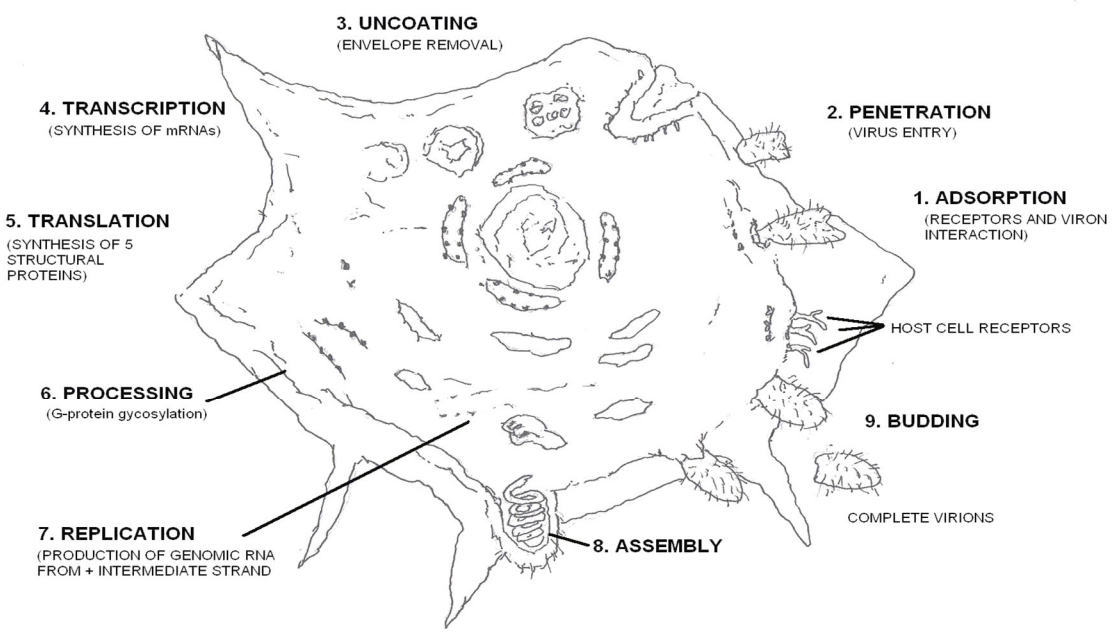
Replication
The first contact between a virus particle and its host cell occurs when an outer viral structure docks with a specific molecule on the cell surface. For example, a glycoprotein called gp120 on the surface of the human immunodeficiency virus (HIV, the cause of acquired immunodeficiency syndrome, or AIDS) virion specifically binds to the CD4 molecule found on certain human T lymphocytes (a type of white blood cell). Most cells that do not have surface CD4 molecules generally cannot be infected by HIV.

After binding to an appropriate cell, a virus must cross the cell membrane. Some viruses accomplish this goal by fusing their lipid envelope to the cell membrane, thus releasing the nucleocapsid into the cytoplasm of the cell.

Other viruses must first be endocytosed (enveloped by a small section of the cell's plasma membrane that pokes into the cell and pinches off to form a bubble-like vesicle called an endosome) before they can cross the cell membrane. Conditions in the endosome allow many viruses to change the shape of one or more of their proteins.

These changes permit the virus either to fuse with the endosomal membrane or to lyse the endosome (cause it to break apart), allowing the nucleocapsid to enter the cell cytoplasm.

CYLCE OF INFECTION AND REPLICATION



Once inside the cell, the virus replicates itself through a series of events. Viral genes direct the production of proteins by the host cellular machinery. The first viral proteins synthesized by some viruses are the enzymes required to copy the viral genome. Using a combination of viral and cellular components, the viral genome can be replicated thousands of times. Late in the replication cycle for many viruses, proteins that make up the capsid are synthesized. These proteins package the viral genetic material to make newly formed nucleocapsids.

To complete the virus replication cycle, viruses must exit the cell. Some viruses bud out of the cell's plasma membrane by a process resembling reverse endocytosis. Other viruses cause the cell to lyse, thereby releasing newly formed virus particles ready to infect other cells.

Still other viruses pass directly from one cell into an adjacent cell without being exposed to the extracellular environment. The virus replication cycle can be as short as a couple of hours for certain small viruses or as long as several days for some large viruses.

Virus Battle

Some viruses kill cells by inflicting severe damage resulting in cell lysis; other viruses cause the cell to kill itself in response to virus infection. This programmed cell suicide is thought to be a host defense mechanism to eliminate infected cells before the virus can complete its replication cycle and spread to other cells. Alternatively, cells may survive virus infection, and the virus can persist for the life of its host. Virtually all people harbor harmless viruses.

Retroviruses
Retroviruses, such as HIV, have RNA that is transcribed into DNA by the viral enzyme reverse transcriptase upon entry into the cell. (The ability of retroviruses to copy RNA into DNA earned them their name because this process is the reverse of the usual transfer of genetic information, from DNA to RNA.) The DNA form of the retrovirus genome is then integrated into the cellular DNA and is referred to as the provirus. The viral genome is replicated every time the host cell replicates its DNA and is thus passed on to daughter cells.

Hepatitis B Virus
Hepatitis B virus can also transcribe RNA to DNA, but this virus packages the DNA version of its genome into virus particles. Unlike retroviruses, hepatitis B virus does not integrate into the host cell DNA.

Viral Infections
Most viral infections cause no symptoms and do not result in disease. For example, only a small percentage of individuals who become infected with Epstein-Barr virus or western equine encephalomyelitis virus ever develop disease symptoms. In contrast, most people who are infected with measles, rabies, or influenza viruses develop the disease. A wide variety of viral and host factors determine the outcome of virus infections. A small genetic variation can produce a virus with increased capacity to cause disease. Such a virus is said to have increased virulence.

Infection
Viruses can enter the body by several routes. Herpes simplex virus and poxviruses enter through the skin by direct contact with virus-containing skin lesions on infected individuals. Ebola, hepatitis B, and HIV can be contracted from infected blood products. Hypodermic needles and animal and insect bites can transmit a variety of viruses through the skin. Viruses that infect through the respiratory tract are usually transmitted by airborne droplets of mucus or saliva from infected individuals who cough or sneeze.

Viruses that enter through the respiratory tract include orthomyxovirus (influenza), rhinovirus and adenovirus (common cold), and varicella-zoster virus (chicken pox). Viruses such as rotavirus, coronavirus, poliovirus, hepatitis A, and some adenoviruses enter the host through the gastrointestinal tract. Sexually transmitted viruses, such as herpes simplex, HIV, and human papilloma viruses (HPV), gain entry through the genitourinary route. Other viruses, including some adenoviruses, echoviruses, Coxsackie viruses, and herpes viruses, can infect through the eye.

Localized or Systemic Infections
Virus infections can be either localized or systemic. The path of virus spread through the body in systemic infections differs among different viruses. Following replication at the initial site of entry, many viruses are spread to their target organs by the bloodstream or the nervous system. The particular cell type can influence the outcome of virus infection. For example, herpes simplex virus undergoes lytic replication in skin cells around the lips but can establish a latent or dormant state in neuron cell bodies (located in ganglia) for extended periods of time. During latency, the viral genome is largely dormant in the cell nucleus until a stimulus such as a sunburn causes the reactivation of latent herpes virus, leading to the lytic replication cycle. Once reactivated, the virus travels from the ganglia back down the nerve to cause a cold sore on the lip near the original site of infection. The herpesvirus genome does not integrate into the host cell genome.

Virus-induced Illnesses
Virus-induced illnesses can be either acute, in which the patient recovers promptly, or chronic, in which the virus remains with the host or the damage caused by the virus is irreparable. For most acute viruses, the time between infection and the onset of disease can vary from three days to three weeks. In contrast, onset of AIDS following infection with HIV takes an average of 7 to 11 years. Several human viruses are likely to be agents of cancer, which can take decades to develop. The precise role of these viruses in human cancers is not well understood, and genetic and environmental factors are likely to contribute to these diseases. But because a number of viruses have been shown to cause tumors in animal models, it is probable that many viruses have a key role in human cancers.

Alphaviruses and Flaviviruses
Some viruses—alphaviruses and flaviviruses, for example—must be able to infect more than one species to complete their life cycles. Eastern equine encephalomyelitis virus, an alphavirus, replicates in mosquitoes and is transmitted to wild birds when the mosquitoes feed. Thus, wild birds and perhaps mammals and reptiles serve as the virus reservoir, and mosquitoes serve as vectors essential to the virus life cycle by ensuring transmission of the virus from one host to another. Horses and people are accidental hosts when they are bitten by an infected mosquito, and they do not play an important role in virus transmission.

Defense
Although viruses cannot be treated with antibiotics, which are effective only against bacteria, the body's immune system has many natural defenses against virus infections. Infected cells produce interferons and other cytokines (soluble components that are largely responsible for regulating the immune response), which can signal adjacent uninfected cells to mount their defenses, enabling uninfected cells to impair virus replication.

Cytokines
Some cytokines can cause a fever in response to viral infection; elevated body temperature retards the growth of some types of viruses. B lymphocytes produce specific antibodies that can bind and inactivate viruses. Cytotoxic T cells recognize virus-infected cells and target them for destruction. However, many viruses have evolved ways to circumvent some of these host defense mechanisms.

The development of antiviral therapies has been thwarted by the difficulty of generating drugs that can distinguish viral processes from cellular processes. Therefore, most treatments for viral diseases simply alleviate symptoms, such as fever, dehydration, and achiness. Nevertheless, antiviral drugs for influenza virus, herpesviruses, and HIV are available, and many others are in the experimental and developmental stages.

Prevention has been a more effective method of controlling virus infections. Viruses that are transmitted by insects or rodent excretions can be controlled with pesticides. Successful vaccines are currently available for poliovirus, influenza, rabies, adenovirus, rubella, yellow fever, measles, mumps, and chicken pox.

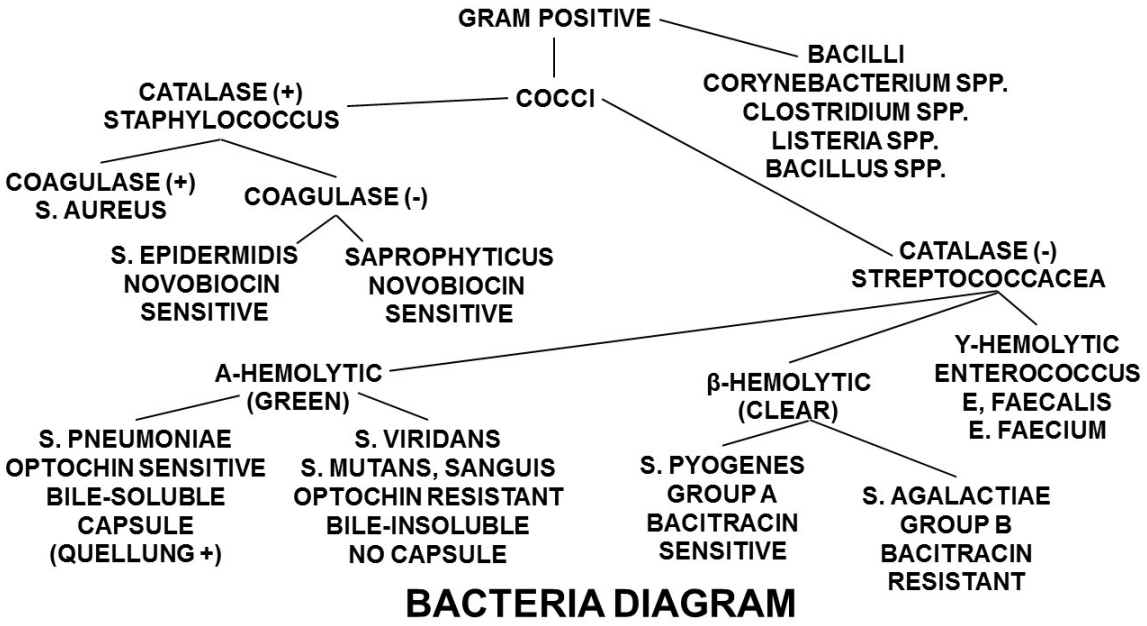
Vaccines are prepared from killed (inactivated) virus, live (attenuated or weakened) virus, or isolated viral proteins (subunits). Each of these types of vaccines elicits an immune response while causing little or no disease, and there are advantages and disadvantages to each. (For a more complete discussion of vaccines, see the Immunization article.)

Vaccination
The principle of vaccination was discovered by British physician Edward Jenner. In 1796 Jenner observed that milkmaids in England who contracted the mild cowpox virus infection from their cows were protected from smallpox, a frequently fatal disease. In 1798 Jenner formally demonstrated that prior infection with cowpox virus protected those that he inoculated with smallpox virus (an experiment that would not meet today's protocol standards because of its use of human subjects).

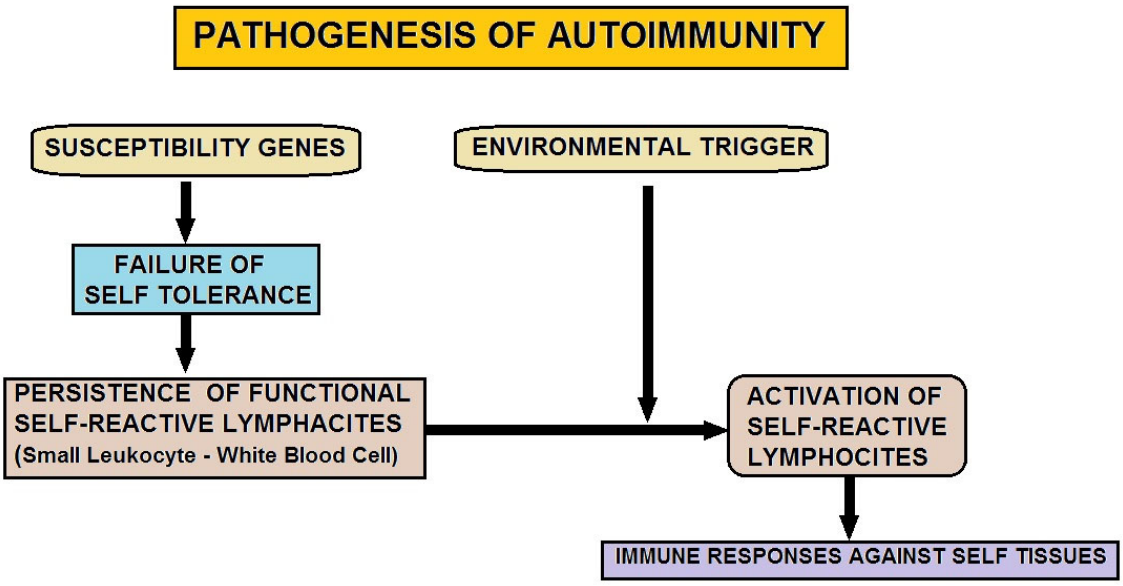
Mutation
Viruses undergo very high rates of mutation (genetic alteration) largely because they lack the repair systems that cells have to safeguard against mutations. A high mutation rate enables the virus to continually adapt to new intracellular environments and to escape from the host immune response.

Co-infection of the same cell with different related viruses allows for genetic re-assortment (exchange of genome segments) and intramolecular recombination. Genetic alterations can alter virulence or allow viruses to gain access to new cell types or new animal hosts.

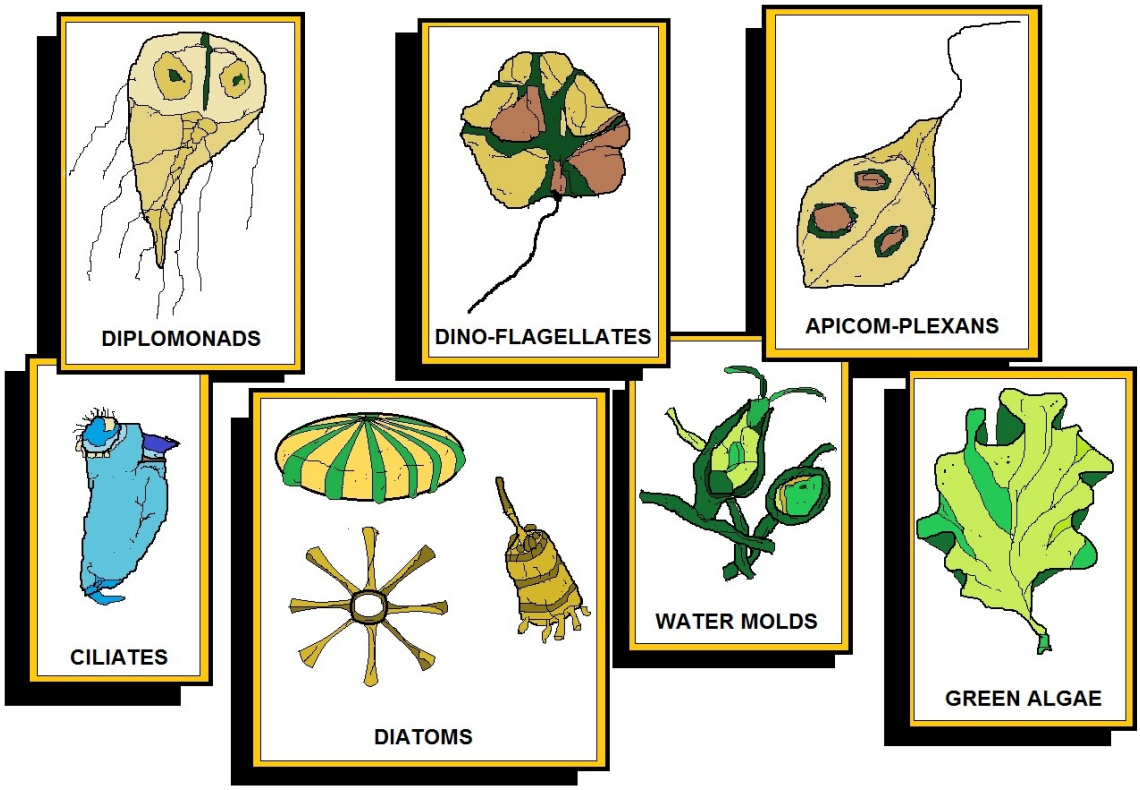
Many scientists believe that HIV is derived from a closely related monkey virus, SIV (simian immunodeficiency virus), that acquired the ability to infect humans. Many of today's emerging viruses may have similar histories.



BACTERIA DIAGRAM



PATHOGENESIS
(The Manner in Which a Disease Develops)

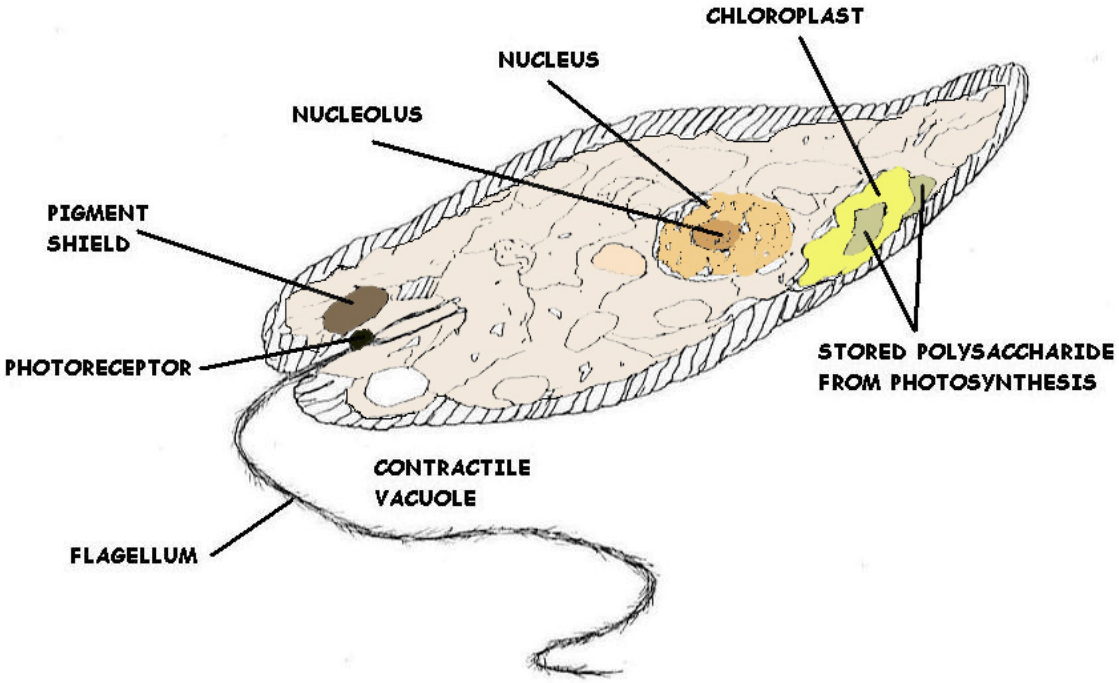


KINGDOM PROTISTA

Protozoa Sub-Section

The diverse assemblage of organisms that carry out all of their life functions within the confines of a single, complex eukaryotic cell are called protozoa.

Paramecium, Euglena, and Amoeba are well-known examples of these major groups of organisms. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. The unicellular photosynthetic protozoa are sometimes also called algae and are addressed elsewhere. This report considers the status of our knowledge of heterotrophic protozoa (protozoa that cannot produce their own food).

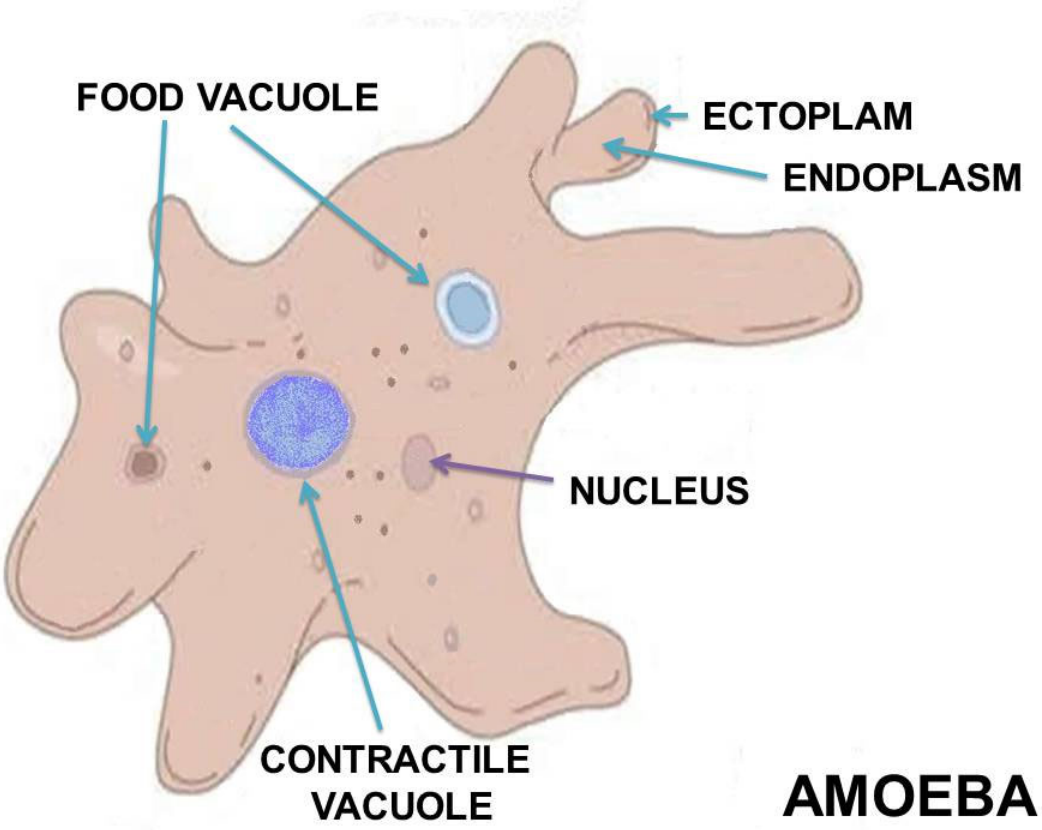


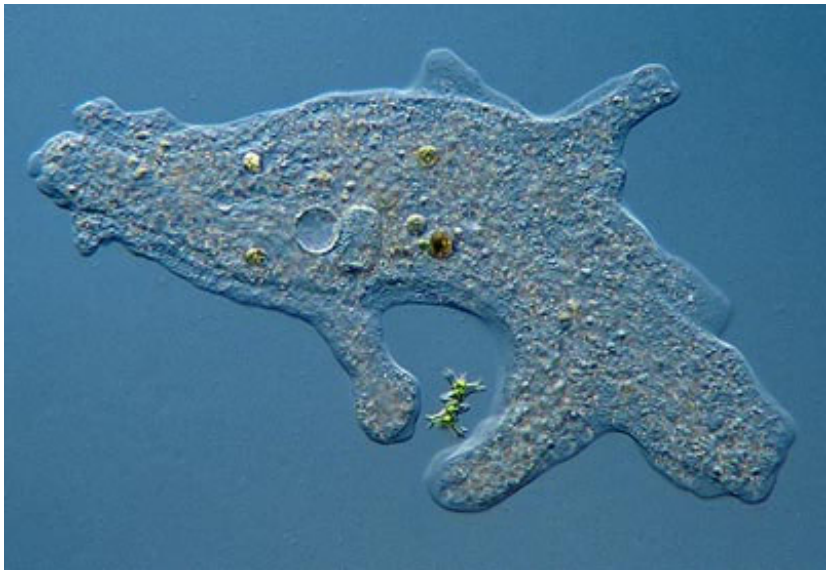
EUGLENA

Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.





Amoeba proteus, pseudopods slowly engulf the small desmid *Staurastrum.*

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey; they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information

Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa

Parasites

Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease

The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following chapters.

Symbionts

Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa

While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

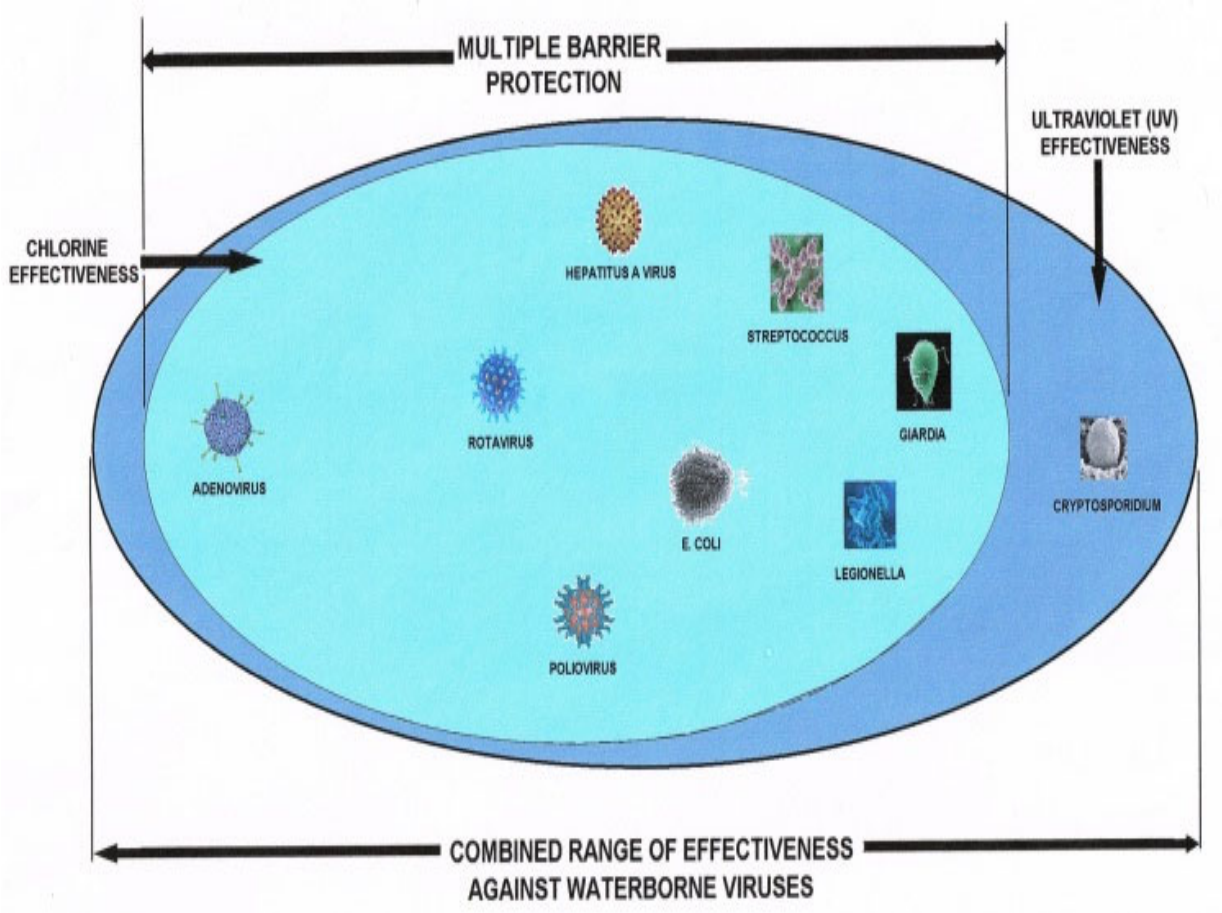
Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

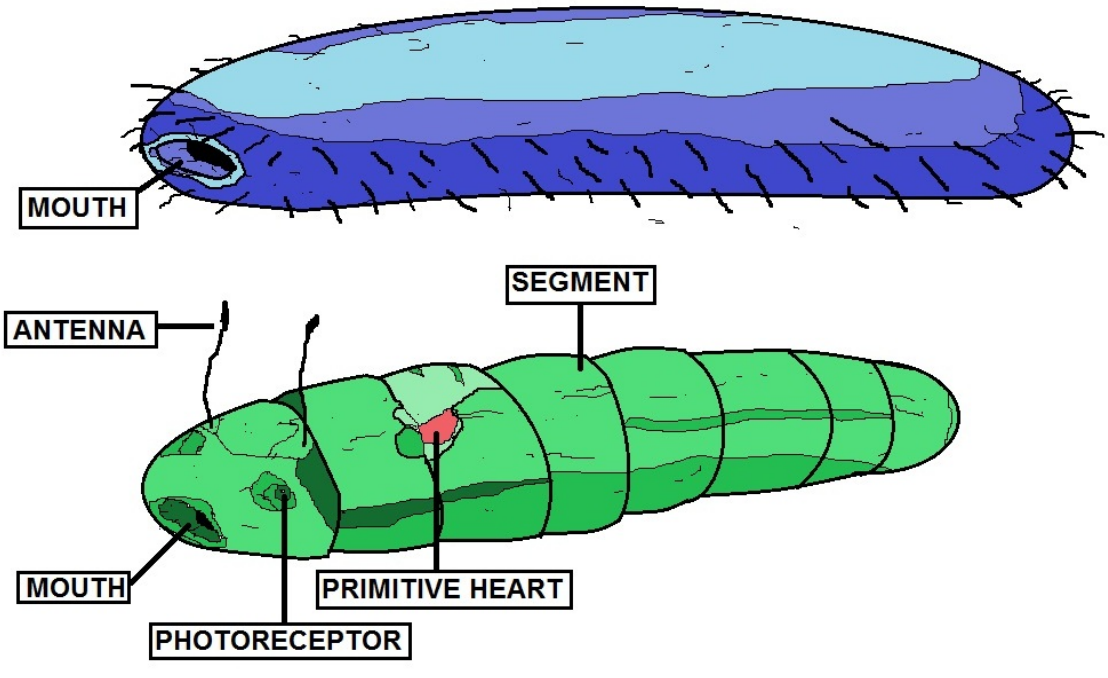
Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great.

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).





THE TWO VERSIONS OF *Urbilateria*

Wastewater Treatment Biology

Four (4) groups of bugs do most of the ***"eating"*** in the activated sludge process. The first group is the bacteria which eat the dissolved organic compounds. The second and third groups of bugs are microorganisms known as the free-swimming and stalked ciliates. These larger bugs eat the bacteria and are heavy enough to settle by gravity. The fourth group is a microorganism, known as Suctoria, which feed on the larger bugs and assist with settling.

The interesting thing about the bacteria that eat the dissolved organics is that they have no mouth. The bacteria have an interesting property; their "fat reserve" is stored on the outside of their body. This fat layer is sticky and is what the organics adhere to.

Once the bacteria have "**contacted**" their food, they start the digestion process. A chemical enzyme is sent out through the cell wall to break up the organic compounds. This enzyme, known as hydrolytic enzyme, breaks the organic molecules into small units which are able to pass through the cell wall of the bacteria.

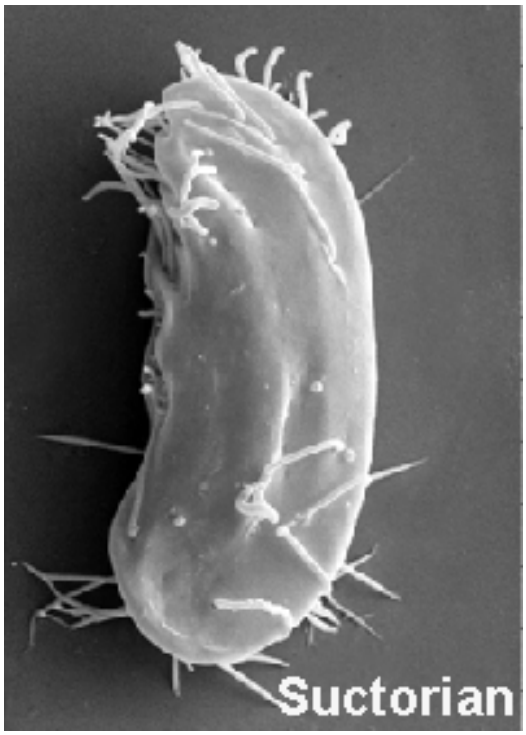
In wastewater treatment, this process of using bacteria-eating-bugs in the presence of oxygen to reduce the organics in water is called activated sludge.

The first step in the process, the contact of the bacteria with the organic compounds, takes about 20 minutes. The second step is the breaking up, ingestion and digestion processes, which takes four (4) to 24 hours.

The fat storage property of the bacteria is also an asset in settling. As the bugs "bump" into each other, the fat on each of them sticks together and causes flocculation of the non-organic solids and biomass.

From the aeration tank, the wastewater, now called mixed liquor, flows to a secondary clarification basin to allow the flocculated biomass of solids to settle out of the water.

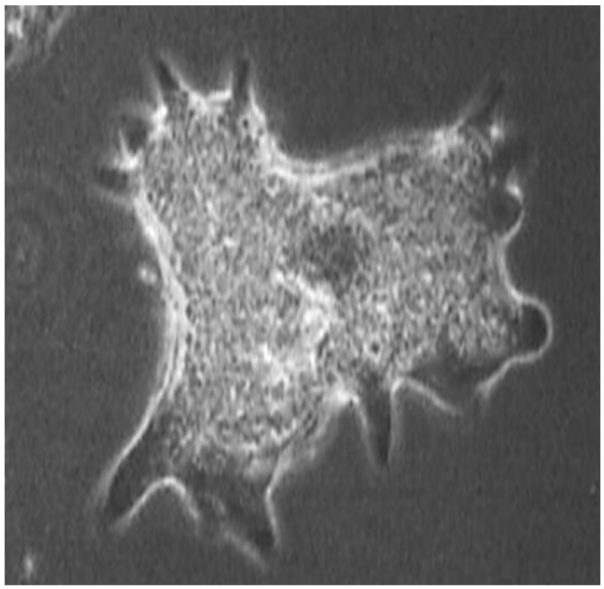
The solids biomass, which is the activated sludge, contains millions of bacteria and other microorganisms, is used again by returning it to the influent of the aeration tank for mixing with the primary effluent and ample amounts of air.



Urostyla or Euplotes

Wastewater Treatment Microlife

Euglypha sp.



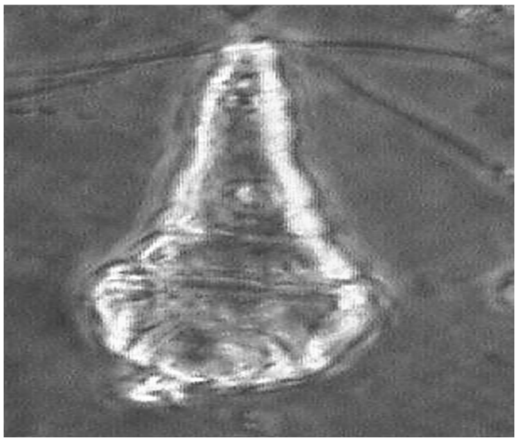
Euglypha (70-100 æm) is a shelled (testate) amoeba. Amoebas have jelly-like bodies. Motion occurs by extending a portion of the body (pseudopodia) outward. Shelled amoebas have a rigid covering which is either secreted or built from sand grains or other extraneous materials. The secreted shell of this Euglypha sp. consists of about 150 oval plates. Its spines project backward from the lower half of the shell.

Euglypha spines may be single or in groups of two or three. The shell has an opening surrounded by 8-11 plates that resemble shark teeth under very high magnification.

The shell of Euglypha is often transparent, allowing the hyaline (watery) body to be seen inside the shell. The pseudopodia extend outward in long, thin, rays when feeding or moving. Euglypha primarily eats bacteria. **Indicator:** Shelled amoebas are common in soil, treatment plants, and stream bottoms where decaying organic matter is present. They adapt to a wide range of conditions and therefore are not good indicator organisms.

Euchlanis sp.

This microscopic animal is a typical rotifer. Euchlanis is a swimmer, using its foot and cilia for locomotion. In common with other rotifers, it has a head rimmed with cilia, a transparent body, and a foot with two strong swimming toes.



The head area, called the "corona," has cilia that beat rhythmically, producing a strong current for feeding or swimming. Euchlanis is an omnivore, meaning that its varied diet includes detritus, bacteria, and small protozoa.

Euchlanis has a glassy shell secreted by its outer skin. The transparent body reveals the brain, stomach, intestines, bladder, and reproductive organs.

A characteristic of rotifers is their mastax, which is a jaw-like device that grinds food as it enters the stomach. At times the action of the mastax resembles the pulsing action of a heart. Rotifers, however, have no circulatory system.

Indicator: Euchlanis is commonly found in activated sludge when effluent quality is good. It requires a continual supply of dissolved oxygen, evidence that aerobic conditions have been sustained.

Protozoan Diseases

Protozoan pathogens are larger than bacteria and viruses but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall, called a "***cyst***." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days. Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne.

Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems, such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times.

For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.

Summary of Common Waterborne Diseases

Name Causative organism Source of organism Disease

Viral gastroenteritis ***Rotavirus*** mostly in young children; Human feces; Diarrhea or vomiting.

Norwalk-like viruses Human feces; also, shellfish; lives in polluted waters; Diarrhea and vomiting.

Salmonellosis ***Salmonella*** (bacterium) Animal or human feces; Diarrhea or vomiting.

Escherichia coli-- E. coli O157:H7 (bacterium) Other *E. coli* organisms. Human feces; Symptoms vary with type caused; gastroenteritis

Typhoid ***Salmonella typhi*** (bacterium) Human feces, urine Inflamed intestine, enlarged spleen, high temperature— sometimes fatal.

Shigellosis ***Shigella*** (bacterium) Human feces Diarrhea.

Cholera ***Vibrio cholerae*** (bacterium) Human feces; also, shellfish; lives in many coastal waters; Vomiting, severe diarrhea, rapid dehydration, mineral loss —high mortality.

Hepatitis A virus Human feces; shellfish grown in polluted waters; Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain — low mortality, lasts up to four months.

Amebiasis ***Entamoeba histolytica*** Human feces; Mild diarrhea, dysentery, (protozoan) extra intestinal infection.

Giardiasis ***Giardia lamblia*** (protozoan) Animal or human feces; Diarrhea, cramps, nausea, and general weakness — lasts one week to months.

Cryptosporidiosis ***Cryptosporidium parvum*** (protozoan) *Animal* or human feces Diarrhea, stomach pain — lasts days to weeks.

Source: Adapted from American Water Works Association, *Introduction to Water Treatment: Principles and Practices of Water Supply Operation*s, Denver CO, 1984.

The Best Method to kill most of these Bugs

Disinfection is usually synonymous with chlorination. That is because chlorine addition is by far the most common form of disinfection used today. In this section, the main emphasis will be on chlorination: how it works, safety, types of chlorine, basic chemistry of chlorine and an introduction to **CT** values. Disinfection is the process of killing microorganisms in water that might cause disease (pathogens).

Disinfection, however, should not be confused with sterilization, which is the destruction of all microorganisms. Disinfection is concerned only with killing pathogens. Cryptosporidium parvum and Giardia lamblia will require proper water treatment techniques.

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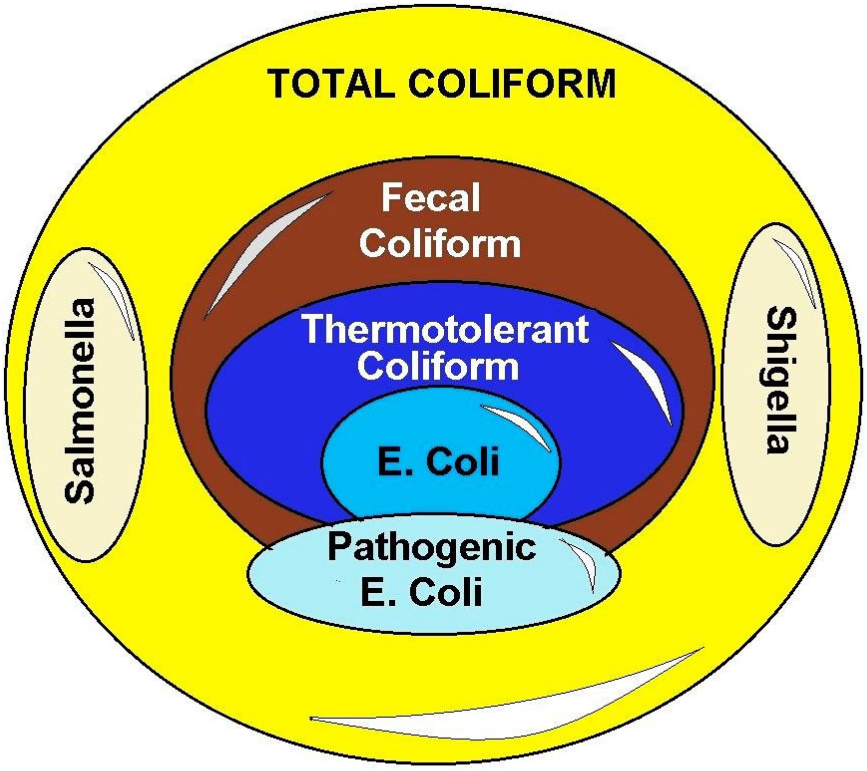
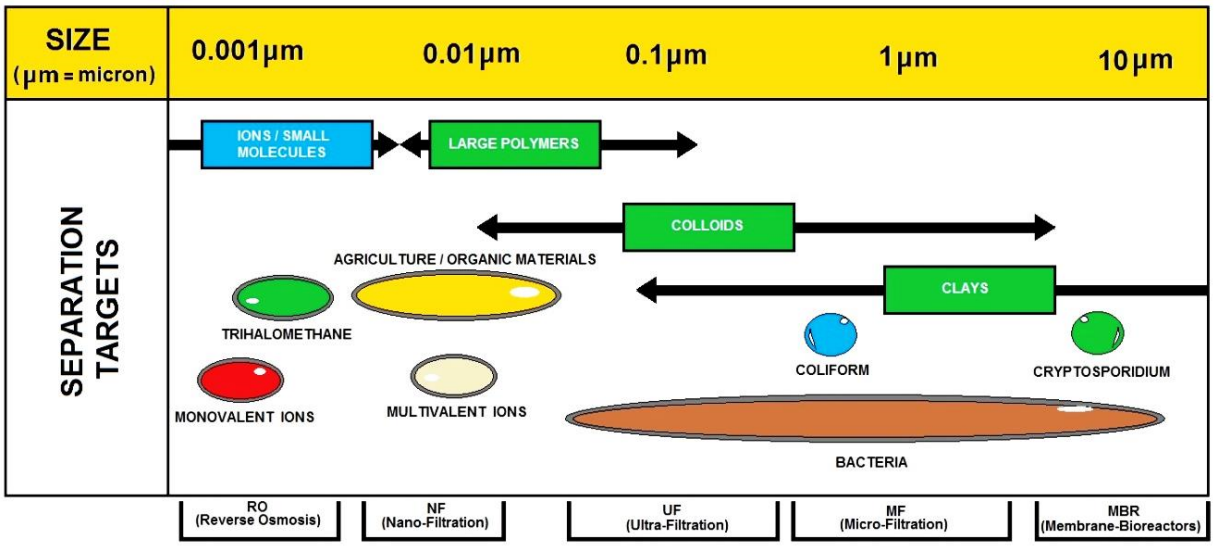


COLIFORM BACTERIA DIAGRAM



REMOVAL METHODS

Chapter 3- Post Quiz Review

Example Question, fill-in-the blank with one correct answer.
_____ is usually credited for dispelling the notion of spontaneous generation and proving that organisms reproduce new organisms.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

Review, you can check your answers at the end of this section.
1. The German scientist _____, a student of Jacob Henle, and the British surgeon Joseph Lister developed techniques for growing cultures of single organisms that allowed the assignment of specific bacteria to specific diseases.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

2. The first experimental transmission of a viral infection was accomplished in about 1880 by the German scientist _____, when he demonstrated that extracts from infected tobacco leaves could transfer tobacco mosaic disease to a new plant, causing spots on the leaves.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

3. Because _____ was unable to isolate a bacterium or fungus from the tobacco leaf extracts, he considered the idea that tobacco mosaic disease might be caused by a soluble agent, but he concluded incorrectly that a new type of bacteria was likely to be the cause.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

4. The Russian scientist _____ extended Mayer's observation and reported in 1892 that the tobacco mosaic agent was small enough to pass through a porcelain filter known to block the passage of bacteria.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

5. In 1917 the French-Canadian scientist _____ discovered that viruses of bacteria, which he named bacteriophage, could make holes in a culture of bacteria.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

6. In 1935 the American biochemist _____ crystallized tobacco mosaic virus to demonstrate that viruses had regular shapes, and in 1939 tobacco mosaic virus was first visualized using the electron microscope.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

7. In 1898 the German bacteriologists Friedrich August Johannes Löffler and Paul F. Frosch (both trained by _____) described foot-and-mouth disease virus as the first filterable agent of animals.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

8. In 1900, the American bacteriologist _____ and colleagues recognized yellow fever virus as the first human filterable agent.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

9. For several decades viruses were referred to as filterable agents, and gradually the term virus (Latin for "_____" or "poison") was employed strictly for this new class of infectious agents.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

10. Through the 1940s and 1950s many critical discoveries were made about viruses through the study of _____ because of the ease with which the bacteria they infect could be grown in the laboratory.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

11. Between 1948 and 1955, scientists at the National Institutes of Health (NIH) and at Johns Hopkins Medical Institutions revolutionized the study of animal viruses by developing _____ that permitted the growth and study of many animal viruses in laboratory dishes.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

12. Louis Pasteur along with _____ developed the germ theory of disease which states that "a specific disease is caused by a specific type of microorganism."
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

13. In 1876, _____ established an experimental procedure to prove the germ theory of disease. This scientific procedure is known as Koch's postulates.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

14. _____ postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

15. _____ observed small empty chambers in the structure of cork with the help of his crude microscope. He called them cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

16. Two German biologists _____ and Thedore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

17. _____ completed the cell theory with the idea that all cells must arise from preexisting cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

18. There's even a species of _____—Deinococcus radiodurans—that can withstand blasts of radiation 1,000 times greater than would kill a human being.
A. Metabolism
B. Cell
C. Mutation
D. Bacteria
E. None of the above

19. “Bacteria” is a singular word. The plural for this word is “bacterium” (**bacter** = rod, staff).
A. True
B. False

20. Bacteria are prokaryotes (Kingdom Monera), which means that they have a large nucleus.
A. True
B. False

21. Bacteria do have one chromosome of single-stranded DNA in a ring. They reproduce by binary fission.
A. True
B. False

22. Most bacteria lack or have very few internal membranes, which means that they don’t have some kinds of organelles (like mitochondria or chloroplasts). Most bacteria are **benign** (**benign** = good, friendly, kind) or beneficial, and only a few are “bad guys” or **pathogens**.
A. True
B. False

23. Kingdom Monera is a very diverse group. All bacteria relatives can do photosynthesis—because they have chloroplasts, because chlorophyll and other needed chemicals are built into their cell membranes.
A. True
B. False

24. These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they’re not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_3, while others are poisoned/killed by it.
A. True
B. False

25. Bacteria fall into a category of life called the Prokaryotes (pro-carry-oats). Prokaryotes' genetic material, or DNA, is not enclosed in a cellular compartment called the nucleus.
A. True
B. False

26. Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes (you-carry-oats), creatures whose cells have nuclei.
A. True
B. False

27. The mitochondria (mite-oh-con-dree-uh) that make energy for your body cells is one example of such an organelle.
A. True
B. False

28. There are less than 200 hundred of species of bacteria, but all of them are basically one of five different shapes.
A. True
B. False

29. Some bacteria are rod - or stick-shaped and are called _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

30. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

31. Most bacteria secrete a covering for themselves which we call a **cell wall**.
A. True
B. False

32. Bacterial cell walls do contain cellulose like plant cell walls do.
A. True
B. False

33. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.
A. True
B. False

34. All antibiotics, like penicillin, prohibit the formation of the chemical cross linkages needed to make peptidoglycan.
A. True
B. False

35. That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.
A. True
B. False

36. However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls.
A. True
B. False

37. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. Dr. Hans Christian Gram, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Heterotopic Gram Count**.
A. True
B. False

38. In this process, the amount of cellulose in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained, thus bacterial cells can be $Gram^{+}$ or $Gram^{-}$.
A. True
B. False

39. $Gram^{+}$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color.
A. True
B. False

40. Human cells have 46 (23 pairs) chromosomes. Each _____ consists of many genes.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

41. A gene is a coiled unit made up of _____ and proteins that codes for, or determines, a particular characteristic of an individual organism.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

42. The cellular material outside the _____; composed of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, centrioles, microtubules, lysosomes and vacoules.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

43. Some eukaryotic cells possess relatively long and thin structures called _____. These are organs of locomotion.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

44. _____ are also organs of locomotion but are shorter and more numerous.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

45. All bacteria are _____.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

46. The _____ of a procaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

47. A typical bacterial _____ contains approximately 10,000 genes.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

48. Semi-liquid, surrounds the chromosome and is contained within the plasma membrane. Within the _____ are located several ribosomes-which are the sites of protein synthesis.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

49. Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei.
A. True
B. False

50. The eukaryotes share a common origin, and are often treated formally as a superkingdom, empire, or domain.
A. True
B. False

51. The name “eukaryotes” comes from the Greek ***eus*** or true and ***karyon*** or nut, referring to the nucleus.
A. True
B. False

52. Protists are eukaryotes because they all have a nucleus.
A. True
B. False

53. Protists have mitochondria, although some have later lost theirs. _____ were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

54. Many Protists have chloroplasts with which they carry on photosynthesis. _____ were derived from photosynthetic cyanobacteria (also prokaryotes) living within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

55. Eukaryotic cells are generally much larger than _____, typically with a thousand times their volumes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

56. Eukaryotic cells have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of _____ and microfilaments, which plays an important role in defining the cell's organization.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

57. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among _____.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

58. Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or _____, can form by budding off of other membranes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

1.E, 2.D, 3.D, 4.C, 5.C, 6.D, 7.B, 8.E, 9.A, 10.B, 11.C, 12.A, 13.A, 14.A, 15.E, 16.B, 17.C, 18.D, 19.B, 20.B, 21.B, 22.A, 23.B, 24.B, 25.A, 26.A, 27.A, 28.B, 29.B, 30. E, 31.A, 32.B, 33.A, 34.B, 35.A, 36.A, 37.B, 38.B, 39.A, 40.C, 41.D, 42.B, 43.A, 44.B, 45.C, 46.E, 47.E, 48.D, 49.A, 50.A, 51.A, 52.A, 53.B, 54.E, 55.C, 56.D, 57.C, 58.A

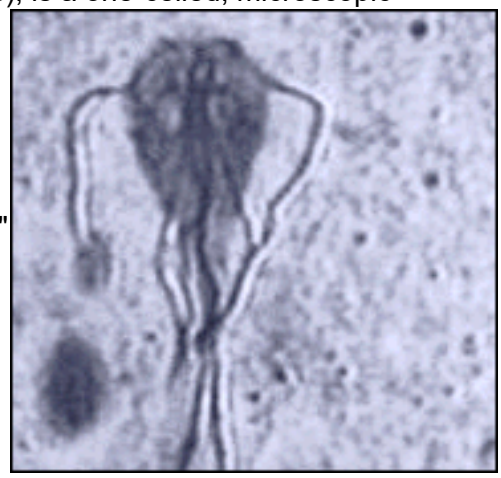
Microorganisms

Contaminant	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Cryptosporidium	zero	TT [3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Giardia lamblia	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Heterotrophic plate count	n/a	TT[3]	HPC has no health effects; it is an analytic method used to measure the variety of bacteria that are common in water. The lower the concentration of bacteria in drinking water, the better maintained the water system is.	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT[3]	Legionnaire's Disease, a type of pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)	zero	5.0%[4]	Not a health threat in itself; it is used to indicate whether other potentially harmful bacteria may be present[5]	Coliforms are naturally present in the environment; as well as feces; fecal coliforms and *E. coli* only come from human and animal fecal waste.
Turbidity	n/a	TT[3]	Turbidity is a measure of the cloudiness of water. It is used to indicate water quality and filtration effectiveness (e.g., whether disease-causing organisms are present). Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.	Soil runoff
Viruses (enteric)	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste

Chapter 4- Giardiasis *Giardia lamblia*

Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella. In Europe, it is sometimes referred to as Lamblia intestinalis.

Giardiasis is the most frequent cause of non-bacterial diarrhea in North America. *Giardia duodenalis*, cause of giardiasis (GEE-are-DYE-uh-sis), is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.

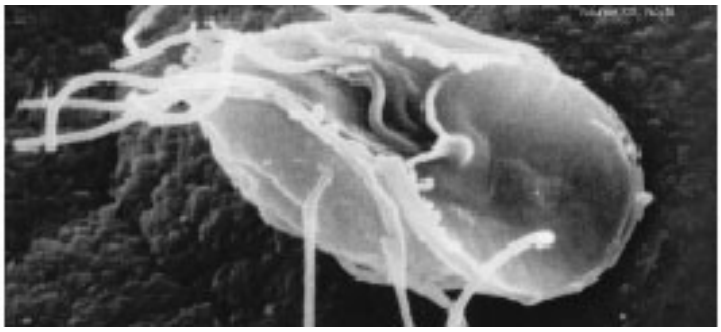


Approximately one week after ingestion of the *Giardia* cysts, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.

It is possible to experience some, not all, of the symptoms, yet still shed cysts and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.

The organism exists in two different forms--a hardy, dormant cyst that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the parasite. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.

Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', one assumption is that the condition of amitochondrialism is not primitive to eukaryotes but instead is a result of reductive evolution.



The microaerophilic Giardia uses these mitosomes in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.

Nature of Disease
Organisms that appear identical to those that cause human illness have been isolated from domestic animals (dogs and cats) and wild animals (beavers and bears). A related but morphologically distinct organism infects rodents, although rodents may be infected with human isolates in the laboratory. Human giardiasis may involve diarrhea within 1 week of ingestion of the cyst, which is the environmental survival form and infective stage of the organism.

Normally illness lasts for 1 to 2 weeks, but there are cases of chronic infections lasting months to years. Chronic cases, both those with defined immune deficiencies and those without, are difficult to treat.

The disease mechanism is unknown, with some investigators reporting that the organism produces a toxin while others are unable to confirm its existence. The organism has been demonstrated inside host cells in the duodenum, but most investigators think this is such an infrequent occurrence that it is not responsible for disease symptoms. Mechanical obstruction of the absorptive surface of the intestine has been proposed as a possible pathogenic mechanism, as has a synergistic relationship with some of the intestinal flora.

Giardia can be excysted, cultured and encysted in vitro; new isolates have bacterial, fungal, and viral symbionts. Classically, the disease was diagnosed by demonstration of the organism in stained fecal smears.

Several strains of *G. lamblia* have been isolated and described through analysis of their proteins and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.

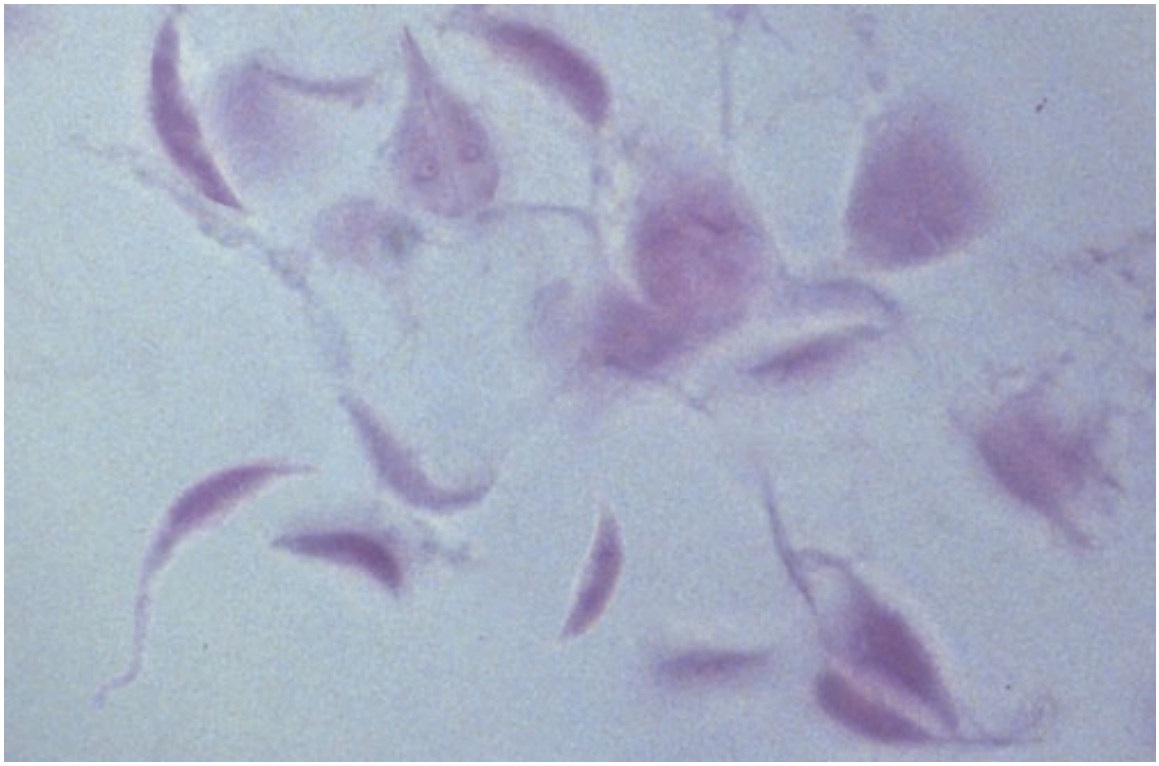
Diagnosis of Human Illness
Giardia lamblia is frequently diagnosed by visualizing the organism, either the trophozoite (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample.

An enzyme linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.

Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the survival of the organism.

Relative Frequency of Disease
Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically. The overall incidence of infection in the United States is estimated at 2% of the population. This disease afflicts many homosexual men, both HIV-positive and HIV-negative individuals. This is presumed to be due to sexual transmission. The disease is also common in child day care centers, especially those in which diapering is done.

Acute outbreaks appear to be common with infants and is not usually associated with water but is related to child care and diaper changing hygiene procedures. When I worked for a major water provider, I would receive 2-3 calls a week about infants diagnosed with Giardiasis. The problem lies with the water provider in that we are obligated to investigate and analyze all water customer complaints and make sure that our water is safe.



This is an example of infectious diarrhea due to Giardia lamblia infection of the small intestine. The small pear-shaped trophozoites live in the duodenum and become infective cysts that are excreted. They produce a watery diarrhea. A useful test for diagnosis of infectious diarrheas is stool examination for ova and parasites.

Course of Disease and Complications
About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.

Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.

Target Populations
Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.

Major Outbreaks
Major outbreaks are associated with contaminated water systems that do not use sand filtration or have a defect in the filtration system.

In April 1988, the Albuquerque Environmental Health Department and the New Mexico Health and Environment Department investigated reports of giardiasis among members of a church youth group in Albuquerque. The first two members to be affected had onset of diarrhea on March 3 and 4, respectively; stool specimens from both were positive for Giardia lamblia cysts. These two persons had only church youth group activities in common.

On August 8, 1983, the Utah Department of Health was notified by the Tooele County Health Department (TCHD) of an outbreak of diarrheal illness in Tooele, Utah, possibly associated with a contaminated public water supply that resulted from flooding during Utah's spring thaw.

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Giardia Images

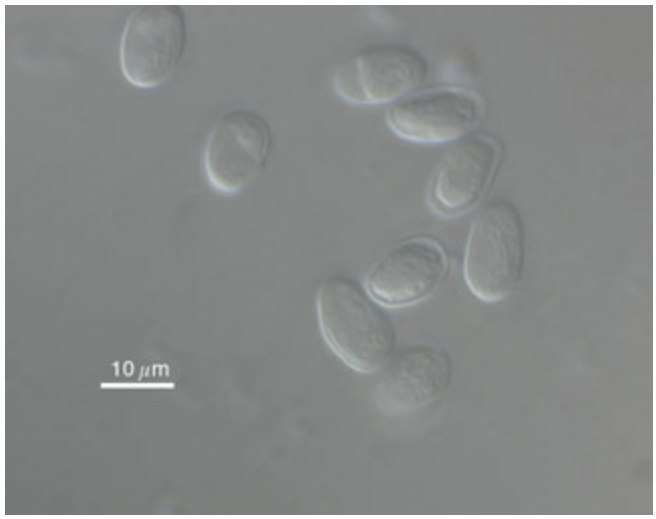


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Differential interference contrast (DIC) image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. Cysts are ovoidal or elipsoidal objects, usually 11-14 microns in length. Cysts may contain as many as 4 nuclei, and residual structures from their trophozoite or vegetative form. These residua include central axonemes, remnants of the striated disk, and remnant median bodies. In some cysts these structures will be indistinct. Scale bar is 10 microns.

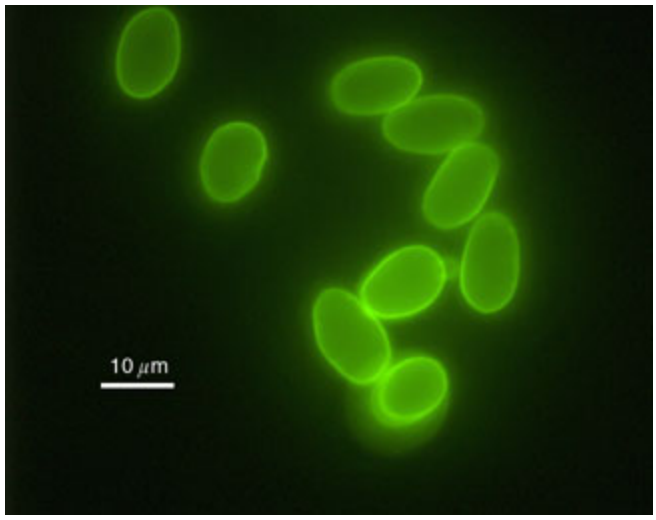


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Immunofluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with commercially available immunofluorescent antibodies. Cysts should have an intense apple green fluorescence on the periphery of their cyst wall, and measure 11-14 microns in length. Scale bar is 10 microns.

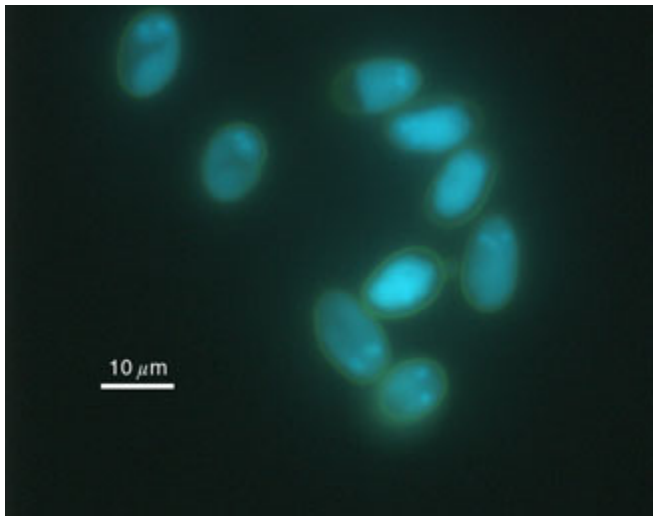


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Fluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with 4,6-diamidino 2-phenyl-indole dihydrochloride (DAPI). DAPI interacts with nucleic acids and stains the nuclei within the cyst. There should be 4 nuclei in each cyst. Cysts that appear to have fewer than 4 stained nuclei may have 4 nuclei with the others not visible in this plane of focus.

Cysts with no nuclei visible, may be dead, may be resistant to DAPI staining, or may be organisms other than *G. lamblia*.

Giardiasis *Giardia lamblia* Review

1. Giardia duodenalis, cause of giardiasis, is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.
A. True
B. False

2. Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella.
A. True
B. False

3. In Europe, Giardia lamblia is sometimes referred to as Lamblia intestinalis.
A. True
B. False

4. Giardiasis is the least frequent cause of non-bacterial diarrhea in North America.
A. True
B. False

5. Approximately one week after ingestion of the Giardia _____, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

6. It is possible to experience some, not all, of the symptoms, yet still shed _____ and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

7. The organism exists in two different forms--a hardy, dormant _____that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

8. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the _____. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

9. Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', which suggest that the condition of _____ is not primitive to eukaryotes but instead is a result of reductive evolution.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

10. The microaerophilic Giardia uses these _____ in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

11. Several strains of G. lamblia have been isolated and described through analysis of their _____ and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

12. Giardia lamblia is frequently diagnosed by visualizing the organism, either the _____ (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

13. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. An _____linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

14. Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the _____ of the organism.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

15. Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically.
A. True
B. False

16. The disease is also common in child day care centers, especially those in which diapering is done.
A. True
B. False

17. Acute outbreaks appear to be common with infants and are not usually associated with water but are related to child care and diaper changing hygiene procedures.
A. True
B. False

18. About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.
A. True
B. False

19. Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment.
A. True
B. False

20. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.
A. True
B. False

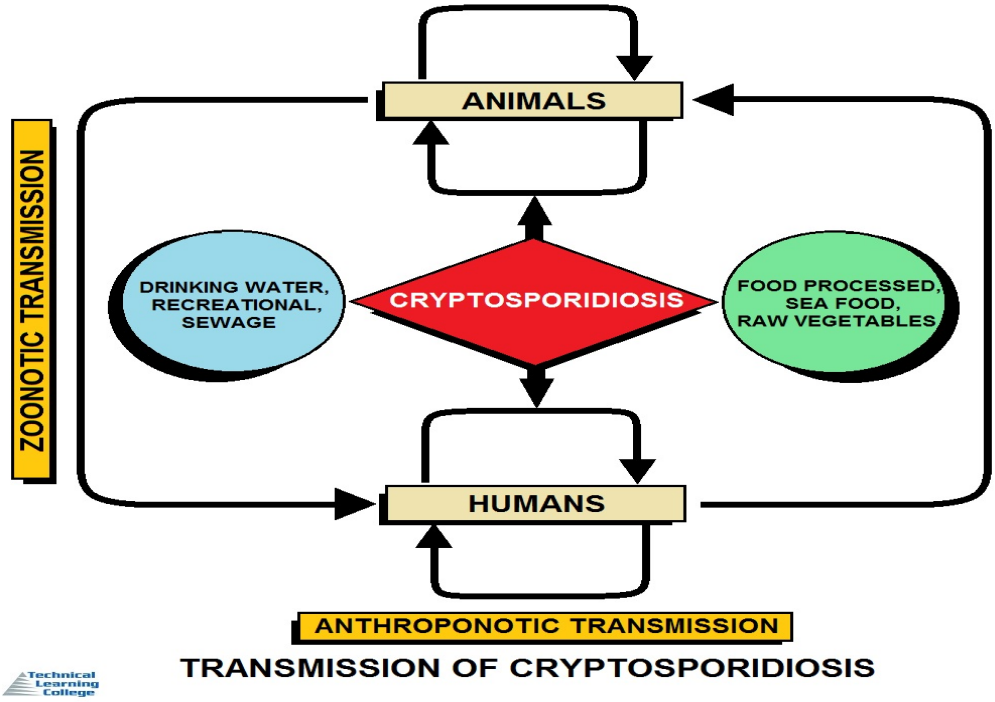
21. Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.
A. True
B. False

1.B, 2.A, 3.A, 4.B,5.A, 6.A, 7.A, 8.C, 9.D, 10.E, 11.D,12.B, 13.E, 14.A, 15.A, 16.A, 17.A, 18.A, 19.A, 20.A, 21.A

Chapter 5 - Cryptosporidiosis *Cryptosporidium*

Section Focus: You will learn the basics of cryptosporidium. At the end of this section, you will be able to describe cryptosporidiosis and cryptosporidium. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: *Cryptosporidium* is a microscopic parasite that causes the diarrheal disease cryptosporidiosis. Both the parasite and the disease are commonly known as "Crypto." There are many species of *Cryptosporidium* that infect animals, some of which also infect humans. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very tolerant to chlorine disinfection. While this parasite can be spread in several different ways, water (drinking water and recreational water) is the most common way to spread the parasite. *Cryptosporidium* is a leading cause of waterborne disease among humans in the United States.



TRANSMISSION OF CRYPTOSPORIDIOSIS

Introduction

Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of *Cryptosporidium parvum*, or the disease it causes, cryptosporidiosis. Today, however, public health and water utility officials are increasingly called on to provide information and make decisions about the control of this protozoan found in public water supplies, recreational water and other areas.

Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death.

Those with CD4 counts above 200 may recover from the symptoms of cryptosporidiosis yet maintain the infection asymptomatically, with symptoms potentially returning if their CD4 count later drops. Other diseases besides AIDS can cause immunosuppression severe enough to lead to chronic cryptosporidiosis. Persons with these diseases should also be concerned about becoming infected. These diseases include congenital agammaglobulinemia, congenital IgA deficiency and cancer. Persons taking corticosteroids, for cancer and bone marrow or organ transplants, also need to be concerned about becoming infected. Even though persons who are taking immunosuppressive drugs may develop chronic and/or severe cryptosporidiosis, the infection usually resolves when these drugs are decreased or stopped. Persons taking immunosuppressive drugs need to consult with their healthcare provider if they believe they have cryptosporidiosis.

Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis. Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.

Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.

AIDS
Patients with AIDS can have a large number of stools per day for months or even years. There is currently no cure for cryptosporidiosis, though drug research is continuing. Patients who suspect they may have cryptosporidiosis should drink extra fluids and may wish to drink oral rehydration therapy liquid, to avoid dehydration.

HIV-Positive Individuals
HIV-positive individuals who suspect they have cryptosporidiosis should contact their healthcare provider. Infected individuals should be advised to wash their hands frequently, especially before preparing food and after going to the toilet. They should also avoid close contact with anyone who has a weakened immune system. Individuals with diarrhea should not swim in public bathing areas while they have diarrhea and for at least 2 weeks after each attack of diarrhea.

Prevention
Washing hands is the most effective means of preventing cryptosporidiosis transmission. For the immunocompromised, sex, including oral sex, that involves possible contact with stool should be avoided. Immunocompromised individuals should also avoid the stool of all animals and wash their hands thoroughly after any contact with animals or the living areas of animals. Immunocompromised persons may also wish to wash, peel, or cook all vegetables and to take extra measures, such as boiling or filtering their drinking water, to ensure its safety.

Life cycle of *Cryptosporidium parvum* and *C. hominis*.

Cryptosporidium stages were reproduced from Juranek DD. Cryptosporidiosis.

In: Strickland GT, editor. Hunter's Tropical Medicine and Emerging Infectious Diseases, 8th ed. Philadelphia: WB Saunders; 2000. Originally adapted from the life cycle that appears in Current WL, Garcia LS. Cryptosporidiosis. Clinc Microbiol Rev 1991;4:325-58.

Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces and possibly other routes such as respiratory secretions.

Transmission of *Cryptosporidium parvum* and *C. hominis* occurs mainly through contact with contaminated water (e.g., drinking or recreational water).

Occasionally food sources, such as chicken salad, may serve as vehicles for transmission. Many outbreaks in the United States have occurred in waterparks, community swimming pools, and day care centers. Zoonotic and anthroponotic transmission of *C. parvum* and anthroponotic transmission of *C. hominis* occur through exposure to infected animals or exposure to water contaminated by feces of infected animals.

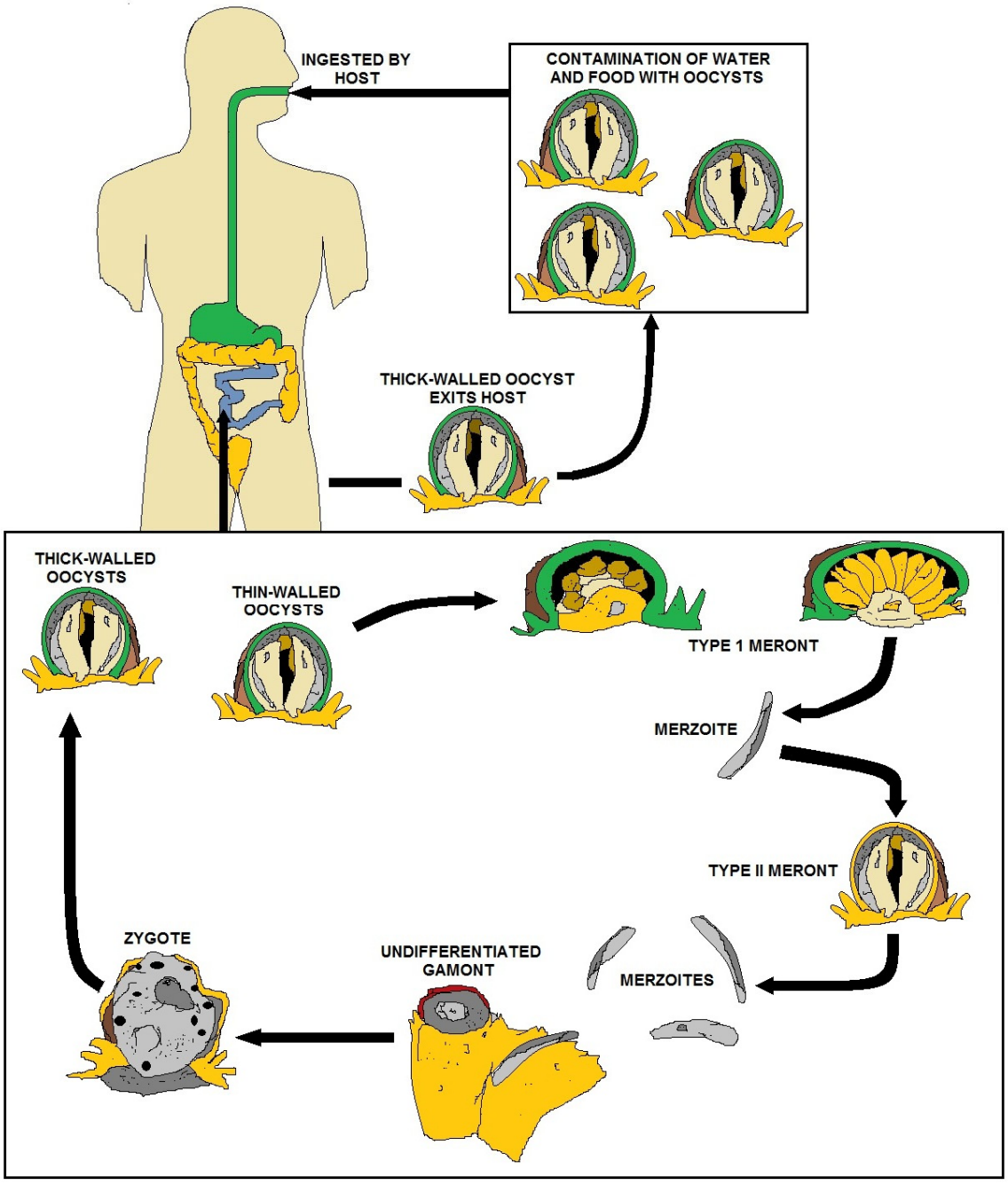
Following ingestion (and possibly inhalation) by a suitable host, excystation occurs. The sporozoites are released and parasitize epithelial cells of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) and then sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts (female).

Upon fertilization of the macrogamonts by the microgametes, oocysts develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host, and the thin-walled oocyst which is primarily involved in autoinfection.

Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission.

Note that oocysts of *Cyclospora cayetanensis*, another important coccidian parasite, are unsporulated at the time of excretion and do not become infective until sporulation is completed.

Refer to the life cycle of *Cyclospora cayentanensis* for further details.



LIFE CYCLE OF CRYPTOSPORIDIOSIS

Cryptosporidiosis Oocysts

Cryptosporidium oocysts

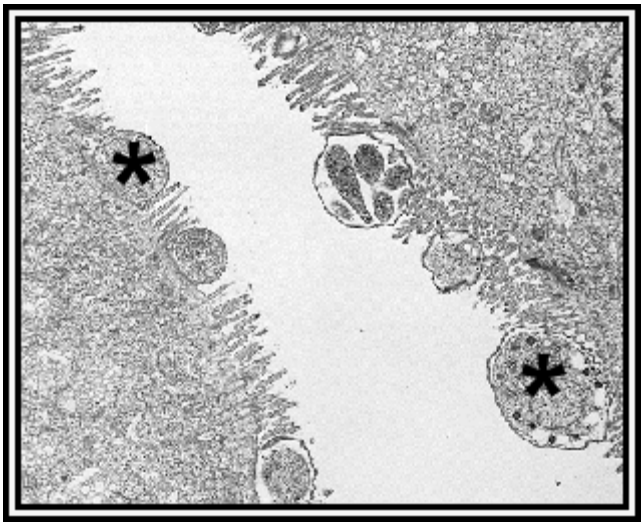
Genus *Cryptosporidium*

Members of the genus *Cryptosporidium* are parasites of the intestinal tracts of fishes, reptiles, birds, and mammals. It seems that members of this genus do not display a high degree of host specificity, so the number of species in this genus remains a matter of some discussion. *Cryptosporidium* isolated from humans is now referred to as *C. parvum*. *Cryptosporidium* infections have been reported from a variety of wild and domesticated animals, and in the last six or seven years literally hundreds of human infections have been reported, including epidemics in several major urban areas in the United States. Cryptosporidiosis is now recognized as an important opportunistic infection, especially in immunocompromised hosts.

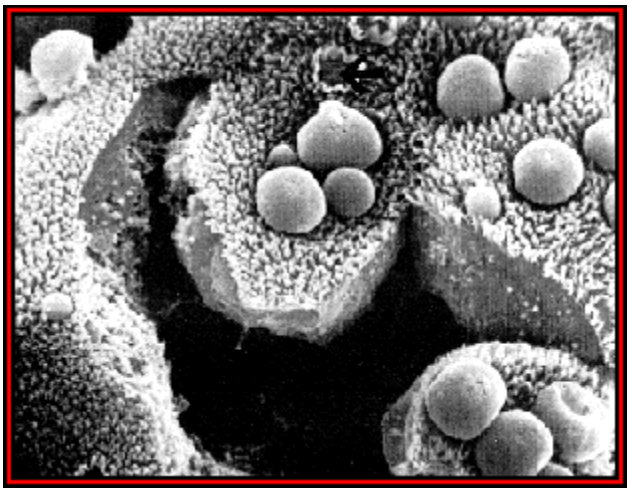
Cryptosporidium is a small parasite, measuring about 3-5 µm. It lives on (or just under) the surface of the cells lining the small intestine, reproduces asexually, and oocysts are passed in the feces. Transmission of the infection occurs via the oocysts. Many human infections have been traced to the contamination of drinking water with oocysts from agricultural "run-off" (i.e., drainage from pastures), so it is considered a zoonosis.

In most patients infected with cryptosporidiosis the infection causes a short term, mild diarrhea. Since such symptoms are associated with a number of ailments, infected individuals may not seek medical treatment, and the infection may subside on its own. Thus, it is difficult to say how many people are infected. On the other hand, in persons with compromised immune systems, this parasite can cause a pronounced, chronic diarrhea; in severe cases the infected individual may produce up to 15 liters/day of stools, and this may go on for weeks or months. Needless to say, such an infection, if not fatal unto itself, can exacerbate other opportunistic infections common in immunocompromised hosts.

A scanning electron micrograph of a broken meront of *Cryptosporidium* showing the merozoites within. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)



An electron micrograph showing several stages of *Cryptosporidium* (two are marked with asterisks) on the intestinal epithelium of a sheep. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)



A scanning electron micrograph of *Cryptosporidium* lining the intestinal tract. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)

How is cryptosporidiosis spread?
Cryptosporidium lives in the intestine of infected humans or animals. Millions of crypto germs can be released in a bowel movement from an infected human or animal.

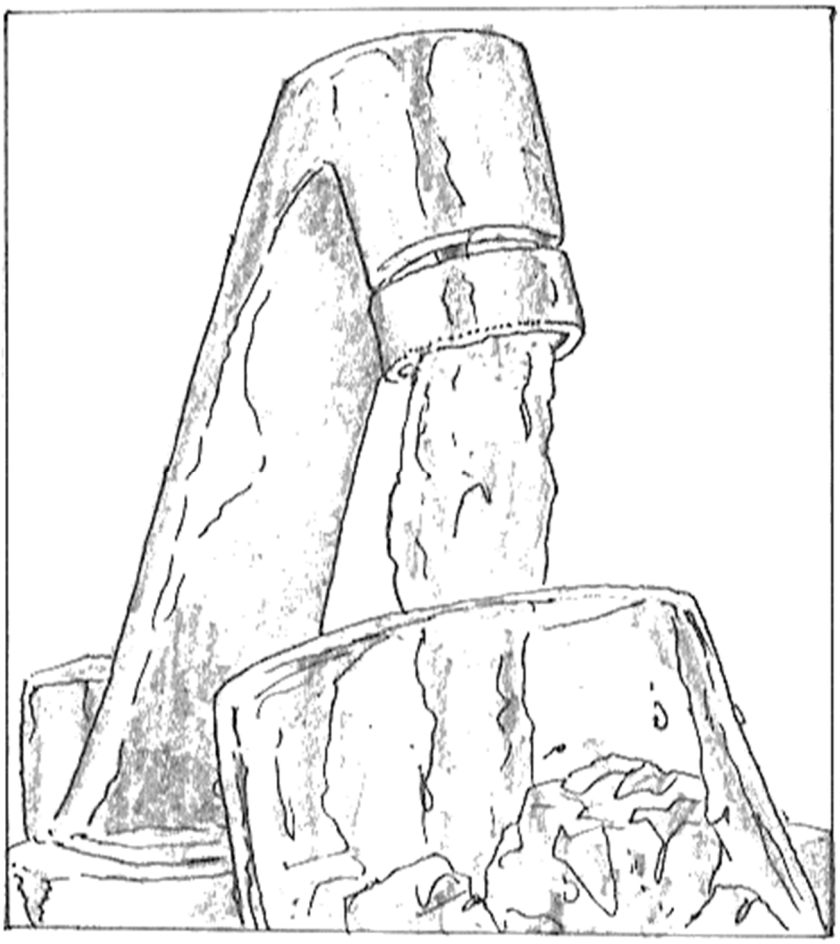
Consequently, *Cryptosporidium* is found in soil, food, water, or surfaces that have been contaminated with infected human or animal feces. If a person swallows the parasite they become infected. You **cannot** become infected through contact with blood. The parasite can be spread by:

- Accidentally putting something into your mouth or swallowing something that has come into contact with feces of a person or animal infected with *Cryptosporidium*.

- Swallowing recreational water contaminated with *Cryptosporidium* (Recreational water includes water in swimming pools, hot tubs, jacuzzis, fountains, lakes, rivers, springs, ponds, or streams that can be contaminated with sewage or feces from humans or animals.) **Note:** *Cryptosporidium* can survive for days in swimming pools with adequate chlorine levels.

- Eating uncooked food contaminated with *Cryptosporidium*. Thoroughly wash with clean, safe water all vegetables and fruits you plan to eat raw.

- Accidentally swallowing *Cryptosporidium* picked up from surfaces (such as bathroom fixtures, changing tables, diaper pails, or toys) contaminated with feces from an infected person.



Key Words

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLVED SOLIDS: Solids in solution that cannot be removed by filtration with a 0.45 micron filter.

What are the Symptoms of Cryptosporidiosis?

The most common symptom of cryptosporidiosis is watery diarrhea. Other symptoms include:

- Dehydration
- Weight loss
- Stomach cramps or pain
- Fever
- Nausea
- Vomiting

Some people with crypto will have no symptoms at all. While the small intestine is the site most commonly affected, *Cryptosporidium* infections could possibly affect other areas of the digestive or the respiratory tract.

How long after infection do symptoms appear?
Symptoms of cryptosporidiosis generally begin 2 to 10 days (average 7 days) after becoming infected with the parasite.

How long will symptoms last?
In persons with healthy immune systems, symptoms usually last about 1 to 2 weeks. The symptoms may go in cycles in which you may seem to get better for a few days, then feel worse again before the illness ends.

If I have been diagnosed with *Cryptosporidium* , should I worry about spreading the infection to others?
Yes, *Cryptosporidium* can be very contagious. Follow these guidelines to avoid spreading the disease to others:

1. Wash your hands with soap and water after using the toilet, changing diapers, and before eating or preparing food.
2. Do not swim in recreational water (pools, hot tubs, lakes or rivers, the ocean, etc.) if you have cryptosporidiosis and for at least 2 weeks after diarrhea stops. You can pass *Cryptosporidium* in your stool and contaminate water for several weeks after your symptoms have ended. This has resulted in outbreaks of cryptosporidiosis among recreational water users.
   Note: Cryptosporidium can be spread in a chlorinated pool because it is resistant to chlorine and, therefore, can live for days in chlorine-treated swimming pools.
3. Avoid fecal exposure during sexual activity.

Who is most at risk for cryptosporidiosis?
People who are most likely to become infected with *Cryptosporidium* include:

- Children who attend day care centers, including diaper-aged children
- Child care workers
- Parents of infected children
- International travelers
- Backpackers, hikers, and campers who drink unfiltered, untreated water
- Swimmers who swallow water while swimming in swimming pools, lakes, rivers, ponds, and streams
- People who drink from shallow, unprotected wells
- People who swallow water from contaminated sources

Contaminated water includes water that has not been boiled or filtered. Several community-wide outbreaks of cryptosporidiosis have been linked to drinking municipal water or recreational water contaminated with *Cryptosporidium.*

Who is most at risk for getting seriously ill with cryptosporidiosis?
Although Crypto can infect all people, some groups are more likely to develop more serious illness.

- Young children and pregnant women may be more susceptible to the dehydration resulting from diarrhea and should drink plenty of fluids while ill.
- If you have a severely weakened immune system, you are at risk for more serious disease. Your symptoms may be more severe and could lead to serious or life-threatening illness. Examples of persons with weakened immune systems include those with HIV/AIDS; cancer and transplant patients who are taking certain immunosuppressive drugs; and those with inherited diseases that affect the immune system.

If you have a severely weakened immune system, talk to your health care provider for additional guidance. You can also call the CDC AIDS HOTLINE toll-free at 1-800-342-2437. Ask for more information on cryptosporidiosis, or go to the CDC fact sheet *Preventing Cryptosporidiosis: A Guide for People with Compromised Immune Systems* available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_ci.htm

What should I do if I think I may have cryptosporidiosis?
If you suspect that you have cryptosporidiosis, see your health care provider.

How is cryptosporidiosis diagnosed?
Your health care provider will ask you to submit stool samples to see if you are infected. Because testing for Crypto can be difficult, you may be asked to submit several stool specimens over several days. Tests for Crypto are not routinely done in most laboratories; therefore, your health care provider should specifically request testing for the parasite.

What is the treatment for cryptosporidiosis?
Although there is no standard treatment for cryptosporidiosis, the symptoms can be treated. Most people who have a healthy immune system will recover without treatment. If you have diarrhea, drink plenty of fluids to prevent dehydration. Rapid loss of fluids from diarrhea may be especially life threatening to babies; therefore, parents should talk to their health care provider about fluid replacement therapy options for infants. Antidiarrheal medicine may help slow down diarrhea, but talk to your health care provider before taking it. A new drug, nitazoxanide, has been approved for treatment of diarrhea caused by *Cryptosporidium* in healthy children less than 12 years old. Consult with your health care provider for more information. People who are in poor health or who have a weakened immune system are at higher risk for more severe and more prolonged illness.

For persons with AIDS, anti-retroviral therapy that improves immune status will also decrease or eliminate symptoms of Crypto. However, even if symptoms disappear, cryptosporidiosis is usually not curable and the symptoms may return if the immune status worsens. See your health care provider to discuss anti-retroviral therapy used to improve your immune status.

How Can I Prevent Cryptosporidiosis?

Practice good hygiene.

1. Wash hands thoroughly with soap and water.
    a. Wash hands after using the toilet and before handling or eating food (especially for persons with diarrhea).
    b. Wash hands after every diaper change, especially if you work with diaper-aged children, even if you are wearing gloves.
2. Protect others by not swimming if you are experiencing diarrhea (essential for children in diapers).

Avoid water that might be contaminated.

1. Do not swallow recreational water
2. Do not drink untreated water from shallow wells, lakes, rivers, springs, ponds, and streams.
3. Do not drink untreated water during community-wide outbreaks of disease caused by contaminated drinking water.
4. Do not use untreated ice or drinking water when traveling in countries where the water supply might be unsafe.

For information on recreational water-related illnesses, visit CDC's Healthy Swimming website at http://www.cdc.gov/healthyswimming.

In the United States, nationally distributed brands of bottled or canned carbonated soft drinks are safe to drink. Commercially packaged non-carbonated soft drinks and fruit juices that do not require refrigeration until after they are opened (those that are stored un-refrigerated on grocery shelves) also are safe.

For information on choosing safe bottled water, see the CDC fact sheet entitled “Preventing Cryptosporidiosis: A Guide to Water Filters and Bottled Water,” available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_water.htm.

If you are unable to avoid using or drinking water that might be contaminated, then you can make the water safe to drink by doing one of the following:

- Heat the water to a rolling boil for at least 1 minute.
  OR
- Use a filter that has an absolute pore size of at least 1 micron or one that has been NSF rated for "cyst removal."

Do not rely on chemicals to disinfect water and kill *Cryptosporidium*. Because it has a thick outer shell, this particular parasite is highly resistant to disinfectants such as chlorine and iodine.

For information on choosing a water filter, see the CDC fact sheet entitled "Preventing Cryptosporidiosis: A Guide to Water Filters and Bottled Water," available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_water.htm.

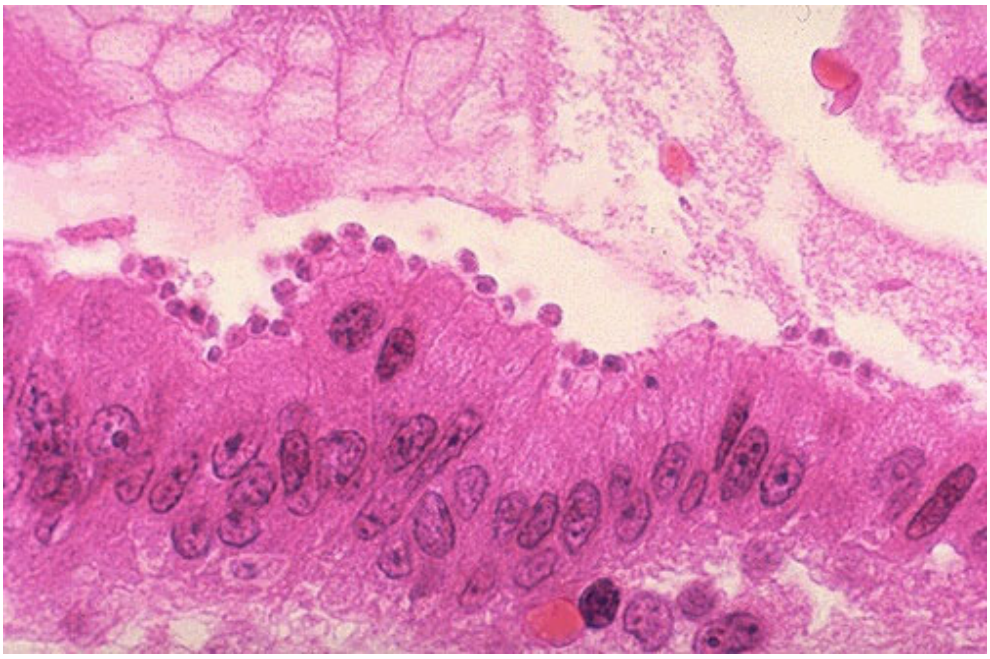
Avoid food that might be contaminated.

1. Wash and/or peel all raw vegetables and fruits before eating.
2. Use safe, uncontaminated water to wash all food that is to be eaten raw.
3. Avoid eating uncooked foods when traveling in countries with minimal water treatment and sanitation systems.

Take extra care when traveling.

If you travel to developing nations, you may be at a greater risk for *Cryptosporidium* infection because of poorer water treatment and food sanitation. Warnings about food, drinks, and swimming are even more important when visiting developing countries. Avoid foods and drinks, in particular raw fruits and vegetables, tap water, or ice made from tap water, unpasteurized milk or dairy products, and items purchased from street vendors. These items may be contaminated with *Cryptosporidium*. Steaming-hot foods, fruits you peel yourself, bottled and canned processed drinks, and hot coffee or hot tea are probably safe. Talk with your health care provider about other guidelines for travel abroad.

Avoid fecal exposure during sexual activity.



This is another infectious agent that is becoming more frequent in immunocompromised patients, particularly those with AIDS. The small round blue organisms at the lumenal border are cryptosporidia. Cryptosporidiosis produces a copious watery diarrhea.

A Guide to Water Filters and Bottled Water

Filtering tap water
Many, but not all, available home water filters remove Cryptosporidium. Some filter designs are more suitable for removal of *Cryptosporidium* than others. Filters that have the words "reverse osmosis" on the label protect against *Cryptosporidium*. Many other types of filters that work by micro-straining also work. Look for a filter that will remove particles that are less than or equal to 1 micron in diameter.

There are two types of these - "absolute 1 micron" filters and "nominal 1 micron" filters. The absolute 1 micron filter will more consistently remove *Cryptosporidium* than a nominal filter. Some nominal 1 micron filters will allow 20% to 30% of 1 micron particles to pass through.

NSF-International
NSF-International (NSF) does independent testing of filters to determine if they remove *Cryptosporidium*. To find out if a particular filter is certified to remove *Cryptosporidium*, you can look for the NSF trademark plus the words "cyst reduction" or "cyst removal" on the product label information. You can also contact the NSF at 789 N. Dixboro Road, Ann Arbor, MI 48105 USA, toll free1-877-867-3435, fax 313-769-0109, email info@nsf.org, or visit their Web site at www.nsf.org/certified/DWTU/.

At their Web site, you can enter the model number of the unit you intend to buy to see if it is on their certified list, or you can look under the section entitled "Reduction claims for drinking water treatment units - Health Effects" and check the box in front of the words "Cyst Reduction." This will display a list of filters tested for their ability to remove *Cryptosporidium*.

Because NSF testing is expensive and voluntary, some filters that may work against *Cryptosporidium* have not been NSF-tested. If you choose to use a product not NSF-certified, select those technologies more likely to reduce *Cryptosporidium*; this includes filters with reverse osmosis and those that have an absolute pore size of 1 micron or smaller.

Package and Label information for purchasing water filters:

Filters designed to remove crypto ***(any of the four messages below on a package label indicate that the filter should be able to remove crypto***	**Filters labeled only with these words may NOT be designed to remove crypto**
Reverse osmosis (with or without NSF testing)	*Nominal* pore size of 1 micron or smaller
Absolute pore size of 1 micron or smaller (with or without NSF testing)	One micron filter
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst removal	Effective against *Giardia*
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst reduction	Effective against parasites
	Carbon filter
	Water purifier
	EPA approved — Caution: EPA does not approve or test filters
	EPA registered — Caution: EPA does not register filters based on their ability to remove Cryptosporidium
	Activated carbon
	Removes chlorine
	Ultraviolet light
	Pentiodide resins
	Water softener

Note: Filters collect germs from water, so someone who is not HIV infected or immune impaired should change the filter cartridges. Anyone changing the cartridges should wear gloves and wash hands afterwards.

Filters may not remove *Cryptosporidium* as well as boiling does because even good brands of filters may sometimes have manufacturing flaws that allow small numbers of *Cryptosporidium* to get in past the filter. Selection of NSF-Certified filters provides additional assurance against such flaws. Also, poor filter maintenance or failure to replace the filter cartridges as recommended by the manufacturer can cause a filter to fail.

If you drink bottled water, read the label and look for this information:

Water so labeled has been processed by method effective against crypto	Water so labeled may not have been processed by method effective against crypto
Reverse osmosis treated	Filtered
Distilled	Micro-filtered
Filtered through an *absolute* 1 micron or smaller filter	Carbon-filtered
"One micron absolute"	Particle-filtered
	Multimedia-filtered
	Ozonated
	Ozone-treated
	Ultraviolet light-treated
	Activated carbon-treated
	Carbon dioxide-treated
	Ion exchange-treated
	Deionized
	Purified
	Chlorinated

Bottled water labels reading "well water," "artesian well water," "spring water," or "mineral water" do not guarantee that the water does not contain crypto. However, water that comes from protected well or protected spring water sources is less likely to contain crypto than bottled water or tap water from less protected sources, such as rivers and lakes.

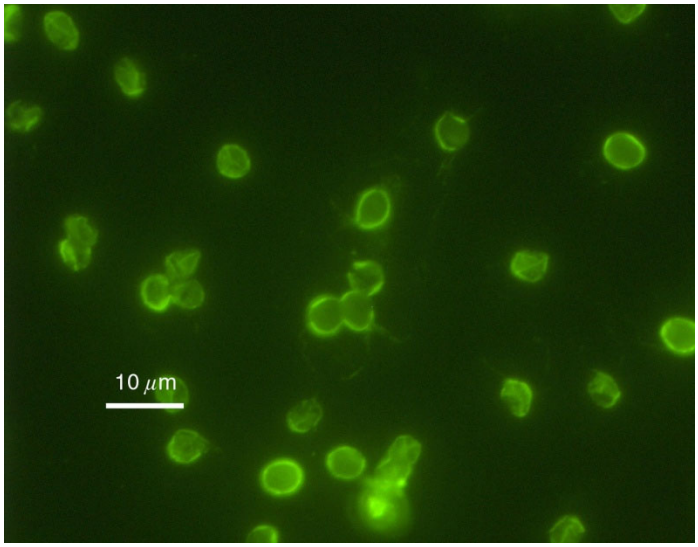
Home distillers: You can remove crypto and other germs from your water with a home distiller. If you use one, you need to carefully store your water as recommended for storing purified water.

Other drinks: Soft drinks and other beverages may or may not contain crypto. You need to know how they were prepared to know if they might contain crypto.

If you consume prepared beverages, look for drinks from which crypto has been removed:

Crypto killed or removed in preparation	Crypto may not be killed or removed in preparation
Canned or bottled soda, seltzer, and fruit drinks	Fountain drinks
Steaming hot (175 degrees F or hotter) tea or coffee	Fruit drinks you mix with tap water from frozen concentrate
Pasteurized drinks	Iced tea or coffee

Juices made from fresh fruit can also be contaminated with crypto. Several people became ill after drinking apple cider made from apples contaminated with crypto. You may wish to avoid unpasteurized juices or fresh juices if you do not know how they were prepared.



Immunofluorescence image of *Cryptosporidium parvum* oocysts, purified from murine fecal material. (Same field of view) Oocysts were stained with commercially available immunofluorescent antibodies. Oocysts should have an intense apple green fluorescence on the periphery of their oocyst wall, and measure 4 to 6 microns in diameter. Scale bar is 10 microns. Photo Credit: H.D.A Lindquist, U.S. EPA.

Chapter 5 - Cryptosporidiosis Review

1. Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of Cryptosporidium parvum, or the disease it causes, cryptosporidiosis.
A. True
B. False

2. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.
A. True
B. False

3. Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible.
A. True
B. False

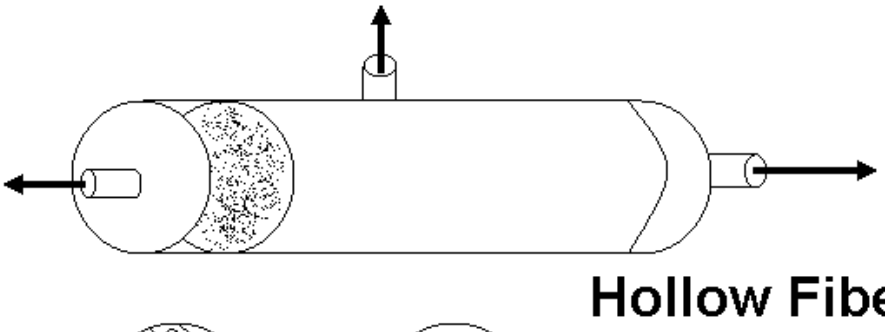
4. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.
A. True
B. False

5. Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death.
A. True
B. False

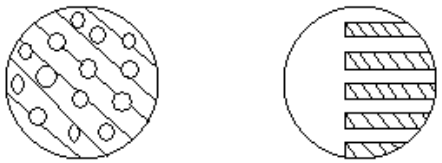
6. Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis.
A. True
B. False

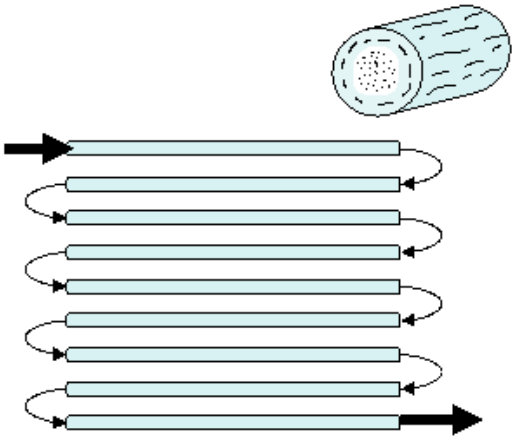
7. Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool.
A. True
B. False

1.A, 2.A, 3.A, 4.A, 5.A, 6.A, 7.A



Hollow Fiber





Tubular

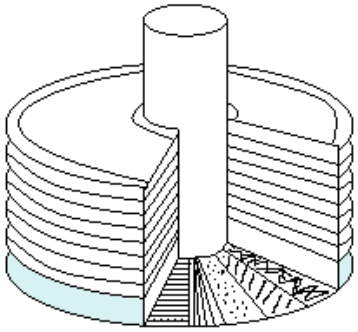
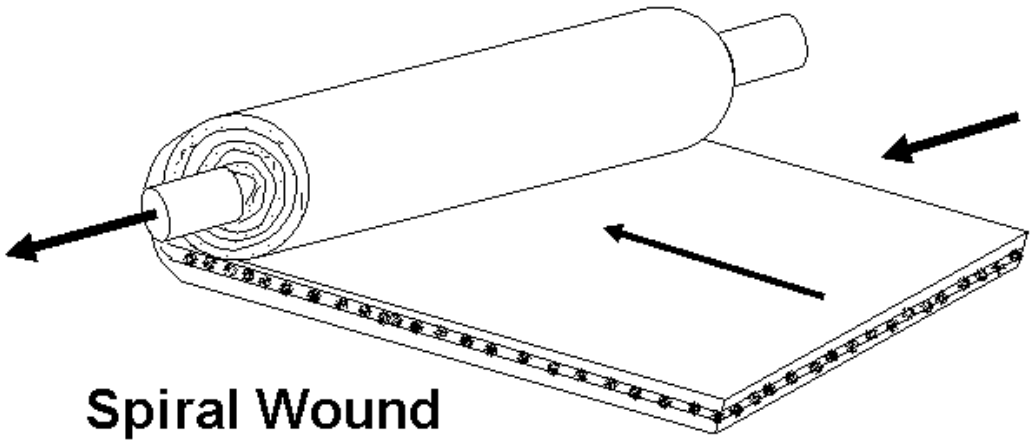


Plate and Frame

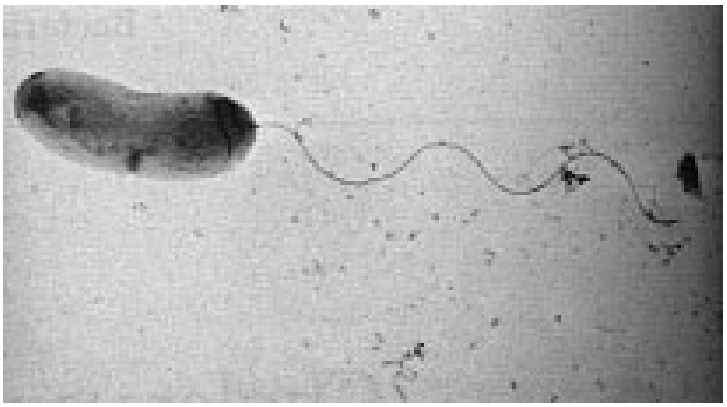


Spiral Wound

Chapter 6 - Cholera *Vibrio cholerae*

Section Focus: You will learn the basics of cholera. At the end of this section, you will be able to describe cholera vibrio. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: Cholera has been very rare in industrialized nations for the last 100 years; however, the disease is still common today in other parts of the world, including the Indian subcontinent and sub-Saharan Africa. Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.



Vibrio cholerae

Cholera, which is derived from a Greek term meaning “flow of bile,” is caused by *Vibrio cholerae* and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of hours of infection. In 1883, Robert Koch discovered *V cholerae* during a cholera outbreak in Egypt. The organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. *V cholerae* O1 or O139 are associated with epidemic cholera. *V cholerae* O1 has 2 major biotypes: classic and El Tor.

Currently, El Tor is the predominant cholera pathogen. Organisms in both biotypes are subdivided into serotypes according to the structure of the O antigen, as follows:

- Serotype Inaba - O antigens A and C
- Serotype Ogawa - O antigens A and B
- Serotype Hikojima - O antigens A, B, and C

How does a person get cholera?
A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water. The cholera bacterium may also live in the environment in brackish rivers and coastal waters.

Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.

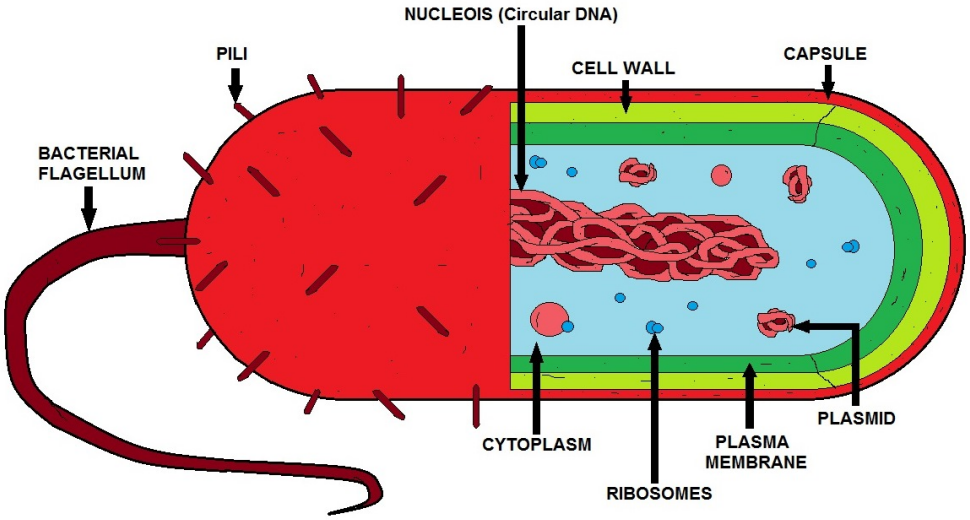


DIAGRAM OF VIBRIO CHOLERA BACTERIA

Vibrio Cholerae Bacterium

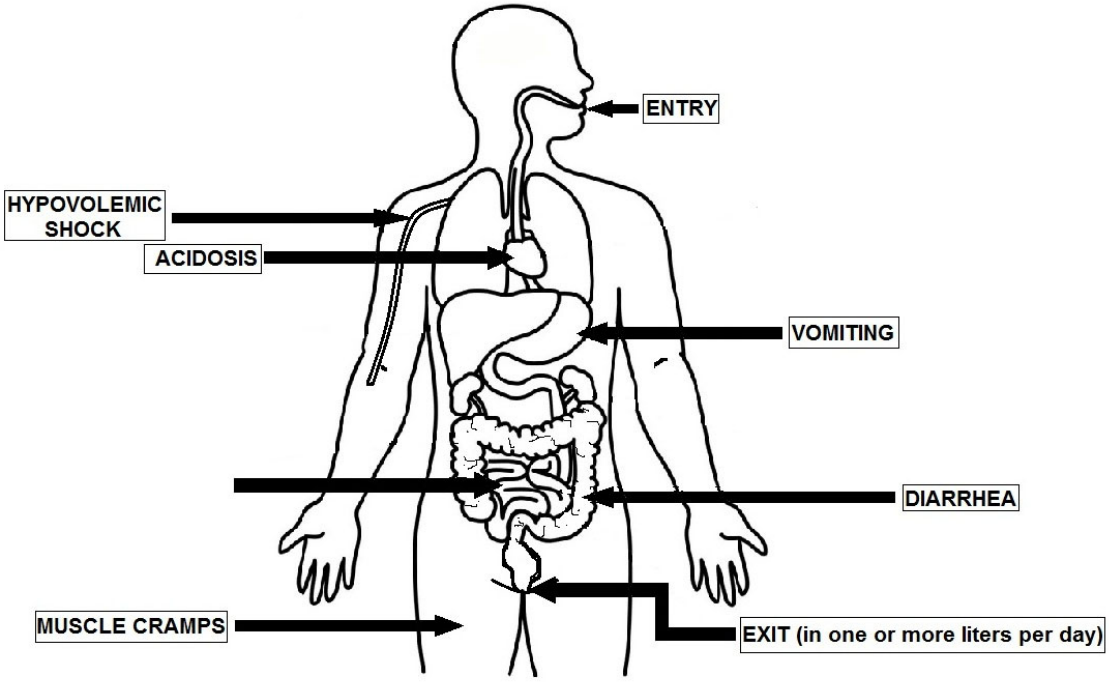
Cholera (also called Asiatic cholera) is a disease of the gastrointestinal tract caused by the Vibrio cholerae bacterium. These bacteria are typically ingested by drinking water contaminated by improper sanitation or by eating improperly cooked fish, especially shellfish. Symptoms include diarrhea, abdominal cramps, nausea, vomiting, and dehydration. Death is generally due to the dehydration caused by the illness. When left untreated, Cholera generally has a high mortality rate. Treatment is typically an aggressive rehydration regimen usually delivered intravenously, which continues until the diarrhea ceases.

* About one million Vibrio cholerae bacteria must be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a weakened immune system, individuals with decreased gastric acidity (as from the use of antacids), or those who are malnourished.

* 1,099,882 cases and 10,453 deaths were reported in the Western Hemisphere between January 1991 and July 1995.

* On average, one case of cholera is reported in the United States every week.
Vibrio cholerae causes disease by producing a toxin that disables the GTPase function of G proteins which are part of G protein-coupled receptors in intestinal cells. This has the effect that the G proteins are locked in the "on position" binding GTP (normally, the G proteins quickly return to "off" by hydrolyzing GTP to GDP). The G proteins then cause adenylate cyclases to produce large amounts of cyclic AMP (cAMP) which results in the loss of fluid and salts across the lining of the gut.

The resulting diarrhea allows the bacterium to spread to other people under unsanitary conditions.



CHOLERA
(vibrio cholerae)

What is the risk for cholera in the United States?
In the United States, cholera was prevalent in the 1800s but has been virtually eliminated by modern sewage and water treatment systems. However, as a result of improved transportation, more persons from the United States travel to parts of Latin America, Africa, or Asia where epidemic cholera is occurring. U.S. travelers to areas with epidemic cholera may be exposed to the cholera bacterium. In addition, travelers may bring contaminated seafood back to the United States; foodborne outbreaks have been caused by contaminated seafood brought into this country by travelers.

Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat. The last major outbreak of cholera in the United States was in 1911. However, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.

What should travelers do to avoid getting cholera?
The risk for cholera is very low for U.S. travelers visiting areas with epidemic cholera. When simple precautions are observed, contracting the disease is unlikely.

All travelers to areas where cholera has occurred should observe the following recommendations:

- Drink only water that you have boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made with boiled water and carbonated, bottled beverages with no ice.
- Eat only foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish, including ceviche.
- Make sure all vegetables are cooked, avoid salads.
- Avoid foods and beverages from street vendors.
- Do not bring perishable seafood back to the United States.

A simple rule of thumb is "***Boil it, cook it, peel it, or forget it***."

Treatment
The objective of treatment is to replace fluid and electrolytes lost through diarrhea. Depending on the condition of the person, oral or intravenous fluid will be given. Tetracycline and other antibiotics may shorten the duration of the symptoms.
Note: Tetracycline is usually not prescribed for children until after all the permanent teeth have come in, because it can permanently discolor teeth that are still forming.

The World Health Organization (WHO) has developed an oral rehydration solution that is cheaper and easier to use than the typical intravenous fluid. This solution of sugar and electrolytes is now being used internationally.

Expectations (prognosis)
Severe dehydration can cause death. Given adequate fluids, most people will make a full recovery.

Complications

- Severe dehydration.

Calling your health care provider
Call your health care provider if profuse watery diarrhea develops.

Call your health care provider if signs of dehydration occur, including rapid pulse (heart rate), dry skin, dry mouth, thirst, "glassy" eyes, lethargy, sunken eyes, no tears, reduced or no urine, and unusual sleepiness or tiredness.

Susceptibility
Recent genetic research has determined that a person's susceptibility to cholera and other diarrheas) is affected by their blood type. Those with type O blood are the most susceptible. Those with type AB are the most resistant, virtually immune. Between these two extremes are the A and B blood types, with type A being more resistant than type B.

Carriers of the cystic fibrosis gene are protected from the severe effects of cholera because they don't lose water as quickly. This explains the high incidence of cystic fibrosis among populations which were formerly exposed to cholera.

Epidemic control and preventive measures
When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an adequate supply of safe drinking water, and good food hygiene. Effective food hygiene measures include cooking food thoroughly and eating it while still hot; preventing cooked foods from being contaminated by contact with raw foods, including water and ice, contaminated surfaces or flies; and avoiding raw fruits or vegetables unless they are first peeled. Washing hands after defecation, and particularly before contact with food or drinking water, is equally important.

Routine treatment of a community with antibiotics, or "mass chemoprophylaxis", has no effect on the spread of cholera, nor does restricting travel and trade between countries or between different regions of a country. Setting up a *cordon sanitaire* at frontiers uses personnel and resources that should be devoted to effective control measures, and hampers collaboration between institutions and countries that should unite their efforts to combat cholera. Because of the severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance, and death. To shorten its duration and severity, antibacterial drugs are beneficial in those with severe disease.

Limited stocks of two oral cholera vaccines that provide high-level protection for several months against cholera caused by *V. cholerae* O1 have recently become available in a few countries. Both are suitable for use by travelers but they have not yet been used on a large scale for public health purposes. Use of this vaccine to prevent or control cholera outbreaks is not recommended because it may give a false sense of security to vaccinated subjects and to health authorities, who may then neglect more effective measures.

Is a vaccine available to prevent cholera?
At the present time, the manufacture and sale of the only licensed cholera vaccine in the United States (Wyeth-Ayerst) has been discontinued. It has not been recommended for travelers because of the brief and incomplete immunity it offers. No cholera vaccination requirements exist for entry or exit in any country.

Two recently developed vaccines for cholera are licensed and available in other countries (Dukoral®, Biotec AB and Mutacol®, Berna). Both vaccines appear to provide a somewhat better immunity and fewer side-effects than the previously available vaccine. However, neither of these two vaccines are recommended for travelers nor are they available in the United States.

History and spread of epidemic cholera
Cholera has smoldered in an endemic fashion on the Indian subcontinent for centuries. There are references to deaths due to dehydrating diarrhea dating back to Hippocrates and Sanskrit writings. Epidemic cholera was described in 1563 by Garcia del Huerto, a Portuguese physician at Goa, India. The mode of transmission of cholera by water was proven in 1849 by John Snow, a London physician. In 1883, Robert Koch successfully isolated the cholera vibrio from the intestinal discharges of cholera patients and proved conclusively that it was the agent of the disease.

The first long-distance spread of cholera to Europe and the Americas began in 1817 and by the early 20th century, six waves of cholera had spread across the world in devastating epidemic fashion. Since then, until the 1960s, the disease contracted, remaining present only in southern Asia. Cholera has been found in only two other animal populations: shellfish and plankton.

Cholera Treatment
Cholera is typically transmitted by either contaminated food or water. With seafood being the usual cause, while in the developing world it is more often water. When consumed, most bacteria do not survive the *stomach acid*. During the passage through the stomach, few surviving bacteria conserve their *energy and stored nutrients* by shutting down much *protein production.* Surviving Cholera *Bacteria* exit the stomach and reach the small intestine, they need to propel themselves through the thick mucus that lines the small intestine to get to the intestinal walls, where they can thrive. V. cholerae bacteria start up production of the hollow cylindrical protein flagellin to make flagella, the cork-screw helical fibers they rotate to propel themselves through the mucus of the small intestine.

If *Cholera* bacteria reach the intestinal wall, they will no longer need the *flagella.* On reaching the intestinal wall, V. cholerae start producing the toxic proteins that give the infected person a watery diarrhea. This carries the multiplying new generations of V. cholerae bacteria out into the drinking water of the next host if proper sanitation measures are not in place.

Antibiotics
Antibiotic treatments for one to three days shorten the course of the disease and reduce the severity of the symptoms. People will recover without them, however, if sufficient hydration is maintained. Doxycycline is typically used first line, although some strains of V. cholerae have shown resistance. Testing for resistance during an outbreak can help determine appropriate future choices. Other antibiotics proven to be effective include cotrimoxazole, erythromycin, tetracycline, chloramphenicol, and furazolidone. Fluoroquinolones, such as norfloxacin, also may be used, but resistance has been reported.

Epidemiology
Cholera affects an estimated 3-5 million people worldwide, and causes 100,000-130,000 deaths a year as of 2010. This occurs mainly in the developing world. In the early 1980s, death rates are believed to have been greater than 3 million a year. It is difficult to calculate exact numbers of cases, as many go unreported due to concerns that an outbreak may have a negative impact on the tourism of a country.

Cholera remains both epidemic and endemic in many areas of the world. Although much is known about the mechanisms behind the spread of cholera, this has not led to a full understanding of what makes cholera outbreaks happen some places and not others. Lack of treatment of human feces and lack of treatment of drinking water greatly facilitate its spread, but bodies of water can serve as a reservoir, and seafood shipped long distances can spread the disease. Cholera was not known in the Americas for most of the 20th century, but it reappeared towards the end of that century and seems likely to persist.

Cholera morbus
The term cholera morbus was used in the 19th and early 20th centuries to describe both nonepidemic cholera and other gastrointestinal diseases (sometimes epidemic) that resembled cholera. The term is not in current use, but is found in many older references. The other diseases are now known collectively as gastroenteritis.

El Tor

In 1961, the "**El Tor**" biotype (distinguished from classic biotypes by the production of hemolysins) reemerged to produce a major epidemic in the Philippines and to initiate a seventh global pandemic. Since then this biotype has spread across Asia, the Middle East, Africa, and more recently, parts of Europe.

There are several characteristics of the El Tor strain that confer upon it a high degree of "epidemic virulence," allowing it to spread across the world as previous strains have done. First, the ratio of cases to carriers is much less than in cholera due to classic biotypes (1: 30-100 for El Tor vs. 1: 2 - 4 for "classic" biotypes). Second, the duration of carriage after infection is longer for the El Tor strain than the classic strains. Third, the El Tor strain survives for longer periods in the extraintestinal environment. Between 1969 and 1974, El Tor replaced the classic strains in the heartland of endemic cholera, the Ganges River Delta of India.

El Tor broke out explosively in Peru in 1991 (after an absence of cholera there for 100 years), and spread rapidly in Central and South America, with recurrent epidemics in 1992 and 1993. From the onset of the epidemic in January 1991 through September 1, 1994, a total of 1,041,422 cases and 9,642 deaths (overall case-fatality rate: 0.9%) were reported from countries in the Western Hemisphere to the Pan American Health Organization. In 1993, the numbers of reported cases and deaths were 204,543 and 2362, respectively.

So far, the United States has been spared except for imported cases, or clusters of infections from imported food. In the United States during 1993 and 1994, 22 and 47 cholera cases were reported to CDC, respectively. Of these, 65 (94%) were associated with foreign travel. In 1982, in Bangladesh, a classic biotype resurfaced with a new capacity to produce more severe illness, and it rapidly replaced the El Tor strain which was thought to be well-entrenched. This classic strain has not yet produced a major outbreak in any other country.

In December, 1992, a large epidemic of cholera began in Bangladesh, and large numbers of people have been involved. The organism has been characterized as *V. cholerae* O139 "Bengal". It is derived genetically from the El Tor pandemic strain but it has changed its antigenic structure such that there is no existing immunity and all ages, even in endemic areas, are susceptible. The epidemic has continued to spread. and *V. cholerae* O139 has affected at least 11 countries in southern Asia. Specific totals for numbers of *V. cholerae* O139 cases are unknown because affected countries do not report infections caused by O1 and O139 separately.

Antigenic Variation and LPS Structure in *Vibrio cholerae*

Antigenic variation plays an important role in the epidemiology and virulence of cholera. The emergence of the Bengal strain, mentioned above, is an example. The flagellar antigens of *V. cholerae* are shared with many water vibrios and therefore are of no use in distinguishing strains causing epidemic cholera. O antigens, however, do distinguish strains of *V. cholerae* into 139 known serotypes. Almost all of these strains of *V. cholerae* are nonvirulent. Until the emergence of the Bengal strain (which is "non-O1") a single serotype, designated O1, has been responsible for epidemic cholera.

However, there are three distinct **O1 biotypes**, named Ogawa, Inaba and Hikojima, and each biotype may display the "classical" or El Tor phenotype. The Bengal strain is a new serological strain with a unique O-antigen which partly explains the lack of residual immunity.

Antigenic Determinants of *Vibrio cholerae*

Serotype	O Antigens
Ogawa	A, B
Inaba	A, C
Hikojima	A, B, C

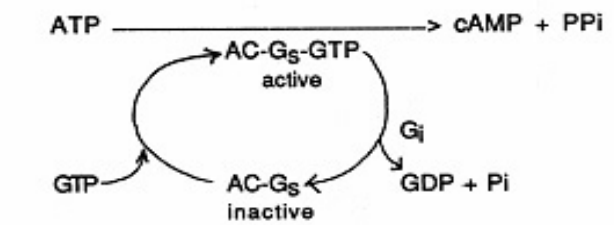
Endotoxin is present in *Vibrio cholerae* as in other Gram-negative bacteria. Fewer details of the chemical structure of *Vibrio cholerae* LPS are known than in the case of *E. coli* and *Salmonella typhimurium*, but some unique properties have been described. Most importantly, variations in LPS occur in vivo and in vitro, which may be correlated with reversion in nature of nonepidemic strains to classic epidemic strains and vice versa.

Cholera Toxin
Cholera toxin activates the adenylate cyclase enzyme in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin.

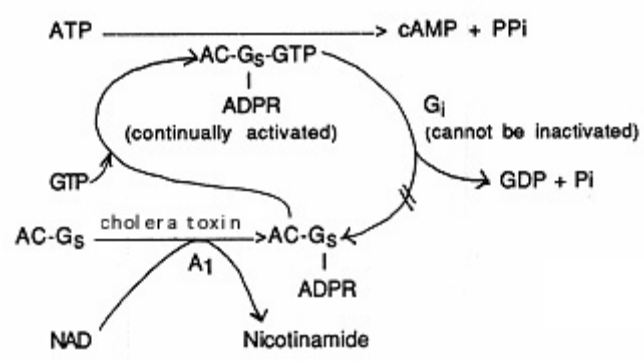
The toxin has been characterized and contains 5 binding (B) subunits of 11,500 daltons, an active (A1) subunit of 23,500 daltons, and a bridging piece (A2) of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells.

Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP.

The normal situation is described as follows.



The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated. This situation can be illustrated as follows.



Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-.

The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes, causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.

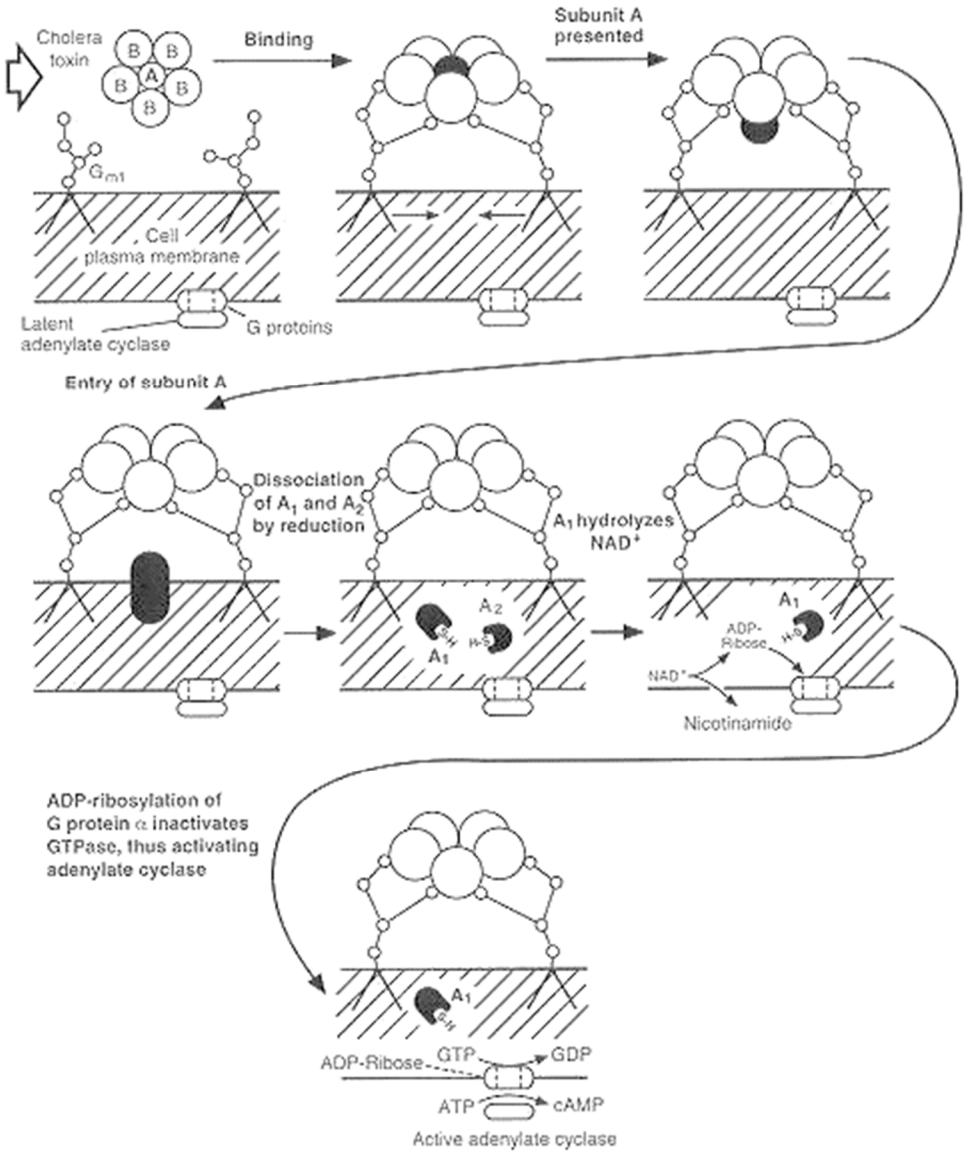
Last Word

E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT toxin is on a plasmid that can be transferred to other *E. coli* strains and probably to other enteric bacteria, as well. Close relationships between the genetic code for LT toxin and the cholera toxin undoubtedly exist but have not been documented as yet.

The genetic information for the toxin in *V. cholerae* is located on the bacterial chromosome. Other bacterial enterotoxins related to cholera toxin have been reported in non-group O *Vibrio* strains and a strain of *Salmonella*.

Enterotoxins, toxins which act in the GI tract, are produced by a wide variety of bacteria.

The family of heat-stable (ST) enterotoxins of *E. coli*, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin. Other enterotoxins, which elicit cytotoxic effects on intestinal epithelial cells, have been described from *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Aeromonas*, *Pseudomonas*, *Shigella*, *V. parahaemolyticus*, *Campylobacter*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium perfringens*, *C. difficile*, and *Staphylococcus aureus*.



Mechanism of action of cholera enterotoxin according to Finkelstein in Baron, Chapter 24. Cholera toxin approaches target cell surface. B subunits bind to oligosaccharide of GM1 ganglioside. Conformational alteration of holotoxin occurs, allowing the presentation of the A subunit to cell surface. The A subunit enters the cell.

The disulfide bond of the A subunit is reduced by intracellular glutathione, freeing A1 and A2. NAD is hydrolyzed by A1, yielding ADP-ribose and nicotinamide. One of the G proteins of adenylate cyclase is ADP-ribosylated, inhibiting the action of GTPase and locking adenylate cyclase in the "on" mode.

Chapter 6 - Cholera Review

1. Cholera, which is derived from a Greek term meaning “Running to the bathroom,” is caused by Vibrio cholerae and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of minutes of infection.
A. True
B. False

2. In 1883, Louis Pasteur discovered V cholerae during a cholera outbreak in Egypt.
A. True
B. False

3. Cholera has been very common in industrialized nations for the last 100 years.
A. True
B. False

4. Cholera is always life-threatening, it is easily prevented and treated with chloramines.
A. True
B. False

5. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.
A. True
B. False

6. The V cholerae organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen.
A. True
B. False

7. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. V cholerae O1 or O139 are associated with epidemic cholera. V cholerae O1 has 2 major biotypes: classic and El Tor.
A. True
B. False

8. Currently, El Leche is the predominant cholera pathogen.
A. True
B. False

9. A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.
A. True
B. False

10. The cholera bacterium may also live in the environment in brackish rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.
A. True
B. False

11. Cholera (also called Asiatic flu) is a disease of the respiratory tract caused by the Vibrio cholerae bacterium. These bacteria are typically ingested by drinking water contaminated by improper sanitation or by eating improperly cooked fish, especially shellfish.
A. True
B. False

12. About one hundred Vibrio cholerae bacteria must be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a strong immune system, individuals with increased gastric acidity, or those who are malnourished.
A. True
B. False

13. Vibrio cholerae causes disease by producing a toxin that disables the _____ of G proteins which are part of G protein-coupled receptors in intestinal cells. This has the effect that the G proteins are locked in the "on position" binding GTP (normally, the G proteins quickly return to "off" by hydrolyzing GTP to GDP).
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

14. The _____ then cause adenylate cyclases to produce large amounts of cyclic AMP (cAMP) which results in the loss of fluid and salts across the lining of the gut.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

15. The resulting diarrhea allows the _____ to spread to other people under unsanitary conditions.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

16. When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an _____ supply of safe drinking water, and good food hygiene.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. None of the above

17. _____ variation plays an important role in the epidemiology and virulence of cholera. The emergence of the Bengal strain is an example.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

18. The _____ of V. cholerae are shared with many water vibrios and therefore are of no use in distinguishing strains causing epidemic cholera.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

19. O antigens, however, do distinguish strains of V. cholerae into 139 known _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

20. Almost all strains of V. cholerae are _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

21. Until the emergence of the Bengal strain (which is "non-O1") a single serotype, designated O1, has been responsible for epidemic cholera. However, there are three distinct O1 biotypes, named Ogawa, Inaba and Hikojima, and each biotype may display the "classical" or El Tor _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

22. E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT _____ is on a plasmid that can be transferred to other E. coli strains and probably to other enteric bacteria, as well.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

23. Close relationships between the genetic code for LT _____ and the cholera toxin undoubtedly exist but have not been documented as yet.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

24. The genetic information for the toxin in V. cholerae is located on the bacterial chromosome. Other bacterial _____ related to cholera toxin have been reported in non-group O Vibrio strains and a strain of Salmonella.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

25. _____, toxins which act in the GI tract, are produced by a wide variety of bacteria. The family of heat-stable (ST) enterotoxins of E. coli, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

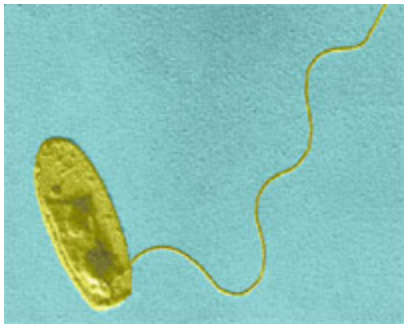
26. Other _____, which elicit cytotoxic effects on intestinal epithelial cells, have been described from Escherichia, Klebsiella, Enterobacter, Citrobacter, Aeromonas, Pseudomonas, Shigella, V. parahaemolyticus, Campylobacter, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, C. difficile, and Staphylococcus aureus.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

1.B, 2.B, 3.B, 4.B, 5.A, 6.A, 7.A, 8.B, 9.A,10.A, 11.B, 12.B, 13.A, 14.B, 15.C, 16.E, 17.D, 18.E, 19.C, 20.B, 21.D,22.A, 23.A, 24.D, 25.D, 26.D

Chapter 7 - Legionnaires' Disease - Legionella

Section Focus: You will learn the basics of legionella. At the end of this section, you will be able to describe legionella /Legionnaire's Disease. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of pneumonia at an American Legion convention led to 29 deaths. The causative agent, what would come to be known as Legionella pneumophila, was isolated and given its own genus. The organisms classified in this genus are Gram-negative bacteria that are considered intracellular parasites. The disease has two distinct forms:

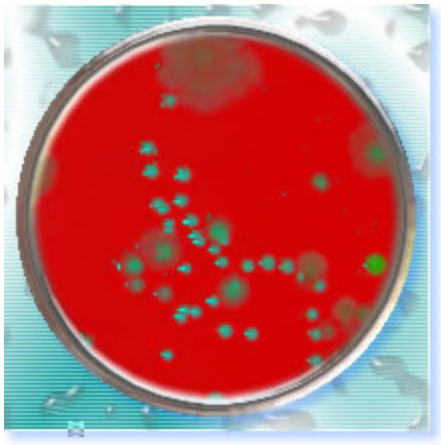


- Legionnaires' disease, the more severe form of infection which includes pneumonia, and
- Pontiac fever, a milder illness.

What have been the water sources for Legionnaires' disease?
The major source is water distribution systems of large buildings, including hotels and hospitals. Cooling towers have long been thought to be a major source for *Legionella*, but new data suggest that this is an overemphasized mode of transmission. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for Legionnaires' disease. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.

Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine aerosol containing Legionella bacteria.

Legionella bacteria are naturally found in environmental water sources such as rivers, lakes and ponds and may colonize man-made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.



How do people contract Legionella?
The most popular theory is that the organism is aerosolized in water and people inhale the droplets containing *Legionella*. However, new evidence suggests that another way of contracting *Legionella* is more common. "Aspiration" is the most common way that bacteria enter into the lungs to cause pneumonia.

Aspiration means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent aspiration is defective in patients who smoke or have lung disease. Aspiration now appears to be the most common mode of transmission.

Legionella may multiply to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.

Within one month, Legionella can multiply, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.

Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a routine maintenance program because equipment may become corroded.

Property owners have been sued for the spread of Legionella, resulting in expensive settlements. Regular monitoring with a battery of DFA monoclonal antibodies for several serogroups and species of Legionella morphologically intact bacteria provides a means for exercising 'reasonable care' to deter potential litigation.

Currently, there are no United States government regulations concerning permissible numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.

Epifluorescence Microscopy DFA Method
The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by ultraviolet light and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.

Most labs will provide a quantitative epifluorescence microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species (listed below).

Legionella anisa	*Legionella bozemanii* sg 1 & 2
Legionella dumoffi	*Legionella feeleii* sg 1 & 2
Legionella gormanii	*Legionella hackeliae* sg 1 & 2
Legionella jordanis	*Legionella longbeachae* sg 1& 2
Legionella maceachernii	*Legionella micdadei*
Legionella oakridgensis	*Legionella parisiensis*
Legionella pneumophila sg 1-14	*Legionella sainthelensi*
Legionella santicrucis	*Legionella wadsworthii*

Heterotrophic bacterial CFU are often inversely proportional to numbers of Legionella in cooling tower samples, in our experience. Routine biocide treatments will not eradicate Legionella bacteria in the environment, only in laboratory studies.

Culture methods are good during outbreaks for biotyping; but culture methods lack sensitivity for routine, quantitative monitoring.

Many factors will inhibit growth or identification of legionella on BCYE with or without antimicrobial agents, heat or acid treatment.

Culture methods will not identify non-culturable legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.

Direct fluorescent antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.

Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly aerobic rods.

The U.S. Environmental Protection Agency and the U.S. Occupational Safety and Health Administration recommend routine maintenance of water-containing equipment. Most State health departments recommend monthly testing for Legionella as part of a routine maintenance program.

As far as we know, there are no federal or state certification programs for laboratories that perform Legionella testing of environmental samples. Therefore, care must be taken when selecting a testing laboratory.

More on Legionnaires' Disease Medical Aspects

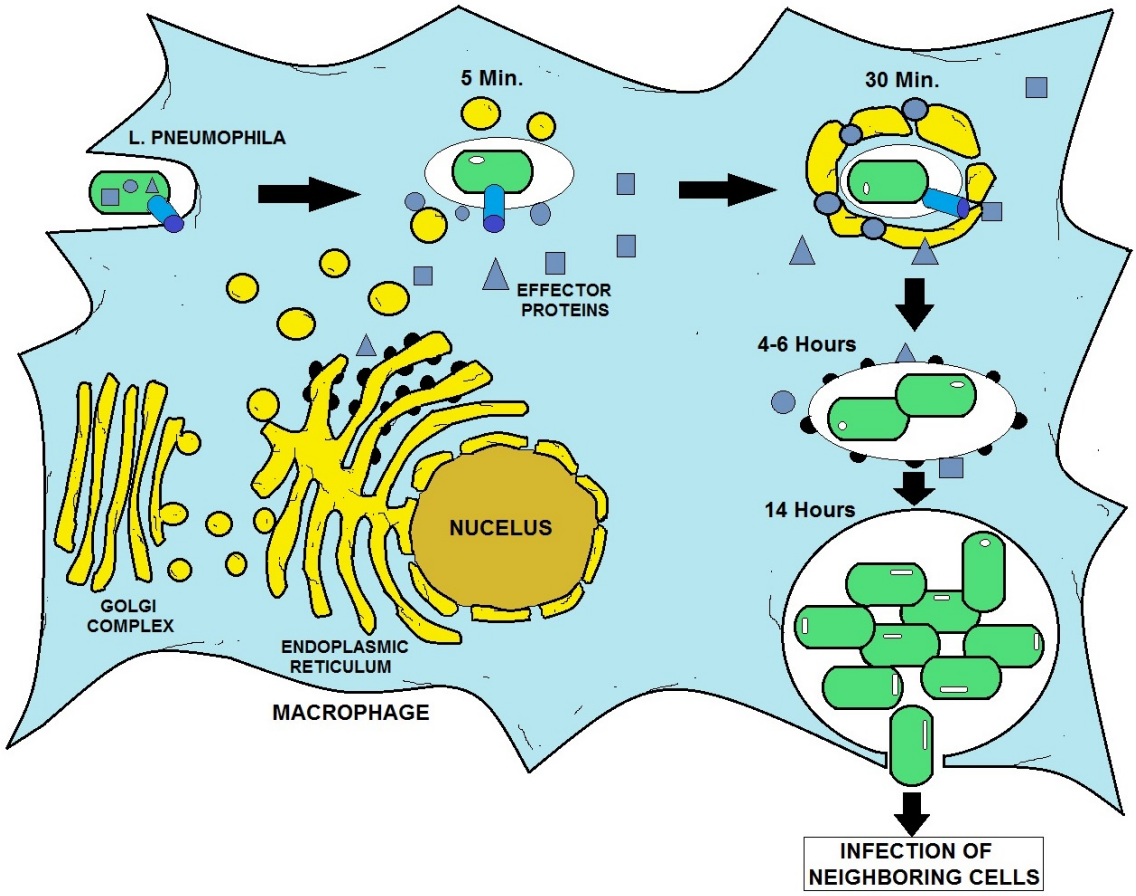
Legionnaires' disease is caused by bacteria that belong to the family Legionellaceae. This family now includes 48 species and over 70 serogroups. Approximately half of these species have been implicated in human disease. Legionella pneumophila is responsible for approximately 90% of infections.

Most cases are caused by L. pneumophila, serogroup 1. Legionella species are small (0.3 to 0.9 μm in width and approximately 2 μm in length) faintly staining Gram-negative rods with polar flagella (except L. oakridgensis). They generally appear as small coccobacilli in infected tissue or secretions.

They are distinguished from other saccharolytic bacteria by their requirement for L-cysteine and iron salts for primary isolation on solid media and by their unique cellular fatty acids and ubiquinones.

They grow well on buffered charcoal yeast extract agar, but it takes about five days to get sufficient growth. When grown on this medium, Legionella colonies appear off-white in color and circular in shape.

Laboratory identification can also include microscopic examination in conjunction with a direct flourescent antibody (DFA) test. Since the initial discovery, many species have been added to the Legionella genus, but only two are within the scope of our discussion.



INFECTION CYCLE OF L. PNEUMOPHILA

L. pneumophila and L. micdadei

L. pneumophila
L. pneumophila is the bacterium associated with Legionnaires' disease and Pontiac fever. Respiratory transmission of this organism can lead to infection, which is usually characterized by a gradual onset of flu-like symptoms.

Patients may experience fever, chills, and a dry cough as part of the early symptoms. Patients can develop severe pneumonia which is not responsive to penicillins or aminoglycosides.

Legionnaires' disease also has the potential to spread into other organ-systems of the body such as the gastrointestinal tract and the central nervous system. Accordingly, patients with advanced infections may experience diarrhea, nausea, disorientation, and confusion.

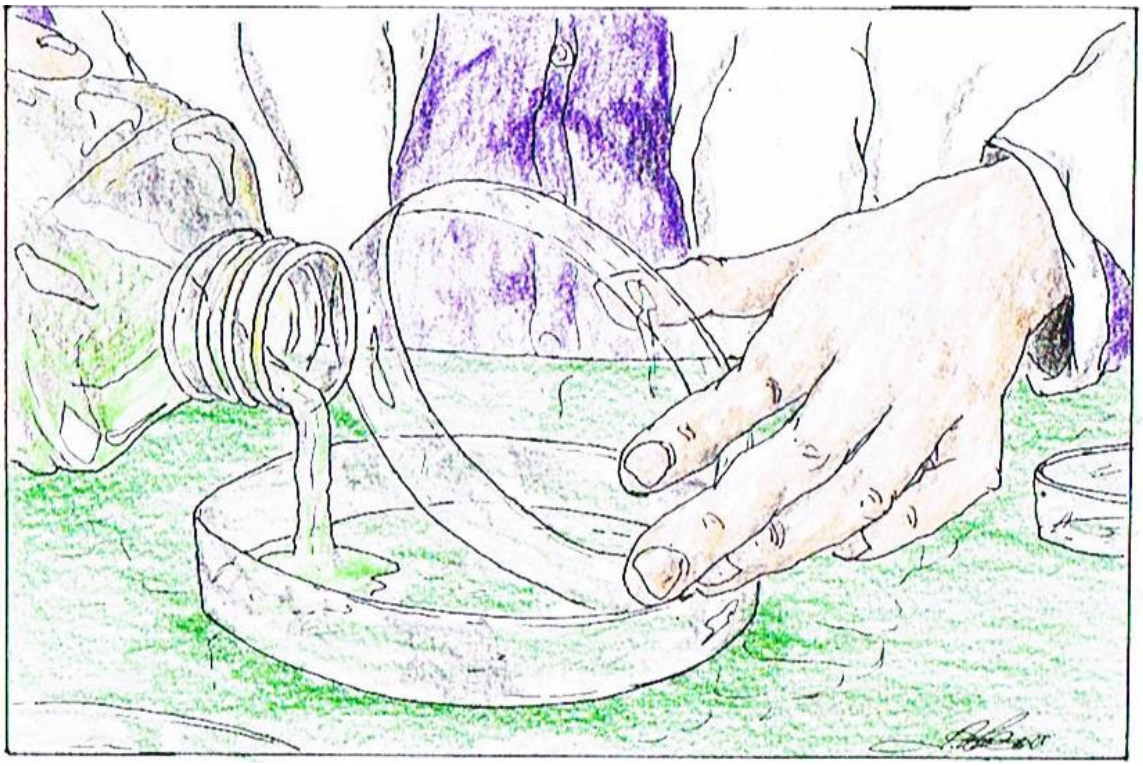
The 1200 or so cases of Legionnaires' disease per year in the United States usually involve middle-aged or immunosuppressed individuals. Pontiac fever is also caused by *L. pneumophila* but does not produce the severity of the symptoms found in Legionnaires' disease.

The flu-like symptoms are still seen in Pontiac fever patients, but pneumonia does not develop and infection does not spread beyond the lungs. Most *L. pneomophila* infections are easily treated with erythromycin.

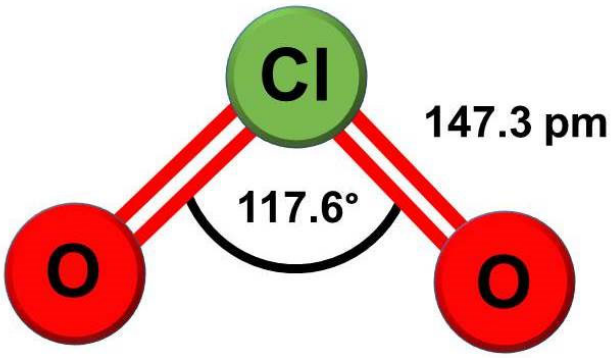
Laboratory Indications
- Beta-lactamase +
- Hippurate hydrolysis +

L. micdadei
L. micdadei is the second most commonly isolated member of *Legionella*. This bacterium can cause the same flu-like symptoms and pneomonia which characterize an *L. pneumophila* infection. Unlike its relative, *L. micdadei* is sensitive to the penicillins because it does not produce beta-lactamase.

Laboratory Indications
- Beta-lactamase -
- Hippurate hydrolysis -
- Acid fast



Chlorine Dioxide



CHLORINE DIOXIDE

Prevention and Control
In the prevention and control of Legionnaires disease (legionella) causing microbes, chlorine dioxide has taken an eminent roll. The specific characteristics of the disinfectant make sure ClO^2 gets the job done where others fail.

Biofilm in the piping can protect legionella from most of the disinfectants.

Chlorine dioxide however removes the biofilm and kills the bacteria, spores and viruses.

Other advantages are:
1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. The required contact time for ClO^2 is lower;
3. Chlorine dioxide has better solubility;
4. Chlorine dioxide does not react with NH3 or NH4+;
5. It destroys THM precursors and increases coagulation;
6. ClO^2 destroys phenols and has no distinct smell.

Hot and cold water systems
The advantages in using chlorine dioxide with hot and cold water systems have already been shown at the descriptions on legionella. There are however more advantages:

1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. Chlorine dioxide is clearly superior to chlorine in the destruction of spores, bacteria's, viruses and other pathogen organisms on an equal residual base (even cryptosporidium and giardia);
3. The required contact time for ClO^2 is lower;
4. Chlorine dioxide has better solubility;
5. No corrosion associated with high chlorine concentrations. Reduces long term maintenance costs;
6. Chlorine dioxide does not react with NH3 or NH4+;
7. It destroys THM precursors and increases coagulation;
8. ClO^2 destroys phenols and has no distinct smell;

9. It is better at removing iron and magnesia compounds than chlorine, especially complex bounds.

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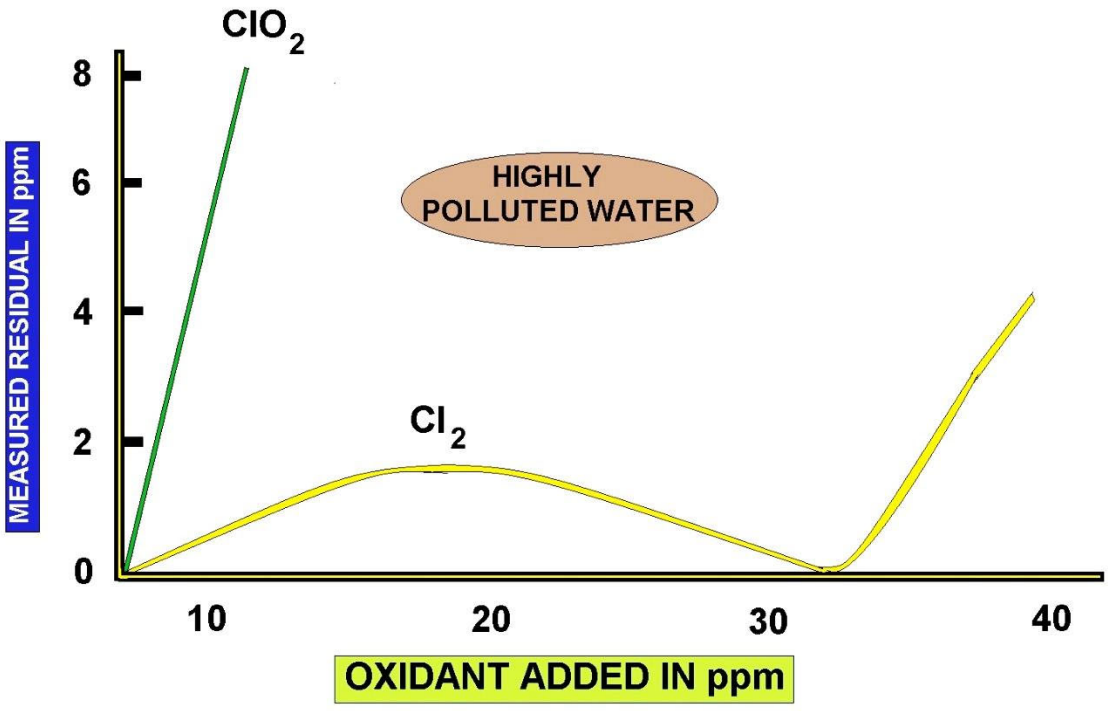


USING CHLORINE DIOXIDE vs CHLORINE

Chapter 7 - Legionella Review

1. The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of _____ at an American Legion convention led to 29 deaths.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

2. The causative agent, what would come to be known as _____, was isolated and given its own genus.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

3. The organisms classified in this genus are Gram-negative bacteria that are considered _____ .
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Intracellular parasites
E. None of the above

4. Legionnaires' disease is the more severe form of infection which includes pneumonia; _____is a milder illness.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

5. The major source is water distribution systems of large buildings including hotels and hospitals. Cooling towers have long been thought to be a major source for _____, but new data suggest that this is an overemphasized mode of transmission.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella
E. None of the above

6. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for _____. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

7. _____ is the most common way that bacteria enter into the lungs to cause pneumonia. It means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent it is defective in patients who smoke or have lung disease.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

8. Legionella may _____ to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

9. Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine _____ containing Legionella bacteria.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

10. Legionella bacteria are _____ in environmental water sources such as rivers, lakes and ponds and may colonise man made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

11. Within one month, Legionella can _____, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

12. Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a _____ because equipment may become corroded.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

13. Currently, there are no United States government regulations concerning _____ numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

14. The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by _____ and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

15. Most labs will provide a _____ microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative epifluorescence

16. Culture methods will not identify _____ legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

17. Direct _____ antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

18. Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly _____.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

19. Legionnaires' disease is caused by bacteria that belong to the family _____.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

20. This family now includes 48 species and over 70 _____. Approximately half of these species have been implicated in human disease.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

21. _____ is responsible for approximately 90% of infections.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

1.B, 2.D, 3.D, 4.C, 5.D, 6.A, 7.B, 8.E, 9.C, 10.D, 11.E, 12.A, 13.B, 14.C, 15.E, 16.B, 17.D, 18.A, 19.C, 20.D, 21.E

Chapter 8- Escherichia Coli *More information in the Appendix*

Section Focus: You will learn the basics of Escherichia coli. At the end of this section, you will be able to describe E.coli. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: *Escherichia coli* (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

Escherichia coli

Two types of pathogenic Escherichia coli, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.

E. coli O157:H7

E. coli O157:H7 (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.

Escherichia coli O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illnesses have been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water.

Consumers can prevent *E. coli* O157:H7 infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully. Because the organism lives in the intestines of healthy cattle, preventive measures on cattle farms and during meat processing are being investigated.

What is *Escherichia coli* O157:H7?

E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.

E. coli O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli.*

Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7.

E. coli is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces.

Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains are capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*.

How does *E. coli* or other fecal coliforms get in the water?
E. coli comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water.

How is water treated to protect me from *E. coli*?
The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli.* Systems using surface water sources are required to disinfect to ensure that all bacterial contamination such as *E. coli*. is inactivated. Systems using ground water sources are not required to disinfect, although many of them do.

How does the U.S. Environmental Protection Agency regulate *E. coli*?
According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the Safe Drinking Water Act. If a system is not a public water system as defined by EPA regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities.

Under the Safe Drinking Water Act, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

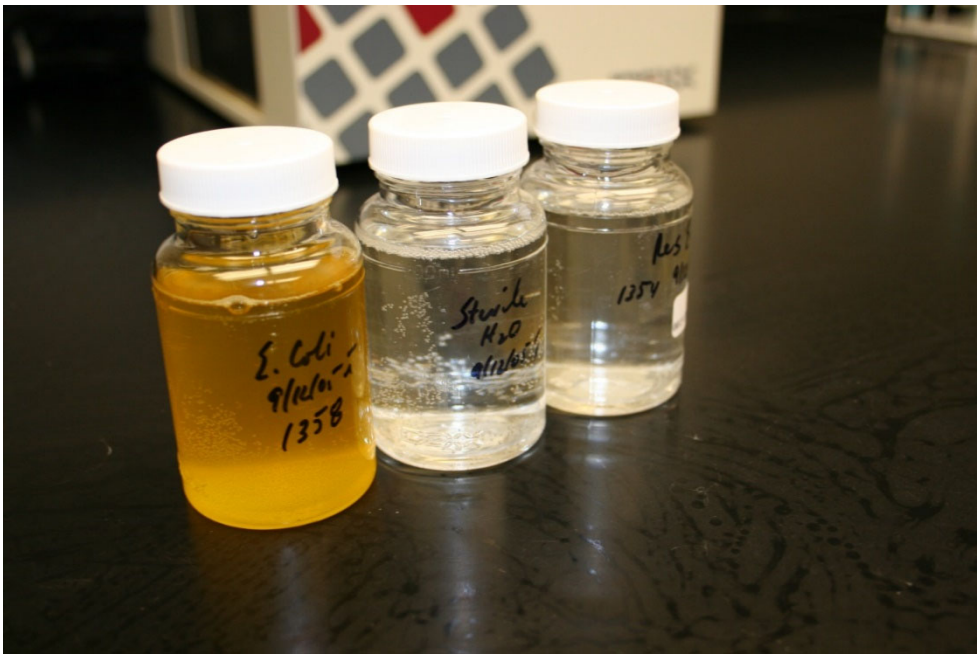
The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last five years.

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection will kill *E. coli* O157:H7.

What can I do to protect myself from *E. coli* O157:H7 in drinking water?
Approximately 89 percent of Americans are receiving water from community water systems that meet all health-based standards. Your public water system is required to notify you if, for any reason, your drinking water is not safe. If you wish to take extra precautions, you can boil your water for one minute at a rolling boil, longer at higher altitudes.

To find out more information about your water, see the Consumer Confidence Report from your local water supplier or contact your local water supplier directly. You can also obtain information about your local water system on the EPA's website at www.epa.gov/safewater/dwinfo.htm.

Positive Tests
If you draw water from a private well, you can contact your state health department to obtain information on how to have your well tested for total coliforms and *E. coli* contamination. If your well tests positive for *E. coli*, there are several steps that you should take: (1) begin boiling all water intended for consumption, (2) disinfect the well according to procedures recommended by your local health department, and (3) monitor your water quality to make certain that the problem does not recur. If the contamination is a recurring problem, you should investigate the feasibility of drilling a new well or install a point-of-entry disinfection unit, which can use chlorine, ultraviolet light, or ozone.



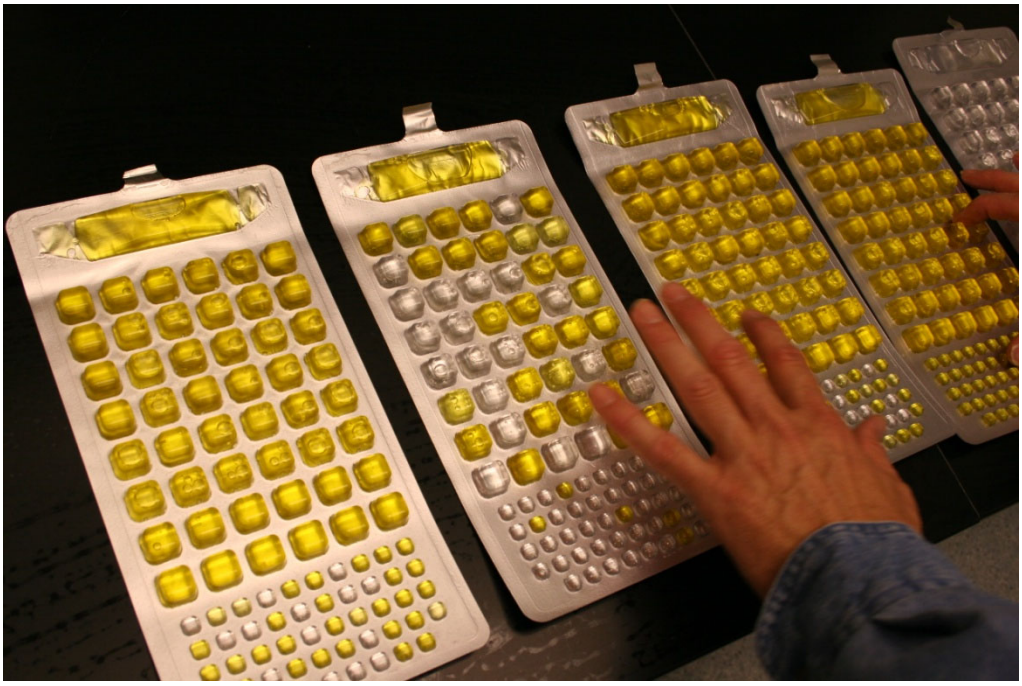
How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment may get into raw milk. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7 can cause infection. Contaminated meat looks and smells normal. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

Among other known sources of infection are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water.

Bacteria in diarrheal stools of infected persons can be passed from one person to another if hygiene or handwashing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected.
Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms.

What illness does *E. coli* O157:H7 cause?
E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Hemorrhagic colitis is the name of the acute disease caused by *E. coli* O157:H7.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.



How is *E. coli* O157:H7 infection diagnosed?
Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not test for *E. coli* O157:H7, so it is important to request that the stool specimen be tested on sorbitol-MacConkey (SMAC) agar for this organism. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli* O157:H7.

How is the illness treated?
Most persons recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (Imodium), should also be avoided. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit.

Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5%.

What are the long-term consequences of infection?
Persons who only have diarrhea usually recover completely. About one-third of persons with hemolytic uremic syndrome have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of persons with hemolytic uremic syndrome have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed.

What can be done to prevent the infection?
E. coli O157:H7 will continue to be an important public health concern as long as it contaminates meat. Preventive measures may reduce the number of cattle that carry it and the contamination of meat during slaughter and grinding. Research into such prevention measures is just beginning.

What can you do to prevent *E. coli* O157:H7 infection?
Cook all ground beef and hamburger thoroughly. Because ground beef can turn brown before disease-causing bacteria are killed, use a digital instant-read meat thermometer to ensure thorough cooking. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 160º F. Persons who cook ground beef without using a thermometer can decrease their risk of illness by not eating ground beef patties that are still pink in the middle.

If you are served an undercooked hamburger or other ground beef product in a restaurant, send it back for further cooking. You may want to ask for a new bun and a clean plate, too. Avoid spreading harmful bacteria in your kitchen. Keep raw meat separate from ready-to-eat foods. Wash hands, counters, and utensils with hot soapy water after they touch raw meat. Never place cooked hamburgers or ground beef on the unwashed plate that held raw patties. Wash meat thermometers in between tests of patties that require further cooking. Drink only pasteurized milk, juice, or cider.

Commercial juice with an extended shelf-life that is sold at room temperature (e.g. juice in cardboard boxes, vacuum sealed juice in glass containers) has been pasteurized, although this is generally not indicated on the label. Juice concentrates are also heated sufficiently to kill pathogens.

Wash fruits and vegetables thoroughly, especially those that will not be cooked. Children under 5 years of age, immunocompromised persons, and the elderly should avoid eating alfalfa sprouts until their safety can be assured. Methods to decontaminate alfalfa seeds and sprouts are being investigated.

Drink municipal water that has been treated with chlorine or other effective disinfectants. Avoid swallowing lake or pool water while swimming. Make sure that persons with diarrhea, especially children, wash their hands carefully with soap after bowel movements to reduce the risk of spreading infection, and that persons wash hands after changing soiled diapers. Anyone with a diarrheal illness should avoid swimming in public pools or lakes, sharing baths with others, and preparing food for others.

Chapter 8 - References

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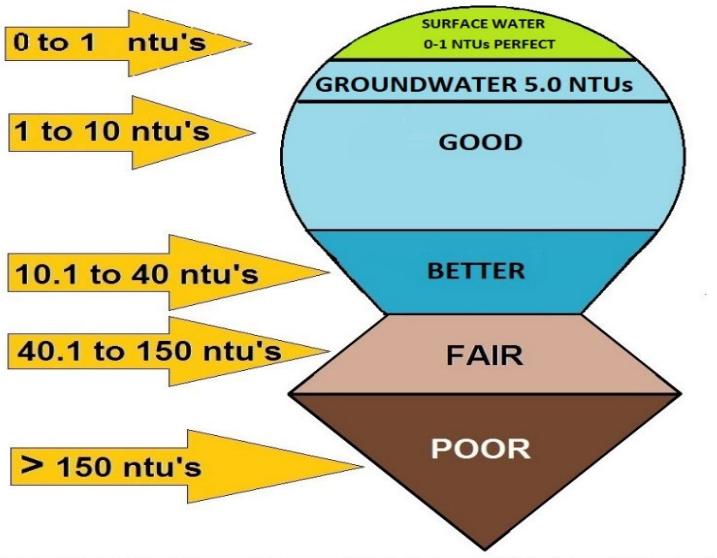
Turbidity

One physical characteristic of water is turbidity. A measure of the cloudiness of water caused by suspended particles. The cloudy appearance of water caused by the presence of tiny particles. High levels of turbidity may interfere with proper water treatment and monitoring. If high quality raw water is low in turbidity, there will be a reduction in water treatment costs. Turbidity is undesirable because it causes health hazards.

An MCL for turbidity established by the EPA because turbidity interferes with disinfection. This characteristic of water changes the most rapidly after a heavy rainfall. The following conditions may cause an inaccurate measure of turbidity; the temperature variation of a sample, a scratched or unclean sample tube in the nephelometer and selecting an incorrect wavelength of a light path.



TURBIDITY PARAMETERS (NTU) FOR WATER QUALITY

Surface Water System Compliance Information
(Depends on Systems and Rule)

- 0.34 NTU in 95% of samples, never to exceed 1.0 NTU spike
- Sample turbidity at each individual filter effluent
- Sample the combined filter turbidity at the clear well
- (Groundwater turbidity = 5.0 NTU)

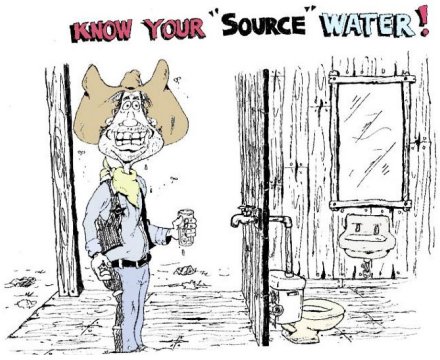
Turbidity Key

- Turbidity can also be measured in ppm (parts per million) and its size is measured in microns. Turbidity can be particles in the water consisting of finely divided solids, larger than molecules, but not visible by the naked eye; ranging in size from .001 to .150mm (1 to 150 microns).

- 0.34 NTU in 95% of surface water samples, never to exceed 1.0 NTU spike

Troubleshooting Table for Sampling Monitoring

Problems
1. Positive Total Coliform.
2. Chlorine taste and odor.
3. Inability to maintain an adequately free chlorine residual at the furthest points of the distribution system or at dead end lines.



Possible Causes
1A. Improper sampling technique.
1B. Contamination entering distribution system.
1C. Inadequate chlorine residual at the sampling site.
1D. Growth of biofilm in the distribution system.
2A. High total chlorine residual and low free residual.
3A. Inadequate chlorine dose at treatment plant.
3B. Problems with chlorine feed equipment.
3C. Ineffective distribution system flushing program.
3D. Growth of biofilm in the distribution system.

Possible Solution
1A. Check distribution system for low pressure conditions, possibly due to line breaks or excessive flows that may result in a backflow problem.
1B. Insure that all staff are properly trained in sampling and transport procedures as described in the TCR.
1C. Check the operation of the chlorination feed system. Refer to issues described in the sections on pumps and hypochlorination systems. Insure that residual test is being performed properly.
1D. Thoroughly flush effected areas of the distribution system. Superchlorination may be necessary in severe cases.
2A. The free residual should be at least 85% of the total residual. Increase the chlorine dose rate to get past the breakpoint in order to destroy some of the combined residual that causes taste and odor problems. Additional system flushing may also be required.
3A. Increase chlorine feed rate at point of application.
3B. Check operation of chlorination equipment.
3C. Review distribution system flushing program and implement improvements to address areas of inadequate chlorine residual.
3D. Increase flushing in area of biofilm problem.

Disinfection Key
- Contact time is required
- 99% or 2 log inactivation of crypto
- 99.9% or 3 log inactivation of giardia lamblia cysts
- 99.99% or 4 log inactivation of enteric viruses
- CT = Concentration of disinfectant x contact time
- The chlorine residual leaving the plant must be = or > 0.2 mg/L and measurable throughout the system.

Pathogenic *Escherichia coli* MLST Database – Clonal Group Definition

Clonal Group	Class	Reference Strain	Comments
0	N/A	TW08017	Undefined Clonal Group
1	atypical B13	TW08889	cluster within E. coli with Boydii 13 (B13) antigen
2	STEC 13	TW08045	includes STEC R:H18 strain
3	EPEC 3	TW06584	O86:H34 strains from infant diarrhea
4	H51	TW08260	cluster with H51 antigen
5	NT-1	TW08997	no common traits
6	EPEC 1	TW06375	Classical EPEC with H6
7	EPEC 4	TW03173	O119:H6 strains, basal to EPEC 1
8	NT-2	TW08983	no common traits
9	NT-3	TW08990	no common traits
10	Shigella 3	TW08837	Reeves Shigella Group 3 including serotypes F1a, F2a, F3a, F3b, F4, F5a FY
11	EHEC 1	TW08264	O157:H7 and relatives including atypical EPEC O55:H7
12	STEC 12	TW00964	STEC O145 strains
13	ETEC P	TW00601	ETEC from pigs including O157:H43 clone
14	EHEC 2	TW00970	O26:H11 and O111:H8 and relatives including RDEC
15	NT-4	TW09177	no common traits
16	EIEC 2	TW01095	Invasive strains with O type 29, 124, 152, 164
17	EPEC 2	TW01120	Classical EPEC with H2 antigen
18	STEC 8	TW04909	Serotype O104:H21, includes Montana outbreak strain G5506
19	STEC 9	TW08580	Serotype O174:H8
20	STEC 10	TW07618	Various serotypes
21	STEC 11	TW07613	Serotype O111:H28
22	EIEC 1	TW01116	Invasive strains with O types 29, 124, 152, 164
23	ECOR A	TW00073	Includes atypical EPEC O111:H12
24	STEC 3	TW08023	Serotype O121:H19
25	Shigella 1	TW07572	Reeves Shigella Group 1 including serotypes F6, D3, D6, D7, D9, D11, D12, B1, B2, B3, B4, B8, B10, B14, B15, B18
26	Shigella 2a	TW02615	Reeves Shigella Group 2 including serotypes D2, D4, B9, B15
27	Shigella 2b	TW01151	Reeves Shigella Group 2 including serotypes B5, B11, B17
28	EPEC 5	TW04892	Atypical EPEC with serotype O111:H9, includes Finland outbreak strain 921
29	Sonnei	TW01150	Shigella sonnei
30	STEC 2	TW01391	Includes serotype O113:H21 and ECOR 30
31	NT-5	TW00676	no common traits

32	NT-6	TW07795	no common traits
33	NT-13	TW09011	no common traits
34	STEC 1	TW01393	STEC with H21 antigen including B2F1
35	SMEC 1	TW02268	Extraintestinal strains including RS218
36	NT-7	TW07608	no common traits
37	Sand 2	TW09237	Environmental E. coli clone
38	UTI 1	TW08018	Uropathogens including CFT073
39	STEC 4	TW07995	Mix of serotypes O121, O116, also includes an EIEC 1758-70
40	NT-8	TW09214	no common traits
41	STEC 14	TW08574	Serotype O174:H2
42	NT-7	TW07612	no common traits
43	STEC	TW01670	serotype O8:H19
44	NT-10	TW10091	no comment traits
45	NT-11	TW08942	no comment traits
46	NT-12	TW09069	no comment traits

Quebec Colony Counter

Chapter 8 - Escherichia Coli Review

1. _____ is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

2. _____ was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

3. Two types of pathogenic _____, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

4. _____ (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

5. _____O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

6. The combination of letters and numbers in the name of the _____ refers to the specific markers found on its surface and distinguishes it from other types of E. coli.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

7. Currently, there are four recognized classes of enterovirulent E. coli (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the _____ (EHEC) strain designated E. coli O157:H7.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

8. E. coli is a normal inhabitant of the intestines of all animals, including humans. When _____ are used, E. coli is the dominant species found in feces.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

9. _____ are bacteria that are associated with human or animal wastes. They usually live in human or animal intestinal tracts, and their presence in drinking water is a strong indication of recent sewage or animal waste contamination.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

10. The water can be treated using _____, ultra-violet light, or ozone, all of which act to kill or inactivate E. coli. Systems using surface water sources are required to disinfect to ensure that all bacterial contamination is inactivated, such as E. coli. Systems using ground water sources are not required to disinfect, although many of them do.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

11. According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the _____.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

12. If a system is not a public water system as defined by EPA regulations, it is not regulated under the _____, although it may be regulated by state or local authorities.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

13. Under the _____, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or E. coli. Both are indicators of contamination with animal waste or human sewage.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

14. The largest public water systems (serving millions of people) must take at least 50 samples per month. Smaller systems must take at least 20 samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last year.
A. True
B. False

15. Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect.
A. True
B. False

16. Disinfection will kill E. coli O157:H7.
A. True
B. False
1.C, 2.C, 3.B, 4.C, 5.B, 6.D, 7.A, 8.E, 9.D, 10.E, 11.A, 12.A, 13.A, 14.B, 15.A, 16.A

Waterborne Diseases

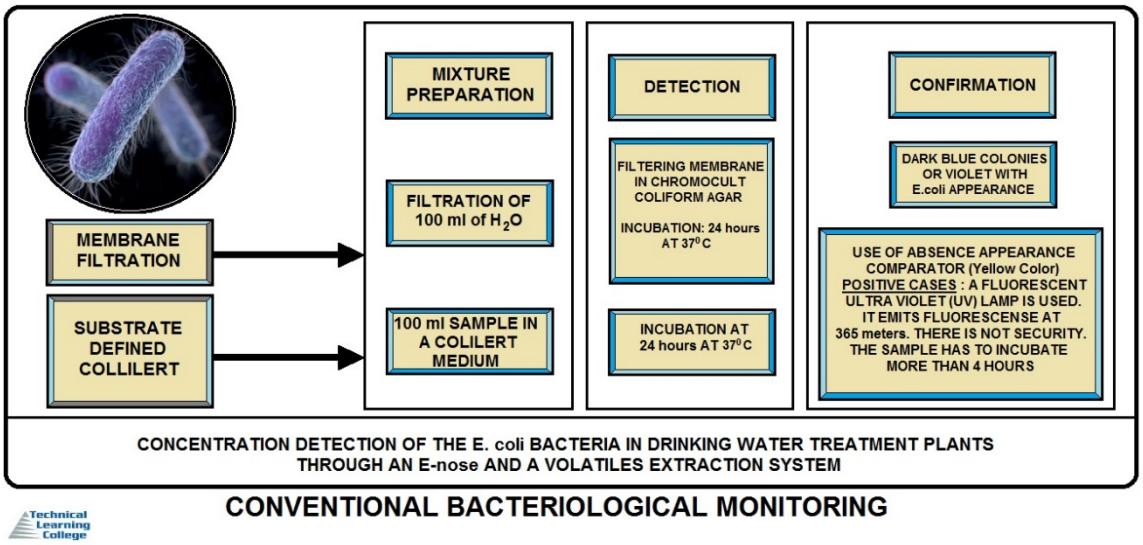
Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) *1	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:
*1 **http://www.cdc.gov/**

Chapter 9- Related Diseases and Associated Illnesses

Section Focus: You will learn the basics of commonly found waterborne diseases that have not been covered. At the end of this section, you will be able to describe common waterborne diseases. There is a post quiz at the end of this section to review your comprehension and a final examination in the Assignment for your contact hours.

Scope/Background: Water providers should be alert to illness patterns and diagnostic clues that might indicate an unusual infectious disease outbreak associated with intentional release of a biologic agent and should report any clusters or findings to their local or state health department.



CONVENTIONAL BACTERIOLOGICAL MONITORING

The covert release of a biologic agent may not have an immediate impact because of the delay between exposure and illness onset, and outbreaks associated with intentional releases might closely resemble naturally occurring outbreaks. Indications of intentional release of a biologic agent include
1) an unusual temporal or geographic clustering of illness (e.g., persons who attended the same public event or gathering) or patients presenting with clinical signs and symptoms that suggest an infectious disease outbreak (e.g., ≥2 patients presenting with an unexplained febrile illness associated with sepsis, pneumonia, respiratory failure, or rash or a botulism-like syndrome with flaccid muscle paralysis, especially if occurring in otherwise healthy persons);
2) an unusual age distribution for common diseases (e.g., an increase in what appears to be a chickenpox-like illness among adult patients, but which might be smallpox); and
3) a large number of cases of acute flaccid paralysis with prominent bulbar palsies, suggestive of a release of *botulinum* toxin.

CDC defines three categories of biologic agents with potential to be used as weapons, based on ease of dissemination or transmission, potential for major public health impact (e.g., high mortality), potential for public panic and social disruption, and requirements for public health preparedness.

Agents of highest concern are *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), variola major (smallpox), *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia), filoviruses (Ebola hemorrhagic fever, Marburg hemorrhagic fever); and arenaviruses (Lassa [Lassa fever], Junin [Argentine hemorrhagic fever], and related viruses). The following summarizes the clinical features of these agents.

Anthrax
A nonspecific prodrome (i.e., fever, dyspnea, cough, and chest discomfort) follows inhalation of infectious spores. Approximately 2--4 days after initial symptoms, sometimes after a brief period of improvement, respiratory failure and hemodynamic collapse ensue. Inhalational anthrax also might include thoracic edema and a widened mediastinum on chest radiograph. Gram-positive bacilli can grow on blood culture, usually 2--3 days after onset of illness.

Cutaneous anthrax follows deposition of the organism onto the skin, occurring particularly on exposed areas of the hands, arms, or face. An area of local edema becomes a pruritic macule or papule, which enlarges and ulcerates after 1--2 days. Small, 1--3 mm vesicles may surround the ulcer. A painless, depressed, black eschar, usually with surrounding local edema, subsequently develops. The syndrome also may include lymphangitis and painful lymphadenopathy.

Plague
Clinical features of pneumonic plague include fever, cough with muco-purulent sputum (gram-negative rods may be seen on gram stain), hemoptysis, and chest pain. A chest radiograph will show evidence of bronchopneumonia.

Botulism
Clinical features include symmetric cranial neuropathies (i.e., drooping eyelids, weakened jaw clench, and difficulty swallowing or speaking), blurred vision or diplopia, symmetric descending weakness in a proximal to distal pattern, and respiratory dysfunction from respiratory muscle paralysis or upper airway obstruction without sensory deficits. Inhalational botulism would have a similar clinical presentation as foodborne botulism; however, the gastrointestinal symptoms that accompany foodborne botulism may be absent.

Smallpox (variola)
The acute clinical symptoms of smallpox resemble other acute viral illnesses, such as influenza, beginning with a 2--4 day nonspecific prodrome of fever and myalgias before rash onset. Several clinical features can help clinicians differentiate varicella (chickenpox) from smallpox. The rash of varicella is most prominent on the trunk and develops in successive groups of lesions over several days, resulting in lesions in various stages of development and resolution. In comparison, the vesicular/pustular rash of smallpox is typically most prominent on the face and extremities, and lesions develop at the same time.

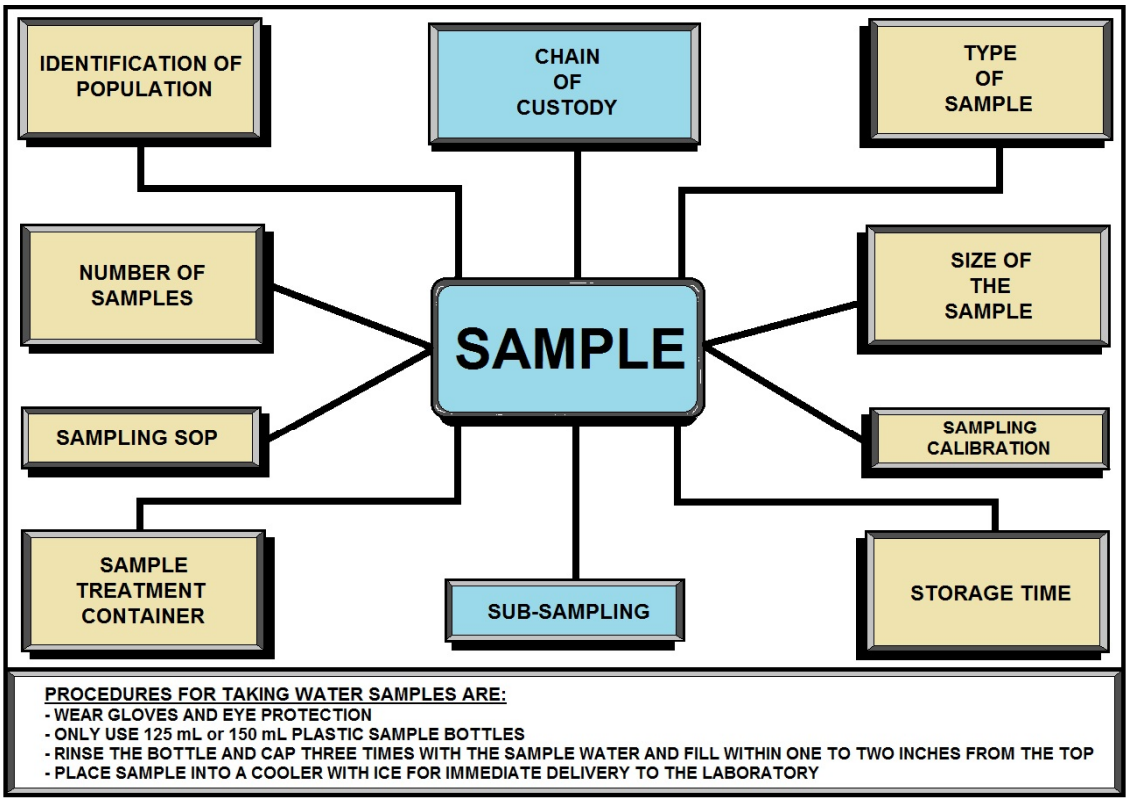
Inhalational tularemia
Inhalation of *F. tularensis* causes an abrupt onset of an acute, nonspecific febrile illness beginning 3--5 days after exposure, with pleuropneumonitis developing in a substantial proportion of cases during subsequent days.

Hemorrhagic fever (such as would be caused by Ebola or Marburg viruses).
After an incubation period of usually 5--10 days (range: 2--19 days), illness is characterized by abrupt onset of fever, myalgia, and headache. Other signs and symptoms include nausea and vomiting, abdominal pain, diarrhea, chest pain, cough, and pharyngitis. A maculopapular rash, prominent on the trunk, develops in most patients approximately 5 days after onset of illness. Bleeding manifestations, such as petechiae, ecchymoses, and hemorrhages, occur as the disease progresses.

Laboratory Personnel
Although unidentified gram-positive bacilli growing on agar may be considered as contaminants and discarded, CDC recommends that these bacilli be treated as a "finding" when they occur in a suspicious clinical setting (e.g., febrile illness in a previously healthy person).

The laboratory should attempt to characterize the organism, such as motility testing, inhibition by penicillin, absence of hemolysis on sheep blood agar, and further biochemical testing or species determination.

An unusually high number of samples, particularly from the same biologic medium (e.g., blood and stool cultures), may alert laboratory personnel to an outbreak. In addition, central laboratories that receive clinical specimens from several sources should be alert to increases in demand or unusual requests for culturing (e.g., uncommon biologic specimens such as cerebrospinal fluid or pulmonary aspirates).



PROPER SAMPLING PROCEDURES (WATER)

Collection of Samples

When collecting or handling specimens, laboratory personnel should:

1) use Biological Safety Level II (BSL-2) or Level III (BSL-3) facilities and practices when working with clinical samples considered potentially infectious;
2) handle all specimens in a BSL-2 laminar flow hood with protective eyewear (e.g., safety glasses or eye shields), use closed-front laboratory coats with cuffed sleeves, and stretch the gloves over the cuffed sleeves;
3) avoid any activity that places persons at risk for infectious exposure, especially activities that might create aerosols or droplet dispersal;
4) decontaminate laboratory benches after each use and dispose of supplies and equipment in proper receptacles;
5) avoid touching mucosal surfaces with their hands (gloved or ungloved), and never eat or drink in the laboratory; and
6) remove and reverse their gloves before leaving the laboratory and dispose of them in a biohazard container, and wash their hands and remove their laboratory coat.

When a laboratory is unable to identify an organism in a clinical specimen, it should be sent to a laboratory where the agent can be characterized, such as the state public health laboratory or, in some large metropolitan areas, the local health department laboratory.

Any clinical specimens suspected to contain variola (smallpox) should be reported to local and state health authorities and then transported to CDC. All variola diagnostics should be conducted at CDC laboratories. Clinical laboratories should report any clusters or findings that could indicate intentional release of a biologic agent to their state and local health departments.

After the terrorist attacks of September 11, state and local health departments initiated various activities to improve surveillance and response, ranging from enhancing communications (between state and local health departments and between public health agencies and health-care providers) to conducting special surveillance projects.

These special projects have included active surveillance for changes in the number of hospital admissions, emergency department visits, and occurrence of specific syndromes. Activities in bioterrorism preparedness and emerging infections over the past few years have better positioned public health agencies to detect and respond to the intentional release of a biologic agent. Immediate review of these activities to identify the most useful and practical approaches will help refine syndrome surveillance efforts in various clinical situations.

Additional information about responding to bioterrorism is available from CDC at <http://www.bt.cdc.gov>; the U.S. Army Medical Research Institute of Infectious Diseases at <http://www.usamriid.army.mil/education/bluebook.html>; the Association for Infection Control Practitioners at <http://www.apic.org>; and the Johns Hopkins Center for Civilian Biodefense at <http://www.hopkins-biodefense.org>.

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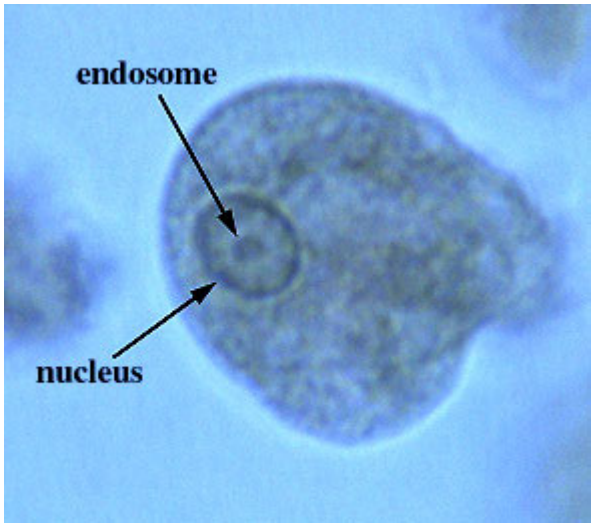
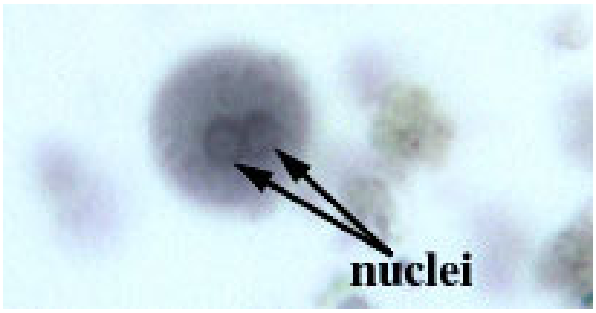
Amebiasis *Entamoeba histolytica Sub*-Section

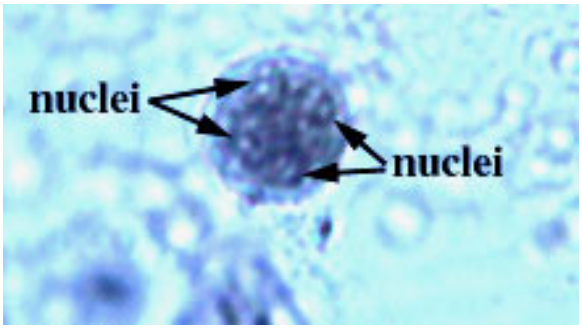
The life cycle of *Entamoeba histolytica* involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and cysts that are passed in the host's feces. Humans are infected by ingesting cysts, most often via food or water contaminated with human fecal material (view diagram of the life cycle). The trophozoites can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, *E. histolytica* is potentially the most pathogenic.



Entamoeba histolytica *trophozoite*



Entamoeba histolytica *immature cyst*



Entamoeba histolytica *mature cyst*

Entamoeba histolytica is an amoeboid protozoan parasite of the intestinal tract and in some cases other visceral organs especially the liver. There are several species in this genus, distinguished by their number of nuclei in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen. *Entamoeba histolytica* has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal crypts, feeding on intestinal contents and host tissue, and multiplying by fission.

Trophozoites
The trophozoites can be carried out in the feces. As the feces pass through the colon they dehydrate. The dehydration of the feces causes the trophozoites to begin the process of encystment. Undigested food is discharged, and the trophozoite condenses and forms a spherical shape to form what is called the pre-cyst, and the cyst wall is secreted. Within the cyst there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst.

The cyst can resist desiccation for 1-2 weeks. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 trophozoites. In some cases the trophozoites secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.

Extensive Tissue Destruction
These can form large abscesses that may allow the parasite to enter the blood stream and be carried to the liver and other organs. In these extra-intestinal sites the trophozoites also can cause extensive tissue destruction. If the intestinal tissue has been invaded the feces can be bloody and diarrheic.

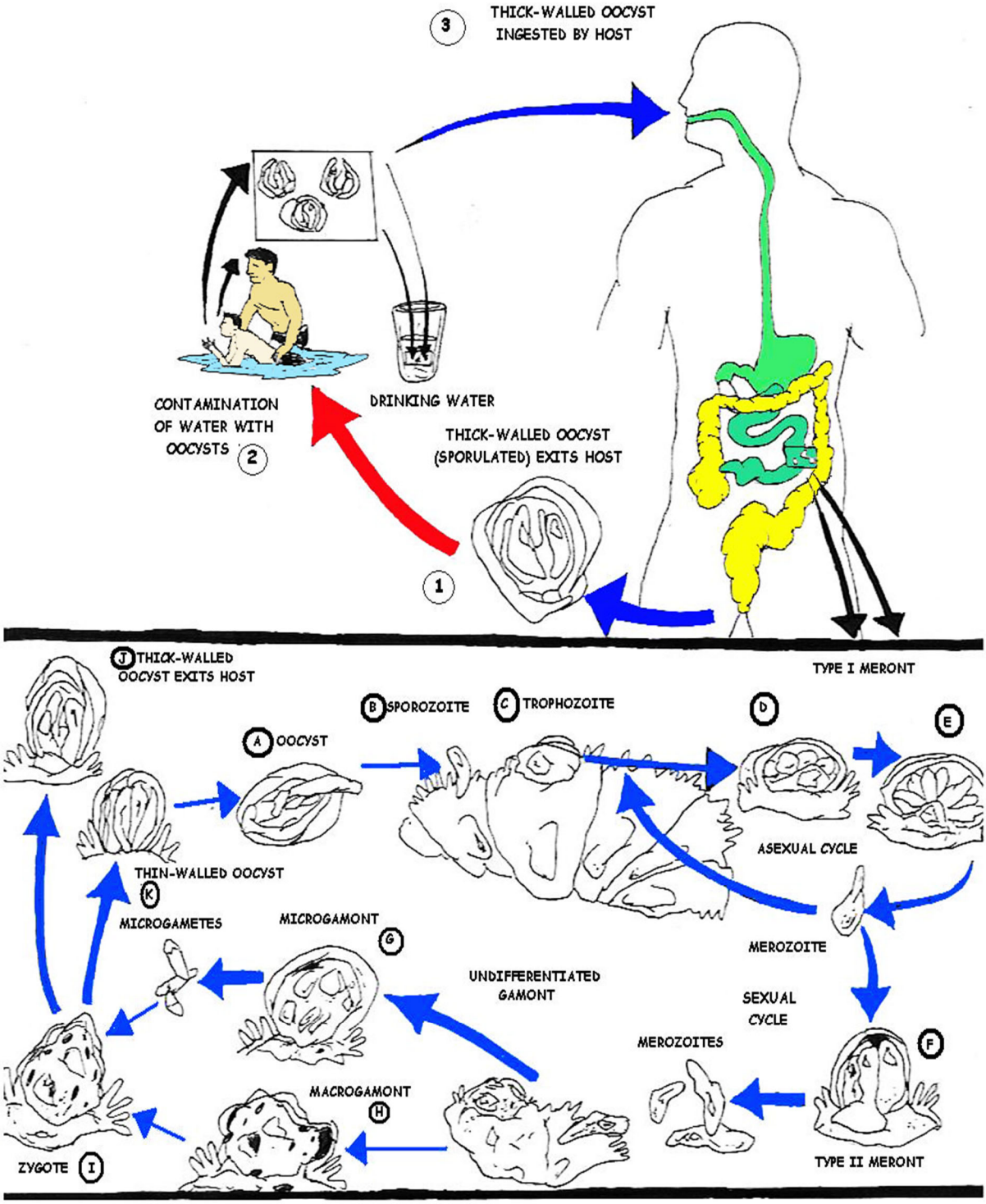
Trophoziotes in diarrheic feces are not stimulated to encyst because the feces are not dehydrating. If they are not encysted they cannot long survive in the external environment. Secondary bacterial infection can complicate an already severe pathology.

Accurate diagnosis of this parasite is important to prevent unnecessary treatment of a non-pathogenic strain, and to ensure treating a pathogenic strain. Definitive diagnosis is based on morphological characteristics of the trophozoites and cysts, the presence of erythrocytes in the trophozoites, and clinical symptoms.

Symptoms of Amoebiasis
In most infected humans the symptoms of "amoebiasis" (or "amebiasis") are intermittent and mild (various gastrointestinal upsets, including colitis and diarrhea). In more severe cases the gastrointestinal tract hemorrhages, resulting in dysentery.

In some cases, the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal.

As with most of the amoebae, infections of *E. histolytica* are often diagnosed by demonstrating cysts or trophozoites in a stool sample.


3
THICK-WALLED OOCYST
INGESTED BY HOST
CONTAMINATION
OF WATER WITH
OOCYSTS
2
DRINKING WATER
THICK-WALLED OOCYST
(SPORULATED) EXITS HOST
1
TYPE I MERONT
J THICK-WALLED
OOCYST EXITS HOST
A OOCYST
B SPOROZOITE
C TROPHOZOITE
D
E
ASEXUAL CYCLE
THIN-WALLED OOCYST
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MICROGAMETES
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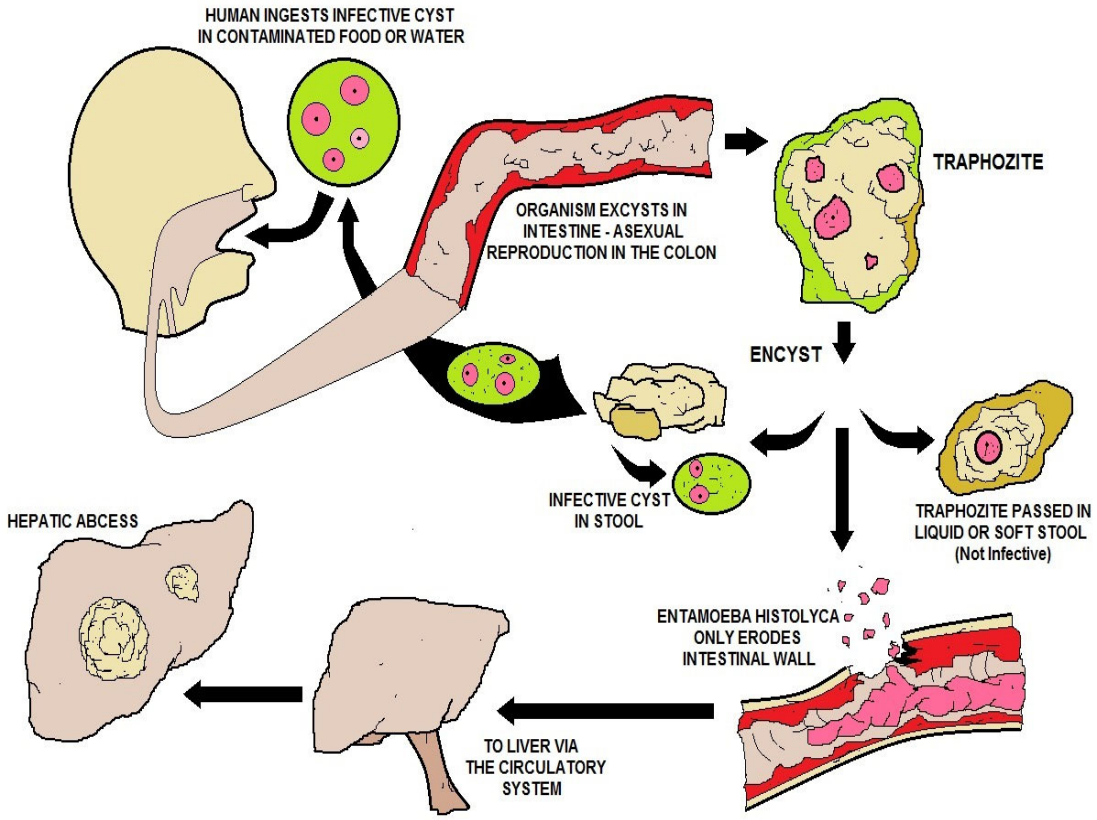
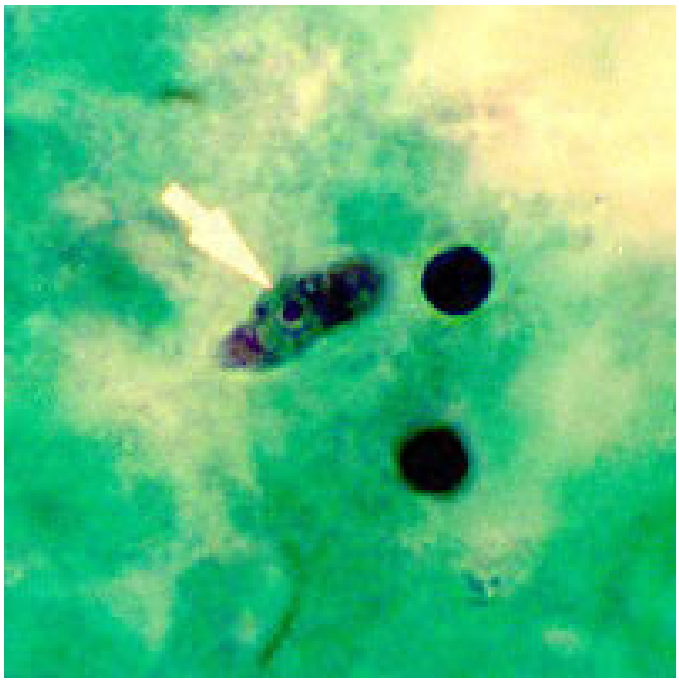


AMEBIASIS

(INFECTIOUS DISEASE BY CAUSED ONE-CELLED PARASITIC ORGANISM: ALSO KNOWN AS DYSENTARY)

Amebic Meningoencephalitis PAM *Naegleria fowleri*

What is primary amebic meningoencephalitis (PAM)?
Primary Amebic Meningoencephalitis (PAM) is a rare and usually deadly disease caused by infection with the ameba (a single-celled organism that constantly changes shape) *Naegleria fowleri*. *[Naegleria fowleri] [Acanthamoeba spp.] [Balamuthia mandrillaris]*



Naegleria fowleri trophozoite in spinal fluid. Trichrome stain. Note the typically large karyosome and the monopodial locomotion. Image contributed by Texas SHD.

What are the symptoms of PAM? What does PAM cause?
Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.

How common is PAM?
The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. *Naegleria* thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.

Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.

Who should be especially careful about PAM?
Cases are usually reported in children and young adults who have had recent exposure to freshwater lakes or streams.

How is PAM spread? How do people get *Naegleria* infection?
The ameba is believed to enter the body through the nose and travel to the brain via the olfactory (smell) nerve. The disease is not spread from person to person.

How do I protect myself from PAM?
To protect yourself against *Naegleria* or any harmful organism that is present in the water:

- Never swim in stagnant or polluted water.
- Do not swim in areas posted as "No Swimming."
- Hold your nose or use nose plugs when jumping or diving into water.
- Avoid swallowing water from rivers, lakes, streams, or stock ponds.
- Use earplugs, swim goggles, or masks if you tend to get ear or eye infections.
- Swim only in properly maintained pools.
- Keep wading pools clean and change the water daily.
- Wash open skin cuts and scrapes with clean water and soap.

What do I do if I think I have PAM?
Seek immediate medical attention and mention any recent fresh water exposure.

How is PAM diagnosed?
The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the patient's spinal cord or frequently after death through the examination of brain tissue.

How are *Naegleria* infections treated?
PAM is a severe illness that does not respond to routine treatments. Aggressive use of some antifungal medications has been successful only in a handful of cases. Intensive supportive care is necessary along with the medication.

Outbreak Information
Health Stream Article - Issue 28 December 2002

Naegleria Deaths In Arizona
Residents of the Arizona towns of Peoria and Glendale have been shocked by the deaths of two five-year old boys from amoebic meningitis caused by Naegleria fowleri. The source of the infections has not been positively established but suspicion has fallen on a small unchlorinated ground water supply operated by a private company.

This supply was taken off-line on 3 November, a boil water notice was issued and 6,000 consumers were warned not to use unboiled tap water for drinking, cooking or bathing. Schools and restaurants in the suspect area were also closed, and residents were advised to drain and clean spas and hyperchlorinate swimming pools.

Supply to the affected area was switched to a chlorinated surface water source, and a flushing program with hyperchlorinated water was carried out to remove possible contamination from the water distribution system.

One of the victims lived in Peoria and the other in the neighboring town of Glendale, some four miles away. They attended separate schools; however the Glendale boy frequently visited his grandparents' home a few blocks from the other boy's residence in Peoria. Both boys became ill on 9 October and died a few days later on 12 and 13 October respectively. Health authorities then began investigating possible common sources of Naegleria exposure including drinking water, pools, bathtubs, spas and fountains.

About 100,000 of Peoria's 120,000 residents receive chlorinated drinking water from the municipal supply. This supply is predominantly drawn from surface water sources but is supplemented by groundwater in times of high demand. As Arizona state law prevents counties from supplying water to areas outside the incorporated municipal zones, the remaining 20,000 residents in the rapidly growing town are served by private water companies which mainly rely on groundwater sources. Some of these companies chlorinate their groundwater supplies and some do not.

The suspect water supply is drawn from a deep aquifer and is not routinely chlorinated, although periodic chlorination has been used after new connections, line breaks or incidents that might allow ingress of microbial contamination.

Tests by the Centers for Disease Control and Prevention have detected N. fowleri in three samples:

· one pre-chlorination water sample from a municipal well that was routinely chlorinated.
· one tank water sample from the suspect unchlorinated groundwater system.
· the refrigerator filter from the home of the grandparents of one of the boys.

The chlorinated well is believed unlikely to be the source of infection as chlorination is effective in killing *N. fowleri*.

Naegleria fowleri is a free living amoeba which is common in the environment and grows optimally at temperatures of 35 to 45 degrees C. Exposure to the organism is believed to be relatively common but infections resulting in illness are rare. The disease was first described in 1965 by Dr. Malcolm Fowler, an Australian pathologist, who identified the amoeba in a patient who had died from meningitis.

Most reported cases of N. fowleri meningitis are associated with swimming in natural surface freshwater bodies, and infection occurs through introduction of the organism into the nasal cavities. Cases are often reported to be associated with jumping or falling into the water, providing conditions where water is forced into the nose at pressure. The amoeba may then penetrate the cribiform plate, a semiporous barrier, and spread to the meninges (the membrane surrounding the brain) and often to the brain tissue itself. The cribiform plate is more permeable in children, making them more susceptible to infection than adults. People with immune deficiencies may also be more prone to infection.

The incubation period is usually 2 to 5 days, and the infection cannot be transmitted from person to person. In early studies, transmission by contaminated dust was suspected as an infection route but this has since been discounted as the organism does not survive desiccation.

N. fowleri meningitis causes non-specific symptoms such as fever, drowsiness, confusion, vomiting, irritability, high pitched crying and convulsions. Similar symptoms also occur in viral and bacterial forms of meningitis which are much more common than the amoebic form. Most cases of *N. fowleri* meningitis are fatal, with only four survivors known among about 100 cases in the US since 1965.

Cases of disease have also been associated with swimming pools where disinfection levels were inadequate, and inhalation of tap water from surface water supplies that have been subject to high temperatures.

The involvement of tap water supplies was first documented in South Australia, where a number of cases occurred in the 1960s and 70s in several towns served by unchlorinated surface water delivered through long above-ground pipelines. About half of the cases in the state did not have a recent history of freshwater swimming, but had intra-nasal exposure to tap water through inhaling or squirting water into the nose.

Investigators found *N. fowleri* in the water supply pipelines, and concluded that the high water temperatures reached in summer provided a suitable environment for growth of the organism. Tap water may also have been the primary source of infections attributed to swimming pools in these towns.

The incidence of disease was greatly reduced by introduction of reliable chlorination facilities along the above-ground pipelines and introduction of chloramination in the 1980s led to virtual elimination of *N. fowleri* from the water supplies.

Cases of disease have also been recorded in Western Australia, Queensland and New South Wales, and *N. fowleri* has been detected in water supplies in each of these states as well as the Northern Territory.

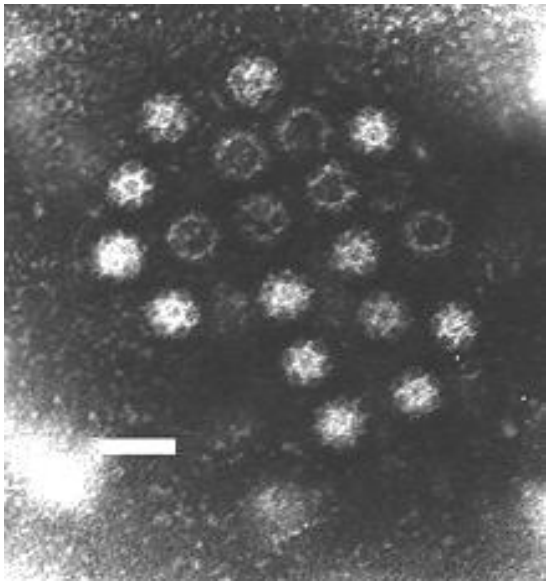
Prior to the incidents in Peoria, *N. fowleri* infections had not been reported to be associated with groundwater supplies. However as the organism may be found in moist soil, it is feasible that the amoeba may penetrate poorly constructed bores or be introduced by occasional contamination events.

Warm water conditions and the absence of free chlorine may then allow it to proliferate in the system. Local health authorities in Arizona are continuing their investigation into the two deaths with assistance from CDC personnel.

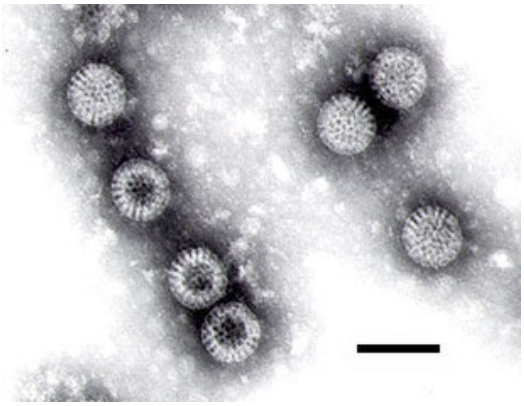
Plans are also underway to install a continuous chlorination plant on the groundwater supply, and some residents have called for the municipality to purchase the private water company and take over its operations.

Calicivirus

See Gastroenteritis section, Norovirus Infection (aka Norwalk virus, calicivirus, viral gastroenteritis)

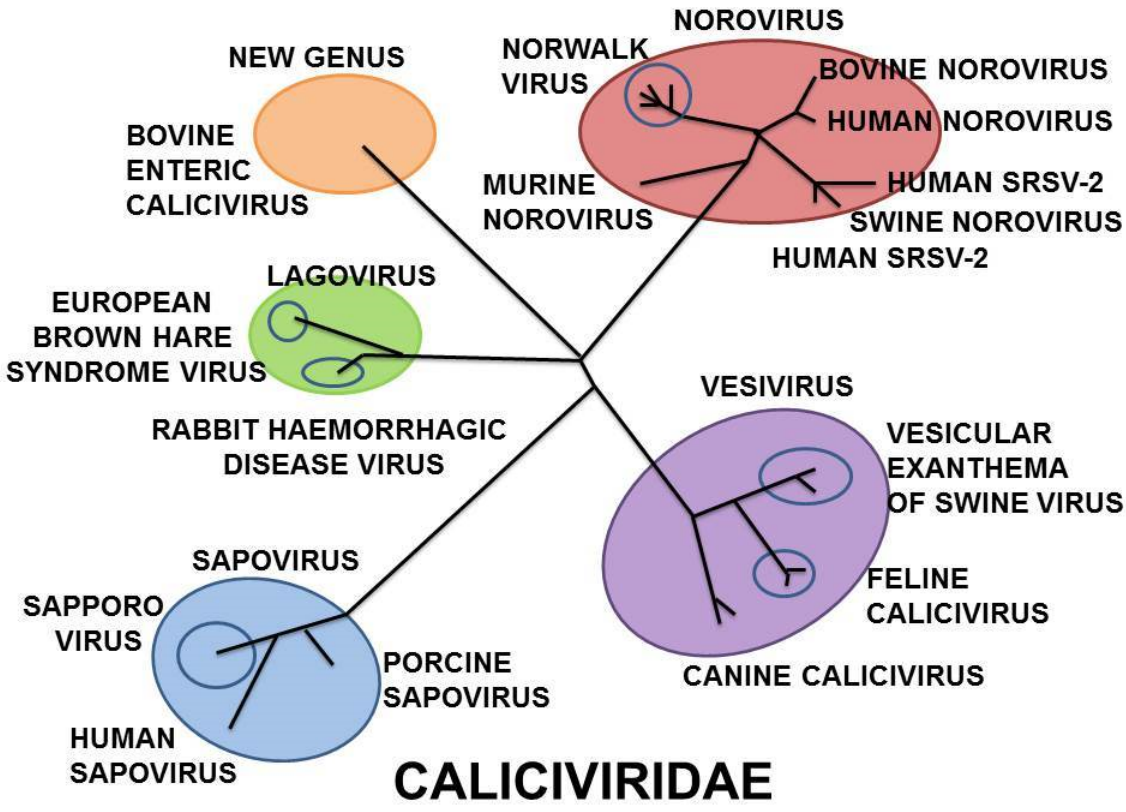


Note the 'Star of David' image exhibited by individual virus particles. This is distinct from the star-like images exhibited by astrovirus particles. Bar = 50 nanometers.
Source: Stool sample from an individual with gastroenteritis.
Method: Negative-stain Transmission Electron Microscopy



Rotovirus
Note the wheel-like appearance of some of the rotavirus particles. The observance of such particles gave the virus its name ('rota' being the Latin word meaning wheel). Bar = 100 nanometers.
Source: Cell culture.
Method: Negative-stain Transmission Electron Microscopy

Photographs and information courtesy from the U.S. EPA and F.P. Williams, U.S. EPA



CALICIVIRIDAE

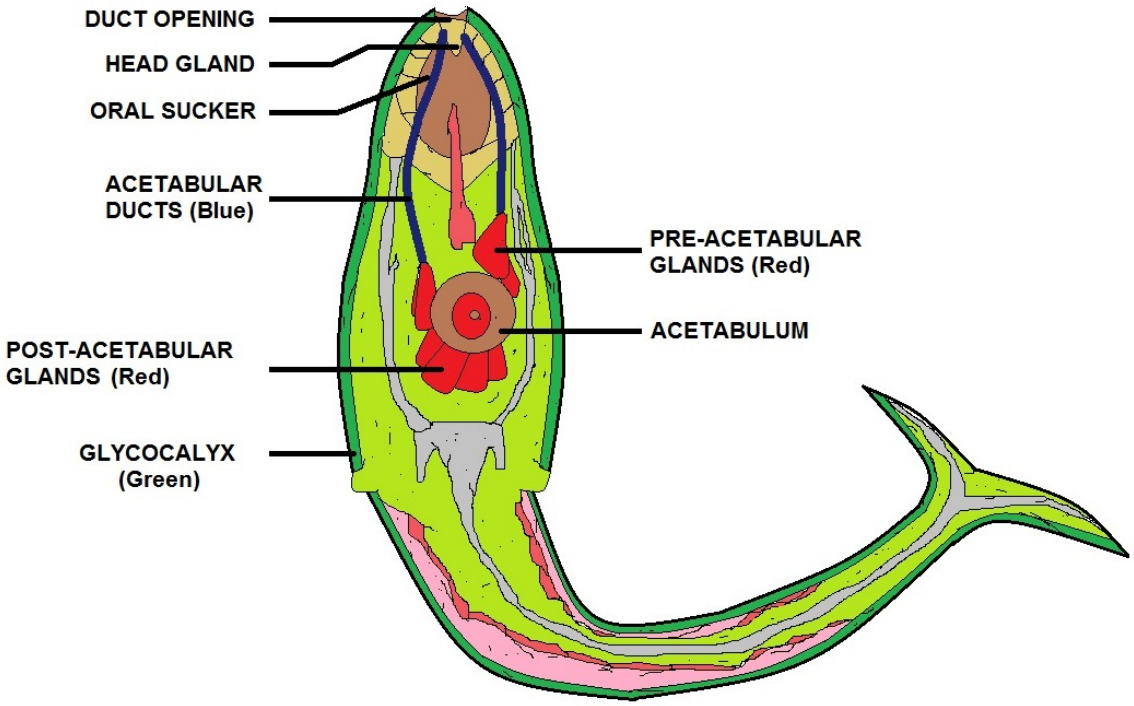


DIAGRAM OF A SCHISTOSOMA

Schistosomes and Other Trematodes

Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except malaria. Almost unknown in industrialized countries, schistosomiasis infects 200 million people in 76 countries of the tropical developing world.

A Flatworm that spends part of its life in a freshwater snail host causes schistosomiasis. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.

Multiplication and Life Cycle
Free-swimming larvae (cercariae) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes). After entering a human, the larvae develop into adult males and females (schistosomes) or hermaphrodites (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.

Cercariae

Pathogenesis
In schistosomiasis, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.

Host Defenses
Host defenses against schistosomiasis include antibody or complement-dependent cellular cytotoxicity and modulation of granulomatous hypersensitivity. The defenses against hermaphroditic flukes are unknown.

Epidemiology
Most infected individuals show no overt disease. In a relatively small proportion of individuals, heavy infections due to repeated exposure to parasitic larvae will lead to the development of clinical manifestations. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other trematodes).

Diagnosis
Diagnosis is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.

Control
As a control measure, exposure to parasite larvae in water and food should be prevented. Treatment with praziquantel is effective.

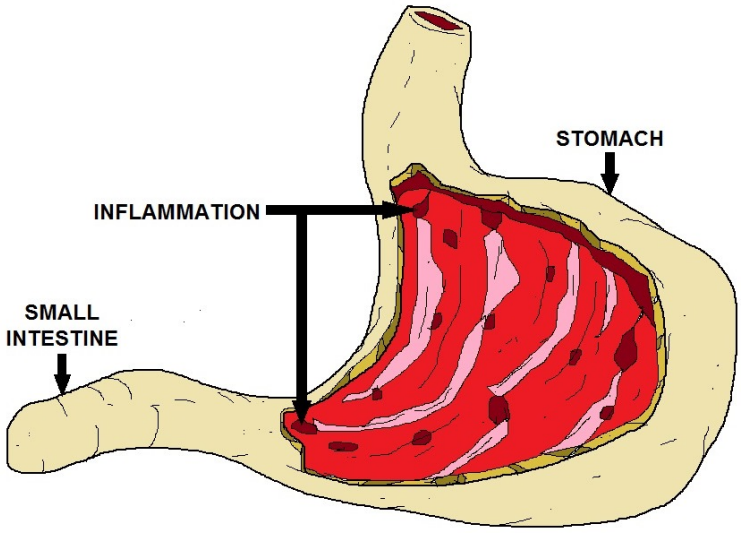
Clinical Manifestations
Signs and symptoms are related largely to the location of the adult worms. Infections with *Schistosoma mansoni* and *S japonicum* (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis.

Schistosoma haematobium (vesical venules) causes dysuria, hema turia, and uremia. *Fasciola hepatica, Clonorchis sinensis,* and *Opisthorchis viverrini* (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice.

Infections with *Paragonimus westermani* (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. *Fasciolopsis buski* (intestines) causes abdominal pain, diarrhea, and edema.

Structure
Trematodes are multicellular eukaryotic helminths.

Gastroenteritis



GASTROENTERITIS

What is Viral Gastroenteritis?

Gastroenteritis means inflammation of the stomach and small and large intestines. Viral gastroenteritis is an infection caused by a variety of viruses that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.

What causes viral gastroenteritis?

Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, caliciviruses, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as *Salmonella* or *Escherichia coli*) or parasites (such as *Giardia*), or by medications or other medical conditions, although the symptoms may be similar. Your doctor can determine if the diarrhea is caused by a virus or by something else.

What are the symptoms of viral gastroenteritis?

The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which virus causes the illness.

Is viral gastroenteritis a serious illness?

For most people, it is not. People who get viral gastroenteritis almost always recover completely without any long-term problems. Gastroenteritis is a serious illness, however, for persons who are unable to drink enough fluids to replace what they lose through vomiting or diarrhea. Infants, young children, and persons who are unable to care for themselves, such as the disabled or elderly, are at risk for dehydration from loss of fluids. Immune compromised persons are at risk for dehydration because they may get a more serious illness, with greater vomiting or diarrhea. They may need to be hospitalized for treatment to correct or prevent dehydration.

Is the illness contagious? How are these viruses spread?
Yes, viral gastroenteritis is contagious. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.

How does food get contaminated by gastroenteritis viruses?
Food may be contaminated by food preparers or handlers who have viral gastroenteritis, especially if they do not wash their hands regularly after using the bathroom. Shellfish may be contaminated by sewage, and persons who eat raw or undercooked shellfish harvested from contaminated waters may get diarrhea. Drinking water can also be contaminated by sewage and be a source of spread of these viruses.

Where and when does viral gastroenteritis occur?
Viral gastroenteritis affects people in all parts of the world. Each virus has its own seasonal activity. For example, in the United States, rotavirus and astrovirus infections occur during the cooler months of the year (October to April), whereas adenovirus infections occur throughout the year. Viral gastroenteritis outbreaks can occur in institutional settings, such as schools, child care facilities, and nursing homes, and can occur in other group settings, such as banquet halls, cruise ships, dormitories, and campgrounds.

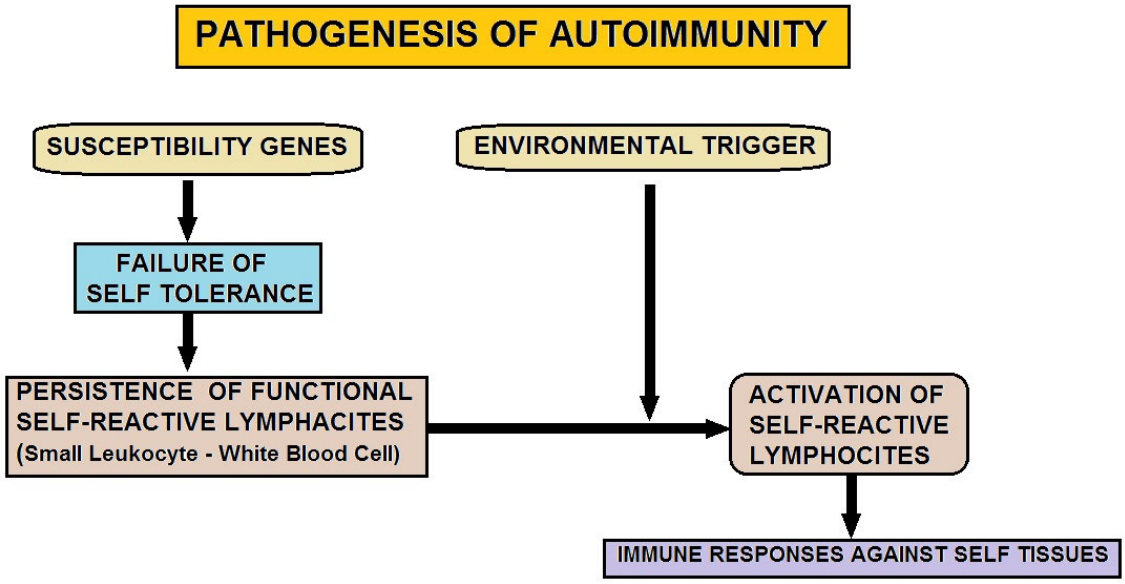
Who gets viral gastroenteritis?
Anyone can get it. Viral gastroenteritis occurs in people of all ages and backgrounds. However, some viruses tend to cause diarrheal disease primarily among people in specific age groups. Rotavirus infection is the most common cause of diarrhea in infants and young children under 5 years old.

Adenoviruses and astroviruses cause diarrhea mostly in young children, but older children and adults can also be affected. Norwalk and Noroviruses are more likely to cause diarrhea in older children and adults.

Gastroenteritis Summary
Gastroenteritis is characterized by inflammation of the gastrointestinal tract that involves both the stomach and the small intestine resulting in some combination of diarrhea, vomiting, and abdominal pain and cramping. Gastroenteritis is unrelated to influenza, it has also been called stomach flu and gastric flu.

Gastroenteritis transmission may occur due to consumption of improperly prepared foods, contaminated water, or via close contact with individuals who are infectious. A person with bacterial gastroenteritis has inflammation of the intestines or stomach caused by a bacterial infection. Common causes of bacterial gastroenteritis include salmonella infection, shigella infection, cholera, Campylobacter enteritis, and pseudomembranous colitis. Gastroenteritis has also been referred to as gastro, stomach bug, and stomach virus.

Rotovirus Information



PATHOGENESIS
(The Manner in Which a Disease Develops)

Clinical Features
Rotavirus is the most common cause of severe diarrhea among children, resulting in the hospitalization of approximately 55,000 children each year in the United States and the death of over 600,000 children annually worldwide. The incubation period for rotavirus disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.

The Virus
A rotavirus has a characteristic wheel-like appearance when viewed by electron microscopy (the name rotavirus is derived from the Latin rota, meaning "wheel"). Rotaviruses are non-enveloped, double-shelled viruses. The genome is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.

Epidemiologic Features
The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces. In the United States and other countries with a temperate climate, the disease has a winter seasonal pattern, with annual epidemics occurring from November to April.

The highest rates of illness occur among infants and young children, and most children in the United States are infected by 2 years of age. Adults can also be infected, though disease tends to be mild.

Diagnosis
Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by enzyme immunoassay or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.

Treatment
For persons with healthy immune systems, rotavirus gastroenteritis is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children with rotavirus gastroenteritis will require hospitalization for intravenous fluids.

Prevention
In 1998, the U.S. Food and Drug Administration approved a live virus vaccine (Rotashield) for use in children. However, the Advisory Committee on Immunization Practices (ACIP) recommended that Rotashield no longer be recommended for infants in the United States because of data that indicated a strong association between Rotashield and intussusception (bowel obstruction) among some infants during the first 1-2 weeks following vaccination.

More information about rotavirus vaccine is available from the National Immunization Program.

Recent Newspaper Article

Norwalk Virus
The Gila County Department of Health is currently investigating an outbreak of viral gastroenteritis in the Globe / Miami area. The outbreak has been laboratory confirmed by the Arizona State Laboratory as Norwalk virus. Please be aware of the following symptoms and recommendations.

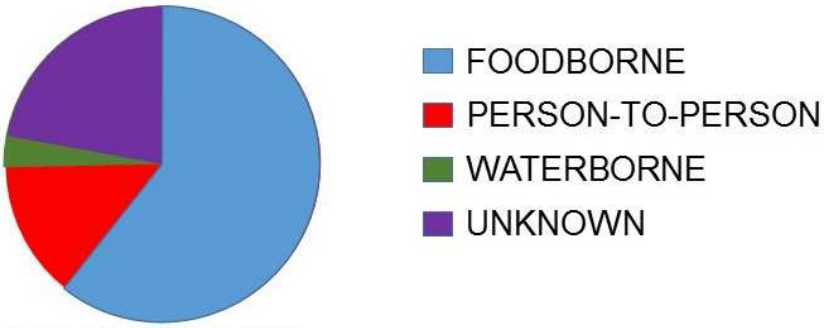
Norwalk Symptoms
Usually a mild to moderate infection that often occurs in outbreaks with clinical symptoms of nausea, vomiting, diarrhea, abdominal pain, low grade fever, or any combination of these symptoms. Gastrointestinal symptoms will characteristically last 24 to 48 hours, resolving on their own.

Mode of Transmission and Communicability
Fecal to oral route is the most likely mode of transmission. This virus is easily transmitted. Patients are communicable during the acute phase of the illness and up to 48 hours after the symptoms resolve.

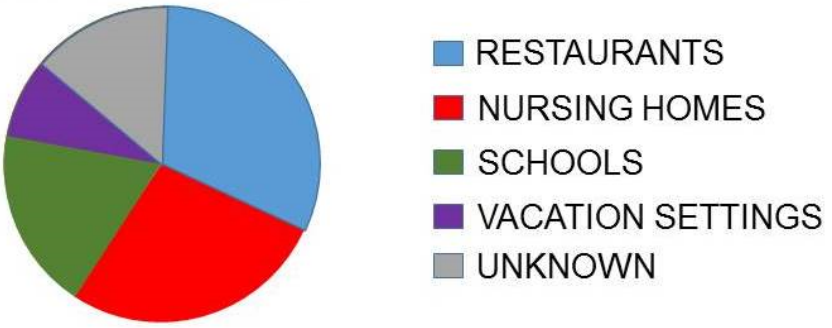
If you are experiencing symptoms consistent with this disease, please exclude yourself from school, work, or any group activity. Hand washing and disinfection are essential to stop the spread of this virus. Anyone experiencing severe complications from this ailment should seek medical attention.

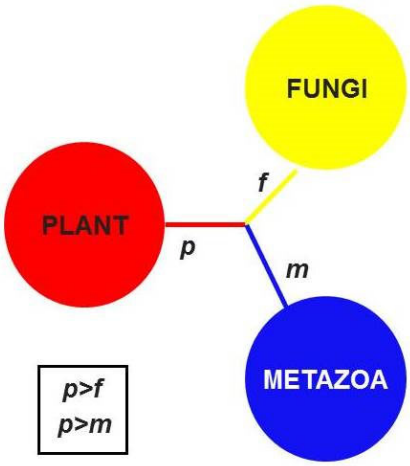
Please report all suspected group outbreaks to the Gila County Department of Health by phone immediately. (928) 425-3189

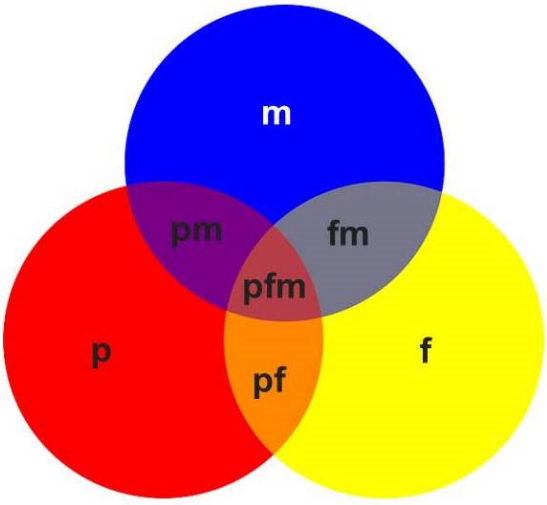
A. SOURCE OF NOROVIRUS



B. SETTING FOR OUTBREAK







METAZOAN AMINO ACID BIOSYNTHESIS

Noroviruses

Noroviruses (genus *Norovirus*, family *Caliciviridae*) are a group of related, single-stranded RNA, non-enveloped viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official genus name for the group of viruses provisionally described as “Norwalk-like viruses” (NLV).

What are the symptoms of illness caused by noroviruses?
The symptoms of norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. The illness is usually brief, with symptoms lasting only about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.

What is the name of the illness caused by noroviruses?
Illness caused by norovirus infection has several names, including:

- Stomach flu – this “stomach flu” is ***not*** related to the flu (or influenza), which is a respiratory illness caused by influenza virus.
- Viral gastroenteritis – the most common name for illness caused by norovirus. Gastroenteritis refers to an inflammation of the stomach and intestines.
- Acute gastroenteritis.
- Non-bacterial gastroenteritis.
- Food poisoning (although there are other causes of food poisoning).
- Calicivirus infection.

How serious is norovirus disease?
Norovirus disease is usually not serious, although people may feel very sick and vomit many times a day. Most people get better within 1 or 2 days, and they have no long-term health effects related to their illness. However, sometimes people are unable to drink enough liquids to replace the liquids they lose because of vomiting and diarrhea. These persons can become dehydrated and may need special medical attention. This problem with dehydration is usually only seen among the very young, the elderly, and persons with weakened immune systems. There is no evidence to suggest that an infected person can become a long-term carrier of norovirus.

How do people become infected with noroviruses?
Noroviruses are found in the stool or vomit of infected people. People can become infected with the virus in several ways, including:

- eating food or drinking liquids that are contaminated with norovirus;
- touching surfaces or objects contaminated with norovirus, and then placing their hand in their mouth;
- having direct contact with another person who is infected and showing symptoms (for example, when caring for someone with illness, or sharing foods or eating utensils with someone who is ill).

Persons working in day-care centers or nursing homes should pay special attention to children or residents who have norovirus illness. This virus is very contagious and can spread rapidly throughout such environments.

When do symptoms appear?
Symptoms of norovirus illness usually begin about 24 to 48 hours after ingestion of the virus, but they can appear as early as 12 hours after exposure.

Are noroviruses contagious?
Noroviruses are very contagious and can spread easily from person to person. Both stool and vomit are infectious. Particular care should be taken with young children in diapers who may have diarrhea.

How long are people contagious?
People infected with norovirus are contagious from the moment they begin feeling ill to at least 3 days after recovery. Some people may be contagious for as long as 2 weeks after recovery. Therefore, it is particularly important for people to use good handwashing and other hygienic practices after they have recently recovered from norovirus illness.

Who gets norovirus infection?
Anyone can become infected with these viruses. There are many different strains of norovirus, which makes it difficult for a person's body to develop long-lasting immunity. Therefore, norovirus illness can recur throughout a person's lifetime. In addition, because of differences in genetic factors, some people are more likely to become infected and develop more severe illness than others.

What treatment is available for people with norovirus infection?
Currently, there is no antiviral medication that works against norovirus and there is no vaccine to prevent infection. Norovirus infection cannot be treated with antibiotics. This is because antibiotics work to fight bacteria and not viruses. Norovirus illness is usually brief in healthy individuals. When people are ill with vomiting and diarrhea, they should drink plenty of fluids to prevent dehydration. Dehydration among young children, the elderly, and the sick can be common, and it is the most serious health effect that can result from norovirus infection. By drinking oral rehydration fluids (ORF), juice, or water, people can reduce their chance of becoming dehydrated. Sports drinks do not replace the nutrients and minerals lost during this illness.

Noroviruses Summary
Noroviruses are a genetically diverse group of single-stranded RNA, non-enveloped viruses in the Caliciviridae family.

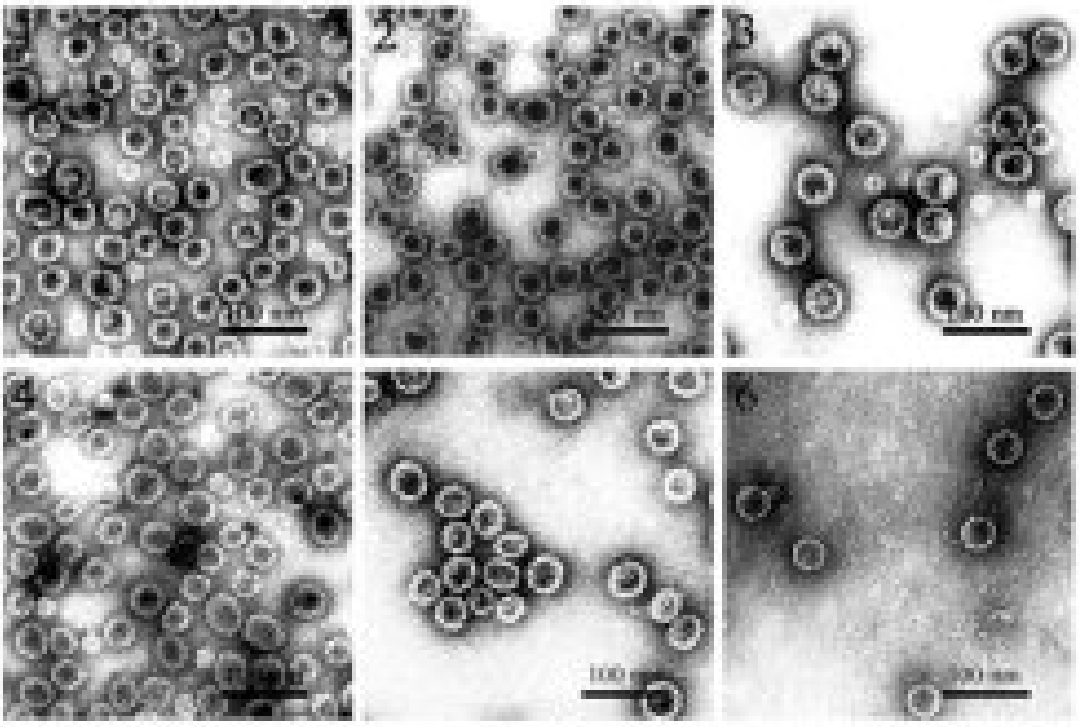
Many norovirus outbreaks have been traced to food that was handled by one infected person. Norovirus is rapidly inactivated by either sufficient heating or by chlorine-based disinfectants, but the virus is less susceptible to alcohols and detergents, as it does not have a lipid envelope. This genus name norovirus is derived from Norwalk virus. Viruses are transmitted by fecally contaminated food or water, by person-to-person contact, and via aerosolization of the virus and subsequent contamination of surfaces. Norovirus are the most common cause of viral gastroenteritis in humans. After infection, immunity to Norovirus is usually incomplete and temporary.

Norovirus infection outbreaks will often occur in closed or semi-closed communities, such as long-term care facilities, overnight camps, hospitals, prisons, dormitories, and cruise ships, where the infection spreads very rapidly either by person-to-person transmission or through contaminated food.

Hepatitis Sub-Section *More information in the Appendix*

Viral hepatitis is a group of diseases of the liver that can be caused by consuming contaminated water or food, using dirty needles or syringes, or practicing unsafe sex.

Scientists have identified six hepatitis viruses, but three - known as A, B and C - cause about 90 percent of acute hepatitis cases in the United States. People infected with hepatitis can experience effects ranging from mild illness to serious liver damage. Many recover completely from an infection, while others become carriers of the disease and can spread it to others unknowingly. It is especially important for women who are pregnant or are trying to become pregnant to get tested for hepatitis.



Typical symptoms of acute hepatitis are:

- fever
- appetite loss
- nausea
- abdominal pain
- jaundice (yellowish color on the skin and eyeballs)

Hepatitis A virus found in human feces; shellfish grown in polluted waters. Yellowed skin, enlarged liver, fever, vomiting, weight loss, and abdominal pain — low mortality, lasts up to four months. Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). Hepatitis A can affect anyone. In the United States, hepatitis A can occur in situations ranging from isolated cases of disease to widespread epidemics.

Each year, an estimated 100 persons die as a result of acute liver failure in the United States due to Hepatitis A. Approximately 30 - 50,000 cases occur yearly in the United States and the direct and indirect costs of these cases exceed $300 million.

The unfortunate aspect of these statistics is that with 21st century medicine, Hepatitis A is totally preventable, and isolated cases, especially outbreaks relegated to food consumption, need not occur.

Viral Hepatitis is a major public health concern in the United States, and a source of significant morbidity and mortality. The Hepatitis A virus or "HAV" is heat stable and will survive for up to a month at ambient temperatures in the environment.

Hepatitis A is a communicable (or contagious) disease that spreads from person to person. (It is not acquired from animals, insects, or other means.) It is transmitted by the "fecal – oral route." This does not mean, or course, that Hepatitis A transmission requires that fecal material from an infectious individual must come in contact directly with the mouth of a susceptible individual. It is almost always true that the virus infects a susceptible individual when he or she ingests it, but it gets to the mouth by an indirect route.

Where and how does hepatitis A virus get into drinking water?
Hepatitis A is found in every part of the United States and throughout the world. When water sources such as private wells are contaminated with feces from infected humans, the water will spread the hepatitis A virus. The virus can enter the water through various ways, including sewage overflows or broken sewage systems.

How do I remove hepatitis A from my drinking water?
Heating water at a full boil for 1 minute (3 minutes if you live in a high altitude) will kill or inactivate the hepatitis A virus. Water should then be stored in a clean container with a lid and refrigerated. Because of the small size of the virus, using a point-of-use filter will not remove it from water.

Most Common Method of Transmission
Food contaminated with the virus is the most common vehicle transmitting Hepatitis A.

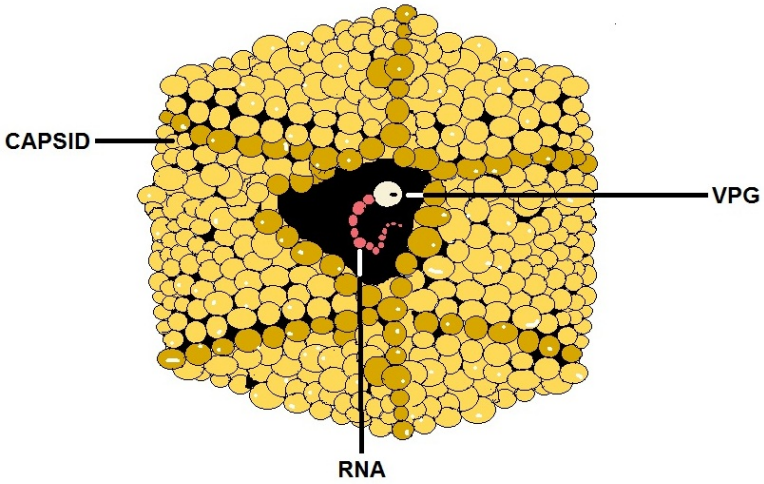
The food preparer or cook is the individual most often contaminating the food. He or she is generally not ill: the peak time of infectivity (i.e., when the most virus is present in the stool of an infectious individual) is during the 2 weeks before illness begins. Hepatitis A is spread almost exclusively through fecal-oral contact, generally from person-to-person, or via contaminated food or water. Outbreaks associated with food have been increasingly implicated as a significant source of Hepatitis A infection.

Such "outbreaks are usually associated with contamination of food during preparation by an HAV-infected food handler."

Indeed, “[v]iral gastroenteritis was reported as the most common food-borne illness in Minnesota from 1984 to 1991, predominantly associated with poor personal hygiene of infected food handlers.”

Although ingestion of contaminated food is the most common means of spread for Hepatitis A, it may also commonly be spread by household contact among families or roommates, sexual contact, by the ingestion of contaminated water, by the ingestion of raw or undercooked fruits and vegetables or shellfish (like oysters), and by direct inoculation from persons sharing illicit drugs.

Children often have asymptomatic or unrecognized infections and can pass the virus through ordinary play, unknown to their parents, who may later become infected from contact with their children.



HEPATITUS A VIRUS

Hepatitis A: is much more common in countries with under-developed sanitation systems. This includes most of the world: an increased transmission rate is seen in all countries other than the United States, Canada, Japan, Australia, New Zealand, and the countries of Western Europe. Within the United States, Native American reservations also experience a greatly increased rate of disease.

Hepatitis B: is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis B virus (HBV), can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.

Hepatitis C: is a liver disease caused by the hepatitis C virus (HCV), which is found in the blood of persons who have the disease. HCV is spread by contact with the blood of an infected person.

Hepatitis D: is a liver disease caused by the hepatitis D virus (HDV), a defective virus that needs the hepatitis B virus to exist. Hepatitis D virus (HDV) is found in the blood of persons infected with the virus.

Hepatitis E: is a liver disease caused by the hepatitis E virus (HEV) transmitted in much the same way as hepatitis A virus. Hepatitis E, however, does not occur often in the United States.

Medical Testing
Hepatitis virus tests require a blood sample. It is not necessary for the patient to withhold food or fluids before any of these tests, unless requested to do so by the physician.

Risks
Risks for these tests are minimal for the patient, but may include slight bleeding from the blood-drawing site, fainting or feeling lightheaded after venipuncture, or hematoma (blood accumulating under the puncture site).

Normal Medical Results
Reference ranges for the antigen/antibody tests are as follows:

- Hepatitis A antibody, IgM: Negative
- Hepatitis B core antibody: Negative
- Hepatitis B e antibody: Negative
- Hepatitis B e-antigen: Negative
- Hepatitis B surface antibody: Varies with clinical circumstance
- (Note: As the presence of anti-HBs indicates past infection with resolution of previous hepatitis B infection, or vaccination against hepatitis B, additional patient history may be necessary for diagnosis.)
- Hepatitis B surface antigen: Negative
- Hepatitis C serology: Negative
- Hepatitis D serology: Negative.

Abnormal Medical Results

Hepatitis A: A single positive anti-HAV test may indicate previous exposure to the virus, but due to the antibody persisting so long in the bloodstream, only evidence of a rising anti-HAV titer confirms hepatitis A.

Determining recent infection rests on identifying the antibody as IgM (associated with recent infection). A negative anti-HAV test rules out hepatitis A.

Hepatitis B: High levels of HBsAg that continue for three or more months after onset of acute infection suggest development of chronic hepatitis or carrier status.

Detection of anti-HBs signals late convalescence or recovery from infection. This antibody remains in the blood to provide immunity to re-infection.

Hepatitis C (non-A, non-B hepatitis): Anti-HBc develops after exposure to hepatitis B.

As an early indicator of acute infection, antibody (IgM) to core antigen (anti-HBc IgM) is rarely detected in chronic infection, so it is useful in distinguishing acute from chronic infection, and hepatitis B from non-A, non-B.

References

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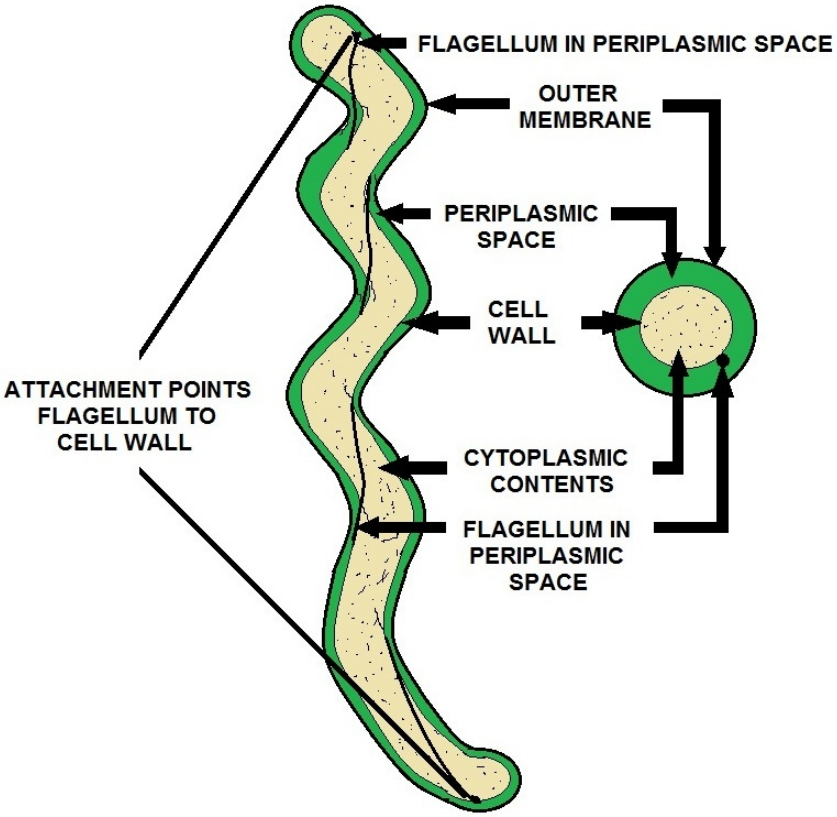
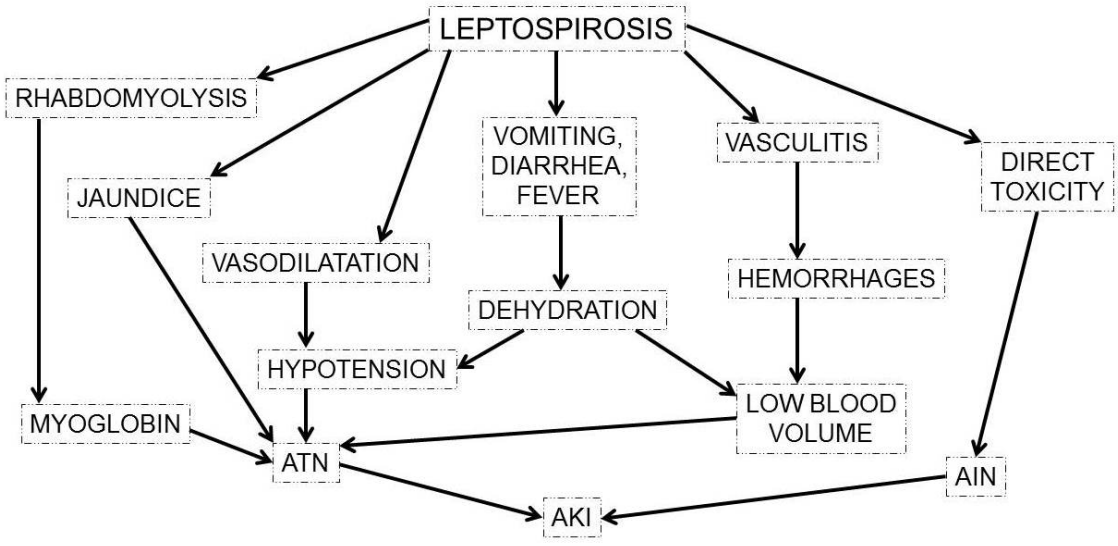
HEPATITUS C VIRUS



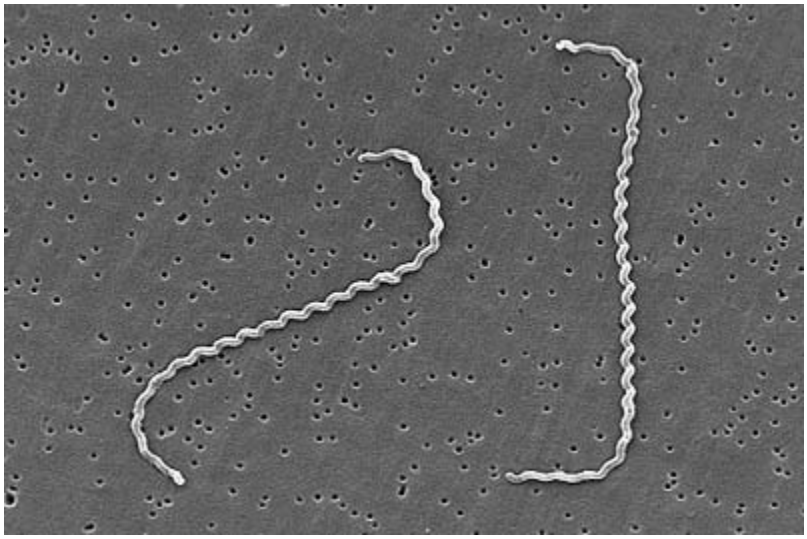
LEPTOSPIRA



PHYSIOPATHOLOGY OF AKI IN LEPTOSPIROSIS

Leptospirosis - Leptospira Sub-Section

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. *Leptospira interrogans* causes leptospirosis, a usually mild febrile illness that may result in liver or kidney failure.



Structure, Classification, and Antigenic Types
Leptospira is a flexible, spiral-shaped, Gram-negative spirochete with internal flagella. *Leptospira interrogans* has many serovars based on cell surface antigens.

How do people get Leptospirosis?
Outbreaks of leptospirosis are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with broken skin.

The disease is not known to be spread from person to person.

Pathogenesis
Leptospira enters the host through mucosa and broken skin, resulting in bacteremia. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.

Host Defenses
Serum antibodies are responsible for host resistance.

Epidemiology
Leptospirosis is a worldwide zoonosis affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.

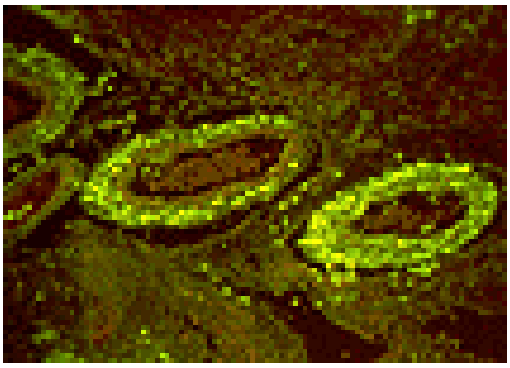
Diagnosis
Clinical diagnosis is usually confirmed by serology. Isolation of spirochetes is possible, but it is time-consuming and requires special media.

Control
Animal vaccination and eradication of rodents are important. Treatment with tetracycline and penicillin G is effective. No human vaccine is available.

Can Leptospirosis be prevented?
The risk of acquiring leptospirosis can be greatly reduced by not swimming or wading in water that might be contaminated with animal urine. Protective clothing or footwear should be worn by those exposed to contaminated water or soil because of their job or recreational activities.

Meningoencephalitis

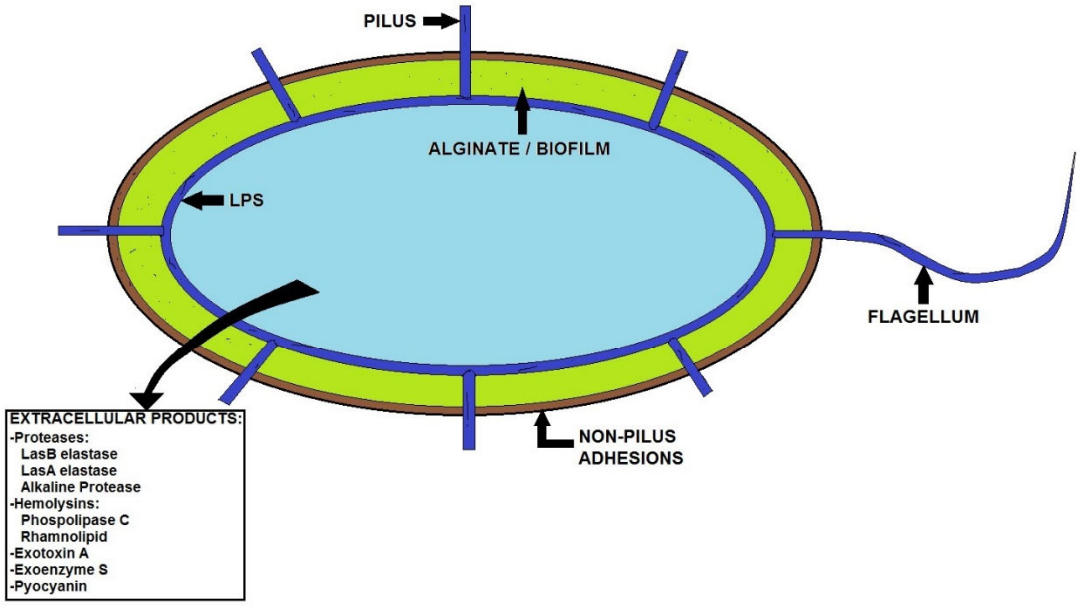
Refer to amoebic meningoencephalitis (PAM), *Naegleria fowleri* and granulomatious amoebic encephalitis (GAE), acanthamoebic keratitis or acanthamoebic uveitis. These organisms are ubiquitous in the environment, in soil, water, and air. Infections in humans are rare and are acquired through water entering the nasal passages (usually during swimming) and by inhalation.



Granulomatous Amoebic Encephalitis due to *Acanthamoeba castellanii*

With immunofluorescent antibody techniques, the amoebae showed distinct fluorescence with anti-*A. castellanii* at 1:20 and 1:50 dilutions, but they were negative or weakly positive with anti-*A. culbertsoni*, *A. polyphaga*, *A. rhysodes*, and *A. astronyxis* sera. Protozoa were also seen in autopsy lung tissue, and identified as *A. castellanii*. There was also necrotizing amoebic panniculitis in subcutaneous, peripancreatic, mesenteric and peri-aortic tissue. There were occasional amoebae in the liver, but not enough to account for all of the patient's liver disease, so part of his liver disease was most likely due to sepsis due to his disseminated amoebiasis.

Pseudomonas Aeruginosa Sub-Section



PSEUDOMONAS AERUGINOSA

(ROD-SHAPED / GRAM NEGATIVE BACTERIUM WHICH CAUSES VARIOUS TYPES OF INFECTIONS)

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner.

Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. *Pseudomonas aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent.

Pseudomonas aeruginosa is primarily a nosocomial pathogen. According to the CDC, the overall incidence of *P. aeruginosa* infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.

Pseudomonas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics. The most serious complication of cystic fibrosis is respiratory tract infection by the ubiquitous bacterium *Pseudomonas aeruginosa*. Cancer and burn patients also commonly suffer serious infections by this organism, as do certain other individuals with immune system deficiencies.

Unlike many environmental bacteria, *P. aeruginosa* has a remarkable capacity to cause disease in susceptible hosts.

It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited. *P. aeruginosa* can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs. This bacterium is also noted for its resistance to many antibiotics.

P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its metabolic capability and environmental versatility. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, and systems involved in sensing and responding to environmental changes.

The typical *Pseudomonas* bacterium in nature might be found in a biofilm, attached to some surface or substrate, or in a planktonic form, as a unicellular organism, actively swimming by means of its flagellum. *Pseudomonas* is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples.
In its natural habitat *Pseudomonas aeruginosa* is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis.

--*Pseudomonas aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *Pseudomonas aeruginosa* consists of acetate for carbon and ammonium sulfate for nitrogen.

--*P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth.

--Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C.

--It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

--*Pseudomonas aeruginosa* has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominance as a nosocomial pathogen.

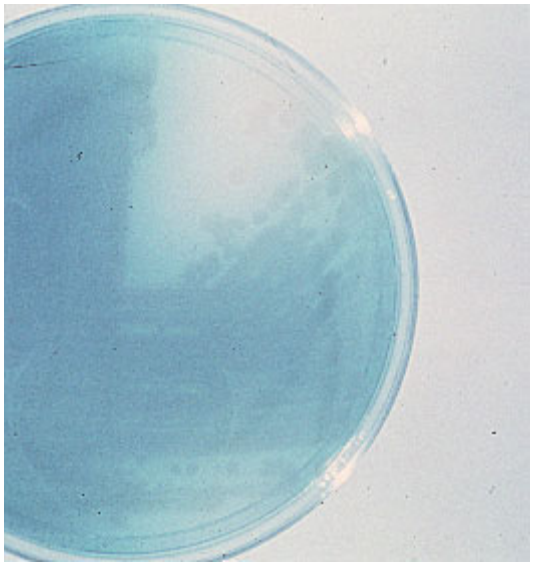
P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large and smooth, with flat edges and an elevated appearance.

Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.

Pseudomonas aeruginosa colonies on agar.

Pyoverdin and the blue pigment Pyocyanin

P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment **pyoverdin** and the blue pigment **pyocyanin**. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus" which is a characteristic of suppurative infections caused by *Pseudomonas aeruginosa.*



The soluble blue pigment pyocyanin is produced by many, but not all, strains of Pseudomonas aeruginosa.

Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally-occurring antibiotics. Moreover, *Pseudomonas* maintains antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against Pseudomonas, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The futility of treating Pseudomonas infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated.

Diagnosis
Diagnosis of P. aeruginosa infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42° C. Fluorescence under ultraviolet light is helpful in early identification of P. aeruginosa colonies. Fluorescence is also used to suggest the presence of P. aeruginosa in wounds.

Pathogenesis
For an opportunistic pathogen such as Pseudomonas aeruginosa, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of Pseudomonas infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis.

Most Pseudomonas infections are both invasive and toxinogenic. The ultimate Pseudomonas infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization; (2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease.

Colonization
Although colonization usually precedes infections by Pseudomonas aeruginosa, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment. It is sometimes present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is relatively low (estimates range from 0 to 24 percent depending on the anatomical locale).

The fimbriae of Pseudomonas will adhere to the epithelial cells of the upper respiratory tract and, by inference, to other epithelial cells as well. These adhesions appear to bind to specific galactose, mannose or sialic acid receptors on epithelial cells.

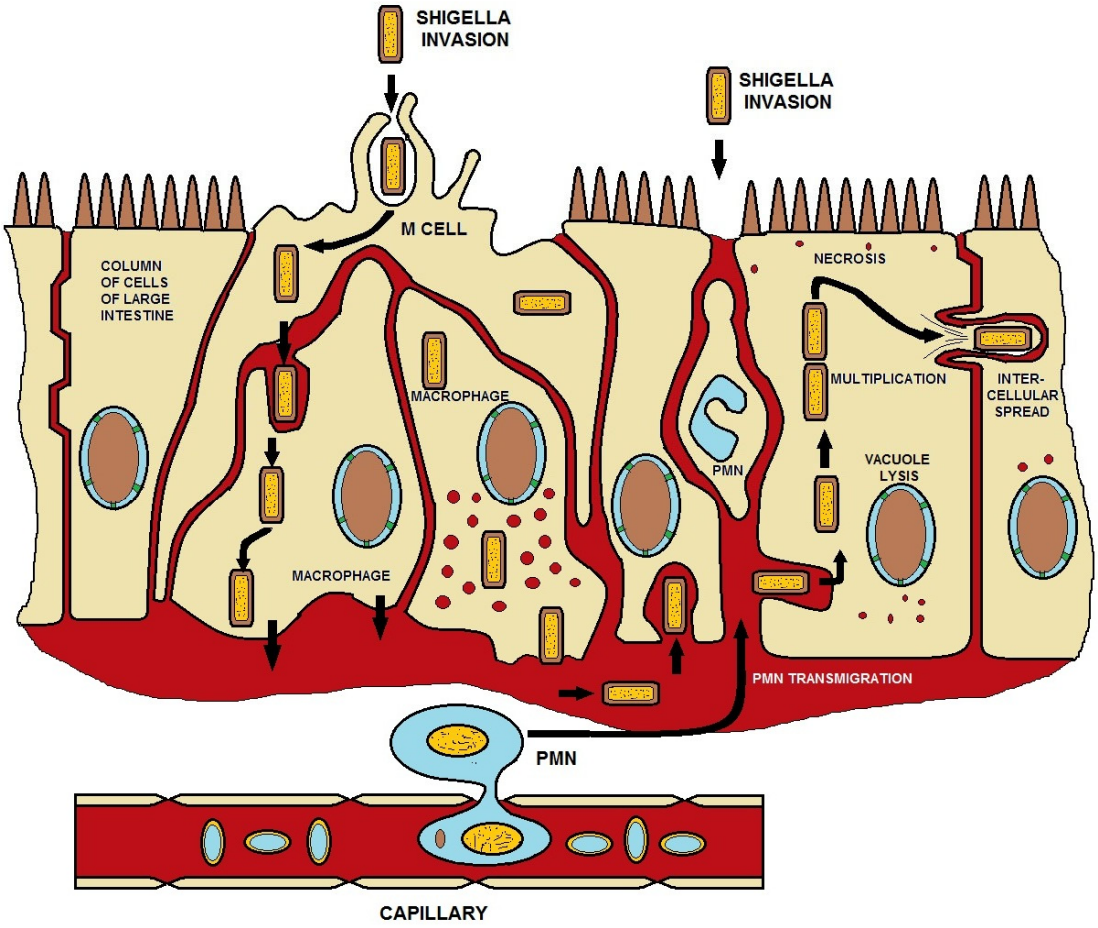
Colonization of the respiratory tract by Pseudomonas requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play a role in colonization of the respiratory tract since P. aeruginosa will adhere to tracheal epithelial cells of mice infected with Influenza virus but not to normal tracheal epithelium.

This has been called opportunistic adherence, and it may be an important step in Pseudomonas keratitis and urinary tract infections, as well as infections of the respiratory tract. The receptor on tracheal epithelial cells for Pseudomonas pili is probably sialic acid (N-acetylneuraminic acid). Mucoid strains, which produce an exopolysaccharide (alginate) have an additional or alternative adhesion which attaches to the tracheobronchial mucin (N-acetylglucosamine).

Besides pili and the mucoid polysaccharide, there are possibly two other cell surface adhesions utilized by Pseudomonas to colonize the respiratory epithelium or mucin. Also, it is likely that surface-bound exoenzyme S could serve as an adhesion for glycolipids on respiratory cells. The mucoid exopolysaccharide produced by P. aeruginosa is a repeating polymer of mannuronic and glucuronic acid referred to as alginate.

Alginate slime forms the matrix of the Pseudomonas biofilm which anchors the cells to their environment and, in medical situations, it protects the bacteria from the host defenses such as lymphocytes, phagocytes, the ciliary action of the respiratory tract, antibodies and complement.

Biofilm mucoid strains of P. aeruginosa are also less susceptible to antibiotics than their planktonic counterparts. Mucoid strains of P. aeruginosa are most often isolated from patients with cystic fibrosis and they are usually found in post mortem lung tissues from such individuals.

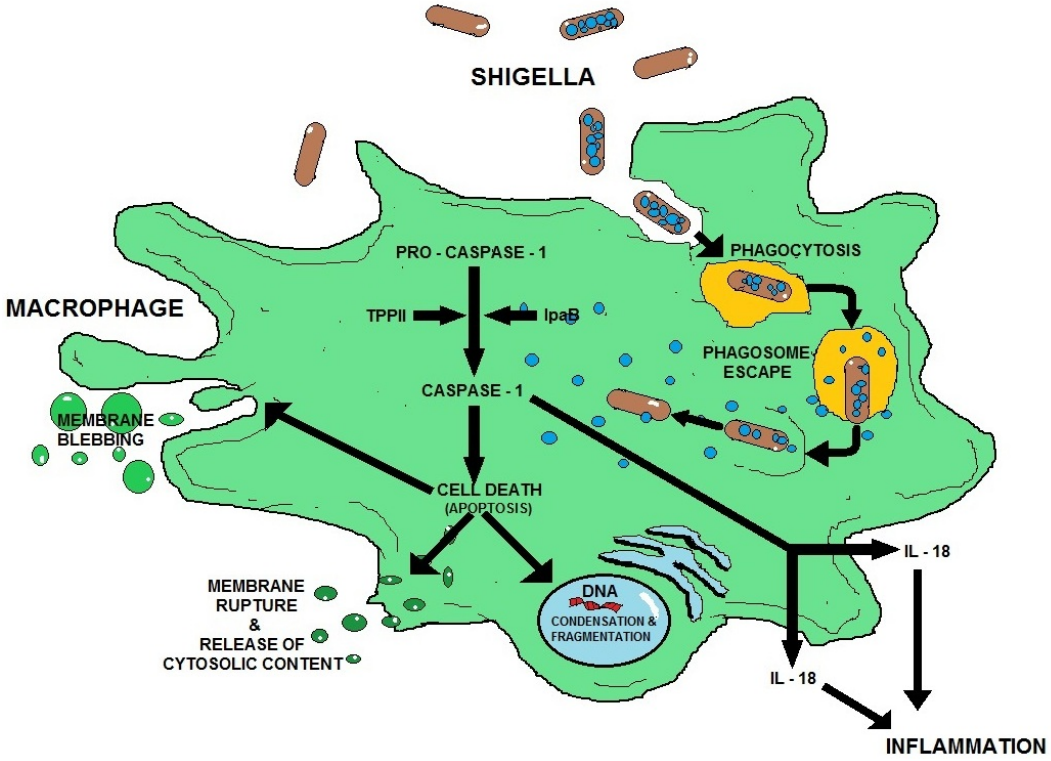


SHIGELLA LIFE CYCLE BEGINS WITH THE PENETRATION OF COLONIC MUCOSA

Shigellosis Shigella Sub-Section

Shigella dysenteriae type 1(or bacillary dysentery) is the only cause of epidemic dysentery. This organism is generally found in the stool of infected individuals, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days. Shigella can even survive in dust particles for six weeks at room temperature.

Infected humans act as host for this particular organism, as well as primates. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. Transmission occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.



SHIGELLOSIS DIAGRAM

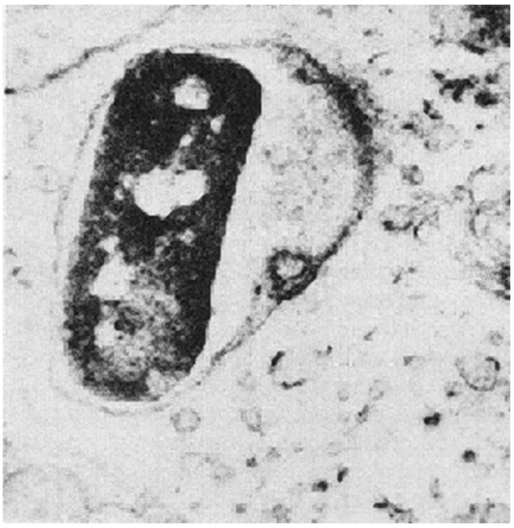
What sort of germ is *Shigella*?
The *Shigella* germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person. *Shigella* were discovered over 100 years ago by a Japanese scientist named Shiga, for whom they are named. There are several different kinds of *Shigella* bacteria: *Shigella sonnei*, also known as "Group D" *Shigella*, accounts for over two-thirds of the shigellosis in the United States. A second type, *Shigella flexneri*, or "group B" *Shigella*, accounts for almost all of the rest. Other types of *Shigella* are rare in this country, though they continue to be important causes of disease in the developing world. One type found in the developing world, *Shigella dysenteriae* type 1, causes deadly epidemics there.

Microbial Characteristics
Shigella dysenteriae is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and lactose and lysine (-). This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.

Shigella dysenteriae is the organism responsible for bacillary dysentery. This disease is most often associated with areas of overcrowding and poor sanitation (developing countries). Illness does, however, tend to be seasonal, happening when it is hot and wet. Symptoms of dysentery due to this organism include mild to severe diarrhea, which is sometimes bloody or watery.

There is also fever and nausea that accompany the diarrhea. Some people, however, also suffer from vomiting and cramping, and some show no symptoms at all. The symptoms of the disease will generally show between 12-96 hours (1-3 days) after becoming infected.

During this incubation period, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.



Micrograph of intra-epithelial membrane-enclosed *Shigella*
(from *Microbiology: Fundamentals and Applications* by R. M. Atlas, p. 609)

How can *Shigella* infections be diagnosed?
Many different kinds of diseases can cause diarrhea and bloody diarrhea, and the treatment depends on which germ is causing the diarrhea. Determining that *Shigella* is the cause of the illness depends on laboratory tests that identify *Shigella* in the stools of an infected person. These tests are sometimes not performed unless the laboratory is instructed specifically to look for the organism. The laboratory can also do special tests to tell which type of *Shigella* the person has and which antibiotics, if any, would be best to treat it.

How can *Shigella* infections be treated?
Shigellosis can usually be treated with antibiotics. The antibiotics commonly used for treatment are ampicillin, trimethoprim/sulfamethoxazole (also known as Bactrim* or Septra*), nalidixic acid, or ciprofloxacin. Appropriate treatment kills the *Shigella* bacteria that might be present in the patient's stools, and shortens the illness. Unfortunately, some *Shigella* bacteria have become resistant to antibiotics and using antibiotics to treat shigellosis can actually make the germs more resistant in the future.

Persons with mild infections will usually recover quickly without antibiotic treatment. Therefore, when many persons in a community are affected by shigellosis, antibiotics are sometimes used selectively to treat only the more severe cases. Antidiarrheal agents such as loperamide (Imodium*) or diphenoxylate with atropine (Lomotil*) are likely to make the illness worse and should be avoided.

Are there long term consequences to a *Shigella* infection?
Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. About 3% of persons who are infected with one type of Shigella, *Shigella flexneri*, will later develop pains in their joints, irritation of the eyes, and painful urination. This is called Reiter's syndrome. It can last for months or years, and can lead to chronic arthritis which is difficult to treat. Reiter's syndrome is caused by a reaction to *Shigella* infection that happens only in people who are genetically predisposed to it.

Once someone has had shigellosis, they are not likely to get infected with that specific type again for at least several years. However, they can still get infected with other types of *Shigella*.

How do people catch *Shigella*?
The *Shigella* bacteria pass from one infected person to the next. *Shigella* are present in the diarrheal stools of infected persons while they are sick and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person.

This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.

Shigella infections may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. *Shigella* infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with shigellosis swims in it.

What can a person do to prevent this illness?
There is no vaccine to prevent shigellosis. However, the spread of *Shigella* from an infected person to other persons can be stopped by frequent and careful handwashing with soap.

Frequent and careful handwashing is important among all age groups. Frequent, supervised handwashing of all children should be followed in day care centers and in homes with children who are not completely toilet-trained (including children in diapers). When possible, young children with a *Shigella* infection who are still in diapers should not be in contact with uninfected children.

People who have shigellosis should not prepare food or pour water for others until they have been shown to no longer be carrying the *Shigella* bacterium.

If a child in diapers has shigellosis, everyone who changes the child's diapers should be sure the diapers are disposed of properly in a closed-lid garbage can, and should wash his or her hands carefully with soap and warm water immediately after changing the diapers. After use, the diaper changing area should be wiped down with a disinfectant such as household bleach, Lysol* or bactericidal wipes.

Basic food safety precautions and regular drinking water treatment prevents shigellosis. At swimming beaches, having enough bathrooms near the swimming area helps keep the water from becoming contaminated.

Simple precautions taken while traveling to the developing world can prevent getting shigellosis. Drink only treated or boiled water, and eat only cooked hot foods or fruits you peel yourself. The same precautions prevent traveler's diarrhea in general.

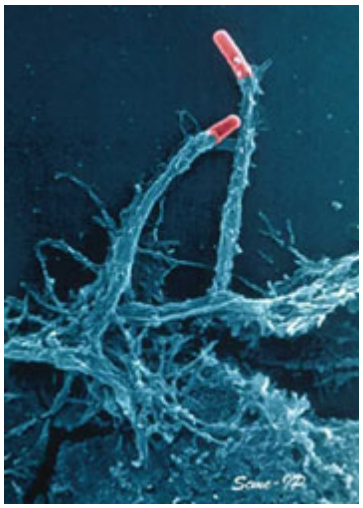
How common is shigellosis?
Every year, about 18,000 cases of shigellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be twenty times greater. Shigellosis is particularly common and causes recurrent problems in settings where hygiene is poor and can sometimes sweep through entire communities. Shigellosis is more common in summer than winter. Children, especially toddlers aged 2 to 4, are the most likely to get shigellosis. Many cases are related to the spread of illness in child-care settings, and many more are the result of the spread of the illness in families with small children.

In the developing world, shigellosis is far more common and is present in most communities most of the time. Chinese scientists have sequenced the genome of a bacterium that is a leading cause of infant mortality in developing countries. About one million people die of *Shigella* infections every year, most of them children. The bacterium *Shigella flexneri* causes sudden and severe diarrhea in humans, known as shigellosis.

New treatments are needed for this highly infectious microbe because antibiotics are often inadequate and drug-resistant strains are on the rise. Currently, no vaccines exist and the World Health Organization considers the development of a vaccine a priority.

The publication of the genome sequence is an important step achieving this goal. The researchers identified regions of DNA linked to the virulence of the organism; these are promising targets for vaccines.

The sequenced *S. flexneri* strain was isolated from a patient with severe acute shigellosis in Beijing in 1984. The bacterium is commonly found in water polluted with human feces. It is transmitted in contaminated food or water and through contact between people. Upon infection, humans develop severe abdominal cramps, fever, and frequent passage of bloody stools.



Highly infectious microbe *Shigella flexneri*. Courtesy P. Sansonetti, Institut Pasteur, Paris, France

The bacterium has about 4,700 genes. The *S. flexneri* genome consists of a chromosome and a smaller DNA structure called a virulence plasmid, which contains genes important in causing disease. The plasmid includes regions that are densely populated with genes called pathogenicity islands.

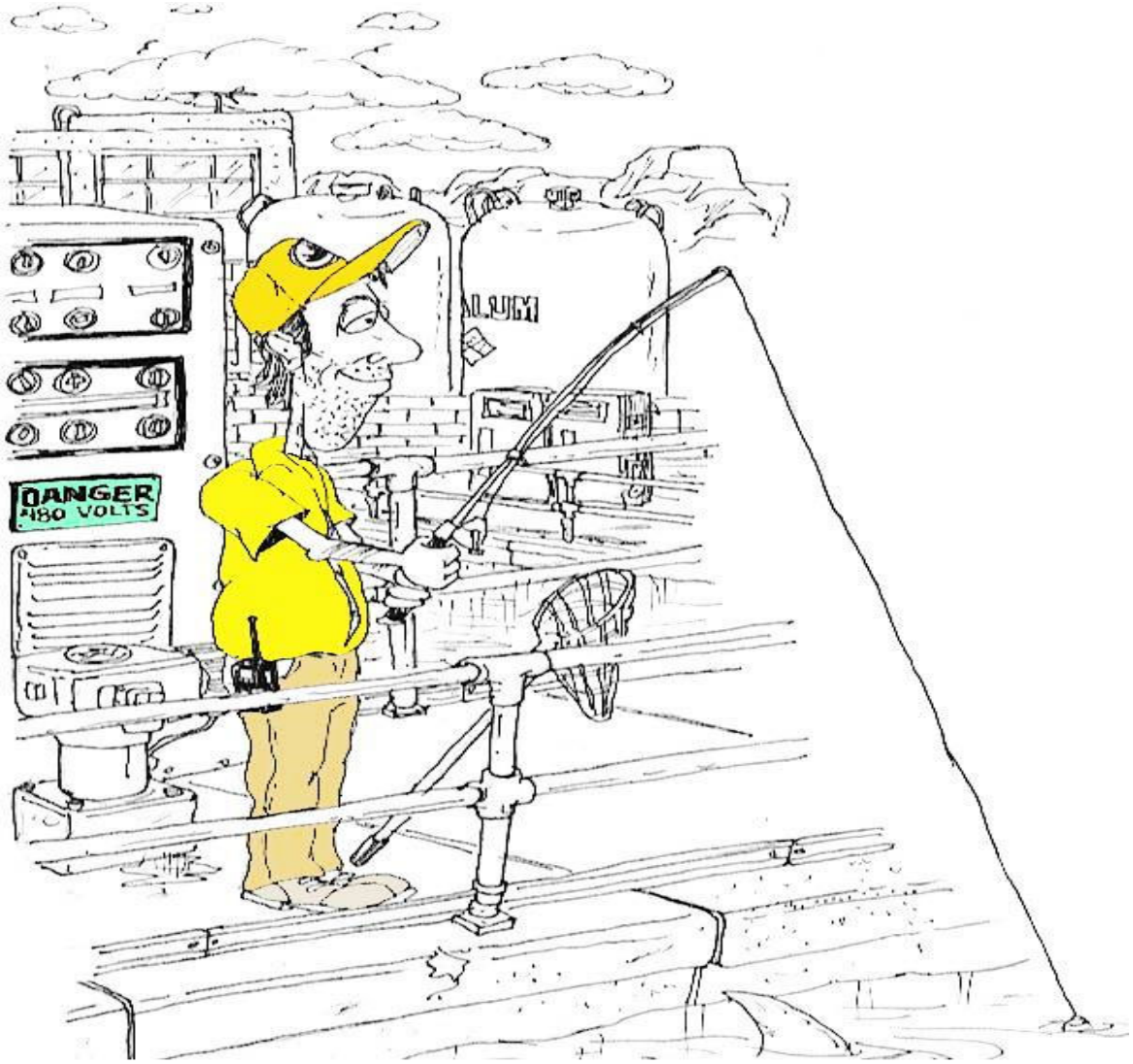
What else can be done to prevent shigellosis?
It is important for the public health department to know about cases of shigellosis. It is important for clinical laboratories to send isolates of *Shigella* to the City, County or State Public Health Laboratory so the specific type can be determined and compared to other *Shigella*. If many cases occur at the same time, it may mean that a restaurant, food or water supply has a problem which needs correction by the public health department. If a number of cases occur in a day-care center, the public health department may need to coordinate efforts to improve handwashing among the staff, children, and their families.

When a community-wide outbreak occurs, a community-wide approach to promote handwashing and basic hygiene among children can stop the outbreak. Improvements in hygiene for vegetables and fruit picking and packing may prevent shigellosis caused by contaminated produce.

Some prevention steps occur every day, without thinking about it. Making municipal water supplies safe and treating sewage are highly effective prevention measures that have been in place for many years.

What is the government doing about shigellosis?
The Centers for Disease Control and Prevention (CDC) monitors the frequency of *Shigella* infections in the country, and assists local and State health departments to investigate outbreaks, determine means of transmission and devise control measures. CDC also conducts research to better understand how to identify and treat shigellosis.

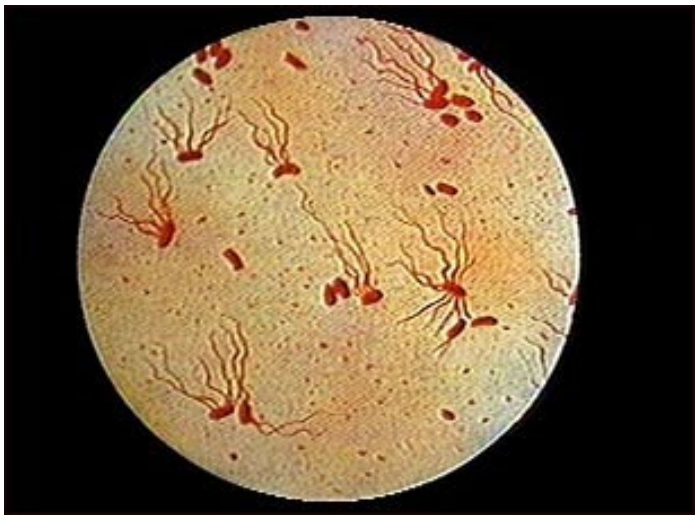
The Food and Drug Administration inspects imported foods, and promotes better food preparation techniques in restaurants and food processing plants. The Environmental Protection Agency regulates and monitors the safety of our drinking water supplies. The government has also maintained active research into the development of a *Shigella* vaccine.


DANGER
480 VOLTS

Typhoid Fever *Salmonella* typhi Sub-Section

Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella* Typhi. In the United States about 400 cases occur each year, and 70% of these are acquired while traveling internationally. Typhoid fever is still common in the developing world, where it affects about 12.5 million persons each year.

Typhoid fever can be prevented and can usually be treated with antibiotics. If you are planning to travel outside the United States, you should know about typhoid fever and what steps you can take to protect yourself.



Salmonella typhi

Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed *S.* Typhi in their feces (stool).

You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding *S.* Typhi or if sewage contaminated with *S.* Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage. Once *S.* Typhi bacteria are eaten or drunk, they multiply and spread into the blood-stream. The body reacts with fever and other signs and symptoms.

In 1885, pioneering American veterinary scientist, Daniel E. Salmon, discovered the first strain of Salmonella from the intestine of a pig. This strain was called Salmonella choleraesuis, the designation that is still used to describe the genus and species of this common human pathogen. Salmonella is a type of bacteria that causes typhoid fever and many other infections of intestinal origin. Typhoid fever, rare in the U.S., is caused by a particular strain designated Salmonella typhi. But illness due to other Salmonella strains, just called "salmonellosis," is common in the U.S. Today, the number of known strains (technically termed "serotypes" or "serovars") of this bacteria total over 2300.

Serotypes
Salmonella serotypes typhimurium and enteritidis are the most common serotypes in the United States. In recent years, concerns have been raised because many strains of Salmonella have become resistant to several of the antibiotics traditionally used to treat it, in both animals and humans.

Getting vaccinated
If you are traveling to a country where typhoid is common, you should consider being vaccinated against typhoid. Visit a doctor or travel clinic to discuss your vaccination options.

Remember that you will need to complete your vaccination at least 1 week before you travel so that the vaccine has time to take effect. Typhoid vaccines lose effectiveness after several years; if you were vaccinated in the past, check with your doctor to see if it is time for a booster vaccination. Taking antibiotics will not prevent typhoid fever; they only help treat it.

Typhoid Fever *Salmonella* typhi Summary
Typhoid fever, also known as Typhoid, is a common worldwide bacterial disease, transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium Salmonella typhi, *Serovar Typhi.*

Salmonella typhi is a Gram-negative short bacillus that is motile due to its peritrichous flagella. Salmonella typhi grows best at 37°C / 98.6°F – human body temperature.

Typhoid fever is unrelated to Typhus. This fever received various names, such as gastric fever, *Abdominal typhus*, infantile remittent fever, slow fever, nervous fever, pythogenic fever, etc.

Typhoid fever is divided into 4 individual stages, each lasting approximately 1 week. In the 1st week, the temperature rises slowly and fever fluctuations are seen with relative bradycardia, malaise, headache, and cough.

According to the text, there is leukopenia, with eosinopenia and relative lymphocytosis, a positive reaction and blood cultures are positive for *Salmonella typhi or paratyphi.*

Tularemia *Francisella tularensis Sub*-Section

What is Tularemia?
Tularemia is a potentially serious illness that occurs naturally in the United States. It is caused by the bacterium *Francisella tularensis* found in animals (especially rodents, rabbits, and hares).

What are the Symptoms of Tularemia?
Symptoms of tularemia could include:

- sudden fever
- chills
- headaches
- diarrhea
- muscle aches
- joint pain
- dry cough
- progressive weakness

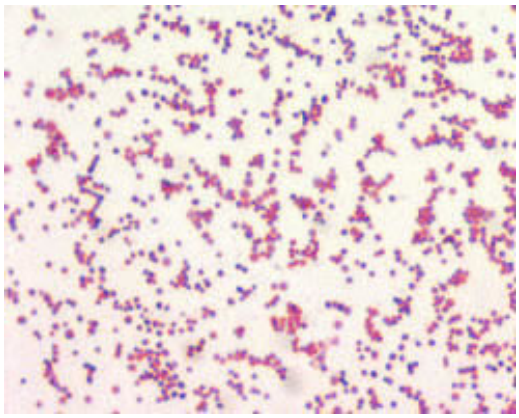
People can also catch pneumonia and develop chest pain,
bloody sputum, and can have trouble breathing, even sometimes stop breathing.

Other symptoms of tularemia depend on how a person was exposed to the tularemia bacteria. These symptoms can include ulcers on the skin or mouth, swollen and painful lymph glands, swollen and painful eyes, and a sore throat.

How Does Tularemia Spread?
People can get tularemia many different ways:

- being bitten by an infected tick, deerfly, or other insect
- handling infected animal carcasses
- eating or drinking contaminated food or water
- breathing in the bacteria, *F. tularensis*

Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated. People who have been exposed to the tularemia bacteria should be treated as soon as possible. The disease can be fatal if it is not treated with the right antibiotics.



F. tularensis
Gram stain

How Soon Do Infected People Get Sick?
Symptoms usually appear 3 to 5 days after exposure to the bacteria, but can take as long as 14 days.

What Should I Do if I Think I Have Tularemia?
Consult your doctor at the first sign of illness. Be sure to let the doctor know if you are pregnant or have a weakened immune system.

How Is Tularemia Treated?
Your doctor will most likely prescribe antibiotics, which must be taken according to the directions supplied with your prescription to ensure the best possible result. Let your doctor know if you have any allergy to antibiotics. A vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States.

What Can I Do To Prevent Becoming Infected with Tularemia?
Tularemia occurs naturally in many parts of the United States. Use insect repellent containing DEET on your skin, or treat clothing with repellent containing permethrin, to prevent insect bites. Wash your hands often, using soap and warm water, especially after handling animal carcasses. Be sure to cook your food thoroughly and that your water is from a safe source.

Note any change in the behavior of your pets (especially rodents, rabbits, and hares) or livestock, and consult a veterinarian if they develop unusual symptoms.

Can Tularemia Be Used As a Weapon?
Francisella tularensis is very infectious. A small number (10-50 or so organisms) can cause disease. If *F. tularensis* were used as a weapon, the bacteria would likely be made airborne for exposure by inhalation. People who inhale an infectious aerosol would generally experience severe respiratory illness, including life-threatening pneumonia and systemic infection, if they are not treated. The bacteria that cause tularemia occur widely in nature and could be isolated and grown in quantity in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication.

F. tularensis is a small Gram-negative aerobic bacillus with two main serotypes: Jellison Type A and Type B. Type A is the more virulent form. The causative agent of the disease was named after Dr. Edward Francis and the location where the organism was discovered, Tulare County, California. Tularemia is frequently spread by direct contact with rabbits, leading to the term "rabbit fever." However, the disease can also be spread by other animals, typically rodents, and by arthropods. It is a primarily rural disease that is found in all 50 states, except Hawaii.

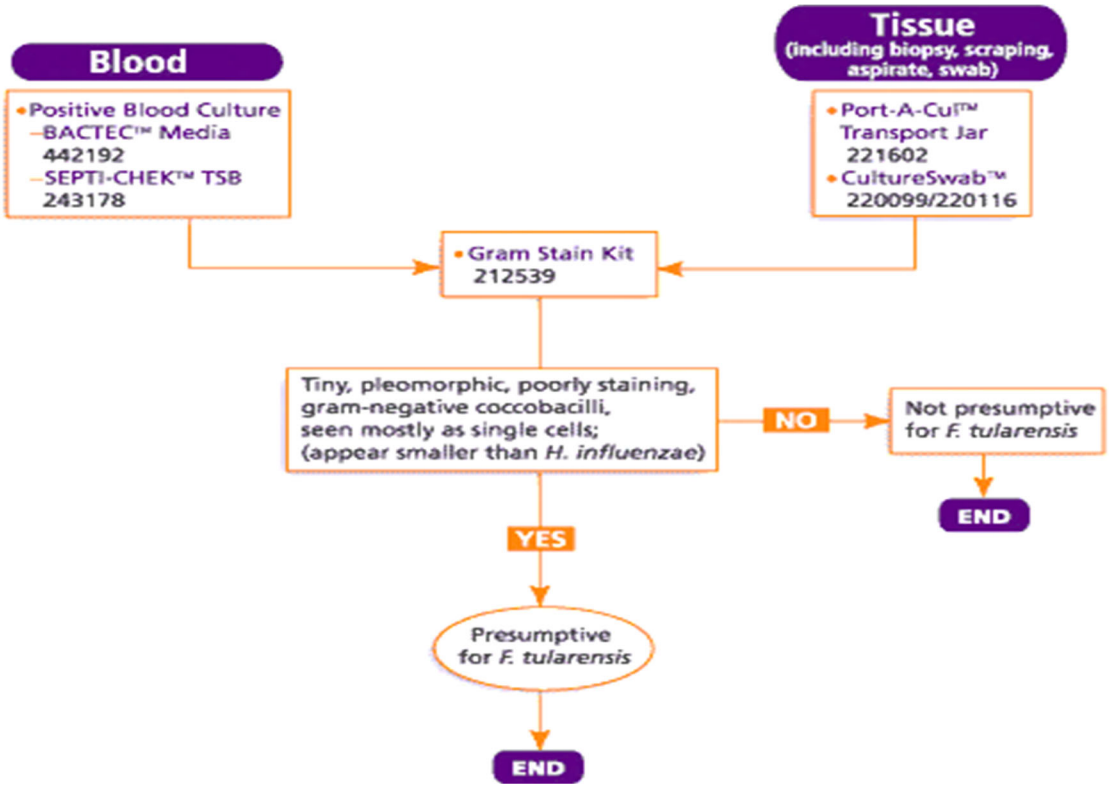
Pathogenesis
Historical commentaries reference the virulence of the disease, indicating that people have been aware of pathogenicity of Francisella for thousands of years. However, there is still much to be learned about this extremely virulent organism. The disease can be contracted by ingestion, inhalation, or by direct skin contact. Tularemia occurs in six different forms: typhoidal, pneumonic, oculoglandular, oropharyngeal, ulceroglandular, and glandular. Clinical diagnosis can be difficult since the disease mimics a slough of other illnesses. Pathogenesis varies greatly depending on mode of infection.

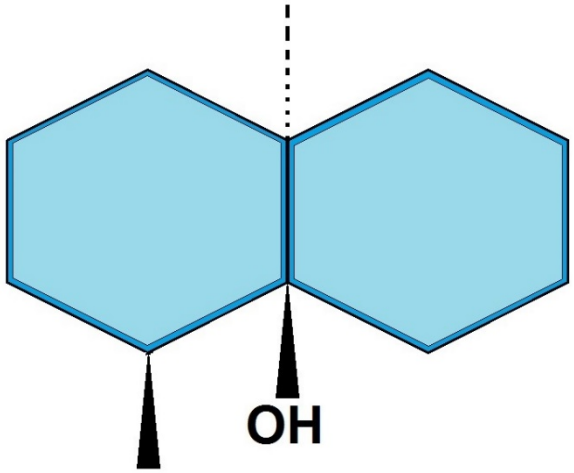
Manifestations
The incubation period is about 3-5 days but it can take as long as two weeks for symptoms to appear. Symptoms vary based on mode of infection, but generally include fever, chills, joint and muscle pain, headache, weakness, and sometimes pneumonia. People who develop pneumonic tularemia experience chest pain, bloody sputum, and difficulty breathing. The disease is easily cured by antibiotic treatment.

Treatment
If infection is suspected, diagnosis can be made based on serological assays since *F. tularensis* is difficult to culture on standard media. Agglutination titers can be performed following the first week of infection and reach a peak during the 4-8 weeks. Infected individuals are normally placed on a regimen of streptomycin or gentamycin for 10-14 days. Beta-lactams are generally ineffective due to beta-lactamase activity.

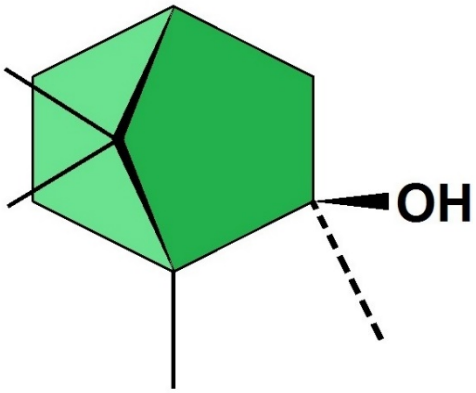
What is CDC Doing About Tularemia?
The CDC operates a national program for bioterrorism preparedness and response that incorporates a broad range of public health partnerships. Other things CDC is doing include:

- Stockpiling antibiotics to treat infected people.
- Coordinating a nation-wide program where states share information about tularemia.
- Creating new education tools and programs for health professionals, the public, and the media.





(-)- GEOSMIN



(-)- METHYLISOBORNEOL

TWO MOST COMMON OCCURING CHEMICALS ATTRIBUTED TO UNPLEASANT TASTE AND ODORS IN DRINKING WATER

MIB and Geosmin Sub-Section

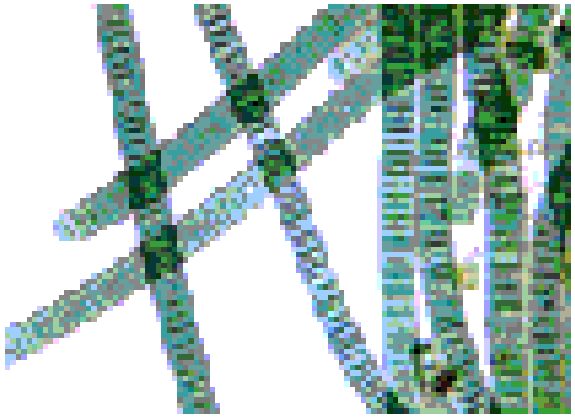
Aesthetics only and not a disease concern
While I was working in the water quality laboratory, we would be overwhelmed by customers calling in and worrying about tastes and odors. While this small section is not really about a waterborne disease, water customers will react to this as if was a disease.

Be prepared
Seasonal occurrences of musty/moldy or earthy tastes and odors may be detected in the system water. Research by laboratories dedicated to this subject, has determined the culprits are naturally occurring algal and fungal (microbiological) by-products. As algae in the canals die, compounds known as Methyl-Isoborneol (MIB) and Geosmin are released into the water.

These stable complex compounds present in parts per trillion are difficult to remove with current technology. The detection of these compounds is dependent upon an individual's olfactory sensitivity. Many people may never detect them, while others who are sensitive may detect the musty/moldy taste and smell at levels below instrument detection levels. Most water providers use activated carbon to adsorb the MIB and Geosmin, thus alleviating the taste and odor.

Earthy-musty tastes and odors are produced by certain cyanobacteria (blue-green algae), actinomycetes, and a few fungi. The substances are produced by actinomycetes and cyanobacteria that cause tastes and odors in drinking-water include geosmin, methyl-isoborneol (MIB), and cardin-4-ene-1-ol. Growing algae produce numerous volatile and nonvolatile organic substances, including aliphatic alcohols, aldehydes, ketones, esters, thioesters, and sulfides.

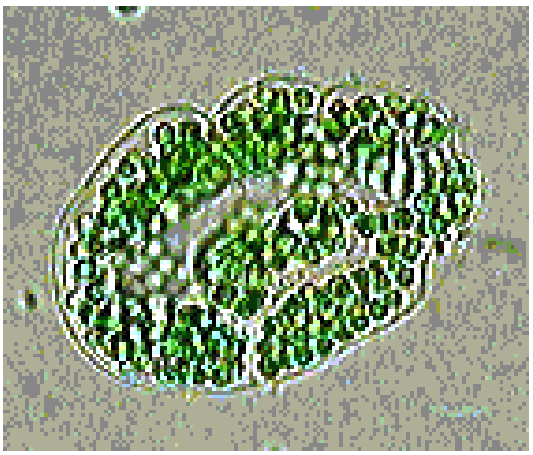
Occasionally, taste and odor problems in water are caused by other bacteria, fungi, zooplankton, and nemathelminthes. Ferrobacteria in water-distribution systems may produce tastes and odors, and some species of Pseudomonas can cause a swampy odor, whereas others can convert sulfur-containing amino acids into hydrogen sulfide, methylthiol, and dimethylpolysulfide.



Oscillatoria chalybea

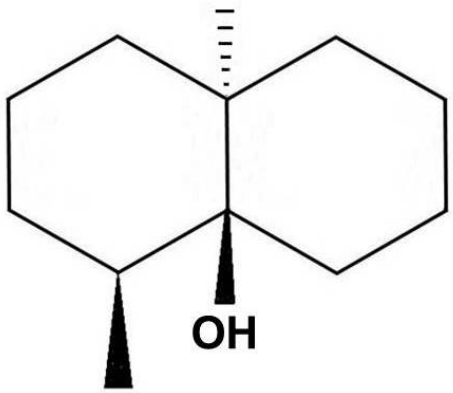
Blooms

Blooms will produce the noxious substances 2-methyl isoborneol (MIB) and geosmin, compounds that are responsible for causing an off-flavor in catfish. MIB and geosmin are often described as smelling like "***sweaty socks***."



Microcystis aeruginosa

Commonly found in lakes and ponds. In the spring, large numbers floating on the water surface produce a blue-tinge. Blooms of this cyanobacterium are also notorious for producing a liver toxin that in large amount can kill fish and livestock.



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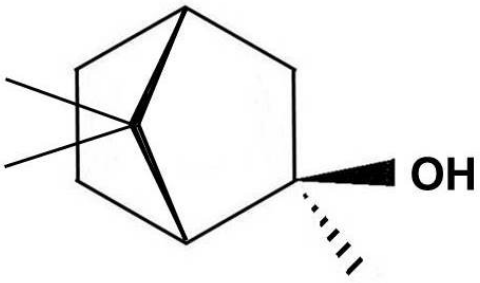


(-)-2- METHYLISOBORNEOL

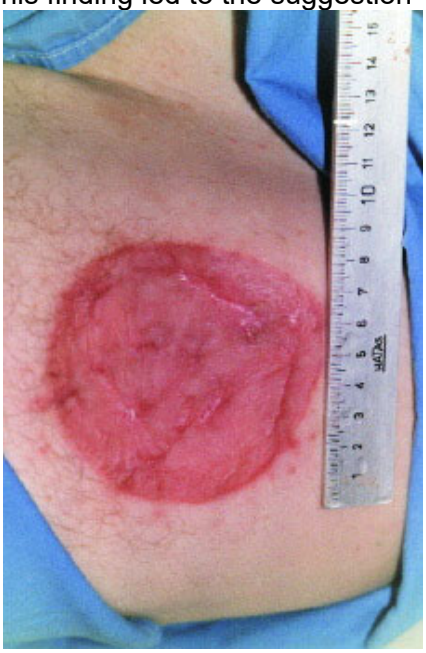
Chemical Related Diseases

Arsenic

Arsenic is a naturally occurring element used since ancient times and has long been known to be toxic to humans. Arsenic in ground water is largely the result of minerals dissolving from weathered rocks and soils. Several types of cancer have been linked to arsenic in water. In 2001 the US Environmental Protection Agency lowered the maximum level of arsenic permitted in drinking water from 50 micrograms per liter (ug/L) to 10 ug/L.

Gastrointestinal and nervous system effects are common and the ingestion of relatively small amounts can result in death. A recent study indicates that arsenic disrupts the activity of glucocorticoids, compounds that have a variety of functions including the regulation of blood sugar.

Interestingly, this same study suggested that arsenic at high levels inhibits those mechanisms that normally suppress tumor production. This finding led to the suggestion that instead of causing cancer arsenic promotes the growth of tumors triggered by other carcinogens. And by the way, arsenic-induced effects appeared at concentrations as low as 2 micrograms per liter. Keep in mind, the new EPA regulation calls for 10 micrograms per liter and the average person ingests 10-15 micrograms per day. These numbers suggest we're getting our minimal daily requirement.



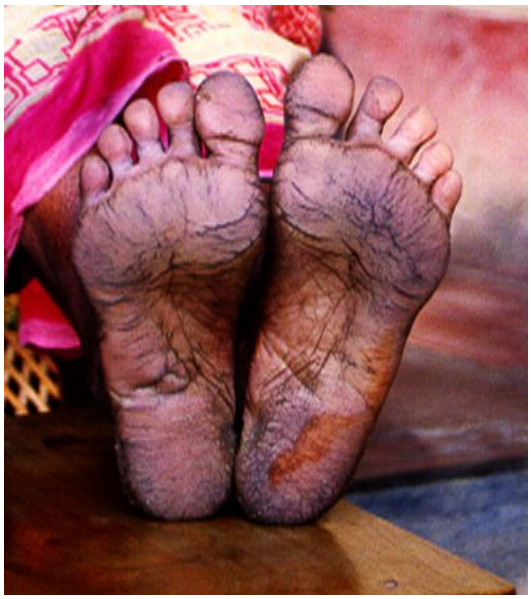
Other epidemiological studies suggest an association between drinking arsenic-tainted water and skin, lung, liver and bladder cancers. A 1999 report by the National Academy of Sciences estimated that daily ingestion of water containing 50 micrograms of arsenic per liter would add about 1 percent to a person's lifetime risk of dying from cancer.

Some studies also found that arsenic harms the central and peripheral nervous systems as well as heart and blood vessels. Arsenic has been associated with birth defects and reproductive problems.

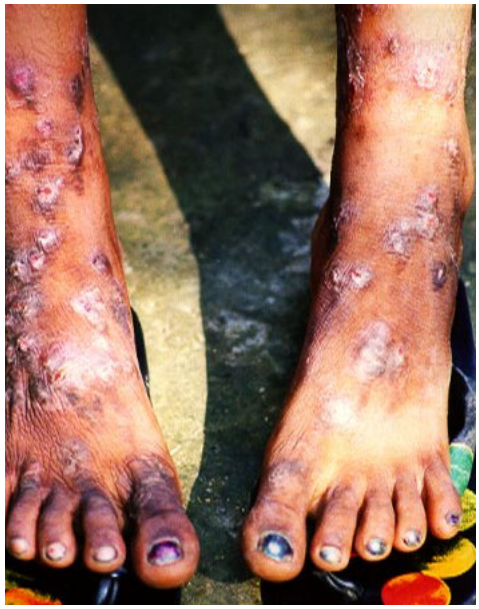
Conservative estimates based on all these data suggest that more than 34 million Americans drink tap water supplied by systems containing average levels of arsenic that pose unacceptable cancer risks. In October 2001, the Environmental Protection Agency implemented new standards for arsenic in drinking water, lowering the maximum acceptable level in parts per billion from 50ppb to 10ppb.

Exposure to higher than average levels of arsenic occurs mostly in the workplace, near hazardous waste sites, or in areas with high natural levels. At high levels, inorganic arsenic can cause death. Exposure to lower levels for a long time can cause a discoloration of the skin and the appearance of small corns or warts.

Arsenic has been found at 1,014 of the 1,598 National Priority List sites identified by the Environmental Protection Agency (EPA).



Keratosis of the feet



Blackfoot disease

What happens to arsenic when it enters the environment?

- Arsenic cannot be destroyed in the environment. It can only change its form.
- Arsenic in air will settle to the ground or is washed out of the air by rain.
- Many arsenic compounds can dissolve in water.
- Fish and shellfish can accumulate arsenic, but the arsenic in fish is mostly in a form that is not harmful.

Is there a medical test to show whether I've been exposed to arsenic?

There are tests to measure the level of arsenic in blood, urine, hair, or fingernails. The urine test is the most reliable test for arsenic exposure within the last few days. Tests on hair and fingernails can measure exposure to high levels of arsenic over the past 6-12 months.

These tests can determine if you have been exposed to above-average levels of arsenic. They cannot predict how the arsenic levels in your body will affect your health.

Blue Baby Syndrome (Methemoglobinemia) Sub-Section

Methemoglobin is an abnormal form of hemoglobin which is unable to transport oxygen. Methemoglobinemia can be an inherited disorder, but it also can be acquired through exposure to chemicals such as nitrates (nitrate-contaminated water), aniline dyes, and potassium chlorate.

Causes, incidence, and risk factors
There are two forms of inheritable methemoglobinemia, Type I and Type II. Most hereditary cases are Type II, and result from a deficiency in the enzyme cytochrome b5 reductase. The other inheritable type, called hemoglobin M disease (Type I), is an autosomal dominant condition (you only need one affected parent to inherit it) characterized by an inability to convert methemoglobin back to hemoglobin. This usually causes few problems.

Acquired by Drinking Water and Other Causes
Exposure to certain chemicals may also cause an increase in the production of methemoglobin. These chemicals include nitrites (used commonly to prevent spoilage of meat), xylocaine, and benzene.

Nitrates and nitrites are nitrogen-oxygen chemical units which combine with various organic and inorganic compounds. The greatest use of nitrates is as a fertilizer. Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia, should be considered as potential nitrate sources. Primary sources of organic nitrates include human sewage and livestock manure, especially from feedlots.

What happens to nitrates/nitrites when they are released to the environment?
Since they are very soluble and do not bind to soils, nitrates have a high potential to migrate to ground water. Because they do not evaporate, nitrates/nitrites are likely to remain in water until consumed by plants or other organisms.

Short-term
Excessive levels of nitrate in drinking water have caused serious illness and sometimes death. The serious illness in infants is due to the conversion of nitrate to nitrite by the body, which can interfere with the oxygen-carrying capacity of the child's blood. This can be an acute condition in which health deteriorates rapidly over a period of days. Symptoms include shortness of breath and blueness of the skin. Long-term: Nitrates and nitrites have the potential to cause the following effects from a lifetime exposure at levels above the MCL: diuresis, increased starchy deposits and hemorrhaging of the spleen.

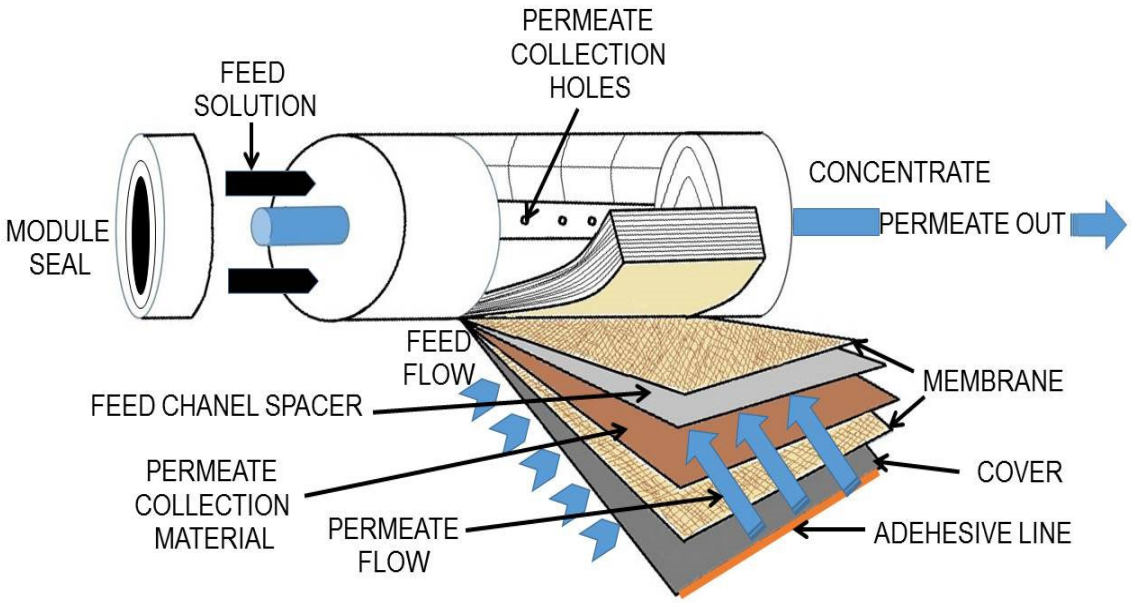
Follow-up
Retest regularly. Nitrate levels greater than 5 mg/L indicate the possibility that agricultural chemicals may be reaching the water source, and pesticide testing is recommended.

Point of Use Filtration
Nitrates cannot be removed from water by such treatments as UV lights, chlorinators, carbon filters, water softeners, iron filters, neutralizers. Nitrates can be removed by properly designed distillers, RO systems, and anion exchange systems.

Whereas distillers and RO systems are only suitable for point of use (i.e., one or two faucets in the home), anion exchange systems remove nitrates from the whole house.

Nitrate specific resin should be used with anion exchange systems to prevent the possibility of a maladjusted or malfunctioning anion exchange system from increasing the nitrate level due to sulfate exchange.

We recommend that persons shopping for nitrate removal systems shop carefully and purchase only from a dealer experienced in nitrate removal.



REVERSE OSMOSIS MEMBRANE

Primary Waterborne Diseases Section - Alphabetical Order

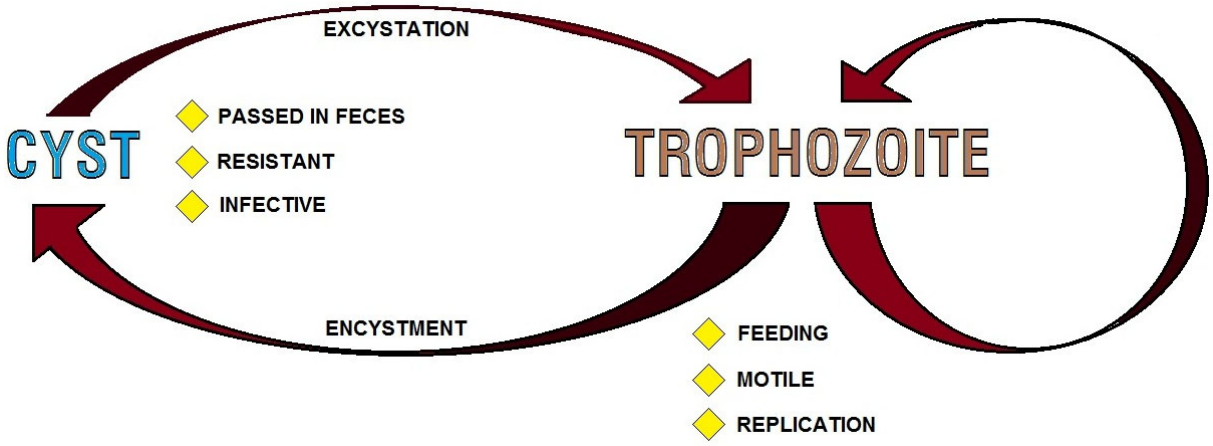
Campylobacter
Campylobacter, the basics. It is a bacterium. It causes diarrheal illness. Campylobacter is primarily associated with poultry, animals, and humans.

Campylobacter prevention: Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Cryptosporidium
Cryptosporidium, the basics. It is a parasite. It causes diarrheal illness known as cryptosporidiosis. It is typically associated with animals and humans, and it can be acquired through consuming fecally contaminated food, contact with fecal contaminated soil and water.

Cryptosporidium, prevention: Prevention strategies for this pathogen include source protection. A CT value of 9,600 is required when dealing with fecal accidents. CT equals a concentration, in parts per million, while time equals a contact time in minutes. Cryptosporidium can also be prevented or eliminated by boiling water for one minute.

Filtration with an "*absolute*" pore size of one micron or smaller can eliminate Cryptosporidium, and reverse osmosis is known to be effective as well.



TYPICAL FECAL-ORAL LIFE CYCLE DIAGRAM

E-Coli Section
Escherichia coli. Escherichia coli O157:H7, the basics. It's a bacteria. There are several pathogenic strains of Escherichia coli, which are classified under enterovirulent E. coli. They are enterohemorrhagic, enteroinvasive, enterotoxigenic, enteropathogenic, and enteroaggregative causes diarrheal illness, and it's classified as an enterohemorrhagic E. coli. In its most severe form, it can cause hemorrhagic colitis. The reservoir for this bacteria are cattle, deer, goats, and sheep. Humans can also be a reservoir. It is typically associated with contaminated food and water.

E. coli O157:H7 prevention: Prevention strategies for this pathogen include source protection, halogenation of water, or boiling water for one minute.

Giardia
Giardia, the basics. It is a parasite. It causes diarrheal illness known as giardiasis. It is typically associated with water. It is the most common pathogen in waterborne outbreaks. It can also be found in soil and food, and humans and animals are the reservoir for this pathogen.

Giardia prevention: Prevention strategies for this pathogen include source protection; filtration, coagulation, and halogenation of drinking water.

Hepatitis A
Hepatitis A, the basics. It is a virus. It causes inflammation of the liver, and the reservoir for Hepatitis A virus is humans.

Hepatitis A, Prevention: Prevention strategies for this pathogen include source protection and adequate disinfection. Fecal matter can protect Hepatitis A virus from chlorine. Additionally, Hepatitis A virus is resistant to combined chlorines, so it is important to have an adequate free chlorine residual.

Legionella
Legionella, the basics. It is a bacterium. It causes a respiratory illness known as Legionellosis. There are two illnesses associated with Legionellosis: the first, Legionnaire's disease, which causes a severe pneumonia, and the second, Pontiac fever, which is a non-pneumonia illness; It is typically an influenza-like illness, and it's less severe. Legionella is naturally found in water, both natural and artificial water sources.

Legionella, prevention: Maintaining hot water systems at or above 50 degrees Centigrade and cold water below 20 degrees Centigrade can prevent or control the proliferation of Legionella in water systems. Hot water in tanks should be maintained between 71 and 77 degrees Centigrade.

Proper recreational water system maintenance and disinfection can prevent the proliferation of Legionella in recreational water systems. It is important to prevent water stagnation. This can be accomplished by eliminating dead ends in distribution systems and in recreational water systems. Additionally, preventing biofilm development is important to control this particular pathogen in water systems.

Norovirus
Norovirus, the basics. It is a virus. It causes diarrheal illness, and humans are the reservoir for this virus.

Norovirus, prevention: Prevention strategies for this pathogen include source protection.

Pseudomonas
Pseudomonas, the basics. It is a bacterium. It is caused by dermal contact with water. It can cause dermatitis, which is an inflammation of the skin, or it can cause otitis, which is an infection of the ear. Pseudomonas is typically associated with soil and water.

Pseudomonas prevention: Proper maintenance and disinfection of recreational water systems is important in preventing Pseudomonas.

Salmonella Typhi
Salmonella typhi, the basics. It is a bacterium. It causes diarrheal illness, also known as typhoid fever. Humans are the reservoir for this pathogen. Salmonella species, the basics. It is a bacterium. It causes diarrheal illness known as salmonellosis.

Humans and animals are the reservoir, and it has typically associated with contaminated food and water. Salmonella species, prevention. Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Salmonella typhi, prevention: Prevention strategies for this pathogen include source protection, chlorination or halogenation of water, and boiling water for one minute.

Schistosomatidae
Schistosomatidae, the basics. It is a parasite. It is acquired through dermal contact, cercarial dermatitis. It is commonly known as swimmer's itch. The reservoir for this pathogen are aquatic snails and birds.

Schistosomatidae prevention: Prevention strategies for this pathogen include eliminating snails with a molluscicide or interrupting the life cycle of the parasite by treating birds with an antihelmetic drug.

Shigella Species
Shigella species, the basics. It is a bacterium. It causes diarrheal illness known as shigellosis. Humans and primates are the reservoir for this pathogen. Shigella species, in the United States two-thirds of the shigellosis in the U.S. is caused by Shigella sonnei, and the remaining one-third is caused by Shigella flexnieri. In developing countries, Shigella dysenteriae is the primary cause of illness associated with this pathogen.

Shigella species prevention: Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Vibrio Cholerae
Vibrio cholerae, the basics. It is a bacterium. It causes diarrheal illness, also known as cholera. It is typically associated with aquatic environments, shell stocks, and human. Vibrio cholerae has also been associated with ship ballast water, and there will be a discussion later on in this presentation of an outbreak associated with ship ballast water.

Vibrio cholerae prevention: Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Waterborne Pathogens and Disease Review

Bacteria, viruses, and protozoan that cause disease are known as pathogens. Most pathogens are generally associated with diseases that cause intestinal illness and affect people in a relatively short amount of time, generally a few days to two weeks. They can cause illness through exposure to small quantities of contaminated water or food, or from direct contact with infected people or animals.

How Diseases are Transmitted

Pathogens that may cause waterborne outbreaks through drinking water have one thing in common: they are spread by the fecal-oral or feces-to-mouth route. Pathogens may get into water and spread when infected humans or animals pass the bacteria, viruses, and protozoa in their stool. For another person to become infected, he or she must take that pathogen in through the mouth. Waterborne pathogens are different from other types of pathogens such as the viruses that cause influenza (the flu) or the bacteria that cause tuberculosis. Influenza virus and tuberculosis bacteria are spread by secretions that are coughed or sneezed into the air by an infected person.

Cryptosporidium→

Human or animal wastes in watersheds, failing septic systems, failing sewage treatment plants or cross-connections of water lines with sewage lines provide the potential for contaminating water with pathogens. The water may not appear to be contaminated because the feces has been broken up, dispersed, and diluted into microscopic particles. These particles, containing pathogens, may remain in the water and be passed to humans or animals unless adequately treated.

Only proper treatment will ensure eliminating the spread of disease. In addition to water, other methods exist for spreading pathogens by the fecal-oral route. The foodborne route is one of the more common methods. A frequent source is a food handler who does not wash his hands after a bowel movement and then handles food with *unclean* hands. The individual who eats feces-contaminated food may become infected and ill. It is interesting to note the majority of foodborne diseases occur in the home, not restaurants.

Day care centers are another common source for spreading pathogens by the fecal-oral route. Here, infected children in diapers may get feces on their fingers, then put their fingers in a friend's mouth or handle toys that other children put into their mouths. The general public and some of the medical community usually refer to diarrhea symptoms as stomach flu.

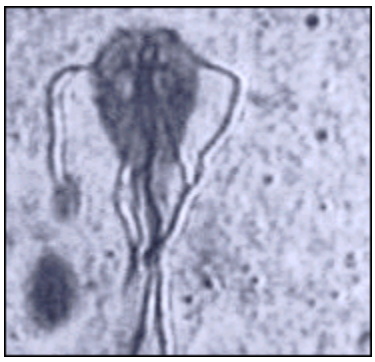
Technically, influenza is an upper respiratory illness and rarely has diarrhea associated with it; therefore, stomach flu is a misleading description for foodborne or waterborne illnesses, yet is accepted by the general public. So the next time you get the stomach flu, you may want to think twice about what you've digested within the past few days.

Chain of Transmission
Water is contaminated with feces. This contamination may be of human or animal origin. The feces must contain pathogens (disease-causing bacteria, viruses or protozoa). If the human or animal source is not infected with a pathogen, no disease will result. The pathogens must survive in the water. This depends on the temperature of the water and the length of time the pathogens are in the water. Some pathogens will survive for only a short time in water, others, such as Giardia or Cryptosporidium, may survive for months.

The pathogens in the water must enter the water system's intake and in numbers sufficient to infect people. The water is either not treated or inadequately treated for the pathogens present. A susceptible person must drink the water that contains the pathogen. Illness (disease) will occur.

This chain lists the events that must occur for the transmission of disease via drinking water. By breaking the chain at any point, the transmission of disease will be prevented.

Bacterial Diseases


Giardia→

Campylobacteriosis is the most common diarrhea illness caused by bacteria. Symptoms include abdominal pain, malaise, fever, nausea and vomiting, and they usually begin three to five days after exposure. The illness is frequently over within two to five days and usually lasts no more than 10 days.

Campylobacteriosis outbreaks have most often been associated with food, especially chicken and unpasteurized milk, as well as un-chlorinated water.

These organisms are also an important cause of travelers' diarrhea. Medical treatment generally is not prescribed for campylobacteriosis because recovery is usually rapid. Cholera, Legionellosis, salmonellosis, shigellosis, and yersiniosis are other bacterial diseases that can be transmitted through water. All bacteria in water are readily killed or inactivated with chlorine or other disinfectants.

Viral-Caused Diseases
Hepatitis A is an example of a common viral disease that may be transmitted through water. The onset is usually abrupt with fever, malaise, loss of appetite, nausea and abdominal discomfort, followed within a few days by jaundice. The disease varies in severity from a mild illness lasting one to two weeks, to a severely disabling disease lasting several months (rare).

Chapter 9- Related Diseases and Associated Illnesses Review

Amebiasis Section Entamoeba histolytica

1. The life cycle of Entamoeba histolytica involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and _____ that are passed in the host's feces.
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

2. Humans are infected by ingesting _____, most often via food or water contaminated with human fecal material (view diagram of the life cycle).
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

3. Entamoeba histolytica has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal _____, feeding on intestinal contents and host tissue, and multiplying by fission.
A. Crypts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

4. The _____ can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, E. histolytica is potentially the most pathogenic.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

5. Entamoeba histolytica is an amoeboid _____ of the intestinal tract and in some cases other visceral organs, especially the liver.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

6. There are several species in this genus, distinguished by their number of _____ in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

7. Within the _____ there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst. The cyst can resist desiccation for 1-2 weeks.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

8. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 _____.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

9. In some cases the _____ secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

10. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal. As with most of the amoebae, infections of E. histolytica are often diagnosed by demonstrating cysts or trophozoites in a stool sample.
A. True
B. False

Amebic Meningoencephalitis PAM Section Naegleria fowleri
11. Primary Amebic Meningoencephalitis (PAM) is a common and usually deadly disease caused by infection with the ameba (a multi-celled organism that maintains the original shape).
A. True
B. False

12. Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.
A. True
B. False

13. The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. Naegleria thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.
A. True
B. False

14. Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.
A. True
B. False

15. The ameba is believed to enter the body through the mouth and travel to the stomach. The disease is easily spread from person to person.
A. True
B. False

16. The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the digestive tract or frequently before death through the examination of digestive lining.
A. True
B. False

17. PAM is a mild illness that responds to routine treatments. Aggressive use of some antifungal medications have always been successful. Intensive supportive care is rarely necessary along with the medication.
A. True
B. False

Schistosomes and Other Trematodes Section
18. Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except _____.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

19. Almost unknown in industrialized countries, _____ infects 200 million people in 76 countries of the tropical developing world.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

20. A Flatworm that spends part of its life in a freshwater snail host causes _____. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

21. Free-swimming larvae (_____) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

22. After entering a human the larvae develop into adult males and females (schistosomes) or _____ (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

23. In _____, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

24. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other _____).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

25. _____ is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. None of the above

26. Signs and symptoms are related largely to the location of the adult worms. Infections with Schistosoma mansoni and S japonicum (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis. Schistosoma haematobium (vesical venules) causes dysuria, hema turia, and uremia. Fasciola hepatica, Clonorchis sinensis, and Opisthorchis viverrini (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice. Infections with Paragonimus westermani (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. Fasciolopsis buski (intestines) causes abdominal pain, diarrhea, and edema.
A. True
B. False

27. Trematodes are single cellular eukaryotic helminths.
A. True
B. False

Gastroenteritis Section
28. _____ means inflammation of the stomach and small and large intestines.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

29. Viral gastroenteritis is an infection caused by a variety of _____ that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

30. Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, _____, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as Salmonella or Escherichia coli) or parasites (such as Giardia), or by medications or other medical conditions, although the symptoms may be similar.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

31. The main symptoms of viral _____ are watery diarrhea and vomiting.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

32. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which _____ causes the illness.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

33. Viral gastroenteritis is _____. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

Rotovirus Information Section
34. The incubation period for _____ disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

35. A _____ has a characteristic wheel-like appearance when viewed by electron microscopy (the name is derived from the Latin rota, meaning "wheel").
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

36. Rotaviruses are nonenveloped, double-shelled viruses. The _____ is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

37. The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the _____, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

38. Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by _____ or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

39. For persons with healthy immune systems, rotavirus _____ is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children will require hospitalization for intravenous fluids.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

Noroviruses Section

40. Noroviruses (genus Norovirus, family Caliciviridae) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official genus name for the group of viruses provisionally described as “Norwalk-like viruses” (NLV).
A. True
B. False

41. The symptoms of norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. The illness is usually brief, with symptoms lasting only about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.
A. True
B. False

42. Persons who are infected with norovirus should not prepare food while they have symptoms and for 3 weeks after they recover from their illness. Food that may have been contaminated by an ill person can be eaten.
A. True
B. False

43. Illness caused by norovirus infection has several names, including stomach flu – this “stomach flu” is **not** related to the flu (or influenza), which is a respiratory illness caused by influenza virus.
A. True
B. False

44. Noroviruses are found in the stool or vomit of infected people. People can become infected with the virus in several ways, including eating food or drinking liquids that are contaminated with norovirus; touching surfaces or objects contaminated with norovirus, and then placing their hand in their mouth; having direct contact with another person who is infected and showing symptoms (for example, when caring for someone with illness, or sharing foods or eating utensils with someone who is ill).
A. True
B. False

45. Persons working in day-care centers or nursing homes should pay special attention to children or residents who have norovirus illness. This virus is very contagious and can spread rapidly throughout such environments.
A. True
B. False

Hepatitis Section

46. Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). Hepatitis A can affect anyone. In the United States, hepatitis A can occur in situations ranging from isolated cases of disease to widespread _____.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

47. Each year, an estimated 100 persons die as a result of _____ in the United States due to Hepatitis A. Approximately 30 - 50,000 cases occur yearly in the United States and the direct and indirect costs of these cases exceed $300 million.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

48. The unfortunate aspect of these statistics is that with 21st century medicine, Hepatitis A is totally _____, and isolated cases, especially outbreaks relegated to food consumption, need not occur.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

49. Viral Hepatitis is a major public health concern in the United States, and a source of significant morbidity and mortality. The Hepatitis A virus or “HAV” is heat stable and will survive for up to a month at _____ in the environment.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

50. Hepatitis A is a _____ (or contagious) disease that spreads from person to person. (It is not acquired from animals, insects, or other means.) It is transmitted by the “fecal – oral route.” This does not mean, or course, that Hepatitis A transmission requires that fecal material from an infectious individual must come in contact directly with the mouth of a susceptible individual. It is almost always true that the virus infects a susceptible individual when he or she ingests it, but it gets to the mouth by an indirect route.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

51. Hepatitis A is found in every part of the United States and throughout the world. When water sources such as private wells are contaminated with feces from infected humans, the water will spread the hepatitis A virus. The _____ can enter the water through various ways, including sewage overflows or broken sewage systems.
A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

52. Hepatitis ____ is a liver disease caused by the hepatitis____ virus, a defective virus that needs the hepatitis B virus to exist. Hepatitis ____ virus is found in the blood of persons infected with the virus.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

53. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus transmitted in much the same way as hepatitis A virus. Hepatitis ____, however, does not occur often in the United States.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

54. Hepatitis ____is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis ____ virus, can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

55. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus, which is found in the blood of persons who have the disease. ____ is spread by contact with the blood of an infected person.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

Leptospirosis Section Leptospira

56. _____ is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus Leptospira. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all.

A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

57. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. Leptospira interrogans causes leptospirosis, a usually mild _____ that may result in liver or kidney failure.

A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

58. Leptospira is a flexible, spiral-shaped, _____ with internal flagella. Leptospira interrogans has many serovars based on cell surface antigens.

A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

59. Outbreaks of _____ are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms.

A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

60. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with _____. The disease is not known to be spread from person to person.

A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

61. Leptospira enters the host through mucosa and broken skin, resulting in _____. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

62. _____ are responsible for host resistance.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

63. Leptospirosis is a worldwide _____ affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

64. Clinical diagnosis is usually confirmed by _____. Isolation of spirochetes is possible, but it is time-consuming and requires special media.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

65. Animal vaccination and eradication of rodents are important. Treatment with _____ and penicillin G is effective. No human vaccine is available.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

Pseudomonas aeruginosa Section

66. Pseudomonas aeruginosa is the _____ of an opportunistic pathogen of humans.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

67. The bacterium almost never infects _____ tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

68. Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, _____ and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

69. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, _____, and burns. The case fatality rate in these patients is 50 percent.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

70. Pseudomonas aeruginosa is primarily a _____. According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Nosocomial pathogen
D. Uncompromised
E. Gastrointestinal infections

71. Unlike many environmental bacteria, P. aeruginosa has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many _____, from water and soil to plant and animal tissues.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Ecological niches
E. None of the above

72. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to _____ where nutrients are limited.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

73. P. aeruginosa can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that _____ the cell membranes and connective tissues in various organs.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

74. This bacterium is also noted for _____to many antibiotics.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. Its resistance

75. P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its _____ and environmental versatility.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

76. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, _____ and systems involved in sensing and responding to environmental changes.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

Shigellosis Section Shigella

77. _____ type 1(or bacillary dysentery) is the only cause of epidemic dysentery.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

78. This organism is generally found in the stool of _____, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

79. _____ can even survive in dust particles for six weeks at room temperature.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

80. Infected humans act as host for this particular organism, as well as _____.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

81. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. _____ occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

82. The _____ germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri

83. A second type, _____, or "group B" Shigella, accounts for almost all of the rest.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

84. Other types of _____ are rare in this country, though they continue to be important causes of disease in the developing world.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

85. One type found in the developing world, _____ type 1, causes deadly epidemics there.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

86. _____ is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

87. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and _____. This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.
Incubation period
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

88. Shigella dysenteriae is the organism responsible for _____.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

89. During this _____, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

90. Most Shigella infections are the result of the _____ from stools or soiled fingers of one person to the mouth of another person. This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

91. _____ may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

92. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. Shigella infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with _____ in it.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

Salmonella typhi Section
93. _____ is a life-threatening illness caused by the bacterium Salmonella Typhi.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

94. _____is still common in the developing world, where it affects about 12.5 million persons each year.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

95. Typhoid fever can be prevented and can usually be treated with _____.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

96. Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called _____, recover from typhoid fever but continue to carry the bacteria. Both ill persons and _____ shed S. Typhi in their feces (stool).
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

97. You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding _____ or if sewage contaminated with S. Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

98. Once S. Typhi bacteria are eaten or drunk, they _____ into the bloodstream. The body reacts with fever and other signs and symptoms.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

1.A, 2.A, 3.A, 4.B, 5.C, 6.D, 7.A, 8.B, 9.B, 10.A, 11.B, 12. A, 13.A, 14.A, 15.B, 16.B, 17.B, 18.C, 19.D, 20.D, 21.A, 22.B, 23.D, 24.E, 25.E, 26.A, 27.B, 28.B, 29.C, 30.D, 31.B, 32. C, 33.A, 34.A, 35.A, 36.C, 37.E, 38.E, 39.D, 40.A, 41.A, 42.B, 43.A, 44.A, 45.A, 46.A, 47.C, 48.B, 49.E, 50.D, 51.E, 52.D, 53.E, 54.B, 55.C, 56.C, 57.E, 58.E, 59.C, 60.E, 61.E, 62.A, 63.B, 64.E, 65.D, 66.C, 67.D, 68.E, 69.B, 70.C, 71.D, 72.D, 73.C, 74.E, 75.B, 76.B, 77.D, 78.E, 79.B, 80.E, 81.E, 82.B, 83.D, 84.B, 85.D, 86.D, 87.A, 88.B, 89.E, 90.C, 91.E, 92.D, 93.B, 94.B, 95.E, 96.D, 97.A, 98.E

Chapter 10- EPA Regulations

Drinking Water Rules and Disease Relationship

Public water systems are regulated under the Safe Drinking Water Act (SDWA) of 1974 and its subsequent 1986 and 1996 amendments (*7--9*). Under SDWA, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants. The 1996 SDWA amendments require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated. The first list was called the drinking water Contaminant Candidate List (CCL). CCL contained 60 contaminants/ contaminant groups, included 10 pathogens, and was published in the *Federal Register* on March 2, 1998 (*10*). A decision concerning whether to regulate ≥5 contaminants from the CCL was required by August 2001.

Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the Surface Water Treatment Rule (SWTR) of 1989 (*11--13*). SWTR covers all water systems that use surface water or groundwater under the direct influence of surface water (Glossary). SWTR is intended to protect against exposure to *Giardia intestinalis*, viruses, and *Legionella*, as well as selected other pathogens. In 1998, the EPA promulgated the Interim Enhanced Surface Water Treatment Rule (IESWTR) (*14*), which provides additional protection against *Cryptosporidium* and other waterborne pathogens for systems that serve ≥10,000 persons.

In 2002, the EPA finalized the Long Term 1 Enhanced SWTR (LT1ESWTR) for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons (*15*). LT1ESWTR was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2001 (*16,17*).

The 1996 Amendments require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; the EPA has proposed the Ground Water Rule (GWR) to meet this mandate (*18*). GWR specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).

This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. GWR does not apply to privately owned wells. Additional protection of groundwater from both chemical and microbial contamination from shallow wells (including cesspools) is expected to be provided as a result of recent revisions to the Underground Injection Control Regulations, published December 7, 1999 (*19*).

To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the Information Collection Rule in 1996 (*20*), which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.

Surface water systems are also required to monitor for the presence of *Cryptosporidium*, *Giardia*, total culturable viruses, and total* and fecal coliforms or *Escherichia coli* ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.

The EPA also made minor changes in 2000 to the Lead and Copper Rule to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same (*21*).

Recreational Water
Regulation of recreational water is determined by state and local governments.

Standards for operating, disinfecting, and filtering public swimming and wading pools are regulated by state and local health departments and, as a result, are varied. In 1986, the EPA established a guideline for microbiological water quality for recreational freshwater (e.g., lakes and ponds) and marine water (*22*). The guideline recommends that the monthly geometric mean concentration of organisms in freshwater should be ≤33/100 mL for enterococci or ≤126/100 mL for *Es. coli*.

States have latitude regarding their guidelines or regulations and can post warning signs to alert potential bathers until water quality improves. Unlike treated venues where disinfection can be used to address problems with microbiological quality of the water, contaminated freshwater can require weeks or months to improve or return to normal. Prompt identification of potential sources of contamination and remedial action is necessary to return bathing water to an appropriate quality for recreational use (*23*).

The EPA's Action Plan for Beaches and Recreational Waters (Beach Watch) was developed as part of the Clean Water Action Plan.† The intent of Beach Watch is to assist state, tribal, and local authorities in strengthening and extending programs that specifically protect users of recreational waters. As part of the Beaches Act of 2000, the U.S. Congress directed the EPA to also develop a new set of guidelines for recreational water based on new water-quality indicators.

Data collected as part of the national WBDO surveillance system are used to describe the epidemiology of waterborne diseases in the United States. Data regarding water systems and deficiencies implicated in these outbreaks are used to assess whether regulations for water treatment and monitoring of water quality are adequate to protect the public against disease. Surveillance also enables identifying etiologic agents and environmental or behavioral risk factors that are responsible for these outbreaks.

This information is used to inform public health and regulatory agencies, water utilities, pool operators, and other stakeholders of new or reemerging trends that might necessitate different interventions and changes in policies and resource allotment.

Data Sources
State, territorial, and local public health agencies have primary responsibility for detecting and investigating WBDOs, and they voluntarily report them to CDC on a standard form (CDC form 52.12, which is available at http:// www.cdc.gov/.

The form solicits data related to 1) characteristics of the outbreak, including person, place, time, and location of the outbreak; 2) results from epidemiological studies conducted; 3) specimen and water sample testing; and 4) factors contributing to the outbreak, including environmental factors, water distribution, and disinfection concerns. Each year, CDC requests reports from state and territorial epidemiologists or from persons designated as WBDO surveillance coordinators.

Additional information regarding water quality and treatment is obtained from the state's drinking water agency as needed. Numerical and text data are abstracted from the outbreak form and supporting documents and are entered into a database before analysis.

Definitions[§]

The unit of analysis for the WBDO surveillance system is an outbreak, not an individual case of a waterborne disease. Two criteria must be met for an event to be defined as a WBDO. First, ≥2 persons must have experienced a similar illness after either ingestion of drinking water or exposure to water encountered in recreational or occupational settings. This criterion is waived for single cases of laboratory-confirmed primary amebic meningoencephalitis and for single cases of chemical poisoning if water-quality data indicate contamination by the chemical. Second, epidemiologic evidence (Table 1) must implicate water as the probable source of the illness.

For drinking water, reported outbreaks caused by contaminated water or ice at the point of use (e.g., a contaminated water faucet or serving container) are not classified as WBDOs.

If primary cases (i.e., among persons exposed to contaminated water) and secondary cases (i.e., among persons who became ill after contact with primary persons) are distinguished on the outbreak report form, only primary cases are included in the total number of cases. If both actual and estimated case counts are included on the outbreak report form, the estimated case count can be used if the population was sampled randomly or the estimated count was calculated by applying the attack rate to a standardized population.

Public water systems, which are classified as either community or noncommunity (Glossary), are regulated under SDWA. Of the approximately 170,000 public water systems in the United States, 113,000 (66.5 %) are noncommunity systems, of which 93,000 are transient systems (i.e., public water systems that regularly serve ≥25 of the same persons for ≥6 months/year [e.g., highway rest stations, restaurants, and parks with their own public water systems]) and 20,000 are nontransient systems (Glossary). A total of 54,000 systems (31.8%) are community systems. Community water systems serve approximately 264 million persons in the United States (96.0% of the U.S. population).

Approximately 11 million persons (4.0%) rely on private or individual water systems (*24,25*) (Glossary). These statistics exclude outbreaks associated with these sources because they are not intended for drinking and are not considered to be public water systems. Also excluded from these statistics are the millions of persons who use noncommunity systems while traveling or working.

In this surveillance system, outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water and other nonpotable sources with or without taps) are also classified as individual systems (Glossary). Sources used for bottled water are also classified as individual systems; bottled water is not regulated by the EPA but is subject to regulation by the Food and Drug Administration (FDA). Each drinking water system associated with a WBDO is classified as having one of the deficiencies in the following list. If >1 deficiency is noted on the outbreak report form, the deficiency that most likely caused the outbreak is noted.

Deficiency classifications are as follows:
1: untreated surface water;
2: untreated groundwater;
3: treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, or inadequate or no filtration);
4: distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, or contamination of a storage facility); and
5: unknown or miscellaneous deficiency (e.g., contaminated bottled water) or water source not intended for drinking (e.g., irrigation water tap).

Recreational waters include swimming pools, wading pools, whirlpools, hot tubs, spas, water parks, interactive fountains, and fresh and marine surface waters. Although the WBDO surveillance system includes whirlpool- and hot tub-associated outbreaks of dermatitis caused by *Pseudomonas aeruginosa*, wound infections resulting from waterborne organisms are not included.

Outbreak Classification ***All related tables and figures are in the rear of this section***
WBDOs reported to the surveillance system are classified according to the strength of the evidence implicating water as the vehicle of transmission (Table 1). The classification scheme (i.e., Classes I--IV) is based on the epidemiologic and water-quality data provided with the outbreak report form. Epidemiologic data are weighted more than water-quality data. Although outbreaks without water-quality data might be included in this summary, reports that lack epidemiologic data were excluded. Outbreaks of dermatitis and single cases of either primary amebic meningoencephalitis or illness resulting from chemical poisoning were not classified according to this scheme. Weighting of epidemiologic data does not preclude the relative importance of both types of data. The purpose of the outbreak system is not only to implicate water as the vehicle for the outbreak, but also to understand the circumstances that led to the outbreak. A classification of I indicates that adequate epidemiologic and water-quality data were reported (Table 1); however, the classification does not necessarily imply whether an investigation was optimally conducted. Likewise, a classification of II, III, or IV should not be interpreted to mean that the investigations were inadequate or incomplete. Outbreaks and the resulting investigations occur under various circumstances, and not all outbreaks can or should be rigorously investigated. In addition, outbreaks that affect fewer persons are more likely to receive a classification of III, rather than I, on the basis of the relatively limited sample size available for analysis.

Results ***All related tables and figures are in the rear of this section***

Outbreaks Associated with Drinking Water

During 1999--2000, a total of 39 outbreaks associated with drinking water were reported by 25 states (see Appendix A for selected case descriptions). One of the 39 outbreaks was a multistate outbreak of *Salmonella* Bareilly that included cases from 10 states. Of the 39 total drinking water outbreaks, 15 outbreaks were reported for 1999 and 24 for 2000. Florida reported the most outbreaks (15) during this period. These 39 outbreaks caused illness among an estimated 2,068 persons; 122 persons were hospitalized, and two died. The median number of persons affected in an outbreak was 13.5 (range: 2--781). Outbreaks peaked during the summer months (Figure 1), June--August.

Nine of the 39 (23.1%) outbreaks were assigned to Class I on the basis of epidemiologic and water-quality data; three (7.7%) were Class II; 25 (64.1%) were Class III; and 1 was Class IV (Table 1). One of two outbreaks associated with a chemical etiology was not assigned a class because that outbreak was a single case of illness resulting from nitrate poisoning associated with consumption of water from a private well. Outbreaks are listed by state (Tables 2 and 3) and are tabulated by the etiologic agent, the water system type (Table 4), and by the type of deficiency and type of water system type (Table 5).

Etiologic Agents
Twenty (51.3%) of the 39 outbreaks were of known infectious etiology; 17 (43.6%) were of unknown etiology; and two (5.1%) were attributed to chemical poisoning. Of the 20 outbreaks with known infectious etiology, seven (35.0%) were caused by parasites; nine (45.0%) were caused by bacteria; and four (20.0%) were caused by viruses (Figure 2) (Appendix A).

Parasites. Seven outbreaks affecting 57 persons were attributed to parasitic infection: six *Giardia* outbreaks and one *Cryptosporidium* outbreak. Six outbreaks of *Giardia* associated with drinking water affected 52 persons from five states: Florida (two outbreaks), New Mexico (one), New Hampshire (one), Minnesota (one), and Colorado (one). These outbreaks occurred in January (one), June (one), July (one), August (one), and September (two). Four outbreaks were associated with well water systems, and two were associated with surface water systems.

Two outbreaks caused by *G. intestinalis* involved possible contamination of wells by animal feces. *G. intestinalis* can infect mammalian hosts, which in turn, can serve as reservoirs for human infection. Water treatment failure was a factor in two other outbreaks of *Giardia*.

Bacteria. Nine outbreaks affecting an estimated 1,166 persons were attributed to bacterial infection: four *Es. coli* O157: H7 outbreaks, one *Campylobacter jejuni*, one *Salmonella* Typhimurium, one *Sa.* Bareilly, and two mixed *Ca. jejuni* and shiga toxin-producing *Es. coli* (O157:H7 or O111) outbreaks. The two outbreaks with multiple pathogens caused the two largest bacterial drinking water outbreaks reported during this study period.

Viruses. During this period, four outbreaks involving viral gastroenteritis were reported. A total of 426 persons reported illness; no hospitalizations or deaths were reported in association with these four viral outbreaks. Three of the four outbreaks occurred in camp facilities in California, New Mexico, and West Virginia. All three water sources were noncommunity groundwater sources.

Chemicals. During 1999, two outbreaks involving chemical contamination were reported. A total of three persons were affected by contamination of drinking water from nitrate and sodium hydroxide.

Unidentified Etiologic Agents. Seventeen outbreaks involving gastroenteritis of unknown etiology were reported from four states, affecting an estimated 416 persons and resulting in five hospitalizations. Testing for certain enteric pathogens (including ova and parasite testing) was attempted in five of the 17 outbreaks. In a June 2000 outbreak affecting 2 persons, stool specimens collected from one person tested negative for *G. intestinalis* but positive for *Blastocystis hominis*.

However, whether *B. hominis* was the cause of the reported illness is unclear, and the pathogenicity of *B. hominis* has been debated in the scientific community (*26*). Stool specimens were negative for parasitic and bacterial enteric pathogens in two outbreaks in Washington (July 1999 and August 1999) and in two Florida outbreaks (March 1999 and April 2000) (Appendix A).

In addition, suspected pathogens were noted in four other outbreak reports submitted. On the basis of symptoms of illness, Norwalk-like virus (NLV) was suspected in an Idaho outbreak among firefighters that caused 65 illnesses and four hospitalizations, but the outbreak was not laboratory-confirmed. *G. intestinalis* was suspected in an April 2000 outbreak in a Florida trailer park affecting 21 persons, on the basis of the incubation period and symptoms reported.

In another outbreak in a Florida trailer park in March 2000 among 19 persons, a bacterial pathogen was suspected as the cause of the outbreak on the basis of the symptoms, which included conjunctivitis and dermatitis in addition to gastroenteritis.

A chemical agent was suspected as the cause of illness among four residents in a Florida apartment building who had a cross-connection between their drinking water and a toilet flush-valve. The residents of the apartment had noted blue tap water before onset of illness on multiple occasions before an improper flush valve in the toilet tank was discovered.

Four outbreaks of gastroenteritis were associated with consumption of untreated water from private wells. These four outbreaks occurred in Florida and affected 3--4 persons each. In July 2000, flooding was a possible contributor to two outbreaks. Water in each of the homes tested positive for coliforms and did not have adequate disinfection.

Water-Quality Data

Water-quality data (i.e., information regarding the presence of coliform bacteria, pathogens, or chemical contaminants) were available for 35 (89.7%) of the 39 drinking water outbreaks. Two reports of outbreaks of confirmed or suspected infectious etiology and two reports of outbreaks of confirmed or suspected chemical etiology did not provide water-quality data.

Of the 36 reports of outbreaks with a suspected or confirmed infectious etiology, 33 outbreaks provided water-quality data. Twenty-six (78.8%) of the 33 outbreaks with a suspected or confirmed infectious etiology reported a positive coliform, total coliform, or fecal coliform result. Organisms also were detected in the water in two of these outbreaks. In August 2000, *Ca. jejuni* was detected in the water in a mixed *Ca. jejuni*/*Es. coli* O157:H7 outbreak in Utah, although shiga toxins were not detected. *Es. coli* O157:H7 was found in the water in a July 2000 California outbreak. In a 2000 Colorado outbreak, the presence of *G. intestinalis* was demonstrated in a sample from the water holding tank, despite the lack of coliform data.

Of the three outbreaks with either a confirmed or suspected chemical etiology, only one demonstrated that the chemical had been directly in the water. Tap water was tested after the health department was notified that an infant had methemoglobinemia. Both fecal coliforms and 28 mg/L of nitrate were detected in the water. For an outbreak where burns and gastroenteritis were reported and linked to a sodium hydroxide spill, a pH test of the water that could indicate whether NaOH or another basic substance had spilled into the water was not documented. However, the environmental assessment indicated the tank contents had emptied into the water. A third suspected chemical outbreak involving a cross-connection between a toilet flush-valve and the drinking water system did not have water-quality data available.

In 11 of the 35 outbreaks, water was not sampled for coliforms until >1 month after the first case associated with the outbreak was reported (range: 5--16 weeks). In four of these 11 outbreaks, the water samples did not test positive for coliforms (fecal or total), chemicals, or pathogens. Instead, these were confirmed as outbreaks by epidemiologic data or by reports that treatment deficiencies had occurred.

Water Systems and Water Sources

Eleven (28.2%) of the 39 drinking water outbreaks were associated with community systems, 11 (28.2%) with noncommunity systems, and 17 (43.6%) with individual water systems (Tables 4 and 5). Ten (25.6%) of the 39 drinking water outbreaks were associated with surface water, including three outbreaks that implicated irrigation water not intended for consumption. Twenty-nine (74.4%) of the 39 drinking water outbreaks, including the outbreak associated with bottled water, were associated with groundwater sources (wells and springs).

Five (45.5%) of the 11 outbreaks associated with community water systems were caused by treatment deficiencies; one (9.0%) outbreak was related to contaminated, untreated groundwater, and five (45.5%) outbreaks were related to problems in the water distribution system. Two of the five distribution system problems were related to cross-connections between the distribution system and an irrigation well.

The third outbreak related to a community water source had a household cross-connection between the toilet water and main kitchen tap. One outbreak of *Cr. parvum* (Florida, December 2000) was related to a repeated history of water main breaks. In another outbreak in Ohio in August 2000, deficiencies in the distribution system of a fairgrounds might have allowed back-siphonage of animal manure into the water used by food and beverage vendors.

Ten (90.1%) of 11 outbreaks associated with noncommunity water systems occurred in groundwater systems. Seven of the 10 groundwater outbreaks were linked to untreated wells, and one of the 10 involved consumption of untreated spring water. Two of the 10 outbreaks were related to treatment deficiencies in water taken from wells or a spring and were associated with outbreaks of NLV and a small round-structured virus. An outbreak associated with *G. intestinalis* related to consumption of surface water occurred when a pump failure and a defective filter cartridge resulted in river water entering the drinking water holding tank without filtration. No information concerning chlorine levels from water samples was provided.

Nine (52.9%) of 17 outbreaks associated with individual water systems occurred in groundwater systems. Eight of these groundwater systems were wells that were not treated routinely; one outbreak of giardiasis occurred when the filtration system for a well was inadvertently turned off. Five (31.3%) of the 16 outbreaks occurred when persons drank water not intended for direct consumption from irrigation systems or when they consumed surface water that had been ineffectively or improperly treated. One (6.3%) of the 16 outbreaks in a system occurred in a home where creek water on the property was directly consumed without treatment.

Of the nine bacterial outbreaks, four occurred in groundwater systems (one was associated with a deficiency in the distribution system, one with a treatment deficiency, and two occurred in untreated systems). Six of seven parasitic outbreaks occurred in groundwater systems: three occurred in untreated systems; two involved problems in the distribution system; and one was related to a treatment deficiency. All four viral outbreaks occurred in noncommunity groundwater systems. Two occurred in untreated wells, and two were related to treatment deficiencies in a spring and well. Two chemical outbreaks were related to treatment deficiencies in well water. Fourteen of the 17 outbreaks of unknown etiology were linked to groundwater systems. Ten of these 14 outbreaks occurred in untreated systems; two were related to distribution system problems, and two were related to treatment deficiencies.

Outbreaks Associated with Recreational Water

During 1999--2000, a total of 23 states reported 59 outbreaks associated with recreational water (Tables 6--9) (see Appendix B for selected case descriptions).

Twenty-three outbreaks were reported for 1999, and 36 for 2000. The states that reported the largest number of outbreaks were Florida (14 outbreaks) and Minnesota (eight outbreaks). These 59 outbreaks affected 2,093 persons and resulted in 25 hospitalizations and four deaths. The median size of the outbreak was 10 persons (range: 1--700).

Of the 59 outbreaks, 36 were outbreaks of gastroenteritis (Tables 6 and 7); 15 were outbreaks of dermatitis (Table 9); four were cases of meningoencephalitis; and the remaining four outbreaks were of leptospirosis, chemical keratitis, acute respiratory infection of unknown etiology, and Pontiac fever (Table 8).

Thirty-one (86.1%) of the 36 outbreaks involving gastroenteritis occurred during the summer months (i.e., June--August). Outbreaks of dermatitis associated with recreational water contact were reported more frequently in February, March, June, and July. The four cases of primary amebic meningoencephalitis occurred in the warmer months (April--October).

Etiologic Agents
Of the 59 recreational water outbreaks, 44 (74.6%) were of known infectious etiology (Tables 6--9). Of the 36 outbreaks involving gastroenteritis, 17 (47.2%) were caused by parasites; nine (25.0%) by bacteria; three (8.3%) by viruses; one (2.8%) by a combination of parasites and bacteria; and the remaining six (16.7%) were of unknown etiology (Figure 4).

Of the 23 nongastroenteritis-related recreational outbreaks, seven were attributed to *P. aeruginosa*, four to free-living amoebae, one to *Leptospira* species, one to *Legionella* species, and one to bromine (Tables 8 and 9). Nine nongastroenteritis-related recreational outbreaks were of unknown etiology, eight of which were suspected but not confirmed to be caused by *P. aeruginosa* or schistosomes. The ninth outbreak of unknown etiology was suspected to be caused by a virus or by *Legionella pneumophila* on the basis of observed symptoms and the epidemiologically implicated vehicle of transmission. Of the 59 recreational water outbreaks, 21 (35.6%) were associated with fresh or surface water, and 37 (62.7%) with treated (e.g., chlorinated) water. Information regarding the water venue for an outbreak of meningoencephalitis was not provided.

Parasites. Sixteen of the 17 parasitic recreational water outbreaks involving gastroenteritis were caused by *Cr. parvum*. The seventeenth outbreak was caused by *G. intestinalis*. Fifteen of the 17 parasitic outbreaks occurred in chlorinated venues; in these outbreaks, inadequate treatment, disrupted chlorine disinfection, or suboptimal pool maintenance were contributing factors to the outbreaks. *Cr. parvum* is highly resistant to chlorine disinfection and can survive for days in adequately chlorinated pools; therefore, suboptimal chlorination of the pool might not be the sole factor contributing to the occurrence of an outbreak.

Three outbreaks of laboratory-confirmed cryptosporidiosis occurred during the 1999 summer swim season. During the 2000 summer swim season, three substantial outbreaks of *Cr. parvum* occurred that were related to swimming in municipal pools. In August 2000, an outbreak occurred in Colorado that affected 112 persons attending a private pool party. In June 2000, the two other cryptosporidiosis outbreaks, one in Ohio affecting 700 persons and the other in Nebraska affecting 225 persons, occurred among members of private swim clubs. In both outbreaks, the protracted nature of the outbreaks during ≥2 months was the result of repeated recontamination of the pools by infected persons continuing to swim; 37 (18%) of 205 persons interviewed in the Nebraska outbreak admitted to swimming while symptomatic, and 32% swam while ill or during the 2 weeks after their illness. Another outbreak (Florida, August 2000) was associated with the outbreak that occurred in Ohio.

A family who were members of the implicated swim club in the Ohio outbreak were vacationing with a sick child. While in a pool in Florida, the infant had two fecal accidents. The resulting outbreak caused five cases of diarrheal illness and two hospitalizations.

Eight other outbreaks of cryptosporidiosis occurred in treated venues during the 2000 swim season. Two outbreaks of gastroenteritis occurred in untreated venues: one in a freshwater lake in Minnesota in July 2000 and one in a Massachusetts pond in July 1999 (Appendix B).

Four cases of laboratory-confirmed primary amebic meningoencephalitis attributed to *Naegleria fowleri* occurred during this 2-year reporting period. All four persons were aged ≤19 years. Three of the persons died from infection after having contact with a pond, lake, or mud hole. The fourth person's freshwater exposure could not be determined; that person had fallen from a jet ski into an unspecified body of water, sustained injuries, and died from an infection shortly after it was detected.

Bacteria. Nine recreational outbreaks involving gastroenteritis were attributed to bacterial pathogens, and five of the nine were linked to freshwater sources. Five cases (Wisconsin, August 1999) of *Es. coli* O157:H7 occurred among persons who had visited the same swimming beach. After a review of potential risk factors, the only common link found was swimming at the implicated beach. The popular beach featured a shallow, dammed area that was used for wading. Total and fecal coliforms were detected in water samples collected before and during the outbreak, although the levels detected did not exceed levels of EPA-recommended guidelines for microbiologic quality of water.

One sample that was tested for *Es. coli* O157:H7 was negative. *Es. coli* O157:H7 was implicated in another outbreak among 36 persons (August 1999) who visited a state park in Washington. *Es. coli* O121:H19 was implicated in an outbreak in a Connecticut community (July 2000). *Shigella sonnei* was implicated in two outbreaks that occurred at swimming beaches in Minnesota (July and August 2000).

Non-freshwater sources were implicated in four bacterial recreational water outbreaks involving gastroenteritis. In March 1999, an outbreak of *Ca. jejuni* was associated with a private pool in Florida that did not have continuous chlorine disinfection and reportedly had ducks swimming in the pool. Outbreaks of *Shigella flexneri* and *Es. coli* O157:H7 (Missouri, September 2000 and Nebraska, June 1999) occurred among children using unchlorinated wading pools. Fecal accidents were factors contributing to the contamination of the water in both outbreaks. *Es. coli* O157:H7 also was implicated as a cause of illness in an outbreak (Florida, September 1999) among two young children who had been playing in ditch water. Both clinical specimens and water samples tested positive for *Es. coli* O157:H7.

Two nongastroenteritis-related recreational water outbreaks were also reported. One outbreak of leptospirosis was reported among 21 persons who participated in an adventure race in Guam in July 2000 (Table 8). These persons reported multiple outdoor exposures, including running through jungles and savannahs, swimming in a river and a reservoir, and bicycling and kayaking in the ocean. *Leptospira* was confirmed by serology, and an epidemiologic investigation demonstrated that swimming in the reservoir, submerging one's head in the water, and swallowing water while swimming were risk factors for illness.

Water samples were not tested, and an environmental assessment of the reservoir was not conducted. The second nongastroenteritis-related recreational water outbreak was an outbreak of Pontiac fever epidemiologically linked to use of a whirlpool at a hotel.

Viruses. During 1999--2000, three outbreaks of NLV (Calicivirus) that affected a total of 202 persons were reported. Two NLV outbreaks occurred in untreated systems; one outbreak of NLV occurred (Idaho, June 1999) at a resort and water park and affected 25 persons. The pool implicated in the investigation was untreated because the source of the pool's water was a natural hot springs that was high in mineral content. The investigators noted that geothermal pools used for swimming are not required to be regulated by public health officials in that locale. The pool implicated by the investigation also had been implicated in a previous outbreak of NLV in June 1996.

Other. During 1999--2000, six recreational water outbreaks involving gastroenteritis of unknown etiology were reported. One outbreak (Florida, August) involved a motel pool that was cloudy and dirty at the time of exposure. Nine persons who swam in this pool and did not share any other common exposure became ill with gastroenteritis. Disinfectant residuals and operation of the filtration system at the time of the investigation were deficient. Problems were also noted with the equipment used for adjusting pH.

Another outbreak (Florida, August 1999) among 38 persons who visited a beach park was attributed to both *Sh. sonnei* and *Cr. parvum* (*28*). Illness was epidemiologically linked to playing in an interactive fountain at the park, ingesting water, and consuming food and beverages at the fountain. The fountain's recirculation, filtration, and disinfection systems were not approved by the health department and were inadequate or not completely operational at the time of its use. Samples of the fountain water tested positive for coliforms but did not test positive for fecal coliforms. Nevertheless, the cause of the outbreak was determined to be the fountain, which was closed until the health department's concerns could be remedied.

Three cases of chemical keratitis (Vermont, February 2000) resulted from exposure to bromine in a hotel swimming pool. Bromine levels were >5 ppm (acceptable bromine levels are 1--3 ppm), and the pH level was >8.5. Patrons who spent time with their heads underwater with their eyes open were affected.

Twelve persons affected in an outbreak (Texas, September 1999) reported symptoms that included exhaustion, sore muscles, headache, chills, and fever after attending a conference at a guest ranch. One woman reported a miscarriage during her illness. Exposure to a hot tub, defined as either immersion or being near the hot tub, was associated with illness. Although clinical specimens (urine, blood, sputum, and throat swabs) were tested for organisms, including *Leg. pneumophila* serogroups 1 and 6, influenza virus, parainfluenza virus, and adenovirus, no infectious agent was identified. No testing for biologic or chemical agents was performed on water samples because the hot tub had already been drained, refilled, and hyperchlorinated before the environmental investigation.

During the 1999--2000 reporting period, 15 outbreaks of dermatitis were identified (Table 9). Three of these outbreaks were associated with swimming in freshwater and were assumed to be cercarial dermatitis caused by contact with the larval form (cercariae) of schistosomes, which are present in freshwater environments. Two of these dermatitis outbreaks occurred in lakes in California that were associated with past cases of cercarial dermatitis. The onset of dermatitis occurred within hours after swimming in the lake and resolved after a limited number of days (median days of illness were 2 and 3 days [range: 2--3 and 3--5 days], respectively).

The 12 remaining outbreaks were associated with pool and hot tub use and affected 5--29 persons each. *P. aeruginosa* was confirmed in clinical isolates in 3 of the 12 outbreaks and was confirmed in water/filter samples in five outbreaks, two of which also had a clinical isolate. In eight of these outbreaks of dermatitis, specific treatment deficiencies or problems were identified. Outbreaks in Arkansas (June 1999), Florida (August 2000), Colorado (December 1999), and Washington (March 2000) were attributed to deficiencies in treatment.

In one outbreak of dermatitis (Maine, February 2000), nine persons reported rash in addition to headache, fever, fatigue, and sore throat (*29*). Swimming in the hot tub or swimming in the pool was a risk factor. The pool and hot tub were on separate filtration systems, and both were used by the majority of persons in the outbreak. Low levels of free chlorine were found in the pool and hot tub, but the presence of chlorinate isocyanurates (chlorine stabilizers) might have influenced measured levels of free chlorine. A clinical isolate of *P. eruginosa* was obtained from an ill person; *P. aeruginosa* also was isolated from the pool filter even after the pool had been cleaned twice.

P. aeruginosa was isolated from clinical specimens and water samples in an outbreak at a Colorado hotel that affected 19 persons in February 1999 (*28*), 13 of whom were children aged <15 years. Symptoms were not limited to rash; they included diarrhea, vomiting, nausea, fever, fatigue, muscle aches, joint pain, swollen lymph nodes, and subcutaneous nodules on hands and feet. Because of the severity and range of symptoms, clinical specimens were examined for enteric bacterial and parasitic pathogens as well as *Legionella* species, *Leptospira* species, and *Entamoeba histolytica* but did not test positive for any of these etiologic agents. Swabs taken from the hot tub floor and rail were positive for *P. aeruginosa* and other *Pseudomonas* species.

Pool and hot tub records indicated that chlorine and pH had declined below the state-mandated levels at the time of exposure. Epidemiologic evidence implicated the hot tub as the likely vehicle of exposure for the outbreak. In both the Colorado outbreak and the Maine outbreak that occurred in February 2000, an offsite contractor had been engaged to monitor disinfectant and pH levels. Insufficient communication between pool staff and the remote monitoring company might have contributed to extended periods of usage with inadequate disinfection.

Outbreaks Associated with Occupational Exposure to Water

Two outbreaks not associated with drinking or recreational water exposure were reported during this period (Table 10). One outbreak of leptospirosis (Hawaii, August 1999) occurred among persons landscaping a pond. Leptospirosis was confirmed serologically for the two persons who had contact with the pond. Both persons reported multiple skin abrasions and were exposed to the pond water for a period of 5--10 days. One of the two persons was hospitalized.

An outbreak of acute respiratory illness occurred among sugar beet processing plant workers (Minnesota, August 2000). Of the 15 cases identified, 13 were hospitalized. Serology for 4 (26.7%) of the 15 persons tested positive for *Leg. pneumophila*; three (20.0%) persons were confirmed positive for *Leg. pneumophila* by sputum polymerase chain reaction (PCR). Fourteen (93.3%) of the 15 persons worked on a crew that had performed high-pressure cleaning in one area of the plant; the fifteenth patient had conducted high-pressure cleaning elsewhere in the plant.

The sources of water for the high-pressure cleaning contained 10^5 colony-forming unit (CFU)/mL of *Leg. pneumophila* and endotoxin levels of 22,200 endotoxin units/mL. Although the attack rate, symptoms, and laboratory findings were consistent with an outbreak of Pontiac fever, endotoxin exposure might have contributed to this outbreak.

Previously Unreported Outbreaks

Three previously unreported drinking water outbreaks that occurred in 1995 and 1997 were submitted during this reporting period (Table 11). An illegal cross-connection (Washington, July 1995) between a domestic water supply and an irrigation system at a plant nursery resulted in contamination of multiple wells in a community. Eighty-seven cases of gastroenteritis were reported, and one hospitalization was recorded. *G. intestinalis* was determined in 33 (52.4%) of 63 stool specimens; *Entamoeba coli* and *B. hominis* were each found in one stool specimen. One (7.1%) of 14 stool specimens that were cultured for *Ca. jejuni* tested positive.

NLV was implicated as the cause of an outbreak (New York, December 1997) of 1,450 cases at a restaurant at a ski resort. Epidemiologic data implicated water or consumption of ice made from water as the cause of the outbreak. The environmental assessment revealed possible problems with the well operation and location. The chlorinator for the well had been malfunctioning and had already been disconnected before the assessment. Testing of the water by the local health department determined that neither a free nor total chlorine residual was detectable in the potable water supply and indicated the presence of fecal coliforms. In addition, the well was located <24 inches away from a stream. During the period the chlorinator was not functioning, the pump for the well had been continuously pumping water. Surface water that might have been introduced into the water supply, plus a deficiency in treatment of the water, played a key role in the outbreak.

Seven persons who were either employees or visitors at a hospital (California, November 1997) were symptomatic for methemoglobinemia in one outbreak. An epidemiologic investigation indicated that the only shared exposure among these persons was a visit to the hospital cafeteria and the consumption of a carbonated beverage with ice from the self-service soda dispenser. The onset of symptoms occurred 1--5 minutes after or while drinking a carbonated beverage. One person was hospitalized, and no deaths occurred. The environmental investigation discovered a cross-connection in the plumbing system that might have allowed water from the cooling tower, which had been recently shock-treated with sodium metaborate, to be drawn into the drinking water system. Sodium metaborate has been associated with nitrate poisoning and methemoglobinemia in past incidents.

Outbreaks Not Classified as WBDOs

Outbreaks attributed to drinking water that was contaminated or potentially contaminated at the point of use rather than at the source or in the distribution system are not classified as WBDOs. Six outbreaks, causing illness among a total of 102 persons, are in this category. None of the six outbreaks reflected a common vehicle of contamination: one outbreak of *Cr. parvum* was epidemiologically associated with ice consumption; a school-based outbreak of *Sh. sonnei* was related to consumption of water from a dispenser stored in a bathroom facility; a third outbreak involved water taken from a garden hose (the water had been stored in an ice chest before consumption at a private residence); and a fourth outbreak was associated with bottled water that might have been contaminated at the point of use.

Two of the six reported point-of-use outbreaks involving a suspected chemical exposure occurred in food service facilities, but water testing was not performed to verify the presence of the chemical; and, because of the relatively limited number of cases associated with these incidents, the epidemiologic information was not adequate to include these incidents as outbreaks.

Data from six other possible or confirmed outbreaks were also not included in this analysis. One confirmed outbreak of leptospirosis was related to travel outside the United States or its territories and therefore was excluded. This outbreak occurred among student travelers who became ill after their return from Ecuador. Three cases of leptospirosis were confirmed by laboratory testing among the cohort, and four additional cases were suspected. Three other outbreaks of *G. intestinalis, Cr. parvum,* and NLV could not be included in the analysis. Although these outbreaks were probably caused by a recreational water exposure, the data provided did not meet the criteria for inclusion (i.e., the outbreaks did not meet the criteria for Classes I--IV).

Two additional outbreaks were excluded because of inadequate information: one outbreak of dermatitis caused by in-home bathing and one potential drinking water outbreak of *Cr. parvum* in a New England community. This outbreak of *Cr. parvum* occurred in a community near another reported community outbreak of *G. intestinalis* and *Cr. parvum* in 1999. The pond implicated in the recreational water outbreak of *G. intestinalis* also served as a surface water source, which was intermittently mixed into the municipal drinking water that supplied the community. However, not all the persons received their drinking water from the municipal water source. Although raw surface water samples later tested positive for *Cr. parvum* by immunomagnetic spectroscopy (IMS), household water samples either tested negative, were not tested, or the results were not provided. The epidemiologic information and water-quality information provided were not conclusive.

Discussion

Considerations Regarding Reported Results

The WBDO surveillance system provides information concerning epidemiologic and etiologic trends in outbreaks. In previous years, a decrease in the number of drinking water-associated outbreaks had been observed. However, the cumulative number of drinking water outbreaks reported for the 1999--2000 period demonstrates a reversal of this trend (Figures 5 and 6). The number of recreational water outbreaks has been gradually increasing for the past 15 years and is at the highest level since CDC began receiving such reports in 1978.

Although the number of outbreaks reported through the surveillance system has increased, the significance of this increase is unclear. Whether this indeed reflects a true increase in the number of outbreaks that occurred in the United States is unknown.

Not all outbreaks are recognized, investigated, and then reported to CDC or the EPA, and studies have not been performed that assess the sensitivity of this system and indicate what percentage of actual outbreaks this system is able to detect. Multiple factors exist that can influence whether WBDOs are recognized and investigated by local, territorial, and state public health agencies: the size of the outbreak; severity of disease caused by the outbreak; public awareness of the outbreak; routine laboratory testing for organisms; requirements for reporting cases of diseases; and resources available to the local health departments for surveillance and investigation of probable outbreaks.

This surveillance system probably underreports the true number of outbreaks because of the multiple steps required before an outbreak is identified and investigated. In addition, changes in the capacity of local, county, and state public health agencies and laboratories to detect an outbreak might influence the numbers of outbreaks reported in each state relative to other states. The states with the majority of outbreaks reported during this period might not be the states where the majority of outbreaks actually occurred. An increase in the number of outbreaks reported could either reflect an actual increase in outbreaks or an improved sensitivity in surveillance practices.

Recognition of WBDOs is also dependent on certain outbreak characteristics; outbreaks associated with serious illness or affecting a substantial number of persons are more likely to receive attention from health authorities. Outbreaks involving acute diseases, including those characterized by a short incubation period, are more readily identified than outbreaks associated with chronic, low-level exposure to an agent (e.g., certain chemicals) or are associated with organisms that have a longer incubation period (e.g., certain parasitic organisms).

Larger Drinking Water Systems

Outbreaks involving larger drinking water systems (e.g., community systems) are more likely to be detected than outbreaks that involve noncommunity systems because these systems serve mostly nonresidential areas and transient populations. Outbreaks associated with individual systems are the most likely to be underreported because they typically involve a limited number of persons. Recreational outbreaks where persons congregate in one venue and then are geographically dispersed can be difficult to document.

The identification of the etiologic agent of a WBDO depends on the timely recognition of the outbreak so that appropriate clinical and environmental samples can be collected. The laboratory involved in the testing of specimens must have the capacity and capability to test for a particular organism. In certain cases, specific tests must be requested. Routine testing of stool specimens at laboratories will include tests for the presence of enteric bacterial pathogens and might also include an ova and parasite examination.

However, *Cr. parvum*, one of the most commonly reported waterborne parasites, is not often included in standard ova and parasite examinations and in certain instances, must be specifically requested (*31*). During 1999--2000, tests for NLV and other possible agents of viral origin were rarely performed or documented in the outbreaks that were reported to CDC. Collection of water-quality data depends primarily on local and state statutory requirements, the availability of investigative personnel, and the technical capacity of the laboratories that test the water. Furthermore, certain outbreaks can substantially alter the relative proportion of cases of waterborne disease attributed to a particular agent. The number of reported cases is typically an approximate figure, and the method and accuracy of the approximation vary among outbreaks.

One key limitation of the data collected as part of the WBDO surveillance system is that the information collected pertains only to outbreaks of waterborne illness. The epidemiologic trends and water-quality concerns observed in outbreaks might not necessarily reflect or correspond with trends associated with endemic waterborne illness.

Epidemiologic Studies
CDC and the EPA are collaborating on a series of epidemiologic studies to assess the magnitude of non-outbreak waterborne illness associated with consumption of municipal drinking water and with exposure to recreational marine and freshwaters.

Outbreaks Associated with Drinking Water
The number of outbreaks reported during 1999 (15) and 2000 (24) is higher than the number reported during 1997 (7) and 1998 (10). As described previously, the number of drinking water outbreaks had declined (*2*,*3*). The increase in reported outbreaks should be carefully interpreted. Although the number of drinking water outbreaks has changed, the total number of persons affected by a drinking water outbreak during 1999--2000 (n = 2,027) is comparable to what was initially reported in 1997--1998 (n = 2,038) and 1995--1996 (n = 2,567) (*2*,*3*).

Changes in surveillance and reporting of outbreaks might have improved detection of outbreaks affecting limited, private systems that in turn, affect a relatively limited number of persons. However, the increase in outbreaks that affect persons in limited, private systems merits further investigation by public health and water-quality agencies.

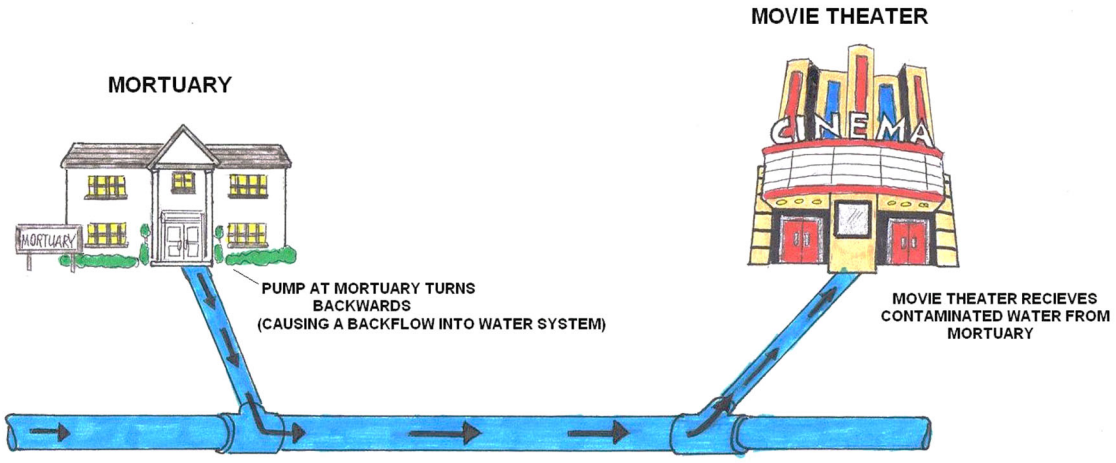
Certain states reported drinking water outbreaks for the first time in >10 years (e.g., Connecticut since 1976 or Utah since 1986). California reported multiple drinking water outbreaks after reporting no outbreaks in 1997 and 1998, and compared with other years during 1990--2000, the number of reported outbreaks in California increased slightly.

The number of outbreaks reported by Florida also increased. Although the numbers of reported outbreaks increased overall, the seasonality of the drinking water outbreaks is consistent with previous years, with the number peaking during the summer months. The observed increase in the number of outbreaks is associated with an increase in outbreaks associated with consumption of untreated water from both surface and groundwater sources, but specifically private wells.

The percentage of drinking water outbreaks associated with surface water during 1999--2000 was 17.9% (i.e., seven outbreaks) (Figure 2). This percentage is higher than the 11.8% reported during 1997--1998 period (i.e., two outbreaks). However, three of the seven surface water outbreaks reported during 1999--2000 were associated with the direct ingestion of surface water without any treatment or with inadequate individual treatment. Two of these outbreaks were associated with consumption of water during outdoor excursions where point-of-use treatment (e.g., filtration or disinfection) might have been attempted and was either inadequate to protect health or was inconsistently or incorrectly applied. The third outbreak occurred after a household had run out of potable water and instead served untreated creek water to their guests.

These three outbreaks illustrate that the public might be unaware that surface water, despite its clarity, is prone to contamination by organisms. Surface waters should not be directly consumed without being treated at the point of use or boiled. Manufacturers of point-of-use devices and the National Sanitation Foundation (NSF) provide information regarding different devices, instructions for use, and their ability to make water safe for human consumption.

The remaining four outbreaks comprise approximately 11% of all drinking water outbreaks, an equivalent percentage to that reported in 1997--1998. These four outbreaks were associated with systems that routinely received treatment. One outbreak of giardiasis occurred at a resort (Colorado, August 2000) served by a noncommunity system. The increased demand for water during the summer, coupled with multiple treatment failures, resulted in the delivery of unfiltered and non-disinfected water to the resort.



Cross-Connections

These multiple failures illustrate the importance of routine maintenance, specifically among noncommunity systems, which do not have consistent demand for water year-round. Two outbreaks (Florida, March 1999 and August 1999) were associated with cross-connections: one to an irrigation well and another to a toilet. Another surface water outbreak (Ohio, August 2000) at a fairgrounds was suspected to have resulted from back-siphonage into the drinking water from an animal manure site. These outbreaks indicate that even when treatment of water at the source is adequate, deficiencies in the distribution system or at the home can result in illness. Such deficiencies are preventable, and the public should be informed of how to detect and avoid creating cross-connections.

Twenty-eight (71.8%) of the 39 outbreaks related to drinking water were associated with groundwater sources. This number is an 87% increase from the number reported in the previous period (i.e., 15). Seventeen of the 28 outbreaks (60.7%) were linked to consumption of untreated groundwater; eight of 28 (28.6%) outbreaks were associated with treatment deficiencies; and three (10.7%) were linked to deficiencies in the distribution system. The observed pattern of deficiencies is contrary to what was observed in the previous reporting period, where the majority of groundwater outbreaks were associated with treatment or distribution system problems.

This pattern indicates that untreated groundwater systems are increasingly associated with outbreaks of illness. Groundwater systems, with the exception of systems influenced by surface water, are not routinely required to use filtration or treatment that would be expected to reduce the number of pathogens in the water. The EPA's pending GWR** is expected to establish multiple barriers in groundwater systems to protect against bacteria and viruses in drinking water from groundwater sources and should establish a targeted strategy to identify groundwater systems at high risk for fecal contamination.

Twenty-six of these 28 groundwater outbreaks had a well as the implicated water source, and two were linked to a spring. The percentages of outbreaks associated with wells and springs were similar during this reporting period to the 1997--1998 period. Although GWR is expected to have public health benefits, these protections extend primarily to community groundwater systems. Of the 26 well-related outbreaks that occurred during the 1999--2000 period, only eight of 26 were associated with community wells. Ten were associated with individual private wells, and eight were associated with noncommunity wells.

These systems would not necessarily benefit from the promulgation of GWR, and therefore, the quality of water in wells remains a public health concern. Approximately 14--15 million households in the United States rely on a private, household well for drinking water each year, and >90,000 new wells are drilled throughout the United States each year. In addition, contamination of a private well is not only a health concern for the household served by the well, but can impact households using other nearby water supplies and could potentially contaminate the aquifer.†† Additional education efforts should be targeted towards well owners, users, well drillers, and local and state drinking water personnel to encourage practices that best ensure safe drinking water for private well users.

Irrigation Waters

Three outbreaks were associated with direct consumption of water from irrigation systems, comprising approximately 8% of drinking water outbreaks (Figure 2). Cross-connections to irrigation systems were implicated as contaminating factors in three other irrigation-related outbreaks. Irrigation waters are not regulated under the Safe Drinking Water Act, because they are typically intended for agricultural purposes, not for human consumption.

Therefore, irrigation water would not be expected to be treated to reduce the level of microorganisms or other contaminants potentially in the water to the same standards as water intended for consumption. In one outbreak, children drank directly from an irrigation canal while playing outside a home. In two other outbreaks, water was directly consumed from an irrigation tap by sports team members. In the first instance, the sports team consumed water from a labeled irrigation tap despite being informed that the water was not intended for consumption. In the second instance, two teams drank from taps on the field because no other source of potable water was available on field.

The multistate outbreak of *Sa.* Bareilly, which was detected through CDC's *Salmonella* Outbreak Surveillance Algorithm (SODA), epidemiologically implicated the consumption of bottled water as a risk factor for illness. This is the first widespread outbreak implicating bottled water in the United States. Previous bottled water outbreaks occurred in New Jersey in 1973 (*33*), Pennsylvania in 1980 (*34*), and in the Northern Mariana Islands in 1993 (*4*). Bottled water standards and regulations, unlike the majority of drinking water standards, are not set and enforced by the EPA, but by the FDA. The FDA regulates bottled water as a packaged food product and bases their bottled water standards on the EPA's tap water standards.

In addition, bottled water might be subject to state and voluntary industry regulation. Bottled water, before this outbreak, had not been identified as a vehicle for transmission of infectious organisms in the United States, although a bottled water outbreak of *Ca. jejuni* associated with consumption of water bottled in Greece was documented during the 1997--1998 surveillance period.

Because of the wide geographic distribution of bottled water products, an outbreak associated with the consumption of bottled water would be difficult to recognize. FDA, EPA, CDC, and the bottled water industry together should address concerns regarding consumption of bottled water and public health.

Overall, the number of outbreaks associated with the five drinking water deficiencies (untreated surface water, untreated groundwater, treatment deficiency, distribution system deficiency, and unknown/miscellaneous deficiency) increased in each category from the 1997--1998 levels. The percentage of outbreaks caused by a treatment deficiency and distribution system problem decreased relative to reported increases in the other three categories.

Although problems with treatment and with distribution systems remain critical concerns for safe drinking water, the public's lack of understanding of the risk associated with consumption of untreated water and the assumption that all water is suitable for consumption is a concern also.

The relative proportion and number of outbreaks associated with different water systems also differs from the figures from the 1997--1998 period (Figure 2). Outbreaks in community systems increased from 8 to 11 outbreaks (37.5% increase); noncommunity outbreaks doubled from 5 to 11; and individual system outbreaks quadrupled, increasing from 4 to 17 outbreaks. However, the proportion of outbreaks in community systems decreased from 47.1% during 1997--1998 to 28.2% during 1999--2000, whereas the relative proportion of outbreaks in individual systems increased from 23.5% during 1997--1998 to 43.6 during 1999--2000. In addition, the number of outbreaks reported that were associated with individual systems during this period is the highest reported level since 1984.

The drinking water quality of community systems, which typically have been the focus of increased EPA regulation, has continually improved. But noncommunity systems and individual systems, which are not regulated to the same extent, are continuing problems. The majority of these individual system outbreaks are linked to currently unregulated groundwater supplies, specifically private wells. The populations served by these systems merit increased attention by public health officials.

Unknown Etiology

The etiologic agent was not identified in 17 (43.6%) of 39 outbreaks (Figure 2). These outbreaks of unknown etiology comprised the largest group of outbreaks, followed by outbreaks caused by bacteria (nine), parasites (seven), viruses (four), and chemicals (two). During 1997--1998, parasites accounted for the largest percentage of the 17 outbreaks (six [35.3%]), followed by unidentified pathogens (five [29.4%]), bacteria (four [23.5%]), chemicals (two [11.6%]), and viruses (zero [0%]). The number of outbreaks per type of agent were increased for all categories during 1999--2000, with the exception of chemicals.

Although the number of reported viral outbreaks increased, indicating an improvement in the availability and usage of laboratory detection methods during previous years, viral outbreaks are probably substantially underreported. Although viruses were suspected in other outbreaks, specifically in those of unknown etiology, testing for viruses was not performed.

The technology for detection of viruses in stool and water samples has improved, but testing for viruses is not widely practiced. Investigators are encouraged to submit clinical specimens to CDC or state laboratories that conduct these tests.

Stool Specimens for Identification
Guidelines for collecting stool specimens for identification of viral organisms are available from CDC. Investigators are also encouraged to contact CDC and EPA regarding testing of water samples.

Only two outbreaks of chemical origin were identified during this surveillance period, the same number as was reported during the 1997--1998 period. One outbreak related to a spill of sodium hydroxide at a community water treatment plant demonstrated the need for safe water treatment practices.

The other outbreak was a single case of methemoglobinemia in an infant who required hospitalization after having been fed boiled water taken from a private well.

Coordination of public health messages is critical; an intervention that was intended to reduce the transmission of infections agents concentrated the chemicals present in the water. These figures, as in the past, probably under represent the actual waterborne chemical poisonings that occur.

Multiple factors can explain the low reporting rate, including the likelihood that:
1) the majority of waterborne chemical poisonings typically occur in private residences and affect a relatively limited number of persons;
2) exposures to chemicals through drinking water might cause illness that is difficult to link to a chemical exposure;
3) the mechanisms for reporting waterborne chemical poisonings to the WBDO surveillance system are not as established for chemicals as they are for WBDOs attributed to infectious agents; and 4) health-care providers and those affected might not as easily recognize chemical poisonings. As a result of these factors, WBDOs of chemical poisonings are less likely to be reported to public health officials.

We Need To Do More
Strengthening the capacity of local and state public health epidemiologists and environmental health specialists to detect and investigate outbreaks remains a priority at CDC and the EPA. As part of that effort, CDC and the EPA should partner with the states, CSTE, and the Association of Public Health Laboratories to develop training materials and online resources that would be useful and easily accessible to local and state public health personnel.

Although no federal regulation exists for monitoring private wells, developing educational materials targeted towards the general public, informing them of ways to maintain the safety and water quality of their wells would be valuable. In addition, health messages regarding the consumption of nonpotable water and appropriate point-of-use treatment should be developed and distributed to the public.

Outbreaks Associated with Recreational Water

Of the 59 recreational WBDOs, those involving gastroenteritis were most frequently reported (n = 36). The 15 outbreaks reported in 1999 and 21 outbreaks reported in 2000 equal or surpass the number reported in 1998, which previously was the highest number of outbreaks involving recreational water-related gastroenteritis reported in one year since the inception of the surveillance system. Together, the outbreaks involving gastroenteritis reported during the 1999--2000 period are higher than the 18 outbreaks documented in the previous reporting period (Figures 7 and 8). Since 1989, the number of gastroenteritis-related outbreaks has been gradually increasing, and this increase is statistically significant (p = 0.01).

Because swimming is essentially a shared water activity or communal bathing, rinsing of soiled bodies and overt fecal accidents cause contamination of the water. Unintentional ingestion of recreational water contaminated with pathogens can then lead to gastrointestinal illness, even in non-outbreak settings (*36,37*). Fresh and marine waters are also subject to other modes of contamination from point sources (i.e., sewage releases), watersheds (i.e., runoff from agriculture and residential areas), and floods.

Outbreaks involving gastroenteritis are more frequently observed during the swimming season, which usually starts on Memorial Day weekend (the last weekend in May) and ends Labor Day weekend (the first weekend in September). However, swimming also occurs year-round in indoor venues and in states with more temperate climates. Outbreaks of illness by month (Figure 3) include two outbreaks that occurred noticeably outside the summer months: one outbreak in a Florida pool in March and another outbreak in an indoor pool in Wisconsin in January.

As during the previous reporting period, *Cr. parvum* accounted for the largest percentage of outbreaks involving gastroenteritis (44.4%), followed by *Es. coli* O157:H7 (11.1%), NLV (8.3%), and *Shigella* (8.3%). An outbreak of *G. intestinalis* was also reported in 1999. The last reported recreational water outbreak of *Giardia* occurred in 1996. Outbreaks of *Ca. jejuni*, *Es. coli* O121:H19, and a mixed *Sh. sonnei*/*Cr. parvum* outbreak were also reported for the first time to the surveillance system. Outbreaks of unknown etiology comprised 16.7% of the recreational water outbreaks involving gastroenteritis.

Twenty-two (61.1%) of the 36 outbreaks of gastroenteritis occurred in treated systems (i.e., pools) that would usually be expected to be chlorinated or disinfected to prevent transmission of infectious agents after unintentional ingestion. However, the term *treated system* might pertain to systems not routinely treated, including wading pools, interactive fountains, and in one case, an untreated pool that was served by a natural hot springs source.

Multiple interrelated factors can impede disinfection in treated venues, including an increased bather load in a pool, high levels of organic material (e.g., fecal material or environmental or skin debris) and ultraviolet light, all of which deplete chlorine residuals that usually maintain protection in the system. In certain outbreaks, fecal material was indicated on the report as a contributing factor to the outbreak; the majority of fecal accidents were attributed to young children who were in or near the water at the time the accident occurred.

Unlike previous years, a substantial number of different bacterial and viral organisms were reported as causing gastrointestinal illness in these treated recreational water venues (Figure 9).

Nevertheless, >66% of these outbreaks were attributed to *Cr. parvum* (Figure 4). Unlike other organisms, which are more susceptible to the levels of chlorine typically found in a pool, *Cr. parvum* is highly chlorine-resistant and requires increased levels of chlorine and longer contact times with chlorine for inactivation. *Cr. parvum* can survive for days in public health-mandated chlorine concentrations required for pools. In addition, its relatively limited size (4--6 μm) can allow it to pass through particulate filtration systems during recirculation of water in the pool.

Because a low number of oocysts might cause illness in a person, even ingestion of a limited amount of water can cause infection. Although the number of *Cr. parvum* outbreaks has been steadily increasing during 1990--2000, multiple explanations could exist for the increase. The properties of the organism, coupled with the popularity of swimming and the tendency of persons to aggregate in larger water venues, increases the likelihood that swimming water can become contaminated and that swimmers will ingest the water and become infected.

However, the increases in outbreaks could be explained by a higher awareness of *Cr. parvum* as a potential cause of illness among swimmers by the public health community and the recreational water industry and, as a result, are more likely to be detected.

The majority of these *Cr. parvum* outbreak investigations noted inadequate pool maintenance. Although low chlorine levels are unlikely to have been the cause of the outbreaks, the frequent reporting of low chlorine levels in these outbreaks indicates a disturbing lack of awareness concerning the role of chlorine and pH control as the major protective barrier against infectious disease transmission in pools.

Inadequate disinfectant levels in any pool increases the risk for transmission of chlorine-sensitive pathogens (e.g., *Es. coli* O157:H7 or *Shigella* species) if an infected swimmer contaminates the pool. Pool operators and staff should be appropriately trained regarding the spread of recreational water illnesses and the critical role of pool maintenance (i.e., disinfection, pH control, and filtration) in preventing WBDOs.[§§]

Gastroenteritis

Fourteen outbreaks involving gastroenteritis after freshwater exposure were reported during 1999--2000, compared with eight during 1997--1998. *Es. coli* O157:H7 accounted for the most outbreaks of known etiology (three), followed by NLV (two), *Shigella* species (two), *Es. coli* O121:H19 (one), *G. intestinalis* (one), and *Cr. parvum* (one). Four outbreaks were of unknown etiology. Certain outbreaks occurred in beach areas that had substantial numbers of families bathing and swimming in the water. Again, a common element noted in these reports was the presence of diaper-aged children in the water, diaper-changing on the beach, and even washing off young children in the water. One incident involved persons who swam in a lake that was had posted signs indicating that the lake was unsafe for swimming.

Reports of infants and children swimming when they have diarrhea is a problem common to both freshwater systems and treated venues. Although health communication messages have been targeted in the past for treated venues, similar messages should be provided to those who swimming in freshwater venues. EPA, as part of the Beaches Action Plan, is developing guidelines and information for users of freshwaters.¶¶

Geothermal Pools and Hot Springs
Geothermal pools and hot springs should be examined closely. In one outbreak, pools in a complex were exempt from public health regulation because they were naturally occurring hot springs and mineral waters. Hot springs, which feature high levels of minerals and elevated temperatures, are potentially ideal venues for microbial growth or contamination. These springs and geothermal pools pose an increased risk to swimmers, compared with treated pools because of their lack of disinfection and filtration. Improved consumer and staff education and supplementary treatment might be necessary to prevent future outbreaks in these enclosed freshwater pools.

Twelve of the 15 outbreaks of dermatitis were associated with hot tub or pool use. The majority of these reports of dermatitis are associated with deficient maintenance and inadequate disinfection of the water. The higher temperatures commonly found in hot tubs deplete disinfectant levels at a more rapid rate; hot tub operators should be encouraged to actively check and maintain adequate disinfectant levels. In addition to rashes, reports have been received of other symptoms.

In Alaska, three of 29 persons reported nausea. In the two Maine outbreaks, persons also reported headache, fatigue, and other symptoms. The Colorado outbreak was notable for its severe symptomatology and an extended duration of illness. Extended and painful rashes associated with *P. aeruginosa* outbreaks are unusual but have been documented (*38,39*).

One report also indicates that a substantial number of children are being affected by these outbreaks. In the Colorado outbreak of *P. aeruginosa*, the persons affected were primarily children, but no indication was provided that age was a risk factor for infection. More remarkable is the observed duration of illness. Certain persons reported chronic illness (i.e., rash, joint pain, abdominal pain, and chest pain) that lasted $\geq$6 weeks. Using remote pool monitoring services in two of these outbreaks underscores the need for training pool staff regarding the role of monitoring service and prompt communication between service and pool operators when problems are detected.

Three outbreaks of dermatitis that occurred after persons swam in fresh or marine water were presumed to be caused by an allergic reaction to the cercariae, the larval form of certain nonhuman species of schistosomes. Cercarial dermatitis was an identified problem in two of these lakes, and signs posted by the health department regarding this problem were ignored by swimmers. The extent of the problem of cercarial dermatitis caused by freshwater exposure is unknown, although it probably occurs more frequently than what is reported to the surveillance system. As schistosomes occur naturally in ecosystems that bring snails and birds or aquatic mammals close together, a substantial number of freshwater lakes in the United States might cause illness among swimmers. Swimmers should pay careful attention to where they swim, avoid shallow swimming areas known to be appropriate snail habitats in lakes associated with cercarial dermatitis, and report any incidents to their local health department to prevent further illnesses.

The four deaths associated with primary amebic meningoencephalitis (PAM) reported during the 1999--2000 period were all linked to freshwater exposure. Typically, these cases are associated with swimming in freshwater bodies in the late summer months because *N. fowleri*, which has been implicated in >90% of the cases reported to CDC, proliferate in warm, stagnant waters. Previous cases of PAM have been reported from states with more temperate climates (e.g., California, Florida, and Texas) or from areas with hot springs.

PAM
The amoebas associated with PAM are believed to enter through the nasal passage. Preventing forceful entry of water up the nasal passages during jumping or diving by holding one's nose or wearing nose plugs could reduce the risk for infection.

Swimming in waters contaminated by animal urine was the likely explanation for an outbreak of leptospirosis among persons participating in an adventure race in Guam. *Leptospira* species can be found frequently in wild animal urine, and can be contracted through inhalation of aerosolized water or ingestion of water while swimming.

Leptospirosis
Leptospirosis can also be acquired through abrasions. In this instance, the exposure was associated with immersion of persons' heads in a body of water while they swam and swallowed water. Although outdoor swimming is not necessarily dangerous, swimmers should be educated regarding the potential risks resulting from swimming in areas that are not secured from wild animal use.

An increased level of bromine, which is used to disinfect pools and hot tubs, caused certain cases of chemical keratitis. Inadequate disinfection of a whirlpool resulted in an outbreak of legionellosis among 20 persons who stayed at a motel. Safe disinfection practices and appropriate pool maintenance protocols should be communicated to operators and managers of facilities that treat recreational water.

Outbreaks Associated with Occupational Exposures to Water
Two outbreaks that do not fit into the previous categories were reported to CDC by Minnesota and Hawaii. Outbreaks associated with exposure to aerosolized water have previously occurred but have not been reported to the WBDO surveillance system. These outbreaks are discussed in this report to demonstrate that water exposures are not limited to ingestion and contact (e.g., through swimming), and these outbreaks are preventable.

Using barrier masks to prevent inhalation of aerosolized water or disinfection of water that is not being used for drinking or swimming purposes could have prevented the respiratory illnesses associated with these two outbreaks.

Conclusion
Data collected as part of the national WBDO surveillance system are used to describe the epidemiology of waterborne diseases in the United States. Data regarding water systems and deficiencies implicated in these outbreaks are used to assess whether regulations for water treatment and monitoring of water quality are adequate to protect the public's health. Identification of the etiologic agents responsible for these outbreaks is also critical because new trends might necessitate different interventions and changes in policies and resource allotment.

Surveillance for waterborne agents and outbreaks occurs primarily at the local and state level. Local and state public health agencies need to detect and recognize WBDOs and implement appropriate prevention and control measures. Improved communication among local and state public health departments, regulatory agencies, water utilities, and recreational water facilities would aid the detection and control of outbreaks.

Share Water-Quality Data
Routine reporting or sharing of water-quality data with the health department is recommended. Other means of improving surveillance at the local, state, and federal level could include the additional review and follow-up of information gathered through other mechanisms (e.g., issuances of boil-water advisories or reports of illness associated with agents thought to be waterborne).

One repeated observation regarding outbreak data collected as part of the WBDO system was that the timely collection of clinical specimens and water samples for testing and commencement of an environmental investigation would have resulted in an improved ability to detect the outbreak's etiologic agent and the source of water contamination.

However, the course of an investigation is influenced by the ability and capacity of public health departments and laboratories to recognize and investigate potential outbreaks of illness. Even when personnel are available to investigate a potential outbreak in a timely manner, a common observation is that investigations cannot always be completed thoroughly. WBDO outbreak investigations typically require input from different disciplines, including infectious disease epidemiology, environmental epidemiology, clinical medicine, sanitation, water engineering, and microbiology. Either further cross-training of existing personnel needs to be implemented or additional personnel and resources need to be made available or linked to those who typically investigate reports of WBDOs.

Epidemiologic Assistance
State health departments can request epidemiologic assistance and laboratory testing from CDC to investigate WBDOs. CDC and the EPA can be consulted regarding engineering and environmental aspects of drinking water and recreational water treatment and regarding collection of large-volume water samples to identify pathogenic viruses and parasites, which require special protocols for their recovery.

Requests for tests for viral organisms should be made to CDC's Viral Gastroenteritis Section, Respiratory and Enterovirus Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases (NCID), at 404-639-3577.

Requests for tests for parasites should be made to CDC's Division of Parasitic Diseases, NCID, at 770-488-7760.

Additional information is available from

- EPA's Safe Drinking Water Hotline at 800-426-4791, on the Internet at http://www.epa.gov/safewater, or by e-mail at hotline-sdwa@epa.gov;
- CDC's NCID website at http://www.cdc.gov/ncidod;
- CDC's Healthy Swimming website at http://ww.cdc.gov/healthyswimming; includes recreational water health communication materials for the general public and pool maintenance staff (e.g., information regarding disinfection, guidelines on response to fecal accidents [*42*], fact sheets concerning recreational water illnesses), and an outbreak investigation toolkit that can be used by public health professionals;
- CDC's Voice and Fax Information System, 888-232-3228 (voice) or 888-232-3299 (fax). Choose cryptosporidiosis in the disease category; and
- for reporting WBDOs, CDC's Division of Parasitic Diseases, NCID, at 770-488-7760 or by fax at 770-488-7761.

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* Total coliforms are considered indicator organisms that typically do not cause disease but might be associated with the presence of other disease-causing organisms. Additional information regarding total coliforms is available at http://www.epa.gov/safewater/dwa/electronic/tcr.pdf.
† Additional information is available at http://www.cleanwater.gov.
§ Additional terms are defined in the glossary.
¶ Additional information is available at http://www.nsf.org.
** Additional information is available at http://www.epa.gov/safewater/gwr.html.
†† Although EPA does not regulate private wells and will not regulate them as part of the proposed GWR, EPA lists recommendations for protecting private water supplies at http://www.epa.gov/safewater/pwells1.html and provides links to other sources of information.
§§ Guidelines for pool operators and other information related to recreational water illnesses is available at http://www.cdc.gov/healthyswimming.
¶¶ Additional information is available at http://www.epa.gov/waterscience/beaches.

Table 1

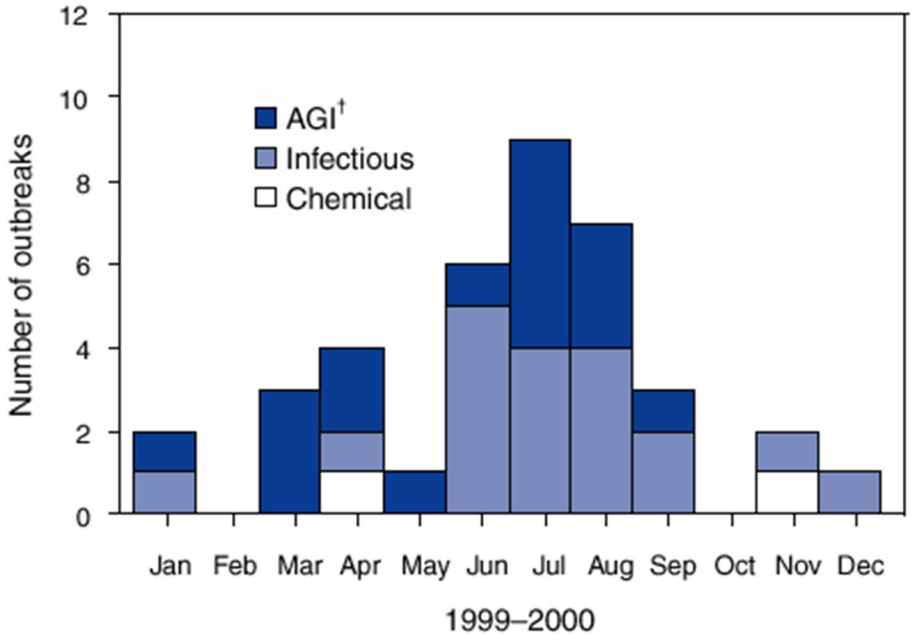
TABLE 1. Classification of investigations of waterborne-disease outbreaks — United States*

Class†	Epidemiologic data	Water-quality data
I	Adequate§ Data were provided regarding exposed and unexposed persons, and the relative risk or odds ratio was ≥2 or the p-value was <0.05	Provided and adequate Historical information or laboratory data (e.g., the history that a chlorinator malfunctioned or a water main broke, no detectable free-chlorine residual, or the presence of coliforms in the water)
II	Adequate	Not provided or inadequate (e.g., stating that a lake was crowded)
III	Provided, but limited Epidemiologic data were provided that did not meet the criteria for Class I, or the claim was made that ill persons had no exposures in common besides water, but no data were provided	Provided and adequate
IV	Provided, but limited	Not provided or inadequate

* Outbreaks of *Pseudomonas* and other water-related dermatitis and single cases of primary amebic meningoencephalitis or of illness resulting from chemical poisoning are not classified according to this scheme.
† On the basis of epidemiologic and water-quality data that were provided on CDC form 52.12.
§ Adequate data were provided to implicate water as the source of the outbreak.

Figure 1

FIGURE 1. Number of waterborne-disease outbreaks associated with drinking water, by etiologic agent and month — United States, 1999–2000 (n = 38)*



* One outbreak of *Salmonella* Bareilly was not included.
† Acute gastrointestinal illness of unknown etiology.

Table 2

TABLE 2. Waterborne-disease outbreaks associated with drinking water — United States, 1999 (n = 15)*

State	Month	Class†	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
California	Jul	III	AGI**	31	Ncom	2	Well	Camp
Florida	Jan	III	AGI	4	Com	2	Well	Community
Florida	Jan	III	*Giardia intestinalis*	2	Ind	2	Well	Household
Florida	Mar	III	AGI	6	Com	4	River/stream	Apartment
Florida	Mar	III	AGI	3	Com	4	Well	Community
Florida	May	III	AGI	3	Ind	2	Well	Household
Florida	Aug	III	AGI††	4	Com	4	River/stream	Apartment
Missouri	Jun	II	*Salmonella* Typhimurium	124	Com	3	Well	Community
New Jersey	Nov	IV	Sodium hydroxide	2	Com	3	Well	Community
New Mexico	Jul	I	Small round-structured virus§§	70	Ncom	3	Spring	Camp
New York	Aug	I	*Escherichia coli* O157:H7, *Campylobacter jejuni*¶¶	781	Ncom	2	Well	Fairgrounds
Texas	Nov	I	*Es. coli* O157:H7	22	Com	3	Well	Community
Washington	Jul	II	AGI	46	Ind	1	River/creek	Household
Washington	Aug	I	AGI	68	Ncom	2	Well	Soccer match
Wisconsin	Apr	NA***	Nitrate	1	Ind	2	Well	Household

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after ingestion of drinking water and 2) epidemiologic evidence that implicates water as the probable source of the illness.

† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

§ Com = community; Ncom = noncommunity; Ind = individual; community and noncommunity water systems are public water systems that serve ≥15 connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).

** Acute gastrointestinal illness of unknown etiology.

†† Unidentified chemical poisoning.

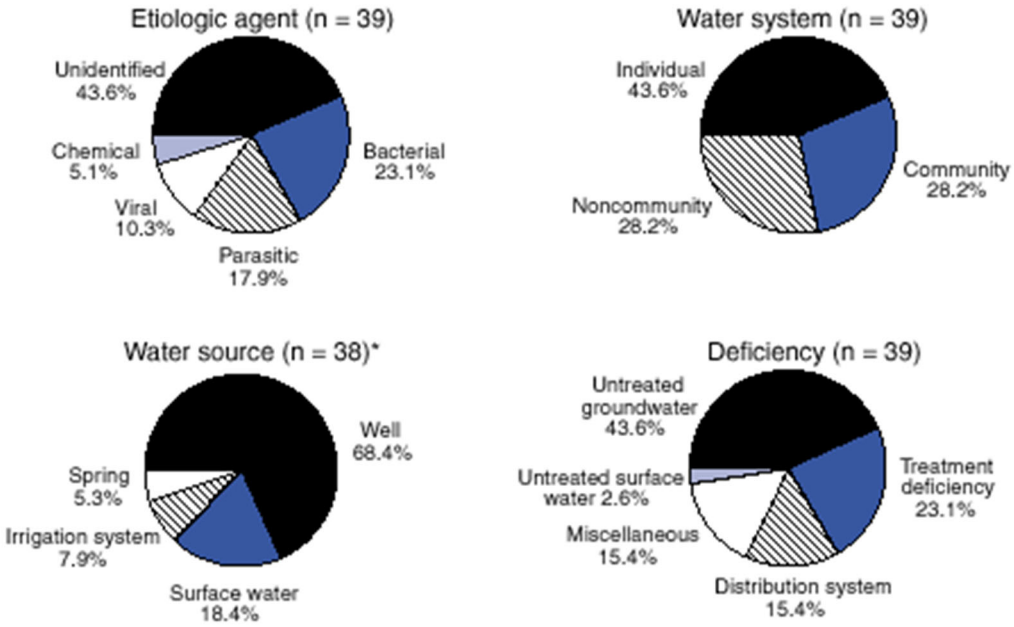
§§ Three persons had stool specimens that tested positive for small round-structured virus, and one person's stool specimen tested positive for *Ca. jejuni*.

¶¶ A total of 126 persons had stool specimens that tested positive for *Es. coli* O157:H7; 43 persons had stool specimens that tested positive for *Ca. jejuni*. One person's stool specimen tested positive for both organisms.

*** Not applicable, see Table 1.

Figure 2

FIGURE 2. Waterborne-disease outbreaks associated with drinking water, by etiologic agent, water system, water source, and deficiency — United States, 1999–2000 (n = 39)



* One outbreak of *Salmonella* Bareilly was not included.

Table 3

TABLE 3. Waterborne-disease outbreaks associated with drinking water — United States, 2000 (n = 24)*

State	Month	Class†	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
California	Jul	I	Norwalk-like virus	147	Ncom	2	Well	Camp
California	Jul	I	*Escherichia coli* O157:H7	5	Ind	5	River/creek	Camp
California	Sep	III	AGI**	63	Ind	5	Irrigation system	Football game
Colorado	Aug	III	*Giardia intestinalis*	27	Ncom	3	River	Resort
Florida	Mar	III	AGI††	19	Com	3	Well	Trailer park
Florida	Apr	III	AGI	21	Com	3	Well	Trailer park
Florida	Apr	I	AGI	71	Ind	2	Well	Community
Florida	Jun	III	AGI§§	2	Ind	2	Well	Household
Florida	Jul	III	AGI	3	Ind	2	Well	Household
Florida	Jul	III	AGI	3	Ind	2	Well	Household
Florida	Aug	III	AGI	4	Ind	2	Well	Household
Florida	Sep	III	*G. intestinalis*	2	Ind	4	Well	Household
Florida	Dec	III	*Cryptosporidium parvum*	5	Com	4	Well	Community
Idaho	Apr	III	*Es. coli* O157:H7	4	Ind	5	Irrigation canal	Household
Idaho	Jun	III	*Campylobacter jejuni*	15	Ncom	2	Spring	Camp
Idaho	Jul	III	AGI	65	Ncom	2	Well	Restaurant
Kansas	Jun	III	Norwalk-like virus	86	Ncom	2	Well	Reception hall
Minnesota	Jun	III	*G. intestinalis*¶¶	12	Ncom	2	Well	Camp
New Hampshire	Sep	III	*G. intestinalis*	5	Ind	3	Well	Household
New Mexico	Jul	II	*G. intestinalis*	4	Ind	5	River	Rafting trip
Ohio	Aug	I	*Es. coli* O157:H7	29	Com	4	Surface water***	Fairgrounds
Utah	Aug	III	*Ca. jejuni*†††	102	Ind	5	Irrigation water	Football camp
West Virginia	Jun	III	Norwalk-like virus	123	Ncom	3	Wells	Camp
Multistate	Apr–Aug	I	*Salmonella* Bareilly	84	Ind	5§§§	Municipal/spring§§§	Wells/bottled water***

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after ingestion of drinking water and 2) epidemiologic evidence that implicates water as the probable source of the illness.

† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

§ Com = community; Ncom = noncommunity; Ind = individual; community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).

** Acute gastrointestinal illness of unknown etiology.

†† Persons also reported rashes in addition to acute gastrointestinal illness.

§§ One person had a stool specimen that tested positive for *Blastocystis hominis*.

¶¶ Eight persons had stool specimens that tested positive for *G. intestinalis*; one stool specimen tested positive for *Dientamoeba fragilis*.

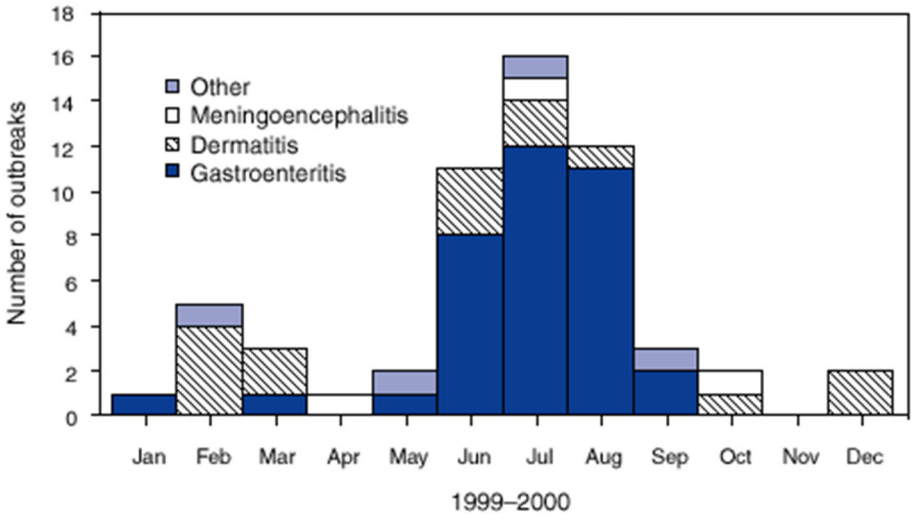
*** Type of water was not specified on report form.

††† Thirty-seven persons had stool specimens that tested positive for *Ca. jejuni*; four persons' stool specimens tested positive for *Es. coli* O157:H7, and three persons had stool that tested positive for *Es. coli* O111.

§§§ The outbreak implicated both drinking water from private wells and springs and water bottled by one facility. The bottling facility used two sources of water.

Figure 3

FIGURE 3. Number of waterborne-disease outbreaks associated with recreational water, by illness and month — United States, 1999–2000 (n = 58)*



* Information regarding the month was not provided for one outbreak of meningoencephalitis.

Table 4

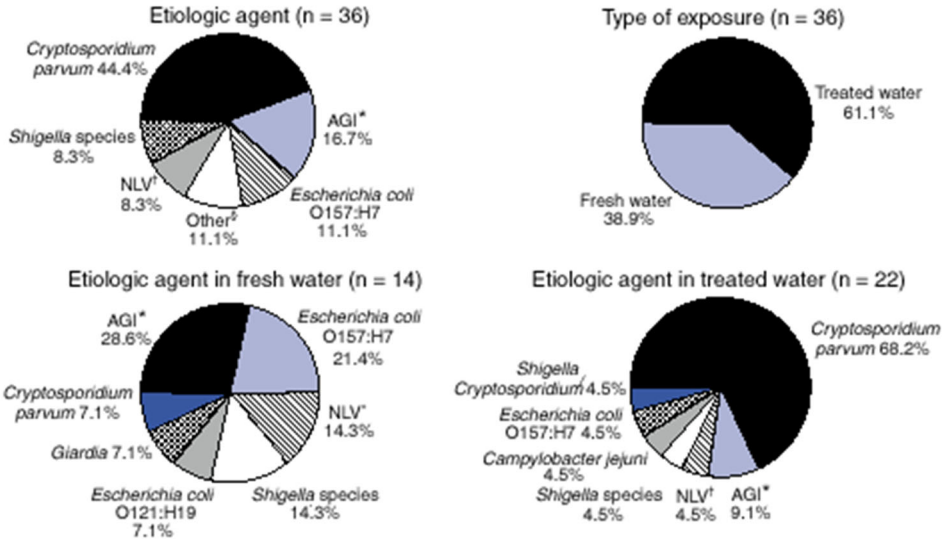
TABLE 4. Waterborne-disease outbreaks associated with drinking water, by etiologic agent and type of water system — United States, 1999–2000 (n = 39)

	Type of water system*							
	Community		Noncommunity		Individual		Total	
Etiologic agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
AGI†	6§	57	3	164	8	195	**17**	**416**
Giardia intestinalis	0	0	2	39	4	13	**6**	**52**
Escherichia coli O157:H7	2	51	0	0	2	9	**4**	**60**
Norwalk-like viruses (NLV)	0	0	3	356	0	0	**3**	**356**
Salmonella species¶	1	124	0	0	1	84	**2**	**208**
Campylobacter jejuni	0	0	1	15	1	102	**2**	**117**
Es. coli O157:H7/*Ca. jejuni*	0	0	1	781	0	0	**1**	**781**
Small round-structured virus	0	0	1	70	0	0	**1**	**70**
Cryptosporidium parvum	1	5	0	0	0	0	**1**	**5**
Sodium hydroxide	1	2	0	0	0	0	**1**	**2**
Nitrate	0	0	0	0	1	1	**1**	**1**
Total	**11**	**239**	**11**	**1,425**	**17**	**404**	**39**	**2,068**
Percentage	**28.2**	**11.6**	**28.2**	**68.9**	**43.6**	**19.5**	**100.0**	**100.0**

* Community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.
† Acute gastrointestinal illness of unknown etiology.
§ One outbreak of four cases was caused by an unidentified chemical.
¶ One outbreak was serotype Typhimurium, and one outbreak was serotype Bareilly.

Figure 4

FIGURE 4. Waterborne-disease outbreaks of gastroenteritis associated with recreational water, by etiologic agent and type of exposure — United States, 1999–2000



* Acute gastrointestinal illness of unknown etiology.
† Norwalk-like virus.
§ These included outbreaks of *Campylobacter jejuni*, *Giardia*, *Escherichia coli* O121:H19 and one mixed *Shigella/Cryptosporidium* outbreak.

Table 5

TABLE 5. Waterborne-disease outbreaks associated with drinking water, by type of deficiency and type of water system — United States, 1999–2000 (n = 39)

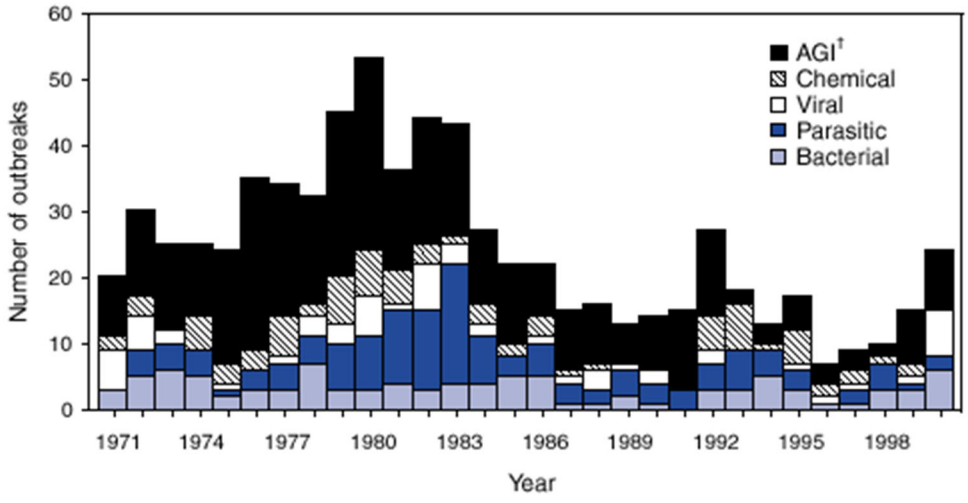
	Type of water system*							
	Community		Noncommunity		Individual		Total	
Type of deficiency†	Outbreaks	%	Outbreaks	%	Outbreaks	%	Outbreaks	%
Untreated surface water	0	0	0	0	1	5.9	**1**	**2.6**
Untreated groundwater	1	9.0	8	72.7	8	47.0	**17**	**43.6**
Inadequate treatment	5	45.5	3	27.3	1	5.9	**9**	**23.1**
Distribution system	5	45.5	0	0	1	5.9	**6**	**15.4**
Miscellaneous or unknown	0	0	0	0	6	35.3	**6**	**15.4**
Total	**11**	**100.0**	**11**	**100.0**	**17**	**100.0**	**39**	**100.0**

* Community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

† Examples of treatment deficiencies include temporary interruption of disinfection, chronically inadequate disinfection, or inadequate or no filtration; examples of distribution system deficiencies include cross-connection, contamination of water mains during construction or repair, or contamination of a storage facility; and examples of unknown or miscellaneous deficiencies include contaminated bottled water.

Figure 5

FIGURE 5. Number of waterborne-disease outbreaks associated with drinking water, by year and etiologic agent — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.
† Acute gastrointestinal illness of unknown etiology.

Table 6

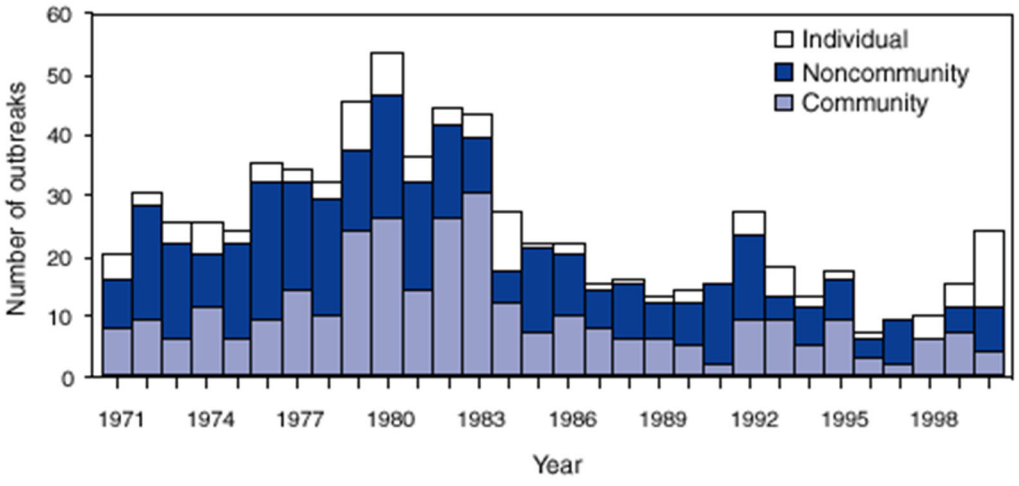
TABLE 6. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 1999 (n = 15)

State	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
California	Jun	III	AGI[†]	Gastroenteritis	23	Pool	Apartment complex
Connecticut	Jul	II	*Escherichia coli* O121:H19	Gastroenteritis	11	Lake	Lake
Florida	Mar	III	*Campylobacter jejuni*	Gastroenteritis	6	Pool	Private home
Florida	Aug	I	*Shigella sonnei*, *Cryptosporidium parvum*[§]	Gastroenteritis	38	Interactive fountain	Beach park
Florida	Aug	IV	*Cr. parvum*	Gastroenteritis	6	Pool	Private home
Florida	Sep	III	*Es. coli* O157:H7	Gastroenteritis	2	Ditch water	Community
Idaho	Jun	IV	Norwalk-like virus	Gastroenteritis	25	Hot springs	Resort
Illinois	Jun	III	AGI	Gastroenteritis	25	Lake	Lake
Massachusetts	Jul	III	*Giardia intestinalis*	Gastroenteritis	18	Pond	Pond
Minnesota	Jul	III	*Cr. parvum*	Gastroenteritis	10	Pool	Trailer park
Nebraska	Jun	IV	*Es. coli* O157:H7	Gastroenteritis	7	Wading pool	Child care center
New York	Jun	II	Norwalk-like virus	Gastroenteritis	168	Lake	County park
Washington	Aug	I	*Es. coli* O157:H7	Gastroenteritis	36	Lake	State park
Wisconsin	Jul	IV	*Cr. parvum*	Gastroenteritis	10	Pool	Municipal pool
Wisconsin	Aug	II	*Es. coli* O157:H7	Gastroenteritis	5	Lake/pond	Swimming beach

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Acute gastrointestinal illness of unknown etiology.
§ Five persons had stool specimens that tested positive for *Shigella sonnei*; two stools tested positive for *Cryptosporidium parvum*.

Figure 6

FIGURE 6. Number of waterborne-disease outbreaks associated with drinking water, by year and type of water system — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.

Table 7

TABLE 7. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 2000 (n = 21)

State	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
Colorado	Aug	I	*Cryptosporidium parvum*	Gastroenteritis	112	Pool	Municipal pool
Florida	May	IV	AGI†	Gastroenteritis	2	Lake	Lake
Florida	Jul	III	AGI	Gastroenteritis	4	Outdoor spring	County park
Florida	Jul	III	*Cr. parvum*	Gastroenteritis	3	Pool	Apartment complex
Florida	Aug	III	*Cr. parvum*	Gastroenteritis	5	Pool	Country club
Florida	Aug	I	*Cr. parvum*	Gastroenteritis	19	Pool	Resort
Florida	Aug	III	AGI	Gastroenteritis	9	Pool	Motel
Florida	Aug	III	*Cr. parvum*	Gastroenteritis	5	Pool	Condominium
Georgia	Jun	II	*Cr. parvum*	Gastroenteritis	36	Pools§	Community
Maine	Jul	II	AGI	Gastroenteritis	32	Lake/pond	Swimming beach
Minnesota	Jul	II	*Cr. parvum*¶	Gastroenteritis	220	Lake	Swimming beach
Minnesota	Jul	IV	*Shigella sonnei***	Gastroenteritis	15	Lake/pond	Swimming beach
Minnesota	Jul	III	*Cr. parvum*	Gastroenteritis	7	Pool	Day camp
Minnesota	Jul	II	*Cr. parvum*	Gastroenteritis	6	Pool	Hotel
Minnesota	Aug	II	*Sh. sonnei*	Gastroenteritis	25	Lake	Public beach
Minnesota	Aug	IV	*Cr. parvum*	Gastroenteritis	4	Pool	Municipal pool
Missouri	Sep	III	*Shigella flexneri*	Gastroenteritis	6	Wading pool	Community
Nebraska	Jun	I	*Cr. parvum*	Gastroenteritis	225	Pools	Community
Ohio	Jun	I	*Cr. parvum*	Gastroenteritis	700	Pool	Private swim club
South Carolina	Jul	IV	*Cr. parvum*	Gastroenteritis	26	Pool	Community
Wisconsin	Jan	IV	Norwalk-like virus	Gastroenteritis	9	Pool	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

† Acute gastrointestinal illness of unknown etiology.

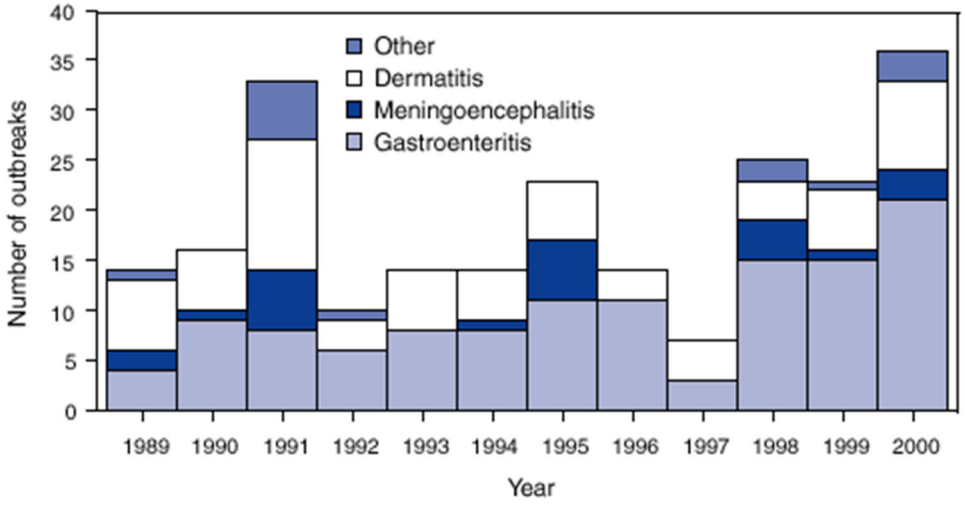
§ Persons swam in a community pool and an inflatable pool.

¶ Seventeen persons had stool specimens that tested positive for *Cr. parvum*. One person had a stool specimen that tested positive for *Giardia intestinalis*. One person had a stool specimen that tested positive for both organisms.

** Fourteen of 15 stool specimens tested positive for *Shigella*; one person tested positive for *Cr. parvum*; and one tested positive for both.

Figure 7

FIGURE 7. Number of waterborne-disease outbreaks associated with recreational water, by year and illness — United States, 1989–2000 (n = 229)*



* The total from previous reports has been corrected from n = 171 to n = 170.

Table 8

TABLE 8. Waterborne-disease outbreaks of meningoencephalitis, keratitis, leptospirosis, and Pontiac fever associated with recreational water — United States, 1999–2000 (n = 8)

State	Year	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
California	2000	Apr	NA†	*Naegleria fowleri*	Meningoencephalitis	1	Mudhole	Mudhole
Florida	1999	Oct	NA	*N. fowleri*	Meningoencephalitis	1	Pond	Pond
Florida	2000	—§	NA	*N. fowleri*	Meningoencephalitis	1	—	—
Guam	2000	Jul	II	*Leptospira interrogans*	Leptospirosis	21	Lake	Adventure race
Vermont	2000	Feb	NA	Bromine	Chemical keratitis	3	Pool	Pool
Texas	1999	Sep	II	Unknown¶	Acute respiratory infection	12	Hot tub	Ranch
Texas	2000	Jul	NA	*N. fowleri*	Meningoencephalitis	1	Lake	Lake
Wisconsin	2000	May	I	*Legionella pneumophila*	Pontiac fever	20	Whirlpool	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Not applicable.
§ The month the outbreak occurred was not reported; the source and setting were not reported.
¶ Clinical specimens tested negative for *Legionella pneumophila* serotypes 1 and 6, adenovirus, influenza virus, and parainfluenza virus.

Figure 8

FIGURE 8. Number of outbreaks involving gastroenteritis associated with recreational water, by year and illness — United States, 1978–2000 (n = 146)

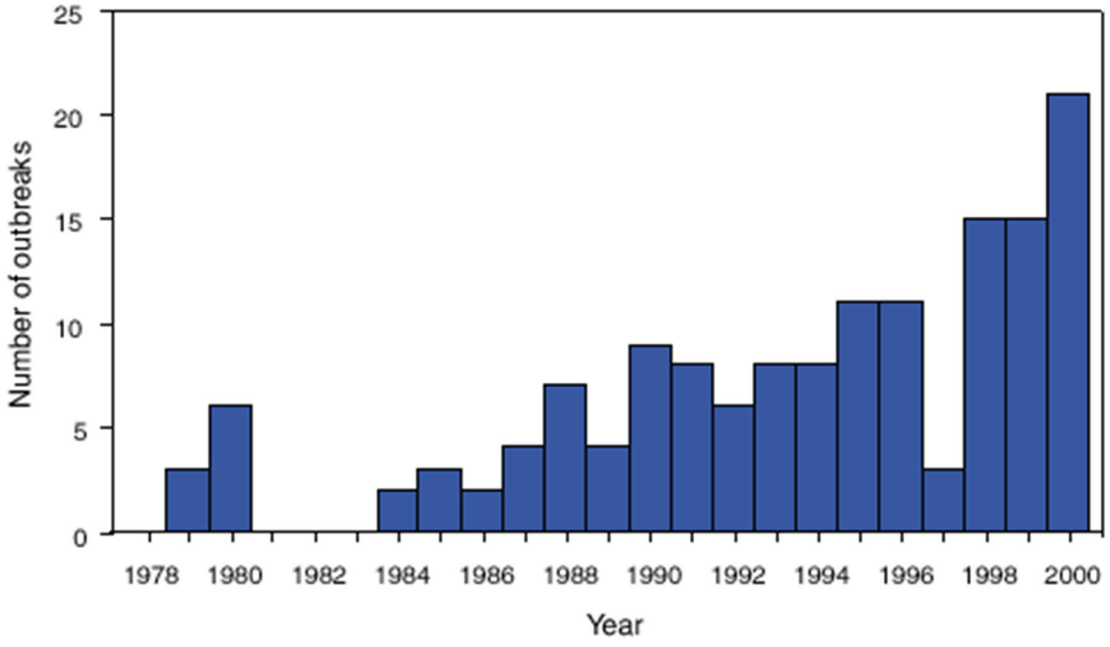


Table 9

TABLE 9. Waterborne-disease outbreaks of dermatitis associated with recreational water — United States, 1999–2000 (n = 15)

State	Year	Month	Class*	Etiologic agent	Number of cases	Source	Setting
Alaska	2000	Oct	NA†	*Pseudomonas aeruginosa*§	29	Pool/hot tub	Hotel
Arkansas	1999	Jun	NA	*P. aeruginosa*§	10	Pool	Community
Arkansas	2000	Feb	NA	*P. aeruginosa*§	26	Pool/ hot tub	Motel
California	2000	Jun	IV	Schistosomes**	6	Pond	Pond
California	2000	Jul	IV	Schistosomes**	4	Pond	Pond
Colorado	1999	Feb	NA	*P. aeruginosa*¶§	19	Hot tub	Hotel
Colorado	1999	Dec	NA	*P. aeruginosa*††	5	Hot tub	Ski lodge
Florida	2000	Aug	NA	*P. aeruginosa*††	6	Hot tub	Apartment complex
Maine	2000	Feb	NA	*P. aeruginosa*¶	9	Hot tub/pool	Hotel
Maine	2000	Mar	NA	*P. aeruginosa*§	11	Hot tub	Hotel
Minnesota	2000	Dec	NA	*P. aeruginosa*¶	16	Hot tub	Private
Oregon	1999	Jul	IV	Schistosomes**	2	Lake	Lake
Vermont	1999	Jun	NA	*P. aeruginosa*††	9	Hot tub	Hotel
Vermont	1999	Feb	NA	*P. aeruginosa*††	11	Hot tub	Vacation home
Washington	2000	Mar	NA	*P. aeruginosa*††	10	Pool/hot tub	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Not applicable.
§ Organism isolated from water.
¶ Laboratory-confirmed case.
** Suspected etiology on the basis of clinical syndrome and setting.
†† Suspected etiology on the basis of clinical syndrome.

Figure 9

FIGURE 9. Number of outbreaks involving gastroenteritis associated with recreational water, by water type — United States, 1985–2000 (n = 135)

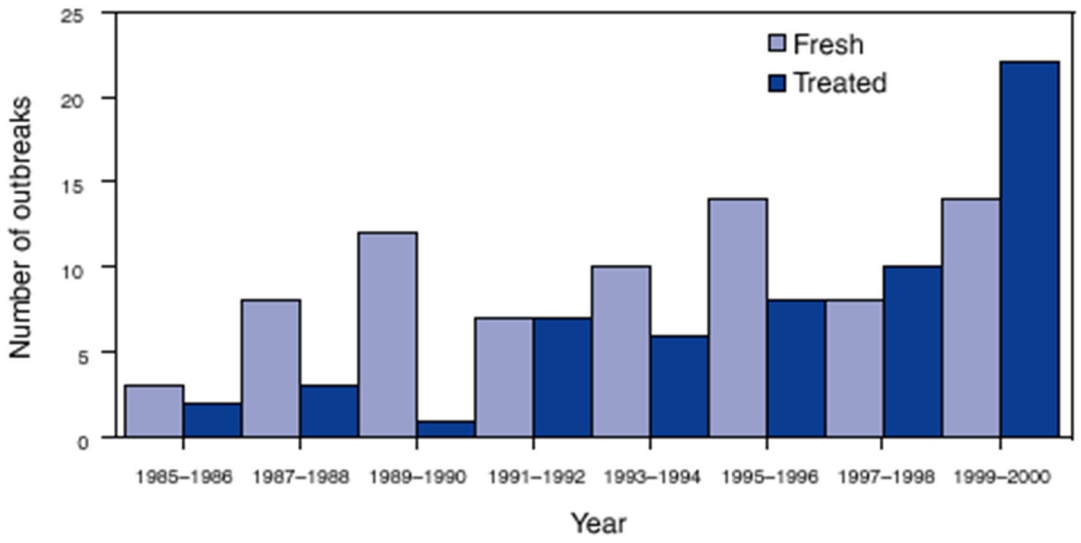


Table 10

TABLE 10. Waterborne-disease outbreaks associated with occupational exposures — United States, 1999–2000 (n = 2)

State	Year	Month	Class*	Etiologic agent	Exposure	Number of cases	Source	Setting
Hawaii	1999	Aug	IV	*Leptospira*	Contact with pond water	2	Pond	Outdoor landscaping
Minnesota	2000	Aug	III	Pontiac fever†	High-pressure cleaning using lagoon water	15	Plant lagoon	Sugar beet plant

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Endotoxin was also isolated from environmental samples; the role of endotoxin is unclear.

Table 11

TABLE 11. Waterborne-disease outbreaks associated with drinking water that were not included in the previous surveillance summaries — United States, 1995–1997 (n = 3)*

State	Year	Month	Class*	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
Washington	1995	Jul	III	*Giardia intestinalis***	87	Com	4	Well	Community
California	1997	Nov	III	Nitrite (sodium metaborite)	7	Com	4	Mixed river/groundwater	Hospital cafeteria
New York	1997	Dec	I	Norwalk-like virus	1,450	Ncom	3	Well	Ski resort

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after either ingestion of drinking water or exposure to water used for recreational purposes and 2) epidemiologic evidence that implicates water as the probable source of illness.
† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
§ Com = community; Ncom = noncommunity; Ind = individual; Community and noncommunity water systems are public water systems that serve ≥15 connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.
¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).
** Thirty-three persons had stool specimens that tested positive for *G. intestinalis*. One specimen tested positive for *Entamoeba coli*. One other specimen tested positive for *Blastocystis hominis*. One cultured specimen tested positive for *Campylobacter jejuni*.

BOX. Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
Safe Drinking Water Act/1974 and 1986 and 1996 amendments	Authorizes EPA to set national standards to protect drinking water and its sources
Total Coliform Rule (TCR)/and Maximum Contaminant Level (MCL)/1989	Requires routine monitoring for total coliforms of all public water systems plus periodic on-site inspections for systems that take <5 samples/month to evaluate and document treatment, storage, distribution network, operation and maintenance, and overall management. Systems that collect ≥40 samples/month (i.e., typically, systems that serve >33,000 persons) violate MCL if >5.0% of the samples collected during each month are positive for total coliforms; systems that collect <40 samples/month violate MCL if two samples during the month are positive for total coliforms. If a system has a total coliform-positive sample, then 1) that sample must be tested for the presence of fecal coliforms or *Escherichia coli*, and 2) three repeat samples must be collected (four, if the system collects ≤1 routine sample/month) within 24 hours and analyzed for total coliforms. If positive, the sample must be analyzed for fecal coliforms or *Es. coli*. In addition, ≥5 routine samples must be collected during the next month of sampling, regardless of system size. For any size system, if two consecutive total coliform-positive samples occur at one site during a month, and one of these samples is also fecal coliform-positive or *Es. coli*-positive, the system has an acute violation of the Maximum Contaminant Level and must notify the state and the public immediately.
Surface Water Treatment Rule (SWTR)/1989	Covers all water systems that use surface water or groundwater under the direct influence of surface water; all systems must disinfect their water, and the majority of systems must filter their water also, unless they meet EPA-specified filter-avoidance criteria that define high-quality source water. Specific requirements include • a combined filter-effluent–performance standard for turbidity (i.e., for rapid granular filters, 0.5 nephelometric turbidity unit [NTU] maximum for 95% of measurements [taken every 4 hours] during a month) and no single NTU reading >5.0; • watershed protection, redundant disinfection capability, and other requirements for unfiltered systems; • a 0.2-mg/L disinfectant residual entering the distribution system; and • maintenance of a detectable disinfectant residual in all parts of the distribution system. This rule requires that all such systems reduce the level of *Giardia* by 99.9% (3-log reduction) and viruses by 99.99% (4-log reduction) through a combination of removal (filtration) and inactivation (disinfection).
Information Collection Rule/ 1996–1998	Requires systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source water quality parameters. Surface water systems are also required to monitor *Cryptosporidium*, *Giardia*, total culturable viruses, and total and fecal coliforms or *Es. coli* ≥1 time/month for 18 months. Results provided information to facilitate development of the Long Term 2 Enhanced SWTR, which is intended to protect against microbial risks by targeting those systems with suboptimal quality source water and to balance the health risks associated with disinfection byproducts and the anticipated Stage 2 Disinfection Byproduct Rule.
Interim Enhanced Surface Water Treatment Rule (IESWTR)/1998	Follow-up to SWTR that covers all public systems using surface water or groundwater under the direct influence of surface water and serving ≥10,000 persons. Key provisions include • a 2-log *Cryptosporidium*-removal requirement for filtered systems; • strengthened combined filter-effluent–turbidity performance standards for systems using conventional filtration treatment or direct filtration (0.3 NTU maximum for 95% of measurements during a month and no single NTU reading >1.0); • individual filter turbidity monitoring provisions;

BOX (*Continued*). Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
	• disinfection profile and benchmark provisions to ensure continued levels of microbial protection while facilities take necessary steps to comply with new disinfection byproduct standards; • revision of the definition of groundwater under the influence of surface water and the watershed-control requirements for unfiltered public water systems to include detection of *Cryptosporidium*; • requirements for covers on newly finished water reservoirs; • sanitary surveys for all surface water systems regardless of size; and • an MCL goal of zero oocysts for *Cryptosporidium*.
Lead and Copper Rule/2000 changes	Streamlines requirements, promotes consistent national implementation, and reduces the burden for water systems.
Long Term 1 Enhanced SWTR (LT1ESWTR)/2002 and the Filter Backwash Recycling Rule (FBRR)/2001	Companion regulations for IESWTR; LT1ESWTR applies to public water systems that use surface water or groundwater under the direct influence of surface water and that serve <10,000 persons. FBRR regulates how treatment plants recycle water that has been used to backwash a filter or that has been extracted from treatment plant sludge. FBRR regulates the point in the treatment plant at which the contaminated recycle water may be introduced, assuring that the water is subject to the entire particle and *Cryptosporidium parvum* removal process.
Long Term 2 Enhanced SWTR (LT2ESWTR)/expected in 2003	Applies to all systems using surface water or groundwater under the influence of surface water; will provide additional protection against *Cryptosporidium*. Systems will be assigned to a treatment category on the basis of their source-water *Cryptosporidium* levels; the category then determines how much additional treatment is required.
Stage 2 Disinfection Byproduct Rule (DBPR)/expected in 2003	Will apply to community water systems and nontransient noncommunity water systems that use an alternative to ultraviolet disinfection or deliver disinfected water; systems will be required to monitor for total trihalomethanes and the sum of five haloacetic acids and comply with MCLs at each monitoring location as a locational running annual average.
Ground Water Rule (GWR) (1996 amendment to EPA's Safe Drinking Water Act)/expected to be finalized in 2003	Applies to public groundwater systems (i.e., systems that have ≥15 service connections, or regularly serve ≥25 persons daily for ≥60 days/year) or any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. Establishes multiple barriers to protect against bacteria and viruses in drinking water from groundwater sources; establishes targeted strategy to identify groundwater systems at high risk for fecal contamination. Key areas include • system sanitary surveys; • hydrogeologic sensitivity assessments for nondisinfected systems; • source-water microbial monitoring by systems that do not disinfect and that draw from hydrogeologically sensitive aquifers or have detected fecal indicators within the system's distribution system; • corrective action by any system with substantial deficiencies or positive microbial samples indicating fecal contamination; and • compliance monitoring for systems that disinfect to ensure that they reliably achieve 4-log (99.99%) inactivation or removal of viruses. GWR does not apply to privately owned wells that serve <25 persons (e.g., individual homeowner wells).

Chapter 10 - Drinking Water Rules and Disease Review

1. The first list was called the drinking water _____. CCL contained 60 contaminants/contaminant groups, included 10 pathogens, and was published in the Federal Register on March 2, 1998. A decision concerning whether to regulate ≥5 contaminants from CCL was required by August 2001.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

2. Public water systems are regulated under the _____and its subsequent 1986 and 1996 amendments. Under it, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

3. The _____ require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

4. Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the _____ of 1989.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

5. _____ covers all water systems that use surface water or groundwater under the direct influence of surface water.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

6. _____ is intended to protect against exposure to Giardia intestinalis, viruses, and Legionella, as well as selected other pathogens.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

7. In 1998, the EPA promulgated the _____, which provides additional protection against Cryptosporidium and other waterborne pathogens for systems that serve ≥10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

8. In 2002, the EPA finalized the _____ for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

9. _____ was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2001.
A. Total Coliform Rule (TCR)
B. Filter Backwash Recycling Rule (FBRR)
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

10. The _____ require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; EPA has proposed the Ground Water Rule (GWR) to meet this mandate.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

11. _____ specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)

12. This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. _____ does not apply to privately owned wells.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

13. To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the _____in 1996, which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

14. _____ are also required to monitor for the presence of Cryptosporidium, Giardia, total culturable viruses, and total* and fecal coliforms or Escherichia coli ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

15. The EPA also made minor changes in 2000 to the _____ to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same.
A. Information Collection Rule
B. Lead and Copper Rule
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

1.D, 2.C, 3.B, 4.E, 5.E, 6.E, 7.C, 8.D, 9.C, 10.B, 11.D, 12.D, 13.A, 14.E, 15.B

A huge high pressure sewer line has ruptured. This looks like a water line break, but it isn't. It is a nightmare and it happens throughout our nation. Most of us will not report this event to the government agencies because we are fearful of the penalties. It seems strange that we are required to report these events but we do not want to lose our jobs or be punished with fines.

We as water/wastewater professionals need to have a better relationship with the governing agencies. I know this is very difficult to develop, hopefully the governing agencies will open their eyes and become reasonable towards us and receive these reports as professionals and treat us as so.

Are you prepared to deal with a terrorist damaging your system? Well the Government believes that this event could happen. Plans for this type of destruction have been found and currently information suggests that the sewer system is the most likely and most vulnerable target in the U.S. Think of the diseases that would spread within a very short period of time. Plus, this event would impact the entire potable water system.

Laboratory Analysis Section

Although development of an acceptable immunomagnetic separation system for *Giardia* lagged behind development of an acceptable system for *Cryptosporidium*, an acceptable system was identified in October 1998, and EPA validated a method for simultaneous detection of *Cryptosporidium* and *Giardia* in February 1999 and developed quality control (QC) acceptance criteria for the method based on this validation study. To avoid confusion with Method 1622, which already had been validated and was in use both domestically and internationally as a stand-alone *Cryptosporidium*-only detection method, EPA designated the new combined procedure EPA Method 1623.

The interlaboratory validated versions of Method 1622 (January 1999; EPA-821-R-99-001) and Method 1623 (April 1999; EPA-821-R-99-006) were used to analyze approximately 3,000 field and QC samples during the Information Collection Rule Supplemental Surveys (ICRSS) between March 1999 and February 2000. Method 1622 was used to analyze samples from March 1999 to mid-July 1999; Method 1623 was used from mid-July 1999 to February 2000. The April 2001 revision of both methods include updated QC acceptance criteria based on analysis of the QC samples analyzed during the ICRSS.

EPA Method 1623 is a performance-based method applicable to the determination of *Cryptosporidium* and *Giardia* in aqueous matrices. EPA Method 1623 requires filtration, immunomagnetic separation of the oocysts and cysts from the material captured, and an immunofluorescence assay for determination of oocyst and cyst concentrations, with confirmation through vital dye staining and differential interference contrast microscopy.

The interlaboratory validation of EPA Method 1623 conducted by the EPA used the Pall Gelman capsule filtration procedure, Dynal immunomagnetic separation (IMS) procedure, and Meridian sample staining procedure are described in this document. Alternate procedures are allowed, provided that required quality control tests are performed and all quality control acceptance criteria in this method are met.

Since the interlaboratory validation of EPA Method 1623, interlaboratory validation studies have been performed to demonstrate the equivalency of modified versions of the method using the following components:

- Whatman Nuclepore CryptTest™ filter
- IDEXX Filta-Max™ filter
- Waterborne Aqua-Glo™ G/C Direct FL antibody stain
- Waterborne Crypt-a-Glo™ and Giardi-a-Glo™ antibody stains

The validation studies for these modified versions of the method met EPA performance-based measurement system Tier 2 validation for nationwide use (see Section 9.1.2 for details), and have been accepted by the EPA as equivalent in performance to the original version of the method validated by the EPA.

The equipment and reagents used in these modified versions of the method are noted in Sections 6 and 7 of the method; the procedures for using these equipment and reagent options are available from the manufacturers.

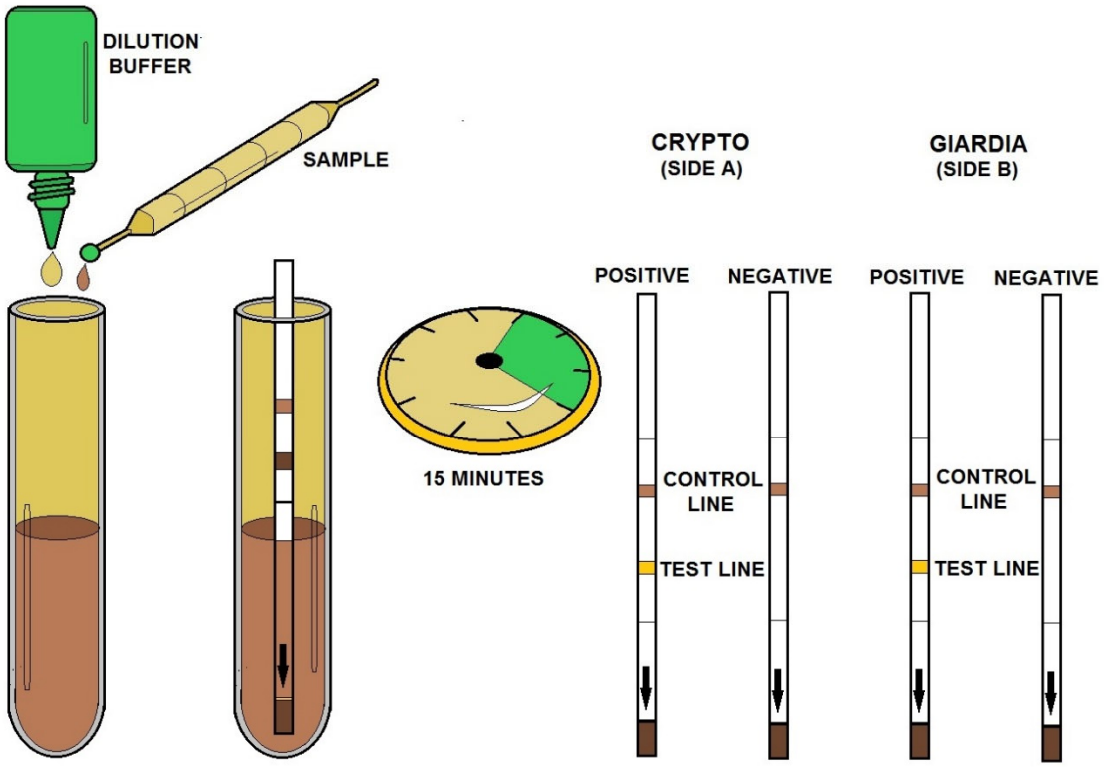
Because this is a performance-based method, other alternative components not listed in the method may be available for evaluation and use by the laboratory. Confirming the acceptable performance of the modified version of the method using alternate components in a single laboratory does not require an interlaboratory validation study be conducted.

However, method modifications validated only in a single laboratory have not undergone sufficient testing to merit inclusion in the method.

Only those modified versions of the method that have been demonstrated as equivalent at multiple laboratories and multiple water sources through a Tier 2 interlaboratory study will be cited in the method.

The EPA initiated an effort in 1996 to identify new and innovative technologies for protozoan monitoring and analysis. After evaluating potential alternatives to the then-current method through literature searches, discussions with research and commercial laboratories, and meetings with experts in the field, the Engineering and Analysis Division within the Office of Science and Technology within the EPA's Office of Water developed draft Method 1622 for *Cryptosporidium* detection in December 1996.

This *Cryptosporidium*-only method was validated through an interlaboratory study in August 1998, and was revised as a final, valid method for detecting *Cryptosporidium* in water in January 1999.



CRYPTO-GIARDIA DUO-STRIP

Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration IMS/FA

1.0 Scope and Application

1.1 This method is for determination of the identity and concentration of *Cryptosporidium* (CAS Registry number 137259-50-8) and *Giardia* (CAS Registry number 137259-49-5) in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. *Cryptosporidium* and *Giardia* may be confirmed using 4',6-diamidino-2-phenylindole (DAPI) staining and differential interference contrast (DIC) microscopy. The method has been validated in surface water, but may be used in other waters, provided the laboratory demonstrates that the method's performance acceptance criteria are met.

1.2 This method is designed to meet the survey and monitoring requirements of the U.S. Environmental Protection Agency (EPA). It is based on laboratory testing of recommendations by a panel of experts convened by EPA. The panel was charged with recommending an improved protocol for recovery and detection of protozoa that could be tested and implemented with minimal additional research.

1.3 This method will not identify the species of *Cryptosporidium* or *Giardia* or the host species of origin, nor can it determine the viability or infectivity of detected oocysts and cysts.

1.4 This method is for use only by persons experienced in the determination of *Cryptosporidium* and *Giardia* by filtration, IMS, and FA. Experienced persons are defined in Section 22.2 as analysts. Laboratories unfamiliar with analyses of environmental samples by the techniques in this method should gain experience using water filtration techniques, IMS, fluorescent antibody staining with monoclonal antibodies, and microscopic examination of biological particulates using bright-field and DIC microscopy.

1.5 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 *CFR* Part 141.27.

2.0 Summary of Method

2.1 A water sample is filtered and the oocysts, cysts, and extraneous materials are retained on the filter. Although EPA has only validated the method using laboratory filtration of bulk water samples shipped from the field, field-filtration also can be used.

2.2 Elution and separation

2.2.1 Materials on the filter are eluted and the eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated.

2.2.2 The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to anti-*Cryptosporidium* and anti-*Giardia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts.

2.3 Enumeration

2.3.1 The oocysts and cysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy.

2.3.2 Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of Cryptosporidium oocysts or Giardia cysts. Potential oocysts or cysts are confirmed through DAPI staining characteristics and DIC microscopy. Oocysts and cysts are identified when the size, shape, color, and morphology agree with specified criteria and examples in a photographic library.

2.3.3 Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

2.4 Quality is assured through reproducible calibration and testing of the filtration, immunomagnetic separation (IMS), staining, and microscopy systems. Detailed information on these tests is provided in Section 9.0.

3.0 Definitions

3.1 *Cryptosporidium* is defined as a protozoan parasite potentially found in water and other media. The six species of *Cryptosporidium* and their potential hosts are *C. parvum* (mammals, including humans); *C. baileyi* and *C. meleagridis* (birds); *C. muris* (rodents); *C. serpentis* (reptiles); and *C. nasorum* (fish).

3.2 *Giardia* is defined as a protozoan parasite potentially found in water and other media. The two species of *Giardia* and their potential hosts are *G. intestinalis* (humans) and *G. muris* (mice).

3.3 Definitions for other terms used in this method are given in the glossary (Section 22.0).

4.0 Contamination, Interferences, and Organism Degradation

4.1 Turbidity caused by inorganic and organic debris can interfere with the concentration, separation, and examination of the sample for *Cryptosporidium* oocysts and *Giardia* cysts. In addition to naturally-occurring debris, such as clays and algae, chemicals, such as iron and alum coagulants and polymers, may be added to finished waters during the treatment process, which may result in additional interference.

4.2 Organisms and debris that autofluoresce or demonstrate non-specific fluorescence, such as algal and yeast cells, when examined by epifluorescent microscopy, may interfere with the detection of oocysts and cysts and contribute to false positives by immunofluorescence assay (FA).

4.3 Solvents, reagents, labware, and other sample-processing hardware may yield artifacts that may cause misinterpretation of microscopic examinations for oocysts and cysts. All materials used shall be demonstrated to be free from interferences under the conditions of analysis by running a method blank (negative control sample) initially and a minimum of every week or after changes in source of reagent water. Specific selection of reagents and purification of solvents and other materials may be required.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the water being sampled. Experience suggests that high levels of algae, bacteria, and other protozoa can interfere in the identification of oocysts and cysts (Reference 20.1).

4.5 Freezing samples, filters, eluates, concentrates, or slides may interfere with the detection and/or identification of oocysts and cysts.

4.6 All equipment should be cleaned according to manufacturers' instructions. Disposable supplies should be used wherever possible.

5.0 Safety

5.1 The biohazard associated with, and the risk of infection from, oocysts and cysts is high in this method because live organisms are handled. This method does not purport to address all of the safety problems associated with its use. It is the responsibility of the laboratory to establish appropriate safety and health practices prior to use of this method. In particular, laboratory staff must know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms while preparing, using, and disposing of sample concentrates, reagents and materials, and while operating sterilization equipment.

5.2 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration regulations regarding the safe handling of the chemicals specified in this method. A reference file of Safety Data Sheets (Formerly Material Safety Data Sheets) should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 20.2 through 20.5.

5.3 Samples may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves and opened in a biological safety cabinet to prevent exposure. Reference materials and standards containing oocysts and cysts must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Do not mouth-pipette.

5.4 Laboratory personnel must change gloves after handling filters and other contaminant-prone equipment and reagents. Gloves must be removed or changed before touching any other laboratory surfaces or equipment.

5.5 Centers for Disease Control (CDC) regulations (42 CFR 72) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. Unless the sample is known or suspected to contain *Cryptosporidium*, *Giardia*, or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by CDC or state regulations, the sample should be shipped in accordance with these regulations.

6.0 Equipment and Supplies

NOTE*: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection equipment for shipment of bulk water samples for laboratory filtration. Collapsible LDPE cubitainer for collection of 10-L bulk sample(s)—Cole Parmer cat. no. U-06100-30 or equivalent. Fill completely to ensure collection of a full 10-L sample. Discard after one use.

6.2 Equipment for sample filtration. Three options have been demonstrated to be acceptable for use with Method 1623. Other options may be used if their acceptability is demonstrated according to the procedures outlined in Section 9.1.2.

6.2.1 Cubitainer spigot to facilitate laboratory filtration of sample (for use with any filtration option)—Cole Parmer cat. no. U-06061-01, or equivalent.

6.2.2 Envirochek™ sampling capsule equipment requirements for use with the procedure described in Section 12.0. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

6.2.2.1 Sampling capsule—Envirochek™, Pall Gelman Laboratory, Ann Arbor, MI, product 12110

6.2.2.2 Laboratory shaker with arms for agitation of sampling capsules

6.2.2.2.1 Laboratory shaker—Lab-Line model 3589, VWR Scientific cat. no. 57039-055, Fisher cat. no. 14260-11, or equivalent

6.2.2.2.2 Side arms for laboratory shaker—Lab-Line Model 3587-4, VWR Scientific cat. no. 57039-045, Fisher cat. no. 14260-13, or equivalent

6.2.3 CrypTest™ capsule filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.3.1 Capsule filter—CrypTest™, Whatman Inc, Clifton, NJ, product no. 610064

6.2.3.2 Cartridge housing—Ametek 5-in. clear polycarbonate, Whatman cat. no. 71503, or equivalent

6.2.3.3 Ultrasonic bath—VWR Model 75T#21811-808, or equivalent

6.2.3.4 Laboratory tubing—Tygon formula R-3603, or equivalent

6.2.4 Filta-Max™ foam filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 50-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.4.1 Foam filter—Filta-Max™, IDEXX, Westbrook, ME. Filter module and membrane: product code FMC 10601; filter membranes (100 pack), product code FMC 10800

NOTE: Check at least one filter per batch to ensure that the filters have not been affected by improper storage or other factors that could result in brittleness or other problems. At a minimum confirm that the test filter expands properly in water before using the batch or shipping filters to the field.

6.2.4.2 Filter processing equipment—Filta-Max starter kit, IDEXX, Westbrook, ME, cat. no. FMC 11002. Includes all equipment required to run and process Filta-Max filter modules (manual wash station (FMC 10102) including plunger head (FMC 12001), elution tubing set (FMC 10301), vacuum set (FMC 10401), filter housing (FMC 10501), and magnetic stirrer (FMC 10901).

6.3 Ancillary sampling equipment

6.3.1 Tubing—Glass, polytetrafluoroethylene (PTFE), high-density polyethylene (HDPE), or other tubing to which oocysts and cysts will not easily adhere—Tygon formula R-3603, or equivalent. If rigid tubing (glass, PTFE, HDPE) is used and the sampling system uses a peristaltic pump, a minimum length of compressible tubing may be used in the pump. Before use, the tubing must be autoclaved, thoroughly rinsed with detergent solution, followed by repeated rinsing with reagent water to minimize sample contamination. Alternately, decontaminate using hypochlorite solution, sodium thiosulfate, and multiple reagent water rinses; dispose of tubing when wear is evident. Dispose of tubing after one use whenever possible.

6.3.2 Flow control valve—0.5 gpm (0.03 L/s), Bertram Controls, Plast-O-Matic cat. no. FC050B½-PV, or equivalent; or 0.4- to 4-Lpm flow meter with valve—Alamo Water Treatment, San Antonio, TX, cat. no. R5310, or equivalent.

6.3.3 Centrifugal pump—Grainger, Springfield, VA, cat. no. 2P613, or equivalent

6.3.4 Flow meter—Sameco cold water totalizer, E. Clark and Associates, Northboro, MA, product no. WFU 10.110, or equivalent.

6.4 Equipment for spiking samples in the laboratory

6.4.1 10-L carboy with bottom delivery port (½")—Cole-Palmer cat. no. 06080-42, or equivalent; calibrate to 10.0 L and mark level with waterproof marker.

6.4.2 Stir bar—Fisher cat. no. 14-511-93, or equivalent.

6.4.3 Stir plate—Fisher cat. no. 14-493-120S, or equivalent.

6.4.4 Hemacytometer—Neubauer type, Hauser Scientific, Horsham, PA, cat. no. 3200 or 1475, or equivalent.

6.4.5 Hemacytometer coverslip—Hauser Scientific, cat. no. 5000 (for hemacytometer cat. no. 3200) or 1461 (for hemacytometer cat. no 1475), or equivalent.

6.4.6 Lens paper without silicone—Fisher cat. no. 11-995, or equivalent.

6.4.7 Polystyrene or polypropylene conical tubes with screw caps—15- and 50-mL.

6.4.8 Equipment required for enumeration of spiking suspensions using membrane filters.

6.4.8.1 Glass microanalysis filter holder—25-mm-diameter, with fritted glass support, Fisher cat. no. 09-753E, or equivalent. Replace stopper with size 8, one-hole rubber stopper, Fisher Cat. No. 14-135M, or equivalent.

6.4.8.2 Three-port vacuum filtration manifold and vacuum source—Fisher Cat. No. 09-753-39A, or equivalent.

6.4.8.3 Cellulose acetate support membrane—1.2-µm-pore-size, 25-mm-diameter, Fisher cat. no. A12SP02500, or equivalent.

6.4.8.4 Polycarbonate track-etch hydrophilic membrane filter—1-µm-pore-size, 25-mm-diameter, Fisher cat. no. K10CP02500, or equivalent.

6.4.8.5 100 × 15 mm polystyrene Petri dishes (bottoms only).

6.4.8.6 60 × 15 mm polystyrene Petri dishes.

6.4.8.7 Glass microscope slides—1 in. × 3 in or 2 in. × 3 in.

6.4.8.8 Coverslips—25 mm^2

6.5 Immunomagnetic separation (IMS) apparatus

6.5.1 Sample mixer—Dynal Inc., Lake Success, NY, cat. no. 947.01, or equivalent.

6.5.2 Magnetic particle concentrator for 10-mL test tubes—Dynal MPC-1® , cat. no. 120.01, or equivalent.

6.5.3 Magnetic particle concentrator for microcentrifuge tubes—Dynal MPC-M®, cat. no. 120.09, or equivalent.

6.5.4 Flat-sided sample tubes—16 × 125 mm Leighton-type tubes with 60 × 10 mm flat-sided magnetic capture area, Dynal L10, cat. no. 740.03, or equivalent.

6.6 Powder-free latex gloves—Fisher cat no. 113945B, or equivalent.

6.7 Graduated cylinders, autoclavable—10-, 100-, and 1000-mL.

6.8 Centrifuges

6.8.1 Centrifuge capable of accepting 15- to 250-mL conical centrifuge tubes and achieving 1500 × G—International Equipment Company, Needham Heights, MA, Centrifuge Size 2, Model K with swinging bucket, or equivalent.

6.8.2 Centrifuge tubes—Conical, graduated, 1.5-, 50-, and 250-mL.

6.9 Microscope

6.9.1 Epifluorescence/differential interference contrast (DIC) with stage and ocular micrometers and 20X (N.A.=0.4) to 100X (N.A.=1.3) objectives—Zeiss™ Axioskop, Olympus™ BH, or equivalent.

6.9.2 Excitation/band-pass filters for immunofluorescence assay (FA)—Zeiss™ 487909 or equivalent, including, 450- to 490-nm exciter filter, 510-nm dicroic beam-splitting mirror, and 515- to 520-nm barrier or suppression filter.

6.9.3 Excitation/band-pass filters for DAPI—Filters cited below (Chroma Technology, Brattleboro, VT), or equivalent.

Microscope model	Fluoro-chrome	Excitation filter (nm)	Dichroic beam-splitting mirror (nm)	Barrier or suppression filter (nm)	Chroma catalog number
Zeiss™ - Axioskop	DAPI (UV)	340-380	400	420	CZ902
Zeiss™ -IM35	DAPI (UV)	340-380	400	420	CZ702
Olympus™ BH	DAPI (UV)	340-380	400	420	11000
			Filter holder		91002
Olympus™ BX	DAPI (UV)	340-380	400	420	11000
			Filter holder		91008
Olympus™ IMT2	DAPI (UV)	340-380	400	420	11000
			Filter holder		91003

6.10 Ancillary equipment for microscopy

6.10.1 Well slides— Spot-On well slides, Dynal cat. no. 740.04; treated, 12-mm diameter well slides, Meridian Diagnostics Inc., Cincinnati, OH, cat. no. R2206; or equivalent.

6.10.2 Glass coverslips—22 × 50 mm.

6.10.3 Nonfluorescing immersion oil.

6.10.4 Micropipette, adjustable: 0- to 10-µL with 0- to 10-µL tips 10- to 100-µL, with 10- to 200-µL tips 100- to 1000-µL with 100- to 1000-µL tips

6.10.5 Forceps—Splinter, fine tip.

6.10.6 Forceps—Blunt-end.

6.10.7 Desiccant—Drierite™ Absorbent, Fisher cat. no. 07-577-1A, or equivalent

6.10.8 Humid chamber—A tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

6.11 Pipettes—Glass or plastic

6.11.1 5-, 10-, and 25-mL.

6.11.2 Pasteur, disposable.

6.12 Balances

6.12.1 Analytical—Capable of weighing 0.1 mg.

6.12.2 Top loading—Capable of weighing 10 mg.

6.13 pH meter

6.14 Incubator—Fisher Scientific Isotemp™, or equivalent.

6.15 Vortex mixer—Fisons Whirlmixer, or equivalent.

6.16 Vacuum source—Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.17 Miscellaneous labware and supplies

6.17.1 Test tubes and rack.

6.17.2 Flasks—Suction, Erlenmeyer, and volumetric, various sizes.

6.17.3 Beakers—Glass or plastic, 5-, 10-, 50-, 100-, 500-, 1000-, and 2000-mL.

6.17.4 Lint-free tissues.

6.18 10- to 15-L graduated container—Fisher cat. no. 02-961-50B, or equivalent; calibrate to 9.0, 9.5, 10.0, 10.5, and 11.0 L and mark levels with waterproof marker.

6.19 Filters for filter-sterilizing reagents—Sterile Acrodisc, 0.45 µm , Gelman Sciences cat no. 4184, or equivalent.

7.0 Reagents and Standards

7.1 Reagents for adjusting pH

7.1.1 Sodium hydroxide (NaOH)—ACS reagent grade, 6.0 N and 1.0 N in reagent water

7.1.2 Hydrochloric acid (HCl)—ACS reagent grade, 6.0 N, 1.0 N, and 0.1 N in reagent water.

NOTE: Due to the low volumes of pH-adjusting reagents used in this method, and the impact that changes in pH have on the immunofluorescence assay, the laboratory should purchase standards at the required normality directly from a vendor. Normality should not be adjusted by the laboratory.

7.2 Solvents—Acetone, glycerol, ethanol, and methanol, ACS reagent grade

7.3 Reagent water—Water in which oocysts and cysts and interfering materials and substances, including magnetic minerals, are not detected by this method.

7.4 Reagents for eluting filters

7.4.1 Reagents for eluting Envirochek™ sampling capsules (Section 6.2.2)

7.4.1.1 Laureth-12—PPG Industries, Gurnee, IL, cat. no. 06194, or equivalent. Store Laureth-12 as a 10% solution in reagent water. Weigh 10 g of Laureth-12 and dissolve using a microwave or hot plate in 90 mL of reagent water. Dispense 10-mL aliquots into sterile vials and store at room temperature for up to 2 months, or in the freezer for up to a year.

7.4.1.2 1 M Tris, pH 7.4—Dissolve 121.1 g Tris (Fisher cat. no. BP152) in 700 mL of reagent water and adjust pH to 7.4 with 1 N HCl or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize through a 0.2-µm membrane into a sterile plastic container and store at room temperature.

7.4.1.3 0.5 M EDTA, 2 Na, pH 8.0—Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate (Fisher cat. no. S311) in 800 mL and adjust pH to 8.0 with 6.0 N HCl or NaOH. Dilute to a final volume of 1000 mL with reagent water and adjust to pH 8.0 with 1.0 N HCl or NaOH.

7.4.1.4 Antifoam A—Sigma Chemical Co. cat. no. A5758, or equivalent

7.4.1.5 Preparation of elution buffer solution—Add the contents of a pre-prepared Laureth-12 vial (Section 7.4.1.1) to a 1000-mL graduated cylinder. Rinse the vial several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution (Section 7.4.1.2), 2 mL of EDTA solution (Section 7.4.1.3), and 150 µL Antifoam A (Section 7.4.1.4). Dilute to 1000 mL with reagent water.

7.4.2 Reagents for eluting CrypTest™ capsule filters (Section 6.2.3). To 900 mL of reagent water add 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 ($12H_2O$) 0.2 g KCl, 0.2 g sodium lauryl sulfate (SDS), 0.2 mL Tween 80, and 0.02 mL Antifoam A (Sigma Chemical Co. cat. no. A5758, or equivalent). Adjust volume to 1 L with reagent water and adjust pH to 7.4 with 1 N NaOH or HCl.

7.4.3 Reagents for eluting Filta-Max™ foam filters (Section 6.2.4)

7.4.3.1 Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4.

7.4.3.2 Tween 20—Sigma Chemical Co. cat. no. P-7949, or equivalent.

7.4.3.3 High-vacuum grease—BDH/Merck. cat. no. 636082B, or equivalent.

7.4.3.4 Preparation of PBST elution buffer. Add the contents of one sachet of PBS to 1.0 L of reagent water. Dissolve by stirring for 30 minutes. Add 100 µL of Tween 20. Mix by stirring for 5 minutes.

7.5 Reagents for immunomagnetic separation (IMS)—Dynabeads® GC-Combo, Dynal cat. nos. 730.02, 730.12, or equivalent.

7.6 Direct antibody labeling reagents for detection of oocysts and cysts. Store reagents at 0 °C to 8 °C and return promptly to this temperature after each use. Do not allow any of the reagents to freeze. The reagents should be protected from exposure to light. Diluted, unused working reagents should be discarded after 48 hours. Discard reagents after the expiration date is reached. The labeling reagents in Sections 7.6.1-7.6.3 have been approved for use with this method.

7.6.1 Merifluor Cryptosporidium/Giardia, Meridian Diagnostics cat. no. 250050, Cincinnati, OH, or equivalent.

7.6.2 Aqua-Glo™ G/C Direct FL, Waterborne cat. no. A100FLR, New Orleans, LA, or equivalent.

7.6.3 Crypt-a-Glo™ and Giardi-a-Glo™, Waterborne cat. nos. A400FLR and A300FLR, respectively, New Orleans, LA, or equivalent.

> **NOTE**: If a laboratory will use multiple types of labeling reagents, the laboratory must demonstrate acceptable performance through an initial precision and recovery test (Section 9.4) for each type, and must perform positive and negative staining controls for each batch of slides stained using each product. However, the laboratory is not required to analyze additional ongoing precision and recovery samples or method blank samples for each type.

7.6.4 Diluent for labeling reagents—Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4. Filter-sterilize (Section 6.19) or autoclave. Discard if growth is detected or after 6 months, whichever comes first.

7.7 4',6-diamidino-2-phenylindole (DAPI) stain—Sigma Chemical Co. cat. no. A5758, or equivalent.

7.7.1 Stock solution—Dissolve 2 mg/mL DAPI in absolute methanol. Prepare volume consistent with minimum use. Store at 0 °C to 8 °C in the dark. Do not allow to freeze. Discard unused solution when positive staining control fails.

7.7.2 Staining solution (1/5000 dilution in PBS [Section 7.6.4])—Add 10 µL of 2 mg/mL DAPI stock solution to 50 mL of PBS. Prepare daily. Store at 0 °C to 8 °C in the dark except when staining. Do not allow to freeze. The solution concentration may be increased up to 1µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.

7.8 Mounting medium

7.8.1 DABCO/glycerol mounting medium (2%)—Dissolve 2 g of DABCO (Sigma Chemical Co. cat no. D-2522, or equivalent) in 95 mL of warm glycerol/PBS (60% glycerol, 40% PBS [Section 7.6.4]). After the DABCO has dissolved completely, adjust the solution volume to 100 mL by adding an appropriate volume of glycerol/PBS solution. Alternately, dissolve the DABCO in 40 mL of PBS, then add azide (1 mL of 100X, or 10% solution), then 60 mL of glycerol.

7.8.2 Mounting medium supplied with Merifluor direct labeling kit (Section 7.6.1)

7.9 Clear fingernail polish or clear fixative, PGC Scientifics, Gaithersburg, MD, cat. no. 60-4890, or equivalent.

7.10 Oocyst and cyst suspensions for spiking

7.10.1 Enumerated spiking suspensions prepared by flow cytometer—not heat-fixed or formalin fixed: Wisconsin State Laboratory of Hygiene Flow Cytometry Unit or equivalent

7.10.2 Materials for manual enumeration of spiking suspensions

7.10.2.1 Purified Cryptosporidium oocyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Sterling Parasitology Laboratory, University of Arizona, Tucson, or equivalent

7.10.2.2 Purified Giardia cyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Waterborne, Inc., New Orleans, LA; Hyperion Research, Medicine Hat, Alberta, Canada; or equivalent

7.10.2.3 Tween-20, 0.01%—Dissolve 1.0 mL of a 10% solution of Tween-20 in 1 L of reagent water

7.10.2.4 Storage procedure—Store oocyst and cyst suspensions at 0 °C to 8 °C, until ready to use; do not allow to freeze

7.11 Additional reagents for enumeration of spiking suspensions using membrane filtration (Section 11.3.6)—Sigmacote® Sigma Company Product No. SL-2, or equivalent

8.0 Sample Collection and Storage

8.1 Samples are collected as bulk samples and shipped to the laboratory for processing through the entire method, or are filtered in the field and shipped to the laboratory for processing from elution (Section 12.2.6) onward. Samples must be shipped via overnight service on the day they are collected. Chill samples as much as possible between collection and shipment by storing in a refrigerator or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment. Samples should be shipped at 0 °C to 8 °C, unless the time required to chill the sample to 8 °C would prevent the sample from being shipped overnight for receipt at the laboratory the day after collection. Samples must not be allowed to freeze. Upon receipt, the laboratory should record the temperature of the samples and store them refrigerated at 0 °C to 8 °C until processed. Results from samples shipped overnight to the laboratory and received at >8 °C should be qualified by the laboratory.

NOTE: See transportation precautions in Section 5.5.

8.2 Sample holding times. Sample processing should be completed as soon as possible by the laboratory. The laboratory should complete sample filtration, elution, concentration, purification, and staining the day the sample is received wherever possible. However, the laboratory is permitted to split up the sample processing steps if processing a sample completely in one day is not possible. If this is necessary, sample processing can be halted after filtration, application of the purified sample onto the slide, or staining. Table 1, in Section 21.0 provides a breakdown of the holding times for each set of steps. Sections 8.2.1 through 8.2.4 provide descriptions of these holding times.

8.2.1 Sample collection and filtration. Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

8.2.2 Sample elution, concentration, and purification. The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.
8.2.3 Staining. The sample must be stained within 72 hours of application of the purified sample to the slide.
8.2.4 Examination. Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.
8.5 Spiking suspension enumeration holding times. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometersorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance (QA) program (Reference 20.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability through performance of the initial precision and recovery (IPR) test (Section 9.4), analysis of spiked samples to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
9.1.1 A test of the microscope used for detection of oocysts and cysts is performed prior to examination of slides. This test is described in Section 10.0.
9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to modify certain method procedures to improve recovery or lower the costs of measurements, provided that all required quality control (QC) tests are performed and all QC acceptance criteria are met. Method procedures that can be modified include front-end techniques, such as filtration or immunomagnetic separation (IMS). The laboratory is not permitted to use an alternate determinative technique to replace immunofluorescence assay in this method (the use of different determinative techniques are considered to be different methods, rather than modified version of this method). However, the laboratory is permitted to modify the immunofluorescence assay procedure, provided that all required QC tests are performed (Section 9.1.2.1) and all QC acceptance criteria are met (see guidance on the use of multiple labeling reagents in Section 7.6).

9.1.2.1 Method modification validation/equivalency demonstration requirements.
9.1.2.1.1 Method modifications at a single laboratory. Each time a modification is made to this method for use in a single laboratory, the laboratory is required to validate the modification according to Tier 1 of EPA's performance-based measurement system (PBMS) (Table 2 and Reference 20.7) to demonstrate that the modification produces results equivalent or superior to results produced by this method as written. Briefly, each time a modification is made to this method, the laboratory is required to demonstrate acceptable modified method performance through the IPR test (Section 9.4). IPR results must meet the QC acceptance criteria in Tables 3 and 4 in Section 21.0, and should be comparable to previous results using the unmodified procedure. Although not required, the laboratory also should perform a matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate the performance of the modified method in at least one real-world matrix before analyzing field samples using the modified method. The laboratory is required to perform MS samples using the modified method at the frequency noted in Section 9.1.8.
9.1.2.1.2 Method modifications for nationwide approval. If the laboratory or a manufacturer seeks EPA approval of a method modification for nationwide use, the laboratory or manufacturer must validate the modification according to Tier 2 of EPA's PBMS (Table 2 and Reference 20.7). Briefly, at least three laboratories must perform IPR tests (Section 9.4) and MS/MSD (Section 9.5) tests using the modified method, and all tests must meet the QC acceptance criteria specified in Tables 3 and 4 in Section 21.0. Upon nationwide approval, laboratories electing to use the modified method still must demonstrate acceptable performance in their own laboratory according to the requirements in Section 9.1.2.1.1.
9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
9.1.2.2.2 A listing of the analyte(s) measured (Cryptosporidium and Giardia).
9.1.2.2.3 9.1.2.2.4 A narrative stating reason(s) for the modification.
9.1.2.2.5 Results from all QC tests comparing the modified method to this method, including: (a) IPR (Section 9.4) (b) MS/MSD (Section 9.5) (c) Analysis of method blanks (Section 9.6) Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:
(a) Sample numbers and other identifiers
(b) Source of spiking suspensions, as well as lot number and date received (Section 7.10)
(c) Spike enumeration date and time
(d) All spiking suspension enumeration counts and calculations (Section 11.0)
(e) Sample spiking dates and times
(f) Volume filtered (Section 12.2.5.2)
(g) Filtration and elution dates and times
(h) Pellet volume, resuspended concentrate volume, resuspended concentrate volume transferred to IMS, and all calculations required to verify the percent of concentrate examined (Section 13.2)
(i) Purification completion dates and times (Section 3.3.3.11)
(j) Staining completion dates and times (Section 14.10)
(k) Staining control results (Section 15.2.1)
(l) All required examination information (Section 15.2.2)

(m) Examination completion dates and times (Section 15.2.4)
(n) Analysis sequence/run chronology
(o) Lot numbers of elution, IMS, and staining reagents
(p) Copies of bench sheets, logbooks, and other recordings of raw data
(q) Data system outputs, and other data to link the raw data to the results reported

9.1.3 The laboratory shall spike a separate sample aliquot from the same source to monitor method performance. This MS test is described in Section 9.5.1.

9.1.4 Analysis of method blanks is required to demonstrate freedom from contamination. The procedures and criteria for analysis of a method blank are described in Section 9.6.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample that the analysis system is in control. These procedures are described in Section 9.7.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Development of accuracy statements is described in Sections 9.5.1.4 and 9.7.3.

9.1.7 The laboratory shall analyze one method blank (Section 9.6) and one OPR sample (Section 9.7) each week during which samples are analyzed if 20 or fewer field samples are analyzed during this period. The laboratory shall analyze one laboratory blank and one OPR sample for every 20 samples if more than 20 samples are analyzed in a week.

9.1.8 The laboratory shall analyze one MS sample (Section 9.5.1) when samples are first received from a utility for which the laboratory has never before analyzed samples. The MS analysis is performed on an additional (second) sample sent from the utility. If the laboratory routinely analyzes samples from 1 or more utilities, 1 MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a given site, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 21st sample from this site, a separate aliquot of this 21st sample must be collected and spiked.

9.2 Micropipette calibration

9.2.1 Micropipettes must be sent to the manufacturer for calibration annually. Alternately, a qualified independent technician specializing in micropipette calibration can be used. Documentation on the precision of the recalibrated micropipette must be obtained from the manufacturer or technician.

9.2.2 Internal and external calibration records must be kept on file in the laboratory's QA logbook.

9.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. Ten replicates should be performed at each weight. Record the weight of the water (assume that 1.00 mL of reagent water weighs 1.00 g) and calculate the relative standard deviation (RSD) for each. If the weight of the reagent water is within 1% of the desired weight (mL) and the RSD of the replicates at each weight is within 1%, then the pipette remains acceptable for use.

9.2.4 If the weight of the reagent water is outside the acceptable limits, consult the manufacturer's instruction manual troubleshooting section and repeat steps

described in Section 9.2.3. If problems with the pipette persist, the laboratory must send the pipette to the manufacturer for recalibration.

9.3 Microscope adjustment and certification: Adjust the microscope as specified in Section 10.0. All of the requirements in Section 10.0 must be met prior to analysis of IPRs, blanks, OPRs, field samples, and MS/MSDs.

9.4 Initial precision and recovery (IPR)—To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.4.1 Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine four reagent water samples spiked with 100 to 500 oocysts and 100 to 500 cysts. If more than one process will be used for filtration and/or separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE: IPR tests must be accompanied by analysis of a method blank (Section 9.6).

9.4.2 Using results of the four analyses, calculate the average percent recovery and the relative standard deviation (RSD) of the recoveries for Cryptosporidium and for Giardia. The RSD is the standard deviation divided by the mean times 100.

9.4.3 Compare RSD and the mean with the corresponding limits for initial precision and recovery in Tables 3 and 4 in Section 21.0. If the RSD and the mean meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If the RSD or the mean falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test (Section 9.4.1).

9.5 Matrix spike (MS) and matrix spike duplicate (MSD):

9.5.1 Matrix spike—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's oocyst and cyst recovery. The MS shall be analyzed according to the frequency in Section 9.1.8.

9.5.1.1 Analyze an unspiked field sample according to the procedures in Sections 12.0 to 15.0. Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine a second field sample aliquot with the number of organisms used in the IPR or OPR tests (Sections 9.4 and 9.7).

9.5.1.2 For each organism, calculate the percent recovery (R) using the following equation.

$$R = 100 \times \frac{Nsp - Ns}{T}$$

where
R is the percent recovery
N_{sp} is the number of oocysts or cysts detected in the spiked sample

N_s is the number of oocysts or cysts detected in the unspiked sample T is the true value of the oocysts or cysts spiked

9.5.1.3 Compare the recovery for each organism with the corresponding limits in Tables 3 and 4 in Section 21.0.

NOTE: Some sample matrices may prevent the acceptance criteria in Tables 3 and 4 from being met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.
9.5.1.4 As part of the QA program for the laboratory, method precision for samples should be assessed and records maintained. After the analysis of five samples for which the spike recovery for each organism passes the tests in Section 9.5.1.3, the laboratory should calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_r). Express the precision assessment as a percent recovery interval from $P - 2\ s_r$ to $P + 2\ s_r$ for each matrix. For example, if P = 80% and s_r = 30%, the accuracy interval is expressed as 20% to 140%. The precision assessment should be updated regularly across all MS samples and stratified by MS samples for each source.
9.5.2 Matrix spike duplicate—MSD analysis is required as part of nationwide approval of a modified version of this method to demonstrate that the modified version of this method produces results equal or superior to results produced by the method as written (Section 9.1.2.1.2). At the same time the laboratory spikes and analyzes the second field sample aliquot in Section 9.5.1.1, the laboratory shall spike and analyze a third, identical field sample aliquot.

NOTE: Matrix spike duplicate samples are only required for Tier 2 validation studies. They are recommended for Tier 1 validation, but not required.

9.5.2.1 For each organism, calculate the percent recovery (R) using the equation in Section 9.5.1.2.

9.5.2.2 Calculate the mean of the number of oocysts or cysts in the MS and MSD (X_{mean}) (= [MS+MSD]/2).

9.5.2.3 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \frac{| NMS - NMSD |}{Xmean}$$

where
RPD is the relative percent difference
N_{MS} is the number of oocysts or cysts detected in the MS
N_{MSD} is the number of oocysts or cysts detected in the MSD
X_{mean} is the mean number of oocysts or cysts detected in the MS and MSD

9.5.2.4 Compare the mean MS/MSD recovery and RPD with the corresponding limits in Tables 3 and 4 in Section 21.0 for each organism.

9.6 Method blank (negative control sample, laboratory blank): Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (Section 9.4) and OPR test (Section 9.7) and prior to analysis of samples for the week to demonstrate freedom from contamination.
9.6.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week (Section 9.1.7) according to the procedures in Sections 12.0 to 15.0. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.
9.6.2 If *Cryptosporidium* oocysts, *Giardia* cysts, or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more oocysts or cysts is assumed to be contaminated and should be recollected, if possible. Any method blank in which oocysts or cysts are not detected is assumed to be uncontaminated and may be reported.

9.7 Ongoing precision and recovery ([OPR]; positive control sample; laboratory control sample): Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water sample spiked with 100 to 500 oocysts and 100 to 500 cysts each week to verify all performance criteria. The laboratory must analyze one OPR sample for every 20 samples if more than 20 samples are analyzed in a week. If multiple method variations are used, separate OPR samples must be prepared for each method variation. Adjustment and/or recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.
9.7.1 Examine the slide from the OPR prior to analysis of samples from the same batch.
9.7.1.1 Using 200X to 400X magnification, more than 50% of the oocysts or cysts must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the organisms. Determine the step or reagent that is causing damage to the organisms. Correct the problem and repeat the OPR test.

9.7.1.2 Identify and enumerate each organism using epifluorescence microscopy. The first three presumptive *Cryptosporidium* oocysts and three *Giardia* cysts identified in the OPR sample must be examined using FITC, DAPI, and DIC, as per Section 15.2, and the detailed characteristics (size, shape, DAPI category, and DIC category) reported on the *Cryptosporidium* and *Giardia* report form, as well as any additional comments on organism appearance, if notable.

9.7.2 For each organism, calculate the percent recovery (R) using the following equation:

$$R = 100 \times \frac{N}{T}$$

where:
R = the percent recovery
N = the number of oocysts or cysts detected
T = the number of oocysts or cysts spiked

9.7.3 Compare the recovery with the limits for ongoing precision and recovery in Tables 3 and 4 in Section 21.0. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, system performance is unacceptable. In this event, there may be a problem with the microscope or with the filtration or separation systems. Troubleshoot the problem using the procedures at Section 9.7.4 as a guide. After assessing the issue, reanalyze the OPR sample. All samples must be associated with an OPR that passes the criteria in Section 21.0. Samples that are not associated with an acceptable OPR must be flagged accordingly.

9.7.4 Troubleshooting. If an OPR sample has failed, and the cause of the failure is not known, the laboratory generally should identify the problem working backward in the analytical process from the microscopic examination to filtration.
9.7.4.1 Microscope system and antibody stain: To determine if the failure of the OPR test is due to changes in the microscope or problems with the antibody stain, re-examine the positive staining control (Section 15.2.1), check Köhler illumination, and check the fluorescence of the fluorescein-labeled monoclonal antibodies (Mabs) and 4',6-diamidino-2-phenylindole (DAPI). If results are unacceptable, re-examine the previously-prepared positive staining control to determine whether the problem is associated with the microscope or the antibody stain.
9.7.4.2 Separation (purification) system: To determine if the failure of the OPR test is attributable to the separation system, check system performance by spiking a 10-mL volume of reagent water with 100 - 500 oocysts and cysts and processing the sample through the IMS, staining, and examination procedures in Sections 13.3 through 15.0.
9.7.4.3 Filtration/elution/concentration system: If the failure of the OPR test is attributable to the filtration/elution/concentration system, check system performance by processing spiked reagent water according to the procedures in Section 12.2 through 13.2.2.1, and filter, stain, and examine the sample concentrate according to Section 11.3.6.

9.7.5 The laboratory should add results that pass the specifications in Section 9.7.3 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from R ¯2 s_r to R + 2 s_r. For example, if R = 95% and s_r = 25%, the accuracy is 45% to 145%.

9.8 The laboratory should periodically analyze an external QC sample, such as a performance evaluation or standard reference material, when available.

The laboratory also should periodically participate in interlaboratory comparison studies using the method.

9.9 The specifications contained in this method can be met if the analytical system is under control. The standards used for initial (Section 9.4) and ongoing (Section 9.7) precision and recovery should be identical, so that the most precise results will be obtained. The microscope in particular will provide the most reproducible results if dedicated to the settings and conditions required for the determination of Cryptosporidium and Giardia by this method.

9.10 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and duplicate spiked samples may be required to determine the precision of the analysis.

10.0 Microscope Calibration and Analyst Verification

10.1 In a room capable of being darkened to near-complete darkness, assemble the microscope, all filters, and attachments. The microscope should be placed on a solid surface free from vibration. Adequate workspace should be provided on either side of the microscope for taking notes and placement of slides and ancillary materials.

10.2 Using the manuals provided with the microscope, all analysts must familiarize themselves with operation of the microscope.

10.3 Microscope adjustment and calibration (adapted from Reference 20.6)

10.3.1 Preparations for adjustment

10.3.1.1 The microscopy portion of this procedure depends upon proper alignment and adjustment of very sophisticated optics. Without proper alignment and adjustment, the microscope will not function at maximal efficiency, and reliable identification and enumeration of oocysts and cysts will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.

10.3.1.2 While microscopes from various vendors are configured somewhat differently, they all operate on the same general physical principles. Therefore, slight deviations or adjustments may be required to make the procedures below work for a particular instrument.

10.3.1.3 The sections below assume that the mercury bulb has not exceeded time limits of operation, that the lamp socket is connected to the lamp house, and that the condenser is adjusted to produce Köhler illumination.

10.3.1.4 Persons with astigmatism should always wear contact lenses or glasses when using the microscope.

CAUTION: In the procedures below, do not touch the quartz portion of the mercury bulb with your bare fingers. Finger oils can cause rapid degradation of the quartz and premature failure of the bulb.
WARNING: Never look at the ultraviolet (UV) light from the mercury lamp, lamp house, or the UV image without a barrier filter in place. UV radiation can cause serious eye damage.

10.3.2 Epifluorescent mercury bulb adjustment: The purpose of this procedure is to ensure even field illumination. This procedure must be followed when the microscope is first used, when replacing bulbs, and if problems such as diminished fluorescence or uneven field illumination are experienced.
10.3.2.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.2.2 Using a prepared microscope slide, adjust the focus so the image in the oculars is sharply defined.
10.3.2.3 Replace the slide with a business card or a piece of lens paper.
10.3.2.4 Close the field diaphragm (iris diaphragm in the microscope base) so only a small point of light is visible on the card. This dot of light indicates the location of the center of the field of view.
10.3.2.5 Mount the mercury lamp house on the microscope without the UV diffuser lens in place and turn on the mercury bulb.
10.3.2.6 Remove the objective in the light path from the nosepiece. A primary (brighter) and secondary image (dimmer) of the mercury bulb arc should appear on the card after focusing the image with the appropriate adjustment.
10.3.2.7 Using the lamp house adjustments, adjust the primary and secondary mercury bulb images so they are side by side (parallel to each other) with the transmitted light dot in between them.
10.3.2.8 Reattach the objective to the nosepiece.
10.3.2.9 Insert the diffuser lens into the light path between the mercury lamp house and the microscope.
10.3.2.10 Turn off the transmitted light and replace the card with a slide of fluorescent material. Check the field for even fluorescent illumination. Adjustment of the diffuser lens probably will be required. Additional slight adjustments as in Section 10.3.2.7 above may be required.
10.3.2.11 Maintain a log of the number of hours the UV bulb has been used. Never use the bulb for longer than it has been rated. Fifty-watt bulbs should not be used longer than 100 hours; 100-watt bulbs should not be used longer than 200 hours.
10.3.3 Transmitted bulb adjustment: The purpose of this procedure is to center the filament and ensure even field illumination. This procedure must be followed when the bulb is changed.
10.3.3.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.3.2 Using a prepared microscope slide and a 40X (or similar) objective, adjust the focus so the image in the oculars is sharply defined.
10.3.3.3 Without the ocular or Bertrand optics in place, view the pupil and filament image at the bottom of the tube.

10.3.3.4 Focus the lamp filament image with the appropriate adjustment on the lamp house.
10.3.3.5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on the lamp house.
10.3.3.6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

10.3.4 Adjustment of the interpupillary distance and oculars for each eye: These adjustments are necessary so that eye strain is reduced to a minimum, and must be made for each individual using the microscope. Section 10.3.4.2 assumes use of a microscope with both oculars adjustable; Section 10.3.4.3 assumes use of a microscope with a single adjustable ocular. The procedure must be followed each time an analyst uses the microscope.

10.3.4.1 Interpupillary distance

10.3.4.1.1 Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.4.1.2 Using both hands, move the oculars closer together or farther apart until a single circle of light is observed while looking through the oculars with both eyes. Note interpupillary distance.

10.3.4.2 Ocular adjustment for microscopes capable of viewing a photographic frame through the viewing binoculars: This procedure assumes both oculars are adjustable.

10.3.4.2.1 Place a card between the right ocular and eye keeping both eyes open. Adjust the correction (focusing) collar on the left ocular by focusing the left ocular until it reads the same as the interpupillary distance. Bring an image located in the center of the field of view into as sharp a focus as possible.

10.3.4.2.2 Transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the same image into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

10.3.4.3 Ocular adjustment for microscopes without binocular capability: This procedure assumes a single focusing ocular. The following procedure assumes that only the right ocular is capable of adjustment.

10.3.4.3.1 Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.

10.3.4.3.2 Transfer the card to between the left eye and ocular. Keeping both eyes open, bring the image for the right eye into sharp focus by adjusting the ocular collar at the top of the ocular without touching the coarse or fine adjustment.

10.3.5 Calibration of an ocular micrometer: This section assumes that a reticle has been installed in one of the oculars by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed, the ocular reticle should be left in place. The more an ocular is manipulated the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is a top lens on the microscope, the calibration procedure must be done for the respective objective at each top lens setting. The procedure must be followed when the microscope is first used and each time the objective is changed.

10.3.5.1 Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.

10.3.5.2 Adjust the stage and ocular with the micrometer so the 0 line on the ocular micrometer is exactly superimposed on the 0 line on the stage micrometer.

10.3.5.3 Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.

10.3.5.4 Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition. For example: Suppose 48 ocular micrometer spaces equal 0.6 mm.

10.3.5.5 Calculate the number of mm/ocular micrometer space. For example:

$$0.6\ mm\ 0.0125\ mm = 48\ ocular\ micrometer\ spaces\ ocular\ micrometer\ space$$

10.3.5.6 Because most measurements of microorganisms are given in µm rather than mm, the value calculated above must be converted to µm by multiplying it by 1000 µm /mm. For example:

0.0125 mm 1,000 µm 12.5 µm x =
ocular micrometer space mm ocular micrometer space

10.3.5.7 Follow the procedure below for each objective. Record the information as shown in the example below and keep the information available at the microscope.

Item no.	Objective power	Description	No. of ocular micrometer spaces	No. of stage micrometer mm[1]	µm/ocular micrometer space[2]
1		10X		N.A.[3]=	
2		20X		N.A.=	
3		40X		N.A.=	
4		100X		N.A.=	

[1]100 µm /mm [2](Stage micrometer length in mm × (1000 µm /mm)) ÷ no. ocular micrometer spaces [3]N.A. refers to numerical aperature. The numerical aperature value is engraved on the barrel of the objective.

10.3.6 Köhler illumination: This section assumes that Köhler illumination will be established for only the 100X oil DIC objective that will be used to identify internal morphological characteristics in Cryptosporidium oocysts and Giardia cysts. If more than one objective is to be used for DIC, then each time the objective is changed, Köhler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Köhler illumination is not properly established, then DIC will not work to its maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore. The procedure must be followed each time an analyst uses the microscope and each time the objective is changed.
10.3.6.1 Place a prepared slide on the microscope stage, place oil on the slide, move the 100X oil objective into place, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.
10.3.6.2 At this point both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.
10.3.6.3 Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the field to the center of the visual field.
10.3.6.4 Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up and down with the condenser focusing knob while you are looking through the binoculars. Once you have accomplished the precise focusing of the radiant field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

10.3.6.5 The aperture diaphragm of the condenser is now adjusted to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

10.3.6.6 After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish DIC

10.4 Protozoa libraries: Each laboratory is encouraged to develop libraries of photographs and drawings for identification of protozoa.

10.4.1 Take color photographs of Cryptosporidium oocysts and Giardia cysts by FA and 4',6-diamidino-2-phenylindole (DAPI) that the analysts (Section 22.2) determine are accurate (Section 15.2).

10.4.2 Similarly, take color photographs of interfering organisms and materials by FA and DAPI that the analysts believe are not Cryptosporidium oocysts or Giardia cysts. Quantify the size, shape, microscope settings, and other characteristics that can be used to differentiate oocysts and cysts from interfering debris and that will result in positive identification of DAPI positive or negative organisms.

10.5 Verification of performance: Until standard reference materials, such as National Institute of Standards and Technology standard reference materials, are available that contain a reliable number of DAPI positive or negative oocysts and cysts, this method shall rely upon the ability of the analyst for identification and enumeration of oocysts and cysts.

10.5.1 At least monthly when microscopic examinations are being performed, the laboratory shall prepare a slide containing 40 to 100 oocysts and 40 to 100 cysts. More than 50% of the oocysts and cysts must be DAPI positive.

10.5.2 Each analyst shall determine the total number of oocysts and cysts and the number that are DAPI positive or negative using the slide prepared in Section 10.5.1.

10.5.3 The total number and the number of DAPI positive or negative oocysts and cysts determined by each analyst (Section 10.5.2.) must be within ±10% of each other. If the number is not within this range, the analysts must identify the source of any variability between analysts' examination criteria, prepare a new slide, and repeat the performance verification (Sections 10.5.1 to 10.5.2).

10.5.4 Document the date, name(s) of analyst(s), number of total, DAPI positive or negative oocysts and cysts determined by the analyst(s), whether the test was passed/failed and the results of attempts before the test was passed.

10.5.5 Only after an analyst has passed the criteria in Section 10.5.3, may oocysts and cysts in QC samples and field samples be identified and enumerated.

11.0 Oocyst and Cyst Suspension Enumeration and Spiking

11.1 This method requires routine analysis of spiked QC samples to demonstrate acceptable initial and ongoing laboratory and method performance (initial precision and recovery samples [Section 9.4], matrix spike and matrix spike duplicate samples [Section 9.5], and ongoing precision and recovery samples [Section 9.7]). The organisms used for these samples must be enumerated to calculate recoveries and precision. EPA recommends that flow cytometry be used for this enumeration, rather than manual techniques. Flow cytometer–sorted spikes generally are characterized by a relative standard deviation of ≤ 2.5%, versus greater variability for manual enumeration techniques (Reference 20.8). Guidance on preparing spiking suspensions using a flow cytometer is provided in Section 11.2. Manual enumeration procedures are provided in Section 11.3. The procedure for spiking bulk samples in the laboratory is provided in Section 11.4.

11.2 Flow cytometry enumeration guidelines. Although it is unlikely that many laboratories performing Method 1623 will have direct access to a flow cytometer for preparing spiking suspensions, flow-sorted suspensions are available from commercial vendors and other sources (Section 7.10.1). The information provided in Sections 11.2.1 through 11.2.4 is simply meant as a guideline for preparing spiking suspensions using a flow cytometer. Laboratories performing flow cytometry must develop and implement detailed standardized protocols for calibration and operation of the flow cytometer.

11.2.1 Spiking suspensions should be prepared using unstained organisms that have not been heat-fixed or formalin-fixed.

11.2.2 Spiking suspensions should be prepared using Cryptosporidium parvum oocysts <3 months old, and Giardia intestinalis cysts <2 weeks old.

11.2.3 Initial calibration. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The

oocyst and cyst levels used for the initial calibration should be the same as the levels used for the spiking suspensions. Each initial calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the laboratory should perform the initial calibration again, until the RSD of the 10 counts is ≤ 2.5%. In addition to counting the organisms, the laboratory also should evaluate the quality of the organisms using DAPI and DIC to confirm that the organisms are in good condition.

11.2.4 Ongoing calibration. When sorting the spiking suspensions for use in QC samples, the laboratory should perform ongoing calibration samples at a 10% frequency, at a minimum. The laboratory should sort the first run and every eleventh sample directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the laboratory should discard the batch.

11.2.5 Method blanks. Depending on the operation of the flow cytometer, method blanks should be prepared and examined at the same frequency as the ongoing calibration samples (Section 11.2.4).

11.2.6 Holding time criteria. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometer-sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory.

11.3 Manual enumeration procedures. Two sets of manual enumerations are required per organism before purified Cryptosporidium oocyst and Giardia cyst stock suspensions (Sections 7.9.2.1 and 7.9.2.2) received from suppliers can be used to spike samples in the laboratory. First, the stock suspension must be diluted and enumerated (Section 11.3.3) to yield a suspension at the appropriate oocyst or cyst concentration for spiking (spiking suspension). Then, 10 aliquots of spiking suspension must be enumerated to calculate a mean spike dose. Spiking suspensions can be enumerated using hemacytometer chamber counting (Section 11.3.4), well slide counting (Section 11.3.5), or membrane filter counting (Section 11.3.6).

11.3.1 Precision criteria. The relative standard deviation (RSD) of the calculated mean spike dose for manually enumerated spiking suspensions must be ≤16% for *Cryptosporidium* and ≤19% for *Giardia* before proceeding (these criteria are based on the pooled RSDs of 105 manual *Cryptosporidium* enumerations and 104 manual *Giardia* enumerations submitted by 20 different laboratories under the EPA Protozoa Performance Evaluation Program).

11.3.2 Holding time criteria. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension or membrane filter to the slides if the

well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

11.3.3 Enumerating and diluting stock suspensions

11.3.3.1 Purified, concentrated stock suspensions (Sections 7.10.2.1 and 7.10.2.2) must be diluted and enumerated before the diluted suspensions are used to spike samples in the laboratory. Stock suspensions should be diluted with reagent water/Tween-20, 0.01% (Section 7.10.2.3), to a concentration of 20 to 50 organisms per large hemacytometer square before proceeding to Section 11.3.3.2.

11.3.3.2 Apply a clean hemacytometer coverslip (Section 6.4.5) to the hemacytometer and load the hemacytometer chamber with 10 µL of vortexed suspension per chamber. If this operation has been properly executed, the liquid should amply fill the entire chamber without bubbles or overflowing into the surrounding moats. Repeat this step with a clean, dry hemacytometer and coverslip if loading has been incorrectly performed. See Section 11.3.3.13, below, for the hemacytometer cleaning procedure.

11.3.3.3 Place the hemacytometer on the microscope stage and allow the oocysts or cysts to settle for 2 minutes Do not attempt to adjust the coverslip, apply clips, or in any way disturb the chamber after it has been filled.

11.3.3.4 Use 200X magnification.

11.3.3.5 Move the chamber so the ruled area is centered underneath it.

11.3.3.6 Move the objective close to the coverslip while watching it from the side of the microscope, rather than through the microscope.

11.3.3.7 Focus up from the coverslip until the hemacytometer ruling appears.

11.3.3.8 At each of the four corners of the chamber is a 1-square-mm area divided into 16 squares in which organisms are to be counted (Figure 1). Beginning with the top row of four squares, count with a hand-tally counter in the directions indicated in Figure 2. Avoid counting organisms twice by counting only those touching the top and left boundary lines. Count each square millimeter in this fashion.

11.3.3.9 Use the following formula to determine the number of organisms per mL of suspension:

11.3.3.10 Record the result on a hemacytometer data sheet.

11.3.3.11 A total of six different hemacytometer chambers must be loaded, counted, and averaged for each suspension to achieve optimal counting accuracy.

11.3.3.12 Based on the hemacytometer counts, the stock suspension should be diluted to a final concentration of between 8000 and 12,000 organisms per mL (80 to 120 organisms per 10 $_{\mu L}$); however, ranges as great as 5000 to 15,000 organisms per mL (50 to 150 organisms per 10 µL) can be used.

NOTE: If the diluted stock suspensions (the spiking suspensions) will be enumerated using hemacytometer chamber counts (Section 11.3.4) or membrane filter counts (Section 11.3.6), then the stock suspensions should be diluted with 0.01% Tween-20. If the spiking suspensions will be enumerated using well slide counts (Section 11.3.3), then the stock suspensions should be diluted in reagent water.

To calculate the volume (in µL) of stock suspension required per mL of reagent water (or reagent water/Tween-20, 0.01%), use the following formula:

required number of organisms x 1000 µLvolume of stock suspension (µL)
required = number of organisms/mL of Stock suspension

If the volume is less than 10 µL , an additional dilution of the stock suspension is recommended before proceeding.

To calculate the dilution factor needed to achieve the required number of organisms per 10 µL , use the following formula:
Total volume (µL) number of organisms required x 10 µL
predicted number of organisms per 10 µL (80 to 120)

To calculate the volume of reagent water (or reagent water/Tween-20, 0.01%) needed, use the following formula:
reagent water volume (µL) = total volume (µL) -stock suspension volume required (µL)

11.3.3.13 After each use, the hemacytometer and coverslip must be cleaned immediately to prevent the organisms and debris from drying on it. Since this apparatus is precisely machined, abrasives cannot be used to clean it, as they will disturb the flooding and volume relationships.

11.3.3.13.1 Rinse the hemacytometer and cover glass first with tap water, then 70% ethanol, and finally with acetone.

11.3.3.13.2 Dry and polish the hemacytometer chamber and cover glass with lens paper. Store it in a secure place.

11.3.3.14 Several factors are known to introduce errors into hemacytometer counts, including:

• Inadequate mixing of suspension before flooding the chamber.
• Irregular filling of the chamber, trapped air bubbles, dust, or oil on the chamber or coverslip.
• Total number of organisms counted is too low to provide statistical confidence in the result
• Error in recording tally.
• Calculation error; failure to consider dilution factor, or area counted.
• Inadequate cleaning and removal of organisms from the previous count.
• Allowing filled chamber to sit too long, so that the chamber suspension dries and concentrates.

11.3.4 Enumerating spiking suspensions using a hemacytometer chamber

NOTE: Spiking suspensions enumerated using a hemacytometer chamber must be used within 24 hours of enumeration.

11.3.4.1 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.4.2 To an appropriate-size beaker containing a stir bar, add enough spiking suspension to perform all spike testing and the enumeration as described. The liquid volume and beaker relationship should be such that a spinning stir bar does not splash the sides of the beaker, the stir bar has unimpeded rotation, and there is enough room to draw sample from the beaker with a 10-µL micropipette without touching the stir bar. Cover the beaker with a watch glass or Petri dish to prevent evaporation between sample withdrawals.

11.3.4.3 Allow the beaker contents to stir for a minimum of 30 minutes before beginning enumeration.

11.3.4.4 While the stir bar is still spinning, remove a 10-µL aliquot and carefully load one side of the hemacytometer. Count all organisms on the platform, at 200X magnification using phase-contrast or darkfield microscopy. The count must include the entire area under the hemacytometer, not just the four outer 1-mm^2 squares. Repeat this procedure nine times. This step allows confirmation of the number of organisms per 10 µL (Section 11.3.3.12). Based on the 10 counts, calculate the mean, standard deviation, and RSD of the counts. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts. Refer to Section 11.3.3.14 for factors that may introduce errors.

Enumerating spiking suspensions using well slides

NOTE: Spiking suspensions enumerated using well slides must be used within 24 hours of application of the spiking suspension to the slides.

11.3.5.1 Remove well slides from cold storage and lay the slides on a flat surface for 15 minutes to allow them to warm to room temperature.

11.3.5.2 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.5.3 Remove a 10-µL aliquot from the spiking suspension and apply it to the center of a well.

11.3.5.4 Before removing subsequent aliquots, cap the tube and gently invert it three times to ensure that the oocysts or cysts are in suspension.

11.3.5.5 Ten wells must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Air-dry the well slides. Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

11.3.5.6 Positive and negative controls must be prepared.

11.3.5.6.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts or cysts to the center of a well and distribute evenly over the well area.

11.3.5.6.2 For the negative control, pipette 50 µL of PBS onto the center of a well and spread it over the well area with a pipette tip.
11.3.5.6.3 Air-dry the control slides.

11.3.5.7 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

11.3.5.8 Follow the manufacturer's instructions (Section 7.6) in applying the stain to the slide.

11.3.5.9 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

11.3.5.10 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with a paper towel or other absorbent material. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

11.3.5.11 Add mounting medium (Section 7.8) to each well.

11.3.5.12 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.

11.3.5.13 Record the date and time that staining was completed. If slides will not be read immediately, store in a humid chamber in the dark at 0 °C to 8 °C until ready for examination.

11.3.5.14 After examination of the 10 wells, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6 Enumeration of spiking suspensions using membrane filters

NOTE: Spiking suspensions enumerated using membrane filters must be used within 24 hours of application of the filters to the slides.

11.3.6.1 Pre-coat the glass funnels with Sigmacote® by placing the funnel in a large Petri dish and applying 5-mL of Sigmacoat® to the funnel opening using a pipette and allowing it to run down the inside of the funnel. Repeat for all funnels to be used. The pooled Sigmacoat® may be returned to the bottle for re-use. Place the funnels at 35 °C or 41 °C for approximately 5 minutes to dry.

11.3.6.2 Place foil around the bottoms of the 100 × 15 mm Petri dishes.

11.3.6.3 Filter-sterilize (Section 6.19) approximately 10 mL of PBS pH

7.2 (Section 7. 9. 4). Dilute detection reagent (Section 7.7) as per manufacturer's instructions using sterile PBS. Multiply the anticipated number of filters to be stained by 100 mL to calculate total volume of stain required. Divide the total volume required by 5 to obtain the microliters of antibody necessary. Subtract the volume of antibody from the total stain volume to obtain the required microliters of sterile PBS to add to the antibody.

11.3.6.4 Label the tops of foil-covered, 60 × 15 mm Petri dishes for 10 spiking suspensions plus positive and negative staining controls and multiple filter blanks controls (one negative control, plus a blank after every five sample filters to control for carry-over). Create a humid chamber by laying damp paper towels on the bottom of a stain tray (the inverted foil-lined Petri dishes will protect filters from light and prevent evaporation during incubation).

11.3.6.5 Place a decontaminated and cleaned filter holder base (Section 6.4.8.1) into each of the three ports of the vacuum manifold (Section 6.4.8.2).

11.3.6.6 Pour approximately 10 mL of 0.01% Tween 20 into a 60 × 15 mm Petri dish.

11.3.6.7 Using forceps, moisten a 1.2-µm cellulose-acetate support membrane (Section 6.4.8.3) in the 0.01% Tween 20 and place it on the fritted glass support of one of the filter bases. Moisten a polycarbonate filter (Section

6.4.8.4) the same way and position it on top of the cellulose-acetate support membrane. Carefully clamp the glass funnel to the loaded filter support. Repeat for the other two filters.

11.3.6.8 Add 5 mL of 0.01% Tween 20 to each of the three filtration units and allow to stand.

11.3.6.9 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.6.10 Using a micropipettor, sequentially remove two, 10-µL aliquots from the spiking suspension and pipet into the 5 mL of 0.01% Tween 20 standing in the unit. Rinse the pipet tip twice after each addition. Apply 10 µL of 0.01% Tween 20 to the third unit to serve as the negative control. Apply vacuum at 2" Hg and allow liquid to drain to miniscus, then close off vacuum. Pipet 10 mL of reagent water into each funnel and drain to miniscus, closing off the vacuum. Repeat the rinse and drain all fluid, close off the vacuum.

11.3.6.11 Pipet 100 mL of diluted antibody to the center of the bottom of a 60 × 15 mm Petri dish for each sample.

11.3.6.12 Unclamp the top funnel and transfer each cellulose acetate support membrane/ polycarbonate filter combination onto the drop of stain using forceps (apply each membrane/filter combination to a different Petri dish containing stain). Roll the filter into the drop to exclude air. Place the small Petri dish containing the filter onto the damp towel and cover with the corresponding labeled foil-covered top. Incubate for approximately 45 minutes at room temperature.

11.3.6.13 Reclamp the top funnels, apply vacuum and rinse each three times, each time with 20 mL of reagent water.

11.3.6.14 Repeat Sections 11.3.6.4 through 11.3.6.10 for the next three samples (if that the diluted spiking suspension has sat less than 15 minutes, reduce the suspension vortex time to 60 seconds). Ten, 10-µL spiking suspension aliquots must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose.

Include a filter blank sample at a frequency of every five samples; rotate the position of filter blank to eventually include all three filter placements.
11.3.6.15 Repeat Sections 11.3.6.4 through 11.3.6.10 until the 10-µL spiking suspensions have been filtered. The last batch should include a 10-µL 0.01 Tween 20 blank control and 20 µL of positive control antigen as a positive staining control.
11.3.6.16 Label slides. After incubation is complete, for each sample, transfer the cellulose acetate filter support and polycarbonate filter from drop of stain and place on fritted glass support. Cycle vacuum on and off briefly to remove excess fluid. Peel the top polycarbonate filter off the supporting filter and place on labeled slide. Discard cellulose acetate filter support. Mount and apply coverslips to the filters immediately to avoid drying.
11.3.6.17 To each slide, add 20 µL of mounting medium (Section 7.8).
11.3.6.18 Apply a coverslip. Seal the edges of the coverslip onto the slide using clear nail polish. (Sealing may be delayed until cover slips are applied to all slides.)

11.3.6.19 Record the date and time that staining was completed. If slides will not be read immediately, store sealed slides in a closed container in the dark at 0 $^{\circ}$C to 8 $^{\circ}$C until ready for examination.

11.3.6.20 After examination of the 10 slides, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6.21 If oocysts or cysts are detected on the filter blanks, modify the rinse procedure to ensure that no carryover occurs and repeat enumeration.

11.4 Procedure for spiking samples in the laboratory with enumerated spiking suspensions.

11.4.1 Arrange a bottom-dispensing container to feed the filter.

11.4.2 For initial precision and recovery (Section 9.4) and ongoing precision and recovery (Section 9.7) samples, fill the container with a volume of reagent water equal to the volume of the field samples analyzed in the analytical batch. For matrix spike samples (Section 9.5), fill the container with the field sample to be spiked. Continuously mix the sample (using a stir bar and stir plate for smaller-volume samples and alternate means for larger-volume samples).

11.4.3 Vortex the spiking suspension(s) (Section 11.2 or Section 11.3) for a minimum of 2 minutes.

11.4.3.1 For flow cytometer–enumerated suspensions (where the entire volume of a spiking suspension tube will be used):

11.4.3.1.1 Add 500 µL of the diluted antifoam to the tube containing the spiking suspension and vortex for 2 minutes.
11.4.3.1.2 Pour the suspension into the sample container.
11.4.3.1.3 Add 20 mL of reagent water to the empty tube, cap, vortex 10 seconds to rinse, and add the rinsate to the carboy.
11.4.3.1.4 Repeat this rinse using another 20 mL of reagent water.
11.4.3.1.5 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet.

11.4.3.1.6 Proceed to Section 11.4.4.

11.4.3.2 For manually enumerated spiking suspensions:

11.4.3.2.1 Rinse a pipette tip with 0.01% Tween-20 once, then rinse with the well-mixed spiking suspension a minimum of five times before pulling an aliquot to be used to spike the container.

11.4.3.2.2 Add the spiking suspension(s) to the carboy, delivering the aliquot below the surface of the water.

11.4.3.2.3 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet. Proceed to Section 11.4.4

11.4.4 Allow the spiking suspensions to mix for approximately 1 minute in the container.

11.4.5 Turn on the pump and allow the flow rate to stabilize. Set flow at the rate designated for the filter being used. As the carboy is depleted, check the flow rate and adjust if necessary.

11.4.6 When the water level approaches the discharge port of the carboy, tilt the container so that it is completely emptied. At that time, turn off the pump and add sufficient reagent water to the container to rinse. Swirl the contents to rinse down the sides.

11.4.7 Turn on the pump. Allow all of the water to flow through the filter and turn off the pump.

12.0 Sample Filtration and Elution

12.1 A water sample is filtered according to the procedures in Section 12.2. Alternate procedures may be used if the laboratory first demonstrates that the alternate procedure provides equivalent or superior performance per Section 9.1.2.

NOTE: Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

12.2 Capsule filtration (adapted from Reference 20.9). This procedure was validated using 10-L sample volumes. Alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

NOTE: The filtration procedures specified in Section 12.2.1 - 12.2.5.3 are specific to laboratory filtration of a bulk sample, and reflect the procedures used during the interlaboratory validation of this method (Reference 20.10). These procedures may require modification if samples will be filtered in the field.

12.2.1 Flow rate adjustment

12.2.1.1 Connect the sampling system, minus the capsule, to a carboy filled with reagent water (Figure 3).

12.2.1.2 Turn on the pump and adjust the flow rate to 2.0 L/min.

12.2.1.3 Allow 2 to 10 L of reagent water to flush the system. Adjust the pump speed as required during this period. Turn off the pump when the flow rate has been adjusted.

12.2.2 Install the capsule filter in the line, securing the inlet and outlet ends with the appropriate clamps/fittings.

12.2.3 Record the sample number, sample turbidity (if not provided with the field sample), sample type, and sample filtration start date and time on a bench sheet.

12.2.4 Filtration

12.2.4.1 Connect the sampling system to the field carboy of sample water, or transfer the sample water to the laboratory carboy used in Section
12.2.1.1. If the sample will be filtered from a field carboy, a spigot (Section 6.2.1) can be used with the carboy to facilitate sample filtration.

NOTE: If the bulk field sample is transferred to a laboratory carboy, the laboratory carboy must be cleaned and disinfected before it is used with another field sample.

12.2.4.2 Place the drain end of the sampling system tubing into an empty graduated container with a capacity of 10 to 15 L, calibrated at 9.0, 9.5, 10.0, 10.5, and 11.0 L (Section 6.18). This container will be used to determine the sample volume filtered. Alternately, connect a flow meter (Section 6.3.4) downstream of the filter, and record the initial meter reading.

12.2.4.3 Allow the carboy discharge tube and capsule to fill with sample water. Vent residual air using the bleed valve/vent port, gently shaking or tapping the capsule, if necessary. Turn on the pump to start water flowing through the filter. Verify that the flow rate is 2 L/min.

12.2.4.4 After all of the sample has passed through the filter, turn off the pump. Allow the pressure to decrease until flow stops. (If the sample was filtered in the field, and excess sample remains in the filter upon receipt in the laboratory, pull the remaining sample volume through the filter before eluting the filter [Section 12.2.6].)

12.2.5 Disassembly

12.2.5.1 Disconnect the inlet end of the capsule filter assembly while maintaining the level of the inlet fitting above the level of the outlet fitting to prevent backwashing and the loss of oocysts and cysts from the filter. Restart the pump and allow as much water to drain as possible. Turn off the pump.

12.2.5.2 Based on the water level in the graduated container or meter reading, record the volume filtered on the bench sheet to the nearest quarter liter. Discard the contents of the graduated container.

12.2.5.3 Loosen the outlet fitting, then cap the inlet and outlet fittings.

12.2.6 Elution

NOTE: The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.

12.2.6.1 Setup

12.2.6.1.1 Assemble the laboratory shaker with the clamps aligned vertically so that the filters will be aligned horizontally. Extend the clamp arms to their maximum distance from the horizontal shaker rods to maximize the shaking action.

12.2.6.1.2 Prepare sufficient elution buffer so that all samples to be eluted that day can be eluted with the same batch of buffer. Elution may require up to 275 mL of buffer per sample.

12.2.6.1.3 Designate at least one 250-mL conical centrifuge tube for each sample and label with the sample number.

12.2.6.2 Elution

12.2.6.2.1 Record the elution date and time on the bench sheet. Using a ring stand or other means, clamp each capsule in a vertical position with the inlet end up. Remove the inlet cap and allow the liquid level to stabilize.

12.2.6.2.2 Pour elution buffer through the inlet fitting. Sufficient elution buffer must be added to cover the pleated white membrane with buffer solution. Replace the inlet cap and clamp the cap in place.

12.2.6.2.3 Securely clamp the capsule in one of the clamps on the laboratory shaker with the bleed valve positioned at the top on a vertical axis (in the 12 o'clock position). Turn on the shaker and set the speed to maximum (approximately 900 rpm). Agitate the capsule for approximately 5 minutes. Time the agitation using a lab timer, rather than the timer on the shaker to ensure accurate time measurement.

12.2.6.2.4 Remove the filter from the shaker, remove the inlet cap, and pour the contents of the capsule into the 250-mL conical centrifuge tube.

12.2.6.2.5 Clamp the capsule vertically with the inlet end up and add sufficient volume of elution buffer through the inlet fitting to cover the pleated membrane. Replace the inlet cap.

12.2.6.2.6 Return the capsule to the shaker with the bleed valve positioned at the 4 o'clock position. Turn on the shaker and agitate the capsule for approximately 5 minutes.

12.2.6.2.7 Remove the filter from the shaker, but leave the elution buffer in the capsule. Re-clamp the capsule to the shaker at the 8 o’clock position. Turn on the shaker and agitate the capsule for a final 5 minutes.

12.2.6.2.8 Remove the filter from the shaker and pour the contents into the 250-mL centrifuge tube. Rinse down the inside of the capsule filter walls with reagent water or elution buffer using a squirt bottle inserted in the inlet end of the capsule. Invert the capsule filter over the centrifuge tube and ensure that as much of the eluate as possible has been transferred.

12.2.7 Proceed to Section 13.0 for concentration and separation (purification).

13.0 Sample Concentration and Separation (Purification)

13.1 During concentration and separation, the filter eluate is concentrated through centrifugation, and the oocysts and cysts in the sample are separated from other particulates through immunomagnetic separation (IMS). Alternate procedures and products may be used if the laboratory first demonstrates equivalent or superior performance as per Section 9.1.2.

13.2 Adjustment of pellet volume

13.2.1 Centrifuge the 250-mL centrifuge tube containing the capsule filter eluate at 1500 × G for 15 minutes. Allow the centrifuge to coast to a stop—do not use the brake. Record the pellet volume (volume of solids) on the bench sheet.

NOTE: Recoveries may be improved if centrifugation force is increased to 2000 × G. However, do not use this higher force if the sample contains sand or other gritty material that may degrade the condition of any oocysts and/or cysts in the sample.

13.2.2 Using a Pasteur pipette, carefully aspirate the supernatant to 5 mL above the pellet. Extra care must be taken to avoid aspirating oocysts and cysts during this step, particularly if the sample is reagent water (e.g. initial or ongoing precision and recovery sample).
13.2.3 If the packed pellet volume is ≤ 0.5 mL, vortex the tube vigorously until pellet is completely resuspended. Swirl the centrifuge tube gently to reduce any foaming after vortexing. Record the resuspended pellet volume on the bench sheet. Proceed to Section 13.3.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts and/or cysts in the sample is not compromised.

13.2.4 If the packed pellet volume is > 0.5 mL, the concentrate needs to be separated into multiple subsamples (a subsample is equivalent to no greater than 0.5 mL of packed pellet material, the recommended maximum amount of particulate material to process through the subsequent purification and examination steps in the method). Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples:

$$\text{total volume (mL) required} = \frac{\text{pellet volume}}{0.5\ \text{mL}} \times 5\ \text{mL}$$

(For example, if the packed pellet volume is 1.2 mL, the total volume required is 12 mL.) Add reagent water to the centrifuge tube to bring the total volume to the level calculated above. Vortex the tube vigorously for 10 to 15 seconds to completely resuspend the pellet. Record the resuspended pellet volume on the bench sheet.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts in the sample is not compromised.

13.2.4.1 Analysis of entire sample. If analysis of the entire sample is required, determine the number of subsamples to be processed independently through the remainder of the method:

13.2.4.1.1 Calculate number of subsamples: Divide the total volume in the centrifuge tube by 5 mL and round up to the nearest integer (for example, if the resuspended volume in Section 13.2.4 is 12 mL, then the number of subsamples would be 12 mL / 5 mL = 2.4, rounded = 3 subsamples).

13.2.4.1.2 Determine volume of resuspended concentrate per subsample. Divide the total volume in the centrifuge tube by the calculated number of subsamples (for

13.2.4.1.3 example, if the resuspended volume in Section 13.2.4 is 12 mL, then the volume to use for each subsample = 12 mL / 3 subsamples = 4 mL).

Process sub-samples through IMS. Proceed to Section 13.3, and transfer aliquots of the resuspended concentrate equivalent to the volume in the previous step to multiple, flat-sided sample tubes in Section 13.3.2.1. Process the sample as multiple, independent subsamples from Section 13.3 onward, including the preparation and examination of separate slides for each aliquot. Record the volume of resuspended concentrate transferred to IMS on the bench sheet (this will be equal to the volume recorded in Section 13.2.4). Also record the number of subsamples processed independently through the method on the bench sheet.

13.2.4.2 Analysis of partial sample. If not all of the concentrate will be examined, proceed to Section 13.3, and transfer one or more 5-mL aliquots of the resuspended concentrate to one or more flat-sided sample tubes in Section 13.3.2.1. Record the volume of resuspended concentrate transferred to IMS on the bench sheet. To determine the volume analyzed, calculate the percent of the concentrate examined using the following formula:

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS}}{\text{total volume of resuspended concentrate in Section 13.2.4}} \times 100\%$$

Then multiply the volume filtered (Section 12.2.5.2) by this percentage to determine the volume analyzed.

13.3 IMS procedure (adapted from Reference 20.11)

NOTE: The IMS procedure should be performed on a bench top with all materials at room temperature, ranging from 15 ^{o}C to 25 ^{o}C.

13.3.1 Preparation and addition of reagents

13.3.1.1 Prepare a 1X dilution of SL-buffer-A from the 10X SL-buffer-A (clear, colorless solution) supplied. Use reagent water (demineralized; Section 7.3) as the diluent. For every 1 mL of 1X SL-buffer-A required, take 100 µL of 10X SL-buffer-A and make up to 1 mL with the diluent water. A volume of 1.5 mL of 1X SL-buffer-A will be required per sample or subsample on which the Dynal IMS procedure is performed.

13.3.1.2 For each sample or subsample (Section 13.2) to be processed through IMS, add 1 mL of the 10X SL-buffer-A (supplied—not the diluted 1X SL-buffer-A) to a flat-sided tube (Section 6.5.4).

13.3.1.3 For each subsample, add 1 mL of the 10X SL-buffer-B (supplied—magenta solution) to the flat-sided tube containing the 10X SL-buffer-A.

13.3.2 Oocyst and cyst capture

13.3.2.1 Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample concentrate from Section 13.2 to the flat-sided tube(s) containing the SL-buffer. If all of the concentrate is used, rinse the centrifuge tube twice with reagent water and add the rinsate to the flat-sided tube containing the concentrate (or to the tube containing the first subsample, if multiple subsamples will be processed). Each of the two rinses should be half the volume needed to bring the total volume in the flat-sided sample tube to 10 mL. (For example, if 5 mL was transferred after resuspension of the pellet, the centrifuge tube would be

rinsed twice with 2.5 mL of reagent water to bring the total volume in the flat-sided tube to 10 mL.) Visually inspect the centrifuge tube after completing the transfer to ensure that no concentrate remains. If multiple subsamples will be processed, bring the volume in the remaining flat-sided tubes to 10 mL with reagent water. Label the flat-sided tube(s) with the sample number (and subsample letters).

13.3.2.2 Vortex the Dynabeads®Crypto-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the sample tube and making sure that there is no residual pellet at the bottom.

13.3.2.3 Add 100 µL of the resuspended Dynabeads®Crypto-Combo (Section 13.3.2.2) to the sample tube(s) containing the water sample concentrate and SL-buffer.

13.3.2.4 Vortex the Dynabeads®Giardia-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the tube and making sure that there is no residual pellet at the bottom.

13.3.2.5 Add 100 µL of the resuspended Dynabeads®Giardia-Combo (Section 13.3.2.4) to the sample tube(s) containing the water sample concentrate, Dynabeads®Crypto-Combo, and SL-buffer.

13.3.2.6 Affix the sample tube(s) to a rotating mixer and rotate at approximately 18 rpm for 1 hour at room temperature.

13.3.2.7 After rotating for 1 hour, remove each sample tube from the mixer and place the tube in the magnetic particle concentrator (MPC-1) with flat side of the tube toward the magnet.

13.3.2.8 Without removing the sample tube from the MPC-1, place the magnet side of the MPC-1 downwards, so the tube is horizontal and the flat side of the tube is facing down.

13.3.2.9 Gently rock the sample tube by hand end-to-end through approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 minutes with approximately one tilt per second.

13.3.2.10 Ensure that the tilting action is continued throughout this period to prevent binding of low-mass, magnetic or magnetizable material. If the sample in the MPC-1 is allowed to stand motionless for more than 10 seconds, repeat Section 13.3.2.9 before continuing to Section 13.3.2.11.

13.3.2.11 Return the MPC-1 to the upright position, sample tube vertical, with cap at top. Immediately remove the cap and, keeping the flat side of the tube on top, pour off all of the supernatant from the tube held in the MPC-1 into a suitable container. Do not shake the tube and do not remove the tube from MPC-1 during this step.

13.3.2.12 Remove the sample tube from the MPC-1 and resuspend the sample in 1-mL 1X SL-buffer-A (prepared from 10X SL-buffer-A stock—supplied). Mix very gently to resuspend all material in the tube. Do not vortex.

13.3.2.13 Quantitatively transfer (transfer followed by two rinses) all the liquid from the sample tube to a labeled, 1.5-mL microcentrifuge tube. Use 1 mL of 1X SL-buffer-A to perform the first rinse and 0.5 mL of reagent water for the second rinse. Liberally rinse down the sides of the Leighton tube before transferring. Allow the flat-sided sample tube to sit for a

minimum of 1 minute after transfer of the second rinse volume, then use a pipette to collect any residual volume that drips down to the bottom of the tube to ensure that as much sample volume is recovered as possible. Ensure that all of the liquid and beads are transferred.

13.3.2.14 Place the microcentrifuge tube into the second magnetic particle concentrator (MPC-M), with its magnetic strip in place.

13.3.2.15 Without removing the microcentrifuge tube from MPC-M, gently rock/roll the tube through 180° by hand. Continue for approximately 1 minute with approximately one 180° roll/rock per second. At the end of this step, the beads should produce a distinct brown dot at the back of the tube.

13.3.2.16 Immediately aspirate the supernatant from the tube and cap held in the MPC-M. If more than one sample is being processed, conduct three 90° rock/roll actions before removing the supernatant from each tube. Take care not to disturb the material attached to the wall of the tube adjacent to the magnet. Do not shake the tube. Do not remove the tube from MPC-M while conducting these steps.

13.3.3 Dissociation of beads/oocyst/cyst complex

NOTE: Two acid dissociations are required.

13.3.3.1 Remove the magnetic strip from the MPC-M.

13.3.3.2 Add 50 µL of 0.1 N HCl, then vortex at the highest setting for approximately 50 seconds.

NOTE: The laboratory should use 0.1-N standards purchased directly from a vendor, rather than adjusting the normality in-house.

13.3.3.3 Place the tube in the MPC-M without the magnetic strip in place and allow to stand in a vertical position for at least 10 minutes at room temperature.

13.3.3.4 Vortex vigorously for approximately 30 seconds.

13.3.3.5 Ensure that all of the sample is at the base of the tube. Place the microcentrifuge tube in the MPC-M.

13.3.3.6 Replace magnetic strip in MPC-M and allow the tube to stand undisturbed for a minimum of 10 seconds.

13.3.3.7 Prepare a well slide for sample screening and label the slide.

13.3.3.8 Add 5 µL of 1.0 N NaOH to the sample wells of two well slides (add 10 µL to the sample well of one well slide if the volume from the two required dissociations will be added to the same slide).

NOTE: The laboratory should use 1.0-N standards purchased directly from a vendor rather than adjusting the normality in-house.

13.3.3.9 Without removing the microcentrifuge tube from the MPC-M, transfer all of the sample from the microcentrifuge tube in the MPC-M to the sample well with the NaOH. Do not disturb the beads at the back wall of the tube. Ensure that all of the fluid is transferred.

13.3.3.10 Do not discard the beads or microcentrifuge tube after transferring the volume from the first acid dissociation to the well slide. Perform the steps in Sections 13.3.3.1 through 13.3.3.9 a second time. The volume from the second dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.

NOTE: If one slide is used, exert extra care when using Dynal Spot-On slides to ensure that the sample stays within the smaller-diameter wells on these slides.

13.3.3.11 Record the date and time the purified sample was applied to the slide(s).

13.3.3.12 Air-dry the sample on the well slide(s). Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

14.0 Sample Staining

NOTE: The sample must be stained within 72 hours of application of the purified sample to the slide.

14.1 Prepare positive and negative controls.

14.1.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts and 200 to 400 cysts to the center of a well.

14.1.2 For the negative control, pipette 50 µL of 150 mM PBS (Section 7.6.4) into the center of a well and spread it over the well area with a pipette tip.

14.1.3 Air-dry the control slides (see Section 13.3.3.12 for guidance).

14.2 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

14.3 Follow manufacturer's instructions in applying stain to slide.

14.4 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

14.5 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.6 Apply 50 µL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.7.2) to each well. Allow to stand at room temperature for a minimum of 1 minute. (The solution concentration may be increased up to 1 µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.)

14.7 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.8 Add mounting medium (Section 7.8) to each well.
14.9 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.
14.10 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

15.0 Examination

NOTE: Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.

15.1 Scanning technique: Scan each well in a systematic fashion. An up-and-down or a side-to-side scanning pattern may be used (Figure 4).
15.2 Examination using immunofluorescence assay (FA), 4',6-diamidino-2-phenylindole (DAPI) staining characteristics, and differential interference contrast (DIC) microscopy. The minimum magnification requirements for each type of examination are noted below.
NOTE: All shape and measurements must be determined using 1000X magnification and reported to the nearest 0.5 µm.

Record examination results for Cryptosporidium oocysts on a Cryptosporidium report form; record examination results for Giardia cysts on a Giardia report form. All oocysts and cysts that meet the criteria specified in Sections 15.2.2 and 15.2.3, less atypical organisms specifically identified as non-target organisms by DIC or DAPI (e.g. possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc), must be reported.

15.2.1 Positive and negative staining control.

15.2.1.1 Each analyst must characterize a minimum of three Cryptosporidium oocysts and three Giardia cysts on the positive staining control slide before examining field sample slides. This characterization must be performed by each analyst during each microscope examination session.

FITC examination must be conducted at a minimum of 200X total magnification, DAPI examination must be conducted at a minimum of 400X, and DIC examination must be conducted at a minimum of 1000X. Size, shape, and DIC and DAPI characteristics of the three Cryptosporidium oocysts and Giardia cysts must be recorded by the analyst on a microscope log. The analyst also must indicate on each sample report form whether the positive staining control was acceptable.

15.2.1.2 Examine the negative staining control to confirm that it does not contain any oocysts or cysts (Section 14.1). Indicate on each sample report form whether the negative staining control was acceptable.

15.2.1.3 If the positive staining control contains oocysts and cysts within the expected range and at the appropriate fluorescence for both FA and DAPI, and the negative staining control does not contain any oocysts or cysts (Section 14.1), proceed to Sections 15.2.2 and 15.2.3.

15.2.2 Sample examination—Cryptosporidium

15.2.2.1 FITC examination (the analyst must use a minimum of 200X total magnification). Use epifluorescence to scan the entire well for apple-green fluorescence of oocyst and cyst shapes. When brilliant apple-green fluorescing ovoid or spherical objects 4 to 6 µm in diameter are observed with brightly highlighted edges, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.2.2), then to DIC (Section 15.2.2.3).

15.2.2.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one of the following characteristics: (a) Light blue internal staining (no distinct nuclei) with a green rim (b) Intense blue internal staining (c) Up to four distinct, sky-blue nuclei Record oocysts in category (a) as DAPI negative; record oocysts in categories (b) and (c) as DAPI positive.

15.2.2.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Cryptosporidium oocysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each apple-green fluorescing object as: (a) An empty Cryptosporidium oocyst (b) A Cryptosporidium oocyst with amorphous structure (c) A Cryptosporidium oocyst with internal structure (one to four sporozoites/oocyst)

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of sporozoites (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics. Although not a defining characteristic, surface oocyst folds may be observed in some specimens.

NOTE: All measurements must be made at 1000X magnification.

15.2.3 Sample examination—Giardia

15.2.3.1 FITC examination (the analyst must use a minimum of 200X total magnification). When brilliant apple-green fluorescing round to oval objects (8 - 18 µm long by 5 - 15 µm wide) are observed, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.3.2) then to DIC (Section 15.2.3.3).

15.2.3.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one or more of the following characteristics: (a) Light blue internal staining (no distinct nuclei) and a green rim (b) Intense blue internal staining (c) Two to four sky-blue nuclei Record cysts in category (a) as DAPI negative; record cysts in categories (b) and (c) as DAPI positive.

15.2.3.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Giardia cysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each object meeting the criteria specified in Sections 15.2.3.1 - 15.2.3.3 as one of the following, based on DIC examination: (a) An empty Giardia cyst (b) A Giardia cyst with amorphous structure (c) A Giardia cyst with one type of internal structure (nuclei, median body, or axonemes), or (d) A Giardia cyst with more than one type of internal structure.

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of nuclei and presence of median body or axonemes (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics.

NOTE: All measurements must be made at 1000X magnification.

15.2.4 Record the date and time that sample examination was completed on the report form. 15.2.5 Report Cryptosporidium and Giardia concentrations as oocysts/L and cysts/L.

16.0 Analysis of Complex Samples

16.1 Some samples may contain high levels (>1000/L) of oocysts and cysts and/or interfering organisms, substances, or materials. Some samples may clog the filter (Section 12.0); others will not allow separation of the oocysts and cysts from the retentate or eluate; and others may contain materials that preclude or confuse microscopic examination.

16.2 If the sample holding time has not been exceeded and a full-volume sample cannot be filtered, dilute an aliquot of sample with reagent water and filter this smaller aliquot (Section 12.0). This dilution must be recorded and reported with the results.

16.3 If the holding times for the sample and for microscopic examination of the cleaned up retentate/eluate have been exceeded, the site should be re-sampled. If this is not possible, the results should be qualified accordingly.

17.0 Method Performance

17.1 Method acceptance criteria are shown in Tables 3 and 4 in Section 21.0. The initial and ongoing precision and recovery criteria are based on the results of spiked reagent water samples analyzed during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike and matrix spike duplicate criteria are based on spiked source water data generated during the interlaboratory validation study of Method 1623 involving 11 laboratories and 11 raw surface water matrices across the U.S. (Reference 20.10).

NOTE: Some sample matrices may prevent the MS acceptance criteria in Tables 3 and 4 to be met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

18.0 Pollution Prevention

18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management

19.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of these requirements can be found in the *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

19.2 Samples, reference materials, and equipment known or suspected to have viable oocysts or cysts attached or contained must be sterilized prior to disposal.

19.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

20.0 References

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21.0 Tables and Figures

Table 1. Method Holding Times (See Section 8.2 for details)

Sample Processing Step	Maximum Allowable Time between Breaks
Collection	
Filtration	
> Up to 96 hours are permitted between sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field) and initiation of elution	
Elution	
	These steps must be completed in 1 working day
Concentration	
Purification	
Application of purified sample to slide	
Drying of sample	
> Up to 72 hours are permitted from application of the purified sample to the slide to staining	
Staining	
> Up to 7 days are permitted between sample staining and examination	
Examination	

Table 2. Tier 1 and Tier 2 Validation/Equivalency Demonstration Requirements

Test	Description	Tier 1 modification(1)	Tier 2 modification(2)
IPR (Section 9.4)	4 replicates of spiked reagent water	Required. Must be accompanied by a method blank.	Required per laboratory
Method blank (Section 9.6)	Unspiked reagent water	Required	Required per laboratory
MS (Section 9.5.1)	Spiked matrix water	Required on each water to which the modification will be applied and on every 20th sample of that water thereafter. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Not required
MS/MSD (Section 9.5)	2 replicates of spiked matrix water	Recommended, but not required. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Required per laboratory. Each laboratory must analyze a different water.

(1) If a modification will be used only in one laboratory, these tests must be performed and the results must meet all of the QC acceptance criteria in the method (these tests also are required the first time a laboratory uses the validated version of the method).
(2) If nationwide approval of a modification is sought for one type of water matrix (such as surface water), a minimum of 3 laboratories must perform the tests and the results from each lab individually must meet all QC acceptance criteria in the method. If more than 3 laboratories are used in a study, a minimum of 75% of the laboratories must meet all QC acceptance criteria.

NOTE: The initial precision and recovery and ongoing precision and recovery (OPR) acceptance criteria listed in Tables 3 and 4 are based on results from 293 Cryptosporidium OPR samples and 186 Giardia OPR samples analyzed by six

laboratories during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike acceptance criteria are based on data generated through interlaboratory validation of Method 1623 (Reference 20.10).

Table 3. Quality Control Acceptance Criteria for *Cryptosporidium*

Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 55
Ongoing precision and recovery (percent)	9.7	11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery[1, 2](as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	13 - 111 61

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries from multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 4.
Quality Control Acceptance Criteria for *Giardia*

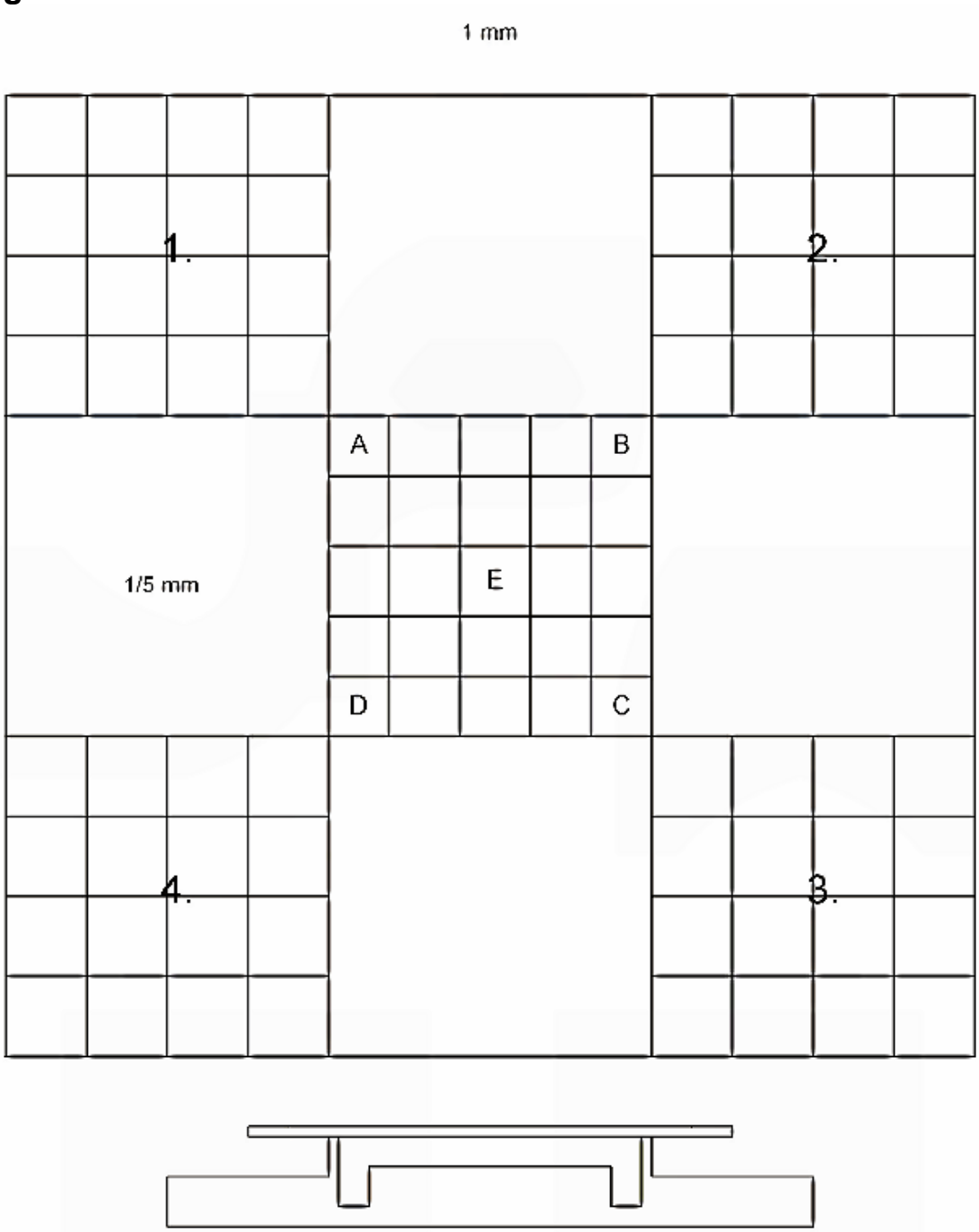
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 49
Ongoing precision and recovery (percent)	9.7	14 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery* (as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	15 - 118 30

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries across multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 5. Distribution of Matrix Spike Recoveries from Multiple Samples Collected from 87 Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13)

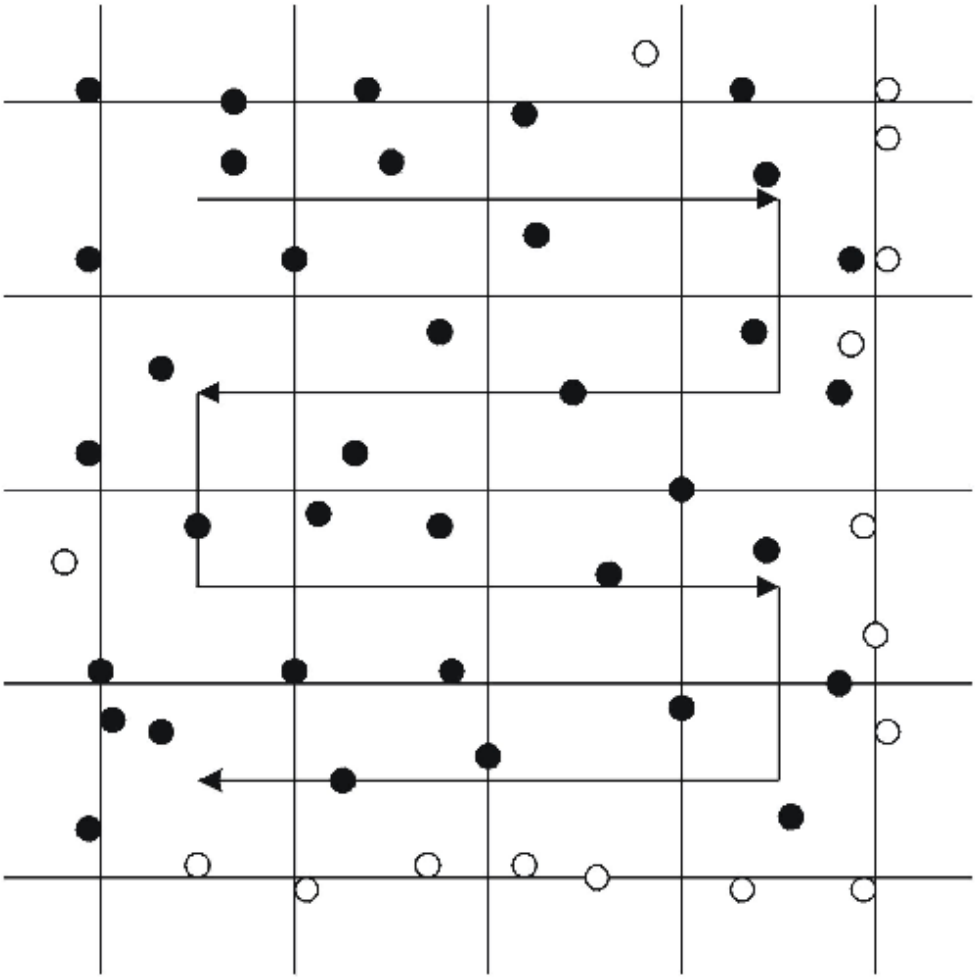
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium* MS Samples in Recovery Range	Percent of 270 *Giardia* MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 50%	18.4%	17.4%
>50% - 60%	17.4%	16.3%
>60% - 70%	11.2%	16.7%
>70% - 80%	8.4%	14.1%
>80% - 90%	2.3%	6.3%
>90%	0.2%	3.7%

Figure 1.



Hemacytometer Platform Ruling. Squares 1, 2, 3, and 4 are used to count stock suspensions of *Cryptosporidium*oocysts and *Giardia* cysts (after Miale, 1967)

Figure 2.



Manner of Counting Oocysts and Cysts in 1 Square mm. Dark organisms are counted and light organisms are omitted (after Miale, 1967).

Figure 3. Laboratory Filtration System

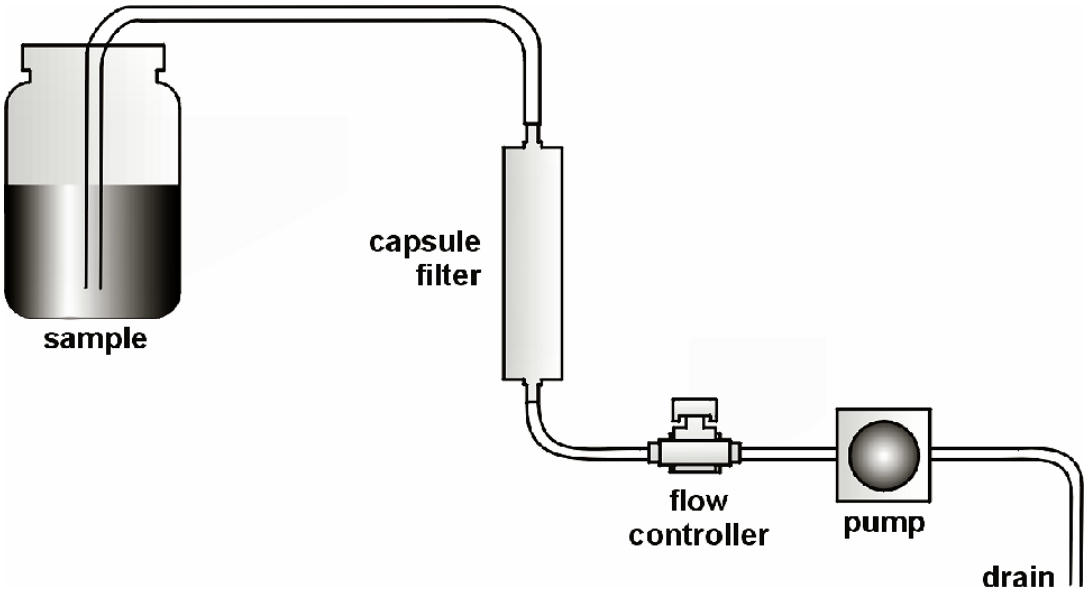
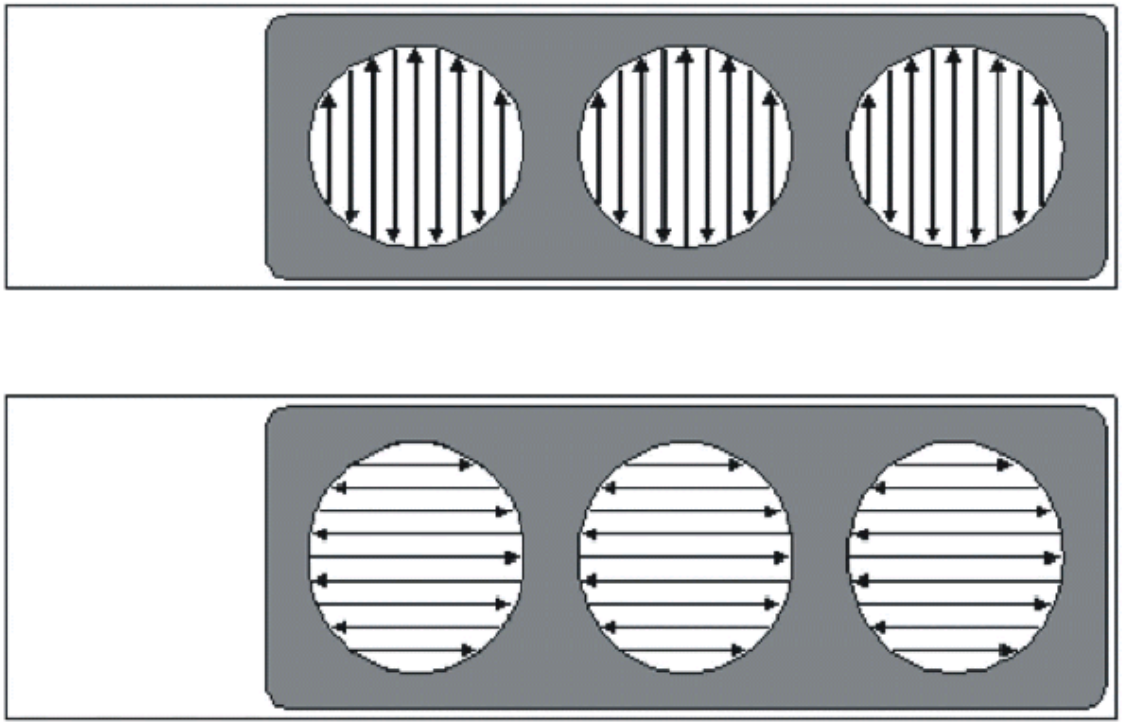
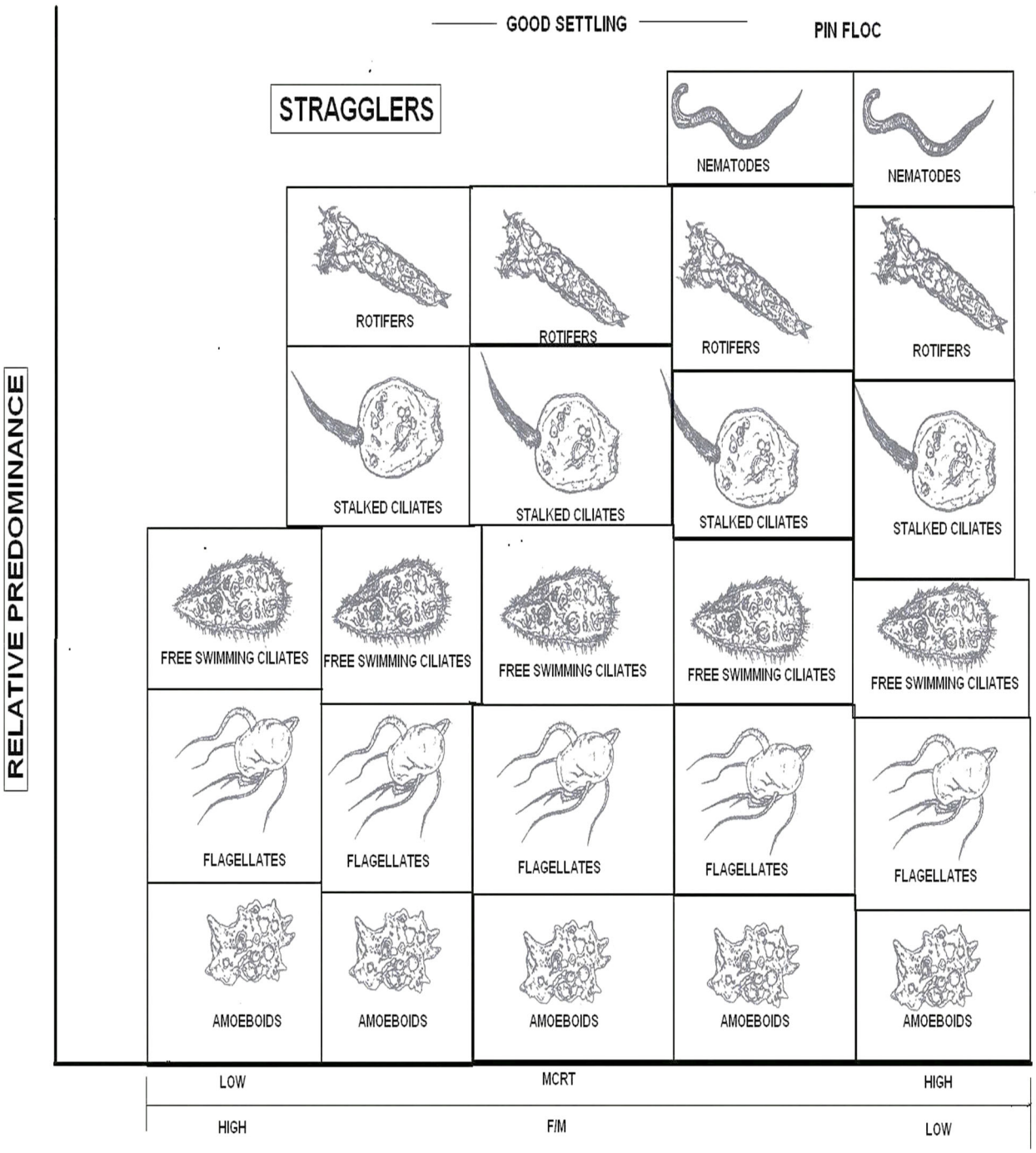


Figure 4. Methods for Scanning a Well Slide





WASTEWATER TREATMENT MICROLIFE

Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using MI agar or MI broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl- β -D-galactopyranoside (MUGal) and a chromogen Indoxyl-β-D-glucuronide (IBDG), are included in the medium to detect the enzymes β -galactosidase and β -glucuronidase, respectively, produced by TC and *E. coli*, respectively.

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. coli* are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (References 16.8 - 16.10), will be used primarily by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human or veterinary), other environmental samples (*e.g.*, aerosols, soil, runoff, or sludge) and/or isolation and separation of transformants though the use of *E. coli lac* Z or *gus* A/*uid* reporter genes (Reference 16.11).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35°C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under long wave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β -galactosidase (Reference 16.8).

3.0 Definitions

3.1 Total coliforms (TC) - In this method, TC are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light (366 nm) after primary culturing on MI agar or broth (See Figure 1.). The fluorescent colonies can be completely blue-white (TC other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-green E. coli colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG (Reference 16.8).

3.2 Escherichia coli - In this method, the E. coli are those bacteria that produce blue colonies under ambient light after primary culturing on MI agar or broth (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under long wave ultraviolet light (366 nm) (Reference 16.8).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies. However, the blue E. coli colonies can often be counted on plates with heavy particulates or high concentrations of total bacteria (See Figures 2 and 3.) (Reference 16.8).

4.2 The presence of some lateral diffusion of blue color away from the target E. coli colonies can affect enumeration and colony picking on plates with high concentrations of E. coli. This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples (Reference 16.8).

4.3 Tiny, flat or peaked pinpoint blue colonies (# 0.5-mm in diameter on filters containing # 200 colonies) may be due to species other than E. coli. These colonies occur occasionally in low numbers and should be excluded from the count of the E. coli colonies, which are usually much larger in size (1-3-mm in diameter). The small colonies have never been observed in the absence of typical E. coli, but, if such should occur, the sample should not be considered E. coli-positive unless at least one colony has been verified by another method [e.g., EC medium with 4-Methylumbelliferyl- β -D-glucuronide (MUG) or API 20E strips] (Reference 16.8).

4.4 Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent TC colonies, may be species other than coliforms. These colonies, which generally occur in low numbers (# 5%) and can usually be distinguished from the TC, should be eliminated from the TC count. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium (Reference 16.8).

5.0 Safety

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization equipment.

5.2 Mouth-pipetting is prohibited.

5.3 Avoid prolonged exposure to long wave or germicidal ultraviolet light.

5.4 Autoclave all contaminated plates and materials at the end of the analysis.

6.0 Equipment and Supplies

6.1 Incubator set at 35°C ± 0.5°C, with approximately 90% humidity if loose-lidded Petri dishes are used.

6.2 Stereoscopic microscope, with magnification of 10-15x, wide-field type.

6.3 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.

6.4 Hand tally.

6.5 Pipet container of stainless steel, aluminum, or Pyrex glass, for pipets.

6.6 Graduated cylinders (100-mL for drinking water), covered with aluminum foil or kraft paper and sterilized.

6.7 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel. These are wrapped with aluminum foil or kraft paper and sterilized.

6.8 Germicidal ultraviolet (254 nm) light box for sanitizing the filter funnels is desirable, but optional.

6.9 Line vacuum, electric vacuum pump, or aspirator is used as a vacuum source. In an emergency, a hand pump or a syringe can be used. Such vacuum-producing devices should be equipped with a check valve to prevent the return flow of air.

6.10 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.

6.11 Safety trap flask, placed between the filter flask and the vacuum source.

6.12 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.

6.13 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.

6.14 Bunsen or Fisher-type burner or electric incinerator unit.

6.15 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mLvolumes).

6.16 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ± 0.02-µm pore size, pretrial or sterilized for 10 minutes at 121°C (15-lb pressure).

6.17 Long wave ultraviolet lamp (366 nm), handheld 4-watt (preferred) or 6-watt, or microscope attachment.

6.18 Dilution water: Sterile phosphate-buffered dilution water, prepared in large volumes (e.g., 1 liter)for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks [Section 9050C in Standard Methods (Reference 16.2)].

6.19 Indelible ink marker for labeling plates.

6.20 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.

6.21 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids, or 15 x 60 mm, glass or plastic, with loose-fitting lids; 15 x 100 mm dishes may also be used.

6.22 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions (if needed). Dilution bottles marked at 90 mL, or tubes marked at 9 mL may be used for 1:10 dilutions.

6.23 Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, for agar preparation.

6.24 Waterbath maintained at 50°C for tempering agar.

6.25 Syringe filter, sterile, disposable, 25-mm diameter, 0.22-µm pore size, to filter cefsulodin for MI agar.

6.26 Syringe, sterile, plastic, disposable, 20-cc capacity. Autoclaved glass syringes are also acceptable.

6.27 Test tubes, sterile, screw-cap, 20 x 150-mm, borosilicate glass or plastic, with lids.

6.28 Sterilization filter units, presterile, disposable, 500- or 1000-mL capacity, 0.2-µm pore size, to filter stock buffer solutions.
6.29 Sterile 47-mm diameter absorbent pads (used with MI broth).
Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.0 Reagents and Standards
7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (Reference 16.1). The agar used in preparation of culture media must be of microbiological grade.
7.2 Whenever possible, use commercial culture media as a means of quality control.
7.3 Purity of Water: Reagent-grade distilled water conforming to Specification D1193, Type II water or better, ASTM Annual Book of Standards (Reference 16.3).
7.4 Buffered Dilution Water (Reference 16.2)
7.4.1 Stock Phosphate Buffer Solution (Reference 16.2):
Potassium Dihydrogen Phosphate (KH_2PO_4) 34.0 g Reagent-Grade Distilled Water 500 mL
7.4.2 Preparation of Stock Buffer Solution: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring volume to 1000 mL with reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.3 $MgCl_2$ Solution (Reference 16.2): Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2C6H_2O$) in one liter of reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.4 Storage of Stock Buffer and $MgCl_2$ Solutions: After sterilization of the stock solutions, store in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears in either stock, the solution should be discarded, and a fresh solution should be prepared.
7.4.5 Working Solution (Final pH 7.0 ± 0.2): Add 1.25 mL phosphate buffer stock (Section 7.4.2) and 5 mL $MgCl_2$ stock (Section 7.4.3) for each liter of reagent-grade distilled water prepared. Mix well, and dispense in appropriate amounts for dilutions in screw-cap dilution bottles or culture tubes, and/or into larger containers for use as rinse water. Autoclave at 121°C (15-lb pressure) for 15 minutes. Longer sterilization times may be needed depending on the container and load size and the amount of time needed for the liquid to reach 121°C.

7.5 MI Agar (Reference 16.8)
7.5.1 Composition:
Proteose Peptone #3 5.0 g
Yeast Extract 3.0 g
β -D-Lactose 1.0 g
4-Methylumbelliferyl- β -D-Galactopyranoside (MUGal)
(Final concentration 100µg/mL) 0.1 g
Indoxyl- β -D-Glucuronide (IBDG)
(Final concentration 320 µg/mL) 0.32 g
NaCl 7.5 g
K_2HPO_4 3.3 g
KH_2PO_4 1.0 g
Sodium Lauryl Sulfate 0.2 g
Sodium Desoxycholate 0.1 g
Agar 15.0 g
Reagent-Grade Distilled Water 1000 mL

7.5.2 Cefsulodin Solution (1 mg / 1 mL): Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-µm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time. Do not save the unused portion.
7.5.3 Preparation: Autoclave the medium for 15 minutes at 121°C (15-lb pressure), and add 5 mL of the freshly-prepared solution of Cefsulodin (5 µg/mL final concentration) per liter of tempered agar medium. Pipet the medium into 9 x 50-mm Petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks. The final pH should be 6.95 ± 0.2.
7.6 MI Broth: The composition of MI broth is the same as MI agar, but without the agar. The final pH of MI broth should be 7.05 ± 0.2. The broth is prepared and sterilized by the same methods described for MI agar in Sections 7.5.1, 7.5.2, and 7.5.3, except that absorbent pads are placed in 9 x 50 mm Petri dishes and saturated with 2-3 mL of MI broth containing 5 :g/mL final concentration of Cefsulodin. Alternately, the broth can be filter-sterilized. Excess broth is poured off before using the plates. Plates should be stored in the refrigerator and discarded after 96 hours (Reference 16.15).

7.7 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or equivalent) (TSA)

7.7.1 ***Composition*****:**
Tryptone 15.0 g
Soytone 5.0 g
NaCl 5.0 g
Agar 15.0 g

7.7.2 ***Preparation*****:** Add the dry ingredients listed above to 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121°C (15-lb pressure) for 15 minutes. Dispense the agar into 9 x 50-mm Petri dishes (5 mL/plate). Incubate the plates for 24 - 48 hours at 35°C to check for contamination. Discard any plates with growth. If > 5% of the plates show contamination, discard all plates, and make new medium. Store at 4°C until needed. The final pH should be 7.3 ± 0.2.

8.0 Sample Collection, Preservation, and Storage

8.1 Water samples are collected in sterile polypropylene sample containers with leakproof lids.

8.2 Sampling procedures are described in detail in Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Reference 16.2) or in the *USEPA Microbiology Methods Manual*, Section II, A (Reference 16.6). Residual chlorine in drinking water (or chlorinated effluent) samples should be neutralized with sodium thiosulfate (1 mL of a 10% solution per liter of water) at the time of collection. Adherence to sample preservation procedures and holding time limits are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.2.1 *Storage Temperature and Handling Conditions:* Ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water from melted ice during transit or storage.

8.2.2 *Holding Time Limitations:* Analyze samples as soon as possible after collection. Drinking water samples should be analyzed within 30 h of collection (Reference 16.13). Do not hold source water samples longer than 6 h between collection and initiation of analyses, and the analyses should be complete within 8 h of sample collection.

9.0 Calibration and Standardization

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits (Reference 16.14).

9.2 Check thermometers at least annually against an NIST-certified thermometer or one traceable to NIST. Check mercury columns for breaks.

10.0 Quality Control (QC)

10.1 Pretest each batch of MI agar or broth for performance (*i.e.*, correct enzyme reactions) with known cultures (*E. coli*, TC, and a non-coliform).

10.2 Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (Reference 16.7).

10.3 Perform specific filtration control tests each time samples are analyzed, and record the results.

10.3.1 *Filter Control*: Place one or more membrane filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the filter(s).

10.3.2 *Phosphate-Buffered Dilution Water Controls*: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the dilution water.

10.3.3 *Agar or Broth Controls*: Place one or more TSA plates and one or more MI agar plates or MI broth pad plates in the incubator for 24 hours at 35°C. Broth pad plates should be incubated *grid-side up*, not inverted like the agar plates. Absence of growth indicates sterility of the plates.

10.4 See recommendations on quality control for microbiological analyses in the "*Manual for the Certification of Laboratories Analyzing Drinking Water*: *Criteria and Procedures; Quality Assurance*" (Reference 16.15) and the *USEPA Microbiology Methods Manual*, part IV, C (Reference 16.6).

11.0 Procedure

11.1 Prepare MI agar or MI broth and TSA as described in Sections 7.5, 7.6, and 7.7. If plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. The crystals that form on MI agar after refrigeration will disappear as the plates warm up (Reference 16.8).

11.2 Label the bottom of the MI agar or MI broth plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar or MI broth sterility control plate(s).

11.3 Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.

11.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.

11.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.

11.6 Shake the sample container vigorously 25 times.

11.7 Measure an appropriate volume (100 mL for drinking water) or dilution of the sample with a sterile pipette or graduated cylinder, and pour it into the funnel. Turn on the vacuum, and leave it on while rinsing the funnel twice with about 30 mL sterile dilution water.

11.8 Remove the funnel from the base of the filter unit. A germicidal ultraviolet (254 nm) light box can be used to hold and sanitize the funnel between filtrations. At least 2 minutes of exposure time is required for funnel decontamination. Protect eyes from UV irradiation with glasses, goggles, or an enclosed UV chamber.

11.9 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the MI agar plate or MI broth pad plate. Slide the filter onto the agar or pad, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar or absorbent pad. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar or pad. Reseat the membrane if non-wetted areas occur due to air bubbles.

11.10 Invert the agar Petri dish, and incubate the plate at 35°C for 24 hours. Pad plates used with MI broth should be incubated grid-side up at 35°C for 24 hours. If loose-lidded plates are used for MI agar or broth, the plates should be placed in a humid chamber.

11.11 Count all blue colonies on each MI plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the E. coli count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete (Reference 16.14).

11.12 Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also E. coli)] (See Figure 1.). Record the data.

11.13 Add any blue, non-fluorescent colonies (if any) found on the same plate to the TC count (Reference 16.8).

12.0 Data Analysis and Calculations

12.1 Use the following general rules to calculate the E. coli or TC per 100 mL of sample:

12.1.1 Select and count filters with # 200 total colonies per plate.

12.1.2 Select and count filter with # 100 target colonies (ideally, 20-80).

12.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^+ (TNTC)" and count the number of E. coli. If both target organisms are $ 200, record the results as "TC^+ EC^+ (TNTC)".

12.1.4 Calculate the final values using the formula:

$$\text{E. coli/100 mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{TC/100 mL} = \frac{\text{Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules (Reference 16.6).

12.3 Report results as E. coli or TC per 100 mL of drinking water.

13.0 Method Performance

13.1 The detection limits of this method are one E. coli and/or one total coliform per sample volume or dilution tested (Reference 16.8).

13.2 The false-positive and false-negative rates for E. coli are both reported to be 4.3% (Reference 16.8).

13.3 The single lab recovery of E. coli is reported (Reference 16.8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (Reference 16.2) and 115% of the R2A spread plate (Reference 16.2). For Klebsiella pneumoniae and Enterobacter aerogenes, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (Reference 16.8), respectively, and 89.3% and 85.8% of the R2A spread plate, respectively.

13.4 The specificities for *E. coli* and total coliforms are reported to be 95.7% and 93.1% (Reference 16.8), respectively.

13.5 The single lab coefficients of variation for *E. coli* and total coliforms are reported to be 25.1% and 17.6% (Reference 16.8), respectively, for a variety of water types.

13.6 In a collaborative study (References 16.4, 16.5, and 16.9), 19 laboratories concurrently analyzed six wastewater-spiked Cincinnati tap water samples, containing 3 different concentrations of *E. coli* (# 10, 11-30, and > 30 per 100 mL).

13.6.1 The single laboratory precision (coefficient of variation), a measure of the repeatability, ranged from 3.3% to 27.3% for *E. coli* and from 2.5% to 5.1% for TC for the six samples tested, while the overall precision (coefficient of variation), a measure of reproducibility, ranged from 8.6% to 40.5% and from 6.9% to 27.7%, respectively. These values are based on $\log_{10}$-transformed data (Reference 16.5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (Reference 16.9) results.

14.0 Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

16.0 References

16.1 American Chemical Society. 1981. Reagent Chemicals. In American Chemical Society Specifications, 6th edition. American Chemical Society, Washington, D.C. For suggestions on the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K. and the United States Pharmacopeia.

16.2 American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition. American Public Health Association, Washington, D.C.

16.3 American Society for Testing and Materials. 1993. Standard Specification for Reagent Water, Designation D1193-91, p. 45-47. In 1993 Annual Book of ASTM Standards: Water and Environmental Technology, Volume 11.01. American Society for Testing and Materials, Philadelphia, PA.

16.4 American Society for Testing and Materials. 1994. Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water, Designation D 2777-86,
p. 31-44. In 1994 Annual Book of ASTM Standards, Section 11: Water and Environmental Technology, Volume 11.01. American Society for Testing and Materials, Philadelphia, PA.

16.5 Association of Official Analytical Chemists. 1989. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis. Journal of the Association of Official Analytical Chemists 72 (4): 694-704.

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17.0 Tables and Figures

Table 1. Statistical Summary of the Collaborative Study Results[1]

Target Organism	Sample Number	*E. coli* Count Category (Range)[2]	Initial n[3]	Final n[4]	S_r[5]	RSD_r[6] (%)	$\bar{\chi}$[7]	S_R[8]	RSD_R[9] (%)	$\frac{RSD_R}{RSD_r}$ Ratio
Escherichia coli	1	Low (≤ 10)	63	63	0.17	27.3	0.64	0.26	40.5	1.49
	2		63	63	0.21	25.0	0.84	0.33	39.0	1.56
	3	Medium (11-30)	63	63	0.10	7.9	1.27	0.15	12.1	1.52
	4		63	60	0.07	5.6	1.32	0.12	9.2	1.65
	5	High (> 30)	63	60	0.06	3.3	1.87	0.16	8.6	2.62
	6		63	63	0.09	4.3	1.99	0.25	12.6	2.91
Total Coliforms	1	Low (≤ 10)	63	63	0.10	4.3	2.35	0.62	26.4	6.11
	2		63	63	0.09	3.8	2.31	0.64	27.7	7.25
	3	Medium (11-30)	63	63	0.11	5.1	2.17	0.47	21.8	4.28
	4		63	57	0.10	3.3	3.07	0.21	6.9	2.08
	5	High (> 30)	63	63	0.15	4.8	3.10	0.43	14.0	2.96
	6		63	63	0.08	2.5	3.14	0.46	14.7	5.97

[1] The values are based on $\log_{10}$ transformed data (Reference 16.5).

[2] The samples were grouped by their *E. coli* count on MI agar into the following categories:
Low (# 10 *E. coli* / 100 mL, samples 1 and 2),
Medium (11-30 *E. coli* / 100 mL, samples 3 and 4), and
High (> 30 *E. coli* / 100 mL, samples 5 and 6).

[3] These values are based on triplicate analyses by each laboratory. The reference laboratory analyzed three sets of samples: the initial and final samples prepared and a sample shipped along with the other 18 lab samples.

[4] These values were obtained after removing outliers by the AOAC procedure (Reference 16.5).

[5] S_r, Single Operator Standard Deviation, a measure of repeatability.

[6] RSD_r, Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability.

[7] $\bar{P}$, The mean of the replicate analyses for all laboratories.

[8] S_R, Overall Standard Deviation, a measure of reproducibility.

[9] RSD_R, Overall Relative Standard Deviation (Coefficient of Variation), a measure of reproducibility.

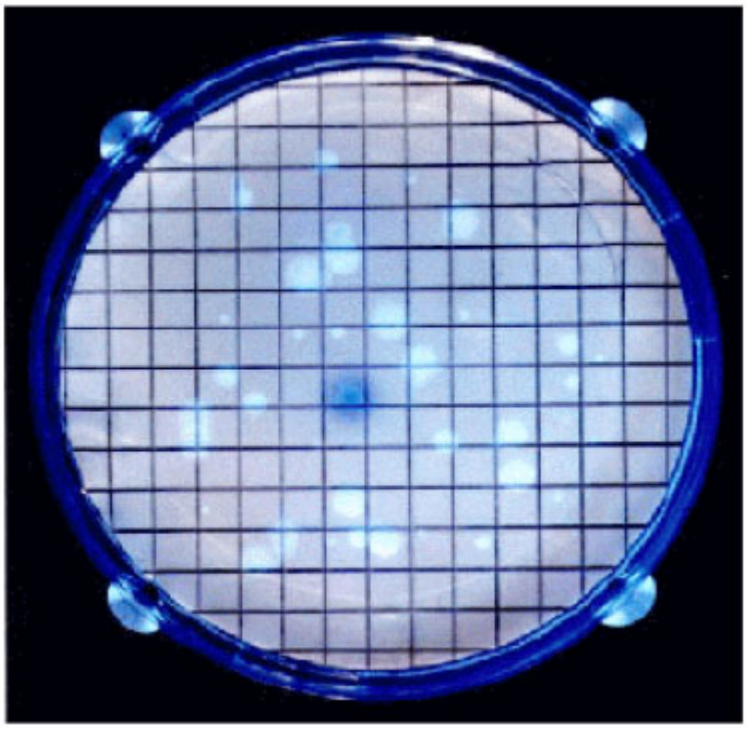


Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked tap water.

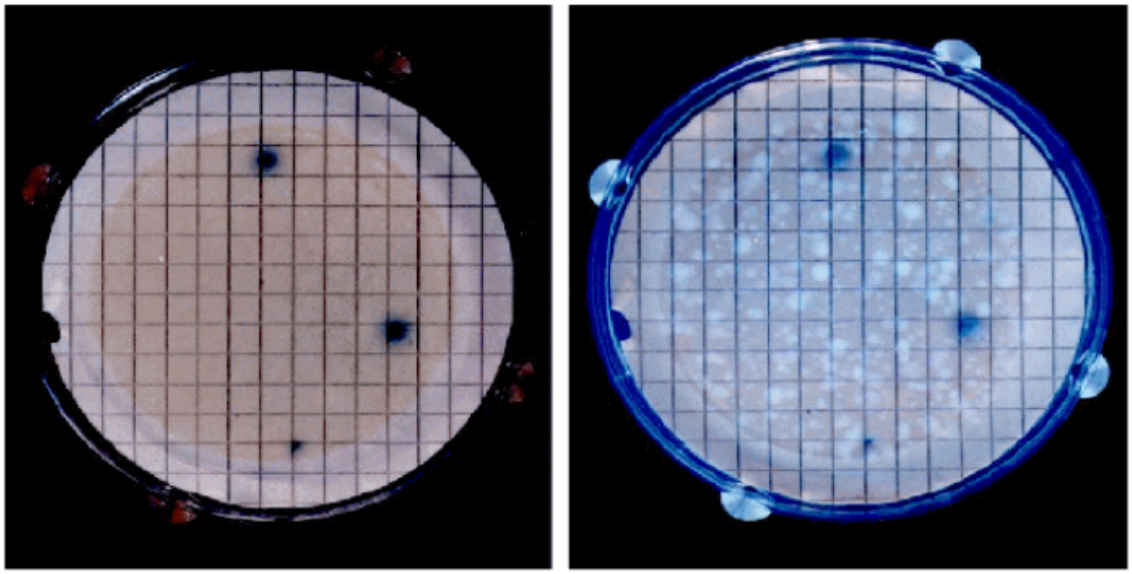


Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other than *E. coli* and non-coliforms are their natural color. Under long wave UV light, all total coliforms, including *E. coli*, are fluorescent, and noncoliforms are non-fluorescent (*i.e.*, they are not visible).

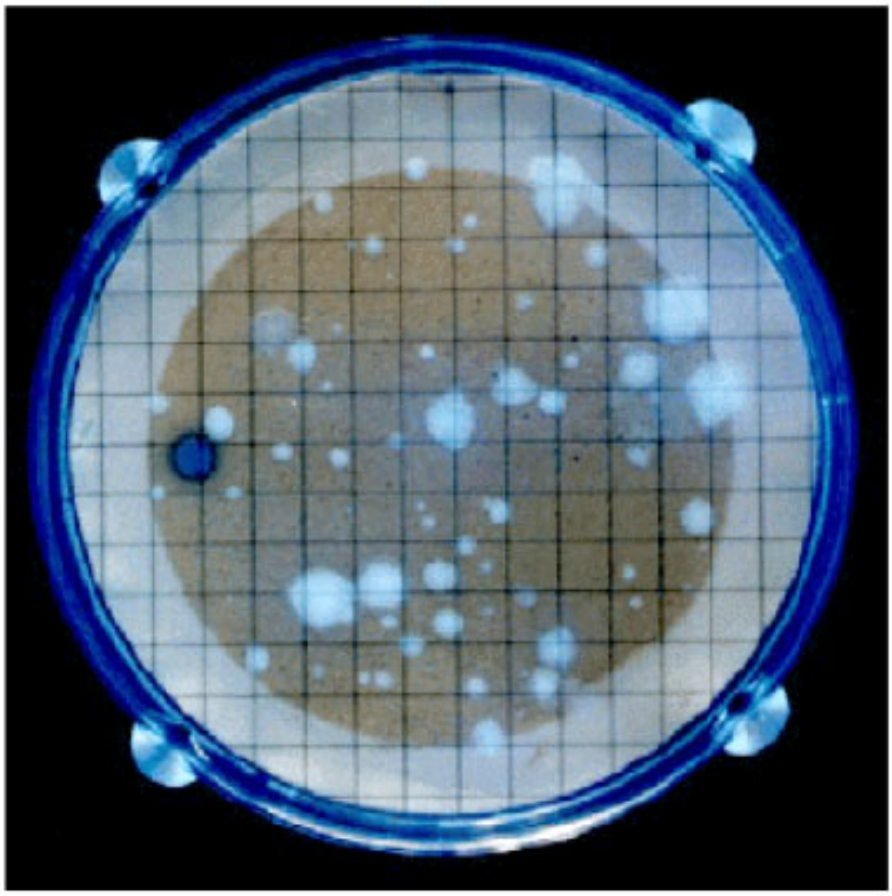
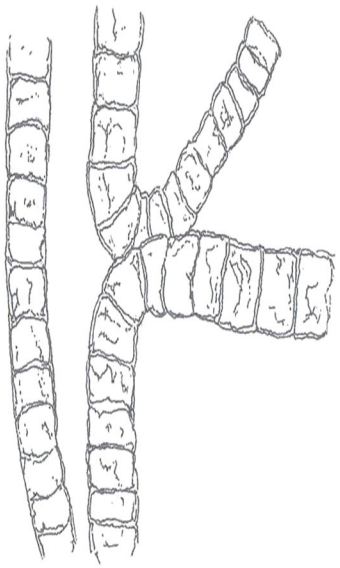
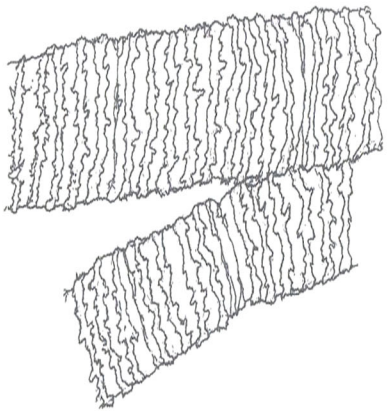


Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked tap water.



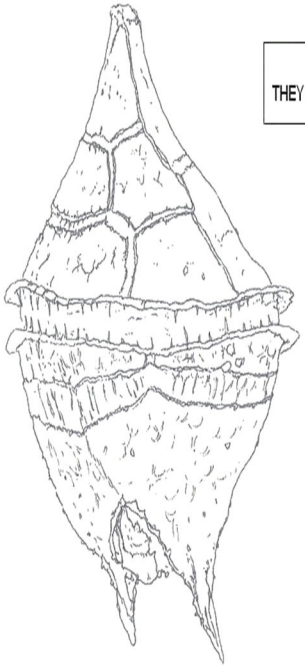
BLUE-GREEN ALGAE

ITS CELLS LACK NUCLEI AND ITS PIGMENT IS SCATTERED.
BLUE-GREEN ALGAE ARE ACTUALLY NOT ALGAE, BUT BACTERIA



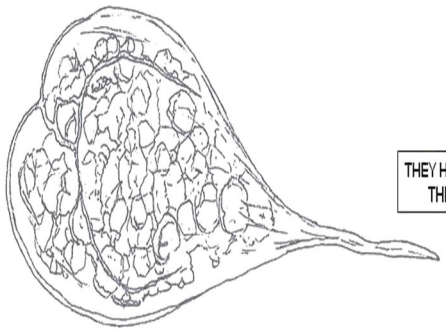
GREEN ALGAE

THEIR CELLS HAVE NUCLEI AND PIGMENT IS DISTINCT.
THEY ARE MOST COMMON ALGAE IN PONDS AND CAN BE MULTICELLULAR



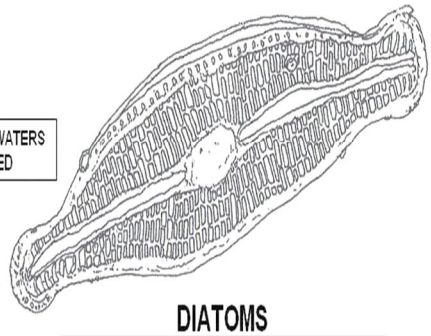
DINOFLAGELLATES

THEY HAVE FLAGELLA AND CAN SWIM IN OPEN WATERS
THEY ARE MICROSCOPIC AND SINGLE-CELLED



EUGLENOIDS

THEY ARE GREEN OR BROWN AND SWIM WITH THEIR FLAGELLUM.
EASY TO SPOT BECAUSE OF THEIR RED EYE. THEY ARE MICROSCOPIC AND SINGLE CELLED



DIATOMS

THEY LOOK LIKE TWO SHELLS THAT FIT TOGETHER.
THEY ARE MICROSCOPIC AND SINGLE CELLED

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

Disclaimer

This method has been validated by the U.S. Environmental Protection Agency through an interlaboratory validation study, and will be proposed for use in drinking water monitoring in the *Federal Register*. This method is not an EPA-approved method until it is promulgated as an approved method in the *Federal Register*.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

Aeromonas is a common genus of bacteria indigenous to surface waters, and may be found in non-chlorinated or low-flow parts of chlorinated water distribution systems. Monitoring their presence in distribution systems is desirable because some aeromonads may be pathogenic and pose a potential human health risk. Method 1605 describes a membrane filtration technique for the detection and enumeration of *Aeromonas* species. This method uses a selective medium that partially inhibits the growth of non-target bacterial species while allowing most species of *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation and the presence of yellow colonies on ampicillin-dextrin agar medium with vancomycin (ADA-V). Yellow colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Safe Drinking Water Act.

Questions concerning this method or its application should be addressed to:

Mary Ann Feige
U.S. EPA Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
26 West Martin Luther King Drive
Cincinnati, OH 45268-1320

Method 1605: *Aeromonas* in Finished Water by Membrane
Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Aeromonas* species in finished water samples. *Aeromonas* is a common genus of bacteria indigenous to surface waters. Its numbers are more likely to be greater during periods of warmer weather and when increased concentrations of organic nutrients are present. It is also more likely to be found in non-chlorinated water distribution systems or low-flow parts of chlorinated systems. Some *Aeromonas* species are opportunistic pathogens.

1.2 This method is adapted from Havelaar et al. (1987) for the enumeration of *Aeromonas* species in finished water by membrane filtration (Reference 15.1). It is a quantitative assay that uses a selective medium which partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation producing yellow colonies. Presumptively positive colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

1.3 This method is designed to meet the finished water monitoring requirements of the U.S. Environmental Protection Agency. *Aeromonas* was included on the Contaminant Candidate List (CCL) (Mar. 2, 1998, 63 *FR* 10274) and in the Revisions to the Unregulated Contaminant Monitoring Proposed Rule (UCMR) (September 17, 1999, 64 FR 50556). Contaminants listed in the UCMR are candidates for future regulation and may be included in a monitoring program for unregulated contaminants. Unregulated contaminant monitoring would be required for large systems and a representative sample of small and medium sized water distribution systems.

1.4 This method was subjected to an interlaboratory validation study involving 11 laboratories and 11 finished drinking water matrices. This method was not validated for other water types. Use of this method and appropriate validation for other water types is the responsibility of the user.

2.0 Summary of Method

2.1 The method provides a direct count of *Aeromonas* species in water based on the growth of yellow colonies on the surface of the membrane filter using a selective medium. A water sample is filtered through 0.45-Fm-pore-size membrane filter. The filter is placed on ampicillin-dextrin agar with vancomycin (ADA-V) and incubated at 35EC ± 0.5EC for 24 ± 2 hours. This medium uses ampicillin and vancomycin to inhibit non-*Aeromonas* species, while allowing most *Aeromonas* species to grow. The medium uses dextrin as a fermentable carbohydrate, and bromothymol blue as an indicator of acidity produced by the fermentation of dextrin. Presumptively identified yellow colonies are counted and confirmed by testing for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose and produce indole.

2.2 The membrane filtration procedure provides a direct count of culturable Aeromonas in water samples that is based on the growth of bacterial colonies on the surface of the membrane filter placed on a selective medium.

2.3 Aeromonas isolates may be archived for further analysis to determine species or hybridization group by inoculating a nutrient agar slant for short term use or shipping, or nutrient broth for freezing.

3.0 Definitions

3.1 Aeromonas are bacteria that are facultative anaerobes, Gram-negative, oxidase-positive, polarly flagellated, and rod shaped. They are classified as members of the family Aeromonadaceae. Demarta et al. (1999) reported 15 Aeromonas species based on 16S rDNA sequences though not all are officially recognized. Some species have been associated with human disease. In this method, Aeromonas are those bacteria that grow on ampicillin-dextrin agar with vancomycin (ADA-V), produce yellow colonies, are oxidase-positive, and have the ability to ferment trehalose and produce indole.

3.2 Definitions for other terms are provided in the glossary at the end of the method (Section 17.3).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material may clog the membrane filter and prevent filtration or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.2 Other ampicillin/vancomycin resistant bacteria that are not aeromonads may be able to grow on this medium. Some of these bacteria may also produce yellow colonies if they are able to produce acid byproducts from the fermentation of dextrin or some other media component, or if they produce a yellow pigment. Enterococcus are reported to produce pinpoint-size yellow colonies on ADA. Confirmation of presumptive Aeromonas colonies is necessary to mitigate false positives.

5.0 Safety

5.1 Some strains of Aeromonas are opportunistic pathogens. Sample containers and waste materials should be autoclaved prior to cleaning or disposal.

5.2 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and other materials.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Safety Data Sheets (SDS) (Formerly Material Safety Data Sheets) (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Equipment for collection and transport of samples to laboratory

- **6.1.1** Autoclavable sample container—Use sterile, non-toxic, glass or plastic containers with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis.
- **6.1.2** Ice chest
- **6.1.3** Ice packs

6.2 Autoclavable dilution bottles—125-mL marked at 99 mL or 90 mL; commercially produced dilution bottles may be used.

6.3 Rinse water bottles

6.4 Sterile plastic or autoclavable glass pipettes with a 2.5% tolerance—to deliver (TD), 1- and 10-mL
6.5 Pipette bulbs or automatic pipetter.
6.6 Autoclavable pipette container (if using glass pipettes).
6.7 Thermometer—with 0.5EC gradations checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
6.8 Inoculating loop—Sterile metal, plastic, or wooden applicator sticks.
6.9 Burner—Flame or electric incinerator for sterilizing metal inoculating loops and forceps.
6.10 Colony counting device—Mechanical, electric or hand tally.
6.11 Hotplate stirrer
6.12 Magnetic stir bar
6.13 Graduated cylinders—100 mL, 500 mL and 1 L, sterile, polypropylene or glass
6.14 Balance—Capable of weighing samples up to 200 g, with a readability of 0.1 g
6.15 Weigh boats
6.16 pH meter
6.17 Turbidimeter (optional)
6.18 Equipment for membrane filter procedure
6.18.1 Incubator—Hot air or water-jacketed microbiological type to maintain a temperature of 35EC ± 0.5EC
6.18.2 Petri dishes—sterile, 50 × 9 mm or other appropriate size
6.18.3 Membrane filtration units (filter base and funnel made of glass, plastic, or stainless steel), wrapped with aluminum foil or Kraft paper, and sterilized by autoclaving.
6.18.4 Vacuum source
6.18.5 Flasks—1-L vacuum filter with appropriate tubing; a filter manifold to hold a number of units is optional
6.18.6 Side-arm flask to place between vacuum source and filtration devices or filter manifold
6.18.7 Membrane filters—Sterile, cellulose ester, white, gridded, 47-mm-diameter with 0.45-Fm pore size (Gelman E04WG04700 or equivalent)
6.18.8 Forceps—Sterile, straight or curved, with smooth tips to handle filters without causing damage
6.18.9 Ethanol or other alcohol in a container to sterilize forceps
6.18.10 Test tubes—125 × 16 mm sterile, screw-cap tube
6.19 Dissecting microscope—Low power (10X to 15X), binocular, illuminated
6.20 Autoclave—Capable of 121EC at 15 psi. Must meet requirements set forth in the Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition. (Reference 15.5)
6.21 Membrane filters (for sterilization purposes)—Sterile with 0.22-Fm pore size (Gelman Acrodisc No. 4192 or equivalent)

7.0 Reagents and Standards
7.1 Purity of reagents and culture media—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents and culture media shall conform to the specifications in Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9050 (Reference 15.2). The agar used in preparation of culture media must be of microbiological grade.

7.2 Purity of water—Reagent-grade water conforming to specifications in Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition (Reference 15.5) or Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9020 (Reference 15.2).

7.3 Phosphate buffered dilution water

7.3.1 Concentrated stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water. Adjust the pH to 7.2 ± 0.5 with 1N sodium hydroxide (NaOH) and dilute to 1 L with reagent-grade water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.2 Magnesium chloride solution—Dissolve 81.1 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$) in reagent-grade water and dilute to 1 L. Autoclave or filter sterilize through a 0.22-Fm-pore-size filter.

7.3.3 Prepare phosphate buffered dilution water by adding 1.25 mL of concentrated stock phosphate buffer solution (Section 7.3.1) and 5.0 mL of magnesium chloride solution (Section 7.3.2) to a 1-L graduated cylinder and adjust final volume to 1 L with reagent-grade water. Prepare a portion of buffered dilution water in 1-L bottles for rinse water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.4 Stored phosphate buffered dilution water should be free from turbidity.

7.4 Ampicillin-dextrin agar with vancomycin (ADA-V)

7.4.1 Preparation of dextrin agar—EPA highly recommends the use of commercial ADA (m-Aeromonas Selective Agar Base [Havelaar]), Section **7.4.1.1**. However, ADA may be prepared by the laboratory (Section 7.4.1.2)

7.4.1.1 Commercial dextrin agar—Tech Pac (distributor, tech@fuse.net), Cincinnati, Ohio; Biolife (www.biolifeit.com) Italiana Srl, 272 Viale Monza, Milan, Italy, Cat. No. 401019 or equivalent. Prepare 1-L of media, according to manufacturer's instructions. Cool to room temperature, and adjust pH to 8.0 using 1N NaOH or 1N HCl. Autoclave for 15 min, cool to 50EC.

7.4.1.2 Laboratory-prepared dextrin agar.

7.4.1.2.1 5.0 g tryptose—Difco cat. no. 0124-17, or equivalent

7.4.1.2.2 11.4 g dextrin—Difco cat. no. 0161-17, or equivalent

7.4.1.2.3 2.0 g yeast extract—Difco cat. no. 0127-17, or equivalent

7.4.1.2.4 3.0 g sodium chloride (NaCl)—Baker cat. no. 3624, or equivalent

7.4.1.2.5 2.0 g potassium chloride (KCl)—Fisher cat. no. P217-500, or equivalent

7.4.1.2.6 0.1 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)—Fishercat. no. M63-500, or equivalent

7.4.1.2.7 0.06 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)—Sigma cat. no. F-2877, or equivalent

7.4.1.2.8 0.08 g bromothymol blue—Baker cat. no. D470, or equivalent

7.4.1.2.9 Sodium deoxycholate—Sigma cat. no. D-6750, or equivalent. Add 100 mg of sodium deoxycholate to 10 mL of reagent water.

7.4.1.2.10 13.0 g agar, bacteriological grade—Fisher cat. no. BP1423-500, or equivalent.

7.4.1.2.11 Add reagents in Sections 7.4.1.2.1 through 7.4.1.2.8 to 1-L of reagent-grade water, stir to dissolve and adjust pH to 8.0 using1N NaOH or 1N HCl. After the pH has been adjusted, add sodium deoxycholate (Section 7.4.1.2.9) and agar (Section7.4.1.2.10) and heat to dissolve. Autoclave for 15 min, cool to 50°C.

7.4.2 Ampicillin, sodium salt—Sigma cat. no. A0166, or equivalent. Add 10 mg of ampicillin, sodium salt to 10 mL reagent water. Prepare on the same day that medium is prepared and filter sterilize through a 0.22-Fm-pore-size filter. Alternatively, use Biolife cat. no. 4240012 prepared according to manufacturer's instructions, taking care to use an appropriate amount of ampicillin for the volume of media being prepared (for example, use two vials for a 1-L batch of ADA-V). Follow manufacturer's instructions for appropriate storage temperature and shelf-life. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.3 Vancomycin hydrochloride—Sigma cat. no. V2002, or equivalent. Add 2 mg of vancomycin hydrochloride to 10 mL of reagent water. Filter sterilize through a 0.22-Fm-pore-size filter. Follow manufacturer's instructions for appropriate storage temperature and time. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.4 After dextrin agar (Section 7.4.1) has been autoclaved and cooled to 50EC, add the sterile ampicillin (Section 7.4.2) and sterile vancomycin hydrochloride solutions (Section 7.4.3).

7.4.5 Add approximately 5 mL of ADA-V per 50 × 9 mm Petri dish and allow to solidify. For larger plates, adjust volume appropriately. ADA-V plates should be stored in a tight fitting container (i.e. sealed plastic bag) at a temperature of 1EC to 5EC for no longer than 14 days.

7.5 Pentahydrate ACS Reagent grade sodium thiosulfate—Fisher cat. no. S445-500, or equivalent. Prepare a 3% stock solution by adding 3 g sodium thiosulfate to 100 mL reagent-grade water.

7.6 Disodium salt of ethylenediaminetetraacetic acid (EDTA)—Sigma cat. no. E 4884, or equivalent. EDTA should only be added to samples if metals in water samples exceed 1.0 mg/L. To prepare stock solution, add 12.4 g EDTA to 80 mL of reagent-grade water. Adjust pH to 8.0 using 10N NaOH. After the pH has been adjusted, bring the volume up to 100 mL with reagent-grade water.

7.7 Positive control culture—Aeromonas hydrophila ATCC #7966; obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, 20110-2209; http://www.atcc.org).

7.8 Negative culture control—Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-Aeromonas bacteria that may grow on ADA-V and to ensure that confirmation test results are appropriate. E. coli (ATCC #25922) is the negative culture control for oxidase, Pseudomonas aeruginosa (ATCC #27853) is the negative culture control for trehalose fermentation, and Bacillus cereus (ATCC #11778) is the negative culture control for indole.

7.9 Nutrient agar—Difco cat. no. 0001-17 or equivalent. Prepare according to manufacturers instructions.

7.10 Oxidase reagents—Dry Slide BBL cat. no. 231746 or equivalent.

7.11 0.5% Trehalose confirmation reagent

7.11.1 Add 5 g trehalose (Sigma cat. no. T0167, or equivalent) to 100 mL water and filter sterilize solution through a filter with 0.22-Fm-pore-size.

7.11.2 Prepare 900 mL purple broth base (Difco cat. no. 0222-17, or equivalent) according to manufacturer's instructions and autoclave.

7.11.3 Aseptically add 100 mL trehalose solution to the cooled 900 mL of purple broth base.

7.11.4 Dispense into 6 mL or larger size tubes and fill approximately half full. Store in refrigerator.

Note: *Alternatively, prepare purple broth base according to manufacturers instructions, add 5 g trehalose per liter, and filter sterilize through a filter with 0.22-*F*m-pore-size.*

7.12 Tryptone broth—Oxoid cat. no. CM0087B, or equivalent. Alternatively, the laboratory may prepare tryptone broth by adding 10 g of tryptone (Difco cat. no. 0123-17 or equivalent) and 5 g of NaCl to 1 L of reagent water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.13 Kovac's reagent—Biomeriuex cat. no. V7050, or equivalent

8.0 Sample Collection, Preservation, and Storage

8.1 Adherence to sample preservation procedures and holding time limits specified in *Standard Methods for the examination of Water and Wastewater* (Reference 15.2) is critical to the production of valid data. Sample results will be considered invalid if those conditions are not met.

8.2 Preparation of sample bottles and sample collection—Samples must be representative of the drinking water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

8.2.1 Use sterile, non-toxic, glass or plastic container (Section 6.1.1) with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis. Sampling procedures are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9060 (Reference 15.2).

8.2.2 Add 1 mL of 3% sodium thiosulfate stock (Section 7.5) per L of sample to sample bottles prior to autoclave sterilization. Alternatively, if using presterilized sample bottles, sodium thiosulfate should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size before adding to the sample bottles.

8.2.3 If metals in the sample exceed 1.0 mg/L, add 3 mL of EDTA stock solution (Section 7.6) per L of sample to sample bottles prior to autoclave sterilization. If using presterilized sample bottles, EDTA should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size.

8.2.4 Collect a minimum of 1-L of sample.

8.3 Sample preservation and handling

8.3.1 Immediately following sample collection, tighten the sample container lid(s) and place the sample container(s) upright in an insulated, plastic-lined storage cooler with ice packs or in a refrigerator to chill prior to packing the cooler for shipment. Do not freeze the sample.

8.3.2 Use enough solidly frozen ice packs to ensure that the samples will arrive at a temperature of 1°C to 10°C. Use a minimum of two ice packs per shipment and add extra ice packs for multiple samples. Place one or more ice packs on each side of the container to stabilize samples.

8.3.3 Samples must be maintained at a temperature of 1°C to 10°C during shipment. Samples must not be frozen.

Note: Sample temperature during shipment is critical. Ice packs must be frozen solid immediately prior to shipment.

8.4 Verify and record sample arrival temperature when received in the laboratory. Refrigerate samples at 1°C to 5°C upon receipt at the laboratory and analyze as soon as possible after collection. Samples must be analyzed within 30 hours of sample collection.

9.0 Quality Control

9.1 Each laboratory that uses Method 1605 is required to operate a formal quality assurance (QA) program. The minimum QA requirements consist of the initial demonstration of capability (IDC) test (Section 9.4), ongoing analysis of spiked reagent water (ODC test, Section 9.8) and spiked finished drinking water samples (MS/MSD, Section 9.7), and analysis of negative culture controls (Section 9.6), dilution/rinse water blanks (Section 9.5), and media sterility checks (Section 9.2.6) as tests of continued acceptable performance. Spiked sample results are compared to acceptance criteria for precision, which are based on data generated during the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished water matrices. The more stringent QA requirements in this method, relative to other, currently used methods for bacterial determination, are an effort to improve overall microbiological QA. The specifications contained in this method can be met if the analytical system is maintained under control. Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections 10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

9.2 General QC requirements—Specific quality control (QC) requirements for Method 1605 are provided below. QA and QC criteria for facilities, personnel, and laboratory equipment, instrumentation, and supplies used in microbiological analyses must be followed according to Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141, Reference 15.2) and the U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fourth Edition (March 1997) (Reference 15.5).

9.2.1 Initial demonstration of capability (IDC). The laboratory shall demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples. The procedure for performing the IDC is described in Section 9.4. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.2.2 Dilution/rinse water blanks. The laboratory shall analyze dilution/rinse water blanks to demonstrate freedom from contamination. The procedures for analysis of dilution/rinse water blanks are described in Section 9.5. At a minimum, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series.

9.2.3 Negative culture controls. The laboratory shall analyze negative culture controls (Section 9.6) to ensure that ADA-V and the confirmation procedures are performing properly. Negative culture controls should be run whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples.
9.2.4 Matrix spike/matix spike duplicate (MS/MSD). The laboratory shall analyze one set of MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples (Section 9.7). Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines. When possible, MS/MSD analyses should be conducted on the same day as ODC samples, using the same spiking procedure and volume.
9.2.4.1 Precision. MS/MSD sample results should meet the precision criteria set forth in Section 12, Table 1.
9.2.4.2 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the number of Aeromonas in the spike is unknown. However, each laboratory should control chart the mean number of Aeromonas per MS/MSD set (adjusted for background) and maintain a record of spike preparation procedures and spike volume. The laboratory should compare number of Aeromonas in MS/MSD samples to results of ODC samples (Section 9.2.5 and 9.8) spiked on the same day. This comparison should help the laboratory recognize when a matrix is interfering with method recovery. If the laboratory observes consistent ODC results from week to week, control charting the MS/MSD results by source may also help to recognize fluctuations in recovery from a particular source.

9.2.5 Ongoing demonstration of capability (ODC). The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate, Section 9.8).
9.2.5.1 Frequency. The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently. No more than one set of ODC samples is required per day, provided that the same equipment (i.e., incubators) are being used for all the samples.
9.2.5.2 Precision. ODC sample results must meet the precision criteria set forth in Section 12, Table 1.
9.2.5.3 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the initial spike dose for ODC samples is unknown.
As a result, each laboratory should control chart the mean number of Aeromonas per ODC sample set and maintain a record of spike preparation procedures and ODC spike volume. Maintaining this information will enable the laboratory to recognize when problems arise. Example: A laboratory that prepares spiking suspensions according to Section 9.3, spikes QC samples with 5 mL of dilution D2, and typically recovers approximately 50 Aeromonas per sample, and maintains a control chart of these counts. If the laboratory continues to prepare spiking suspensions the same way, but the number of Aeromonas counted declines noticeably (e.g. 20 Aeromonas per sample), then there may be a problem with the media, reagents, or the spiking suspension.
9.2.6 Media sterility checks. The laboratory shall test media sterility by incubating one unit (tube or plate) from each batch of medium (ADA-V, nutrient agar slant, nutrient agar, streak plate, trehalose, and tryptone) at 35°C ± 0.5°C for 24 ± 2 hours and observing for growth.

9.2.7 Analyst colony counting variability. If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one ODC sample per month (Section 9.9), at a minimum.

9.2.8 Record maintenance. The laboratory shall maintain records to define the quality of data that are generated. The laboratory shall maintain a record of the date and results of all QC sample analyses described in Section 9.2. A record of media sterility check, dilution/rinse water blank, analyst counting variability, IDC, ODC, and MS/MSD sample results must be maintained. Laboratories shall maintain reagent and material lot numbers along with samples analyzed using each of the lots. Laboratories shall also maintain media preparation records.

9.2.9 Performance studies. The laboratory should periodically analyze external QC samples, such as performance evaluation (PE) samples, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

9.2.10 Autoclave sterilization verification. At a minimum, the laboratory shall verify autoclave sterilization according to the procedure in Section 9.10 on a monthly basis.

9.2.11 Culture maintenance. The laboratory should use 24 ± 2 hour-old nutrient agar slant cultures for preparation of IDC, ODC, and MS/MSD spiking suspension dilutions. The laboratory should use 22 to 72 hour-old nutrient agar slant cultures to inoculate ADA-V streak plates for analysis of negative culture controls. With regard to the preparation of subcultures, it is recommended that a maximum of three passages be prepared to help avoid contamination. After three passages, start a new subculture from the frozen stock.

9.2.12 Method modification.

9.2.12.1 Membrane filtration. Because recovery criteria are not available for this method, laboratories are not permitted to modify the membrane filtration procedures (Section 10.1 through Section 10.10.) or ADA-V media.

9.2.12.2 Confirmation procedures. The confirmation procedures in Section 10.11 may be modified, provided that the laboratory demonstrates the ability to generate acceptable performance by performing an IDC test (Section 9.2.1) and the appropriate negative culture control test(s) (Section 9.2.3) before analyzing any field samples using the modified confirmation. 100% of the colonies submitted to confirmation from IDC and negative culture control samples must give the appropriate confirmation response. These tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2) and media sterility checks (Section 9.2.6).

9.3 Preparation of Aeromonas spiking suspension for use in spiking IDC, ODC, and MS/MSD samples—This dilution scheme is adapted from Standard Methods for the Examination of Water and Wastewater, 19th Edition, Section 9020 B (Reference 15.9). This entire process should be performed quickly to avoid loss of viable organisms. See Section 16, Flowchart 1, for an example of this dilution scheme. Please note: Provided that all QC acceptance criteria are met and the recommended target range of 20 - 60 CFU per plate are typically observed, laboratories may prepare QC spiking suspensions using commercial products or other procedures such as growing bacteria in a broth, measuring optical density, and spiking each test sample with an equivalent volume.

9.3.1 Inoculate Aeromonas hydrophila (ATCC #7966) onto the entire surface of several nutrient agar slants with a slope approximately 6.3 cm long in a 125 × 16 mm screw-cap tube. Incubate for 24 ± 2 hours at 35°C ± 0.5°C.

9.3.2 From the slant that has the best growth, prepare serial dilutions using four dilution bottles with 99 mL of sterile buffered dilution water (A, B, C and D below in Sections 9.3.3 and 9.3.4) and one dilution bottle containing 90-mL of sterile buffered dilution water (D2 below in Section 9.3.5).

9.3.3 Pipette 1 mL of buffered dilution water from bottle "A" to one of the slants. Emulsify the growth on the slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar. Pipette the suspension back into dilution bottle "A." Repeat this procedure a second time to remove any remaining growth on the agar slant, without disturbing the agar.

9.3.4 Make serial dilutions as follows:

9.3.4.1 Shake bottle "A" vigorously and pipette 1 mL to bottle "B"

9.3.4.2 Shake bottle "B" vigorously and pipette 1 mL to bottle "C"

9.3.4.3 Shake bottle "C" vigorously and pipette 1 mL to bottle "D"

9.3.4.4 Shake bottle "D" vigorously and pipette 10 mL to bottle "D2"; this should result in a final dilution of approximately 10 CFU / mL.

9.3.5 Filter 1- to 5-mL portions in triplicate from bottles "D" and "D2" according to the procedure in Section 10 to determine the number of CFU in the dilutions. The recommended target dilution and spike volume is one that produces 20 to 60 colonies per ADA-V plate. (It may be difficult to count plates with more than 60 colonies due to crowding.) Dilutions should be stored at 1EC to 5EC and may be used throughout the day they are prepared. However, it should be noted that the QC acceptance criteria were established using dilutions that were prepared immediately prior to spiking samples.

9.3.6 Analysts may practice the dilution scheme by placing filters on nutrient agar plates instead of ADA-V plates. After a growth pattern is determined and the analyst can accurately determine the target concentrations, dilutions from Section 9.3.5 may be used for spiking IDC, ODC, and MS/MSD samples. However, multiple dilutions should be analyzed in replicate when new cultures are received from an outside source to ensure that the analyst can accurately spike target concentrations.

Note: If it is more convenient for your laboratory, an acceptable alternative to the dilution scheme presented in Section 9.3, is to pipette 11 mL of dilution D into a dilution bottle D2, which contains 99 mL of dilution water. There should be approximately 10^{10} Aeromonas hydrophila CFU per slant. Therefore, dilution bottles "A" through "D2" should contain approximately 10^{10}, 10^{8}, 10^{6}, 10^{4}, and 10^{3} CFU per dilution bottle, respectively. Depending on the growing conditions, these numbers may vary. As a result, until experience has been gained, more dilutions may need to be filtered to determine the appropriate dilution.

9.4 Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.4.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.4.2 For each of the four IDC test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.
9.4.3 Process IDC test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per IDC test sample to the confirmation procedures in Section 10.11.
9.4.4 Using all four IDC sample results, compute the relative standard deviation (RSD) of Aeromonas CFU per 100 mL. (See glossary for definition of RSD.) Compare the RSD with the corresponding limits for IDC (Section 12). If the RSD meets the acceptance criteria, the system performance is acceptable and analysis of samples may begin. If the RSD falls outside the range, system performance is unacceptable. In this event, identify and correct the problem and repeat the test.
9.5 Dilution/rinse water blanks—On an ongoing basis, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series. For example, if a laboratory plans to run 30 samples during a filtration series, a dilution/rinse water blank should be processed at the beginning, middle, and end of the filtration series.
9.5.1 Process 100-mL dilution/rinse water blanks according to the procedures in Section 10, as appropriate.
9.5.2 No growth should appear in dilution/rinse water blanks. If growth appears, prepare new dilution/rinse water and reanalyze a 100-mL dilution/rinse water blank. If colonies are present after analyzing the new dilution/rinse water, assess laboratory technique and reagents. If growth in dilution/rinse water blank(s) is presumptively positive, all associated sample results should be discarded and sources re-sampled immediately.

9.6 Negative culture controls— Negative controls should be run whenever a new batch of medium or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples. Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate. *E. coli* is (ATCC #25922) the negative culture control for oxidase, *Pseudomonas aeruginosa* (ATCC #27853) is the negative culture control for trehalose fermentation, and *Bacillus cereus* (ATCC #11778) is the negative culture control for indole.
9.6.1 Using pure cultures obtained from a qualified outside source (Sections 7.7 and 7.8), inoculate negative culture controls onto nutrient agar slants and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Alternatively, nutrient agar slants may be inoculated up to 72 hours in advance. If nutrient agar slants will be incubated for more than 24 ± 2 hours, consider incubation at room temperature to ensure that the slants do not dry out prior to use.

9.6.2 For each negative culture control, place a membrane filter on an ADA-V plate, streak onto the filter, taking care not to break the filter, and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Streaking on a filter will give the laboratory a more realistic example of the appearance of these organisms in field samples. Although not recommended, laboratories may streak directly onto the ADA-V (without the filter).

9.6.3 For each ADA-V negative culture control plate, pick a single colony, streak the colony onto a plate of nutrient agar medium (Section 7.9), and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies. *Please note: Bacillus cereus typically grows only at the point of inoculation on ADA-V or not at all. If Bacillus cereus did not grow on the ADA-V plate, inoculate the streak plate from the nutrient agar slant that was originally used to inoculate the ADA-V plate.*

9.6.4 Negative culture control confirmation procedures

9.6.4.1 Oxidase negative culture control—From the streak plate, submit a single *E. coli* colony to the oxidase confirmation procedure described in Section 10.11.

9.6.4.2 Trehalose negative culture control—From the streak plate, submit a single *Pseudomonas aeruginosa* colony to the trehalose confirmation procedure described in Section 10.11.

9.6.4.3 Indole negative culture control—From the streak plate, submit a single *Bacillus cereus* colony to the indole confirmation procedure described in Section 10.11.

9.6.5 If any of the negative culture controls result in a positive confirmation, prepare, check and/or replace the associated media, reagents, and/or respective control organism and reanalyze the appropriate negative culture control(s). All presumptively positive colonies that have been archived from field samples (10 per sample) should be confirmed using media/reagents that exhibit the appropriate negative culture control response.

9.7 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall analyze MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples. Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines.

9.7.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Sections 9.3.1 through 9.3.4.

9.7.2 For each of the 500-mL MS and MSD test samples, spike enough volume of the appropriate dilution to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.7.3 Process MS/MSD test samples and an unspiked finished drinking water sample according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. (If the filter clogs during filtration, follow the instructions in Section 10, making sure to filter the same volume for both the MS and MSD. The same QC acceptance criteria apply.) Submit 10 colonies per IDC test sample to the confirmation procedures in Section 10.11.

Note: If results exceed the optimum range because of “background” target colonies (as indicated by the results of the unspiked matrix sample), the MS/MSD should be repeated and a smaller volume of sample, for example 200-mL, should be spiked.

9.7.4 For the MS and MSD test samples, calculate the number of confirmed Aeromonas CFU per 100 mL according to Section 11 and adjust based on any background Aeromonas observed in the unspiked sample.

9.7.5 Calculate the relative percent difference (RPD) using the following equation:

$$RPD = 100 \frac{| X_{MS} - X_{MSD} |}{X_{mean}}$$

where **RPD** is the relative percent difference

$\mathbf{X_{MS}}$ is the number of confirmed Aeromonas per 100 mL in the MS sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)

$\mathbf{X_{MSD}}$ is the number of confirmed Aeromonas per 100 mL in the MSD sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)

$\mathbf{X_{mean}}$ is the mean number of confirmed Aeromonas per 100 mL in the MS and MSD

9.7.6 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of finished water samples from this source may continue. If the MS/MSD results are unacceptable and the ODC sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. If the MS/MSD results are unacceptable, all associated field data should be flagged.

9.8 Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate). The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently.

9.8.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.8.2 For each of the 500-mL positive control (PC) and positive control duplicate (PC/PCD) test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.8.3 Process PC/PCD test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per PC/PCD test sample to the confirmation procedures in Section 10.11.

9.8.4 Calculate the relative percent difference (RPD) using the following equation:

$$PRD = 100 \frac{| X - X_{PCD} |}{X_{mean}}$$

where
RPD is the relative percent difference
X_{PC} is the number of confirmed Aeromonas per 100 mL in the PC sample
X_{PCD} is the number of confirmed Aeromonas per 100 mL in the PCD sampleX_{mean} is the mean number of confirmed Aeromonas per 100 mL in the PC and PCD samples

9.8.5 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of samples may continue. If RPD falls outside the range, system performance is unacceptable. Identify and correct the problem and perform another ODC test before continuing with the analysis of field samples.
9.8.6 As part of the QA program for the laboratory, method precision for ODC samples should be charted and records retained.

9.9 Analyst colony counting variability—If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one positive field sample per month. Compare each analyst's count of the target colonies. Counts should fall within 10% between analysts. If counts fail to fall within 10% of each other, analysts should perform additional sets of counts, until the number of target colonies counted fall within 10% between analysts for at least three consecutive samples. If there are no positive samples, an MS, MSD, or ODC sample can be used for this determination (MS or MSD are preferable to ODC samples, since they may have other background growth).

9.10 Autoclave sterilization verification—Verify autoclave sterilization monthly by placing Bacillus stearothermophilus spore suspensions or strips inside glassware. Sterilize at 121°C for 15 minutes. Place in trypticase soy broth tubes and incubate at 55°C for 48 hours. Check for growth to verify that sterilization was adequate. If sterilization was inadequate, determine appropriate time for autoclave sterilization. Filter sterilization may be used provided that these same QC steps are instituted for the filtrate.

10.0 Procedure
10.1 The membrane filter (MF) procedure with ampicillin-dextrin agar with vancomycin (ADA-V) is used to enumerate Aeromonas in finished waters.
10.2 Label each Petri dish with sample identification, preparation date, and analysis start date/time.
10.3 Use a sterile MF unit assembly (Section 6.18.3) at the beginning of each filtration series. The laboratory must sanitize each MF unit between filtrations by using a UV sanitizer, flowing steam, or boiling water for 2 min. A filtration series ends when 30 minutes or more elapse between sample filtrations.

10.4 Sterilize forceps with alcohol. Flame off excess alcohol. Using sterile forceps, place the MF (grid side up) over the sterilized funnel. Carefully place the top half of the filtration unit over the funnel and lock it in place.
10.5 Shake the sample bottle vigorously approximately 25 times to distribute the bacteria uniformly. Using aseptic technique, transfer one, 500-mL aliquot of sample to a single funnel. Use a graduated cylinder with a “to deliver” tolerance of approximately 2.5%.
Note: Laboratories must filter the entire 500-mL sample volume unless the filter clogs. If the filter clogs, a minimum of 100 mL of sample must be filtered, which may require multiple filtrations. If less than 500 mL are filtered and analyzed due to filter clogging, measure the residual, unfiltered volume to determine the volume filtered, and adjust the reporting limit accordingly.
10.6 Filter each sample under partial vacuum through a sterile membrane filter. Rinse the funnel after each sample filtration by filtering three, 30-mL portions of sterile buffered dilution water, being sure to thoroughly rinse the sides of the funnel.
10.7 Upon completion of the final rinse, disengage the vacuum and remove the funnel.
10.8 Using sterile forceps, immediately remove the MF and place it grid-side-up on the ADA-V medium with a rolling motion to avoid trapping air under the filter. Reseat the membrane filter if bubbles occur. Place the inverted Petri dishes in the 35°C ± 0.5°C incubator within 30 minutes of preparation. Sterilize forceps and sanitize the MF unit between the analysis of each sample.
10.9 After 24 ± 2 hours of incubation at 35°C ± 0.5°C, count and record yellow colonies under magnification using a dissecting microscope.
10.10 Isolation of a yellow colony on ampicillin-dextrin agar with vancomycin (ADA-V) should be considered presumptively positive for Aeromonas.
10.11 Confirmation—All presumptive colonies, up to ten per sample, must be submitted to confirmation. In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. If the result for any confirmation procedure is negative, no further confirmation steps are necessary. Slight variations in color and morphology may be present between different Aeromonas species grown on ADA-V medium. The colonies selected for confirmation should be representative of all yellow (presumptively positive) colony morphology types on ADA-V plate. For example, if 30 bright yellow colonies and 20 dull yellow colonies are observed, then 6 bright yellow and 4 dull yellow colonies should be submitted to confirmation.
Note: It is important to record the number of colonies of each presumptively positive morphological type so that the final density of Aeromonas can be reported based on percent confirmation of each morphological type. Also, the laboratory may submit more than ten presumptively positive colonies to the confirmation step.
10.11.1 Nutrient agar streak plate. To confirm as Aeromonas, pick a colony and streak the colony onto a plate of nutrient agar medium (Section 7.9) and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies.
10.11.2 Oxidase confirmation. Apply a very small amount of a discreet colony from the nutrient agar to the oxidase dry slide using a wooden or plastic applicator. Do not use iron or other reactive wire because it may cause false positive reactions. Also, do not transfer any medium with the culture material, as this could lead to inconsistent results. A blue/purple color reaction within 10

seconds is considered a positive oxidase test. For commercially-prepared reagent, adhere to manufacturer's expiration date. Freshly-made solutions should be used within one week. Please note: This method was validated using nutrient agar, if the oxidase reagent is to be dropped directly on colonies, use tryptic soy agar plates because nutrient agar plates give inconsistent results. The use of tryptic soy agar plates for streaking (Section 10.11.1) has not been validated and is considered a method modification and, as a result, the laboratory must demonstrate acceptable performance for the QC analyses described in Section 9.2.12.

Note: Timing of the color reaction is critical, as some Gram-positive bacteria may give false positives after 10 seconds. Also, it is important to put just a small amount of the colony on the oxidase dry slide or saturated pad, as too much bacteria can also cause a false positive oxidase test.

10.11.3 Trehalose confirmation. If the oxidase test is positive, then test for trehalose fermentation. Trehalose fermentation is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of 0.5% trehalose in purple broth base (Section 7.11) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. A change in color of the medium from purple to yellow is considered a positive for trehalose fermentation.

10.11.4 Indole confirmation. If the oxidase and trehalose tests are positive, then test for indole production. (If the laboratory prefers, the indole confirmation procedure may be started on the same day as the trehalose confirmation.) Indole production is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of tryptone broth (Section 7.12) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. After incubation, add 0.2 to 0.3 mL (4 to 6 drops) of Kovac's test reagent (Section 7.13) to each tube, let stand for approximately 10 minutes and observe results. A pink to red color in the surface layer constitutes a positive indole test. The original color of the Kovac's reagent indicates a negative indole test. An orange color probably indicates the presence of skatole, a breakdown product of indole, and is considered a positive result.

10.11.5 If a colony is oxidase, trehalose, and indole positive, report as a confirmed Aeromonas and archive the colony for further identification.

Note: If samples are to be archived for further analysis to determine species or hybridization group, from the nutrient agar plate (Section 10.11.1), inoculate a nutrient agar slant for short term use or shipment to another laboratory.

11.0 Data Analysis and Calculations

11.1 See Standard Methods for the Examination of Water and Wastewater (Reference 15.2) for general counting rules. The density of Aeromonas determined by the membrane filter (MF) procedure is calculated by direct identification and enumeration of yellow colonies by a dissecting microscope (Section 6.19) followed by oxidase, trehalose, and indole confirmation. Bacterial density is recorded as presumptive Aeromonas colony forming units (CFU) per 100 mL of sample and confirmed Aeromonas CFU per 100 mL.

11.2 Counting colonies on ADA-V

11.2.1 Record the number of presumptive Aeromonas CFU/100mL. If there is more than one morphological type that is considered to be presumptively positive, record the number of presumptive positives for each morphological type, as well as the total number of presumptive positives.

11.2.2 If there are more than 200 colonies, including background colonies, report results as too numerous to count (TNTC) and resample. If the filter is TNTC with more background colonies than presumptive aeromonads, split the 500 mL resample between 3 or 4 filters in order to better differentiate the colony morphology types. If the filter is TNTC with mostly aeromonads, a minimum of three dilutions (e.g. 100 mL, 10 mL and 1 mL) should be analyzed.
11.2.3 If the colonies are not discrete and appear to be growing together, report results as confluent growth (CG) and resample.

11.3 Confirmation and calculation of Aeromonas density
11.3.1 In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. For the final density of confirmed Aeromonas, adjust the initial, presumptive count based on the positive confirmation percentage for each presumptively positive morphological type and report as confirmed CFU per 100 mL.
11.3.2 Calculate the number of positive confirmations for each presumptively positive morphological type from all filters of a given sample using the following equation:

$$\left(\frac{\text{Number positively confirmed}}{\text{Number submitted to confirmation}} \text{ X Number of presumptive positives}\right) = A$$

$$A \text{ X } \frac{100}{\text{mL filtered}} = \text{Confirmed Aeromonas /100 mL}$$

11.3.3 Record the number of confirmed Aeromonas per 100 mL for each colony morphology.
11.3.4 Sum the number of confirmed Aeromonas per 100 mL for all presumptively positive colony types (Section 11.3.2) and report as the density of confirmed Aeromonas per 100 mL.
11.3.5 Example 1: In this example, 500 mL of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 1 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Bright yellow, round, opaque	30	6	6	6
Type B: Dull yellow, oval, translucent	20	4	3	3
Total number of confirmed *Aeromonas* per sample:				**9 per 100 mL**

Example 1 results in 9 confirmed *Aeromonas* / 100 mL.
11.3.6 Example 2: In this example, ***200 mL*** of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 2 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Dull yellow, round, opaque	40	5	5	20
Type B: Dull yellow, round, translucent	40	5	3	12
Total number of confirmed *Aeromonas* per sample:				**32 per 100 mL**

Example 2 results in 32 confirmed *Aeromonas* / 100 mL.
11.3.7 If there were no presumptively positive colonies or if none of the presumptive colonies are confirmed, then report the results as less than the detection limit (DL) in CFU per 100 mL based on sample volume filtered. If less than 500 mL are filtered, then adjust the reporting limit per 100 mL accordingly. The DL may be calculated as follows:

DL per 100 mL = 100 / volume filtered CFU per 100mL

11.3.7.1 Example 3: If 500 mL of sample was filtered and there were no confirmed colonies, then report as <0.2 CFU/100 mL.
11.3.7.2 Example 4: If 100 mL of sample was filtered and there were no confirmed colonies, then report as <1.0 CFU/100 mL.

12.0 Method Performance
12.1 Specificity of media **12.1.1** Please refer to Section 16, Table 2, for results of *Aeromonas* growth after 24 hours on ADA at 30EC and 35EC and ADA-V at 35EC .
12.1.2 ADA-V was able to support the growth of the *Aeromonas* species (*hydrophila, caviae,* and *veronii/sobria*) most often associated with human disease.
12.1.3 Efforts continue to identify colonies which give a presumptive positive on the ADA-V media but do not confirm.
12.2 The QC acceptance criteria listed in Table 1, below are based on data generated through the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished drinking water matrices. Detailed method QC procedures applicable to these criteria are discussed in Section 9.

Table 1. QC Acceptance Criteria for Method 1605 QC specification	**Maximum acceptable precision**
Initial demonstration of capability (IDC): This test will require the analysis of 4 spiked reagent water samples	RSD = 22%
Ongoing demonstration of capability (ODC): This test will require the analysis of 2 spiked reagent water samples	RPD = 37%
Matrix spike/matrix spike duplicate (MS/MSD) precision: This test will require the analysis of 2 spiked finished water (matrix) samples	RPD = 48%

13.0 Pollution Prevention
13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.
13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.
14.0 Waste Management
14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known or suspected of having bacterial contamination from this work must be sterilized prior to disposal.

14.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both of which are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 References

15.1 Havelaar, A.H., M. During, and J.F.M. Versteegh. 1987. Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. Journal of Applied Microbiology. 62:279-287.

15.2 Standard Methods for the Examination of Water and Wastewater. 1998. 20th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.

15.3 Demarta, A., M. Tonolla, A. Caminada, N. Ruggeri, and R. Peduzzi. 1999. Signature region within the 16S rDNA sequences of *Aeromonas popoffii.* FEMS Microbiol. Letts. 172:239-246.

15.4 Annual Book of ASTM Standards, Vol. 11.01. American Society for Testing and Materials. Philadelphia, PA 19103.

15.5 Manual for the Certification of Laboratories Analyzing Drinking Water. 1997. 4th Edition. EPA-815-B-97-001. Office of Ground Water and Drinking Water. U.S. EPA.

15.6 Moyer, N. P. 1996. Isolation and enumeration of aeromonads. *In*: The Genus *Aeromonas*. Eds. B. Austin, M. Altwegg, P. Gosling, and S. Joseph. John Wiley and Sons publisher, Chichester, U.K.

15.7 Reagent Chemicals, American Chemical Society Specifications. American Chemical Society, Washington, D.C.

15.8 Handfield, M., P. Simard, and R. Letarte. 1996. Differential media for quantitative recovery of waterborne *Aeromonas hydrophila*. Applied Environmental Microbiology 62:3544-3547.

15.9 Standard Methods for the Examination of Water and Wastewater. 1995. 19th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.

15.10 Janda, J.M. and S.L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Journal of Clinical Infectious diseases. 27:332-344.

16.0 Tables and Flowcharts

Collection #	Hybridization group	*Aeromonas*species	ADA at 30°C	ADA at 35°C	ADA-V at 35°C
ATCC 7966	Group 1	*hydrophila*	+	+	+
ATCC 35654	Group 1	*hydrophila*	+	+	+
AMC 12723-W	Group 1	*hydrophila*	+	+	+
ATCC 51108	Group 2	*bestiarum*	+	+	+
AMC 14228-V	Group 2	*bestiarum*	+	+	+
ATCC 33658[1]	Group 3	*salmonicida*/*salmonicida*	-	-	NA
AMC 15228-V	Group 3	*salmonicida*	+	+	+
ATCC 15468	Group 4	*caviae*	+	+	+
MML 1685-E	Group 4	*caviae*	+	+	+
ATCC 33907	Group 5	*media*	-	-	NA
AMC Leftwich	Group 5	*media*	-	-	NA
ATCC 23309[1]	Group 6	*eucrenophila*	+	-	NA
ATCC 35993	Group 7	*sobria*	+	+	+
Muldoon SMHC	Group 7	*sobria*	+	+	+
ATCC 9071	Group 8	*veronii/sobria*	+	+	+
AMC 1123-W	Group 8	*veronii/sobria*	+	+	+
ATCC 43700	Group 12	*schubertii*	+[2]	+[5]	+[5]
AMC 1108-W	Group 12	*schubertii*	+	-	NA
ATCC 49657[3]	unknown	*trota*	-	-	NA
NMRI 206	unknown	*trota*	-	-	NA
ATCC 51208	unknown	*allosaccharophila*	+	+	+
ATCC 49568	Group 9	*jandaei*	+	+	+
AS 14	Group 9	*jandaei*	+	+	+
ATCC 35622	Group 10	*veronii/veronii*	+	+	+
WR 4659	Group 10	*veronii/veronii*	+	+	+
CECT 4342	Group 11	*encheleia*	+	-	NA
LMG 17541[4]	unknown	*popoffii*	+	+	+
AMC (ATCC 35941)	unknown	ornithine positive	-	-	NA
AMC (ATCC 43946)	unknown	Group 501	+	+	+
CDC 0434-84	Group 3	Motile Group 3	+	+	+

(1) Respective *Aeromonas* cultures grew on ADA medium when streaked, but not when filtered.

(2) Respective Aeromonas cultures grew when streaked on ADA medium at 30°C, however filtration was not performed with these cultures.(3) Respective Aeromonas cultures did not grow on ADA medium when streaked.(4) Respective Aeromonas cultures grew poorly on ADA medium at both temperatures and on ADA-V at 35°C. The same pattern of poor growth was also observed on non-selective media.(5) Respective Aeromonas cultures grew poorly on ADA and ADA-V medium at 35°C. The same pattern of poor growth was also observed on non-selective media.
Results: A Based on ADA results, it was assumed that the culture would not grow on ADA-V at 35°C.
+ positive growth
-No growth

ATCC = American Type Culture Collection, Manassas, VA Other cultures were obtained from Amy Carnahan, University of Maryland. Serial dilutions representing approximately 10-200 CFU were filtered and the membrane placed on ADA or ADA-V medium as described in Section 10. Additional membranes representing the same dilution for each of the respective cultures were placed on brain heart infusion agar as a control.

17.3 Definitions

Confirmed colonies—Presumptively positive colonies that test positive for oxidase, ferment trehalose, and produce indole

Dilution/rinse water blank—A 100-mL aliquot of dilution/rinse water that is treated exactly as a sample and carried through all portions of the procedure until determined to be negative or positive. The Dilution/rinse water blank is used to determine if the sample has become contaminated by the introduction of a foreign microorganism through poor technique.

Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples.

Must—This action, activity, or procedural step is required.

Negative culture control—A non-*Aeromonas* bacteria processed to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate.

Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate).

Positive control—A 500-mL reagent water spiked with 20 - 80 CFU of Aeromonas. The positive control is analyzed exactly like a sample. Its purpose is to ensure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Presumptive positive colonies—Colonies that are yellow on ADA-V.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean.

Selective medium—A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of the target bacteria.

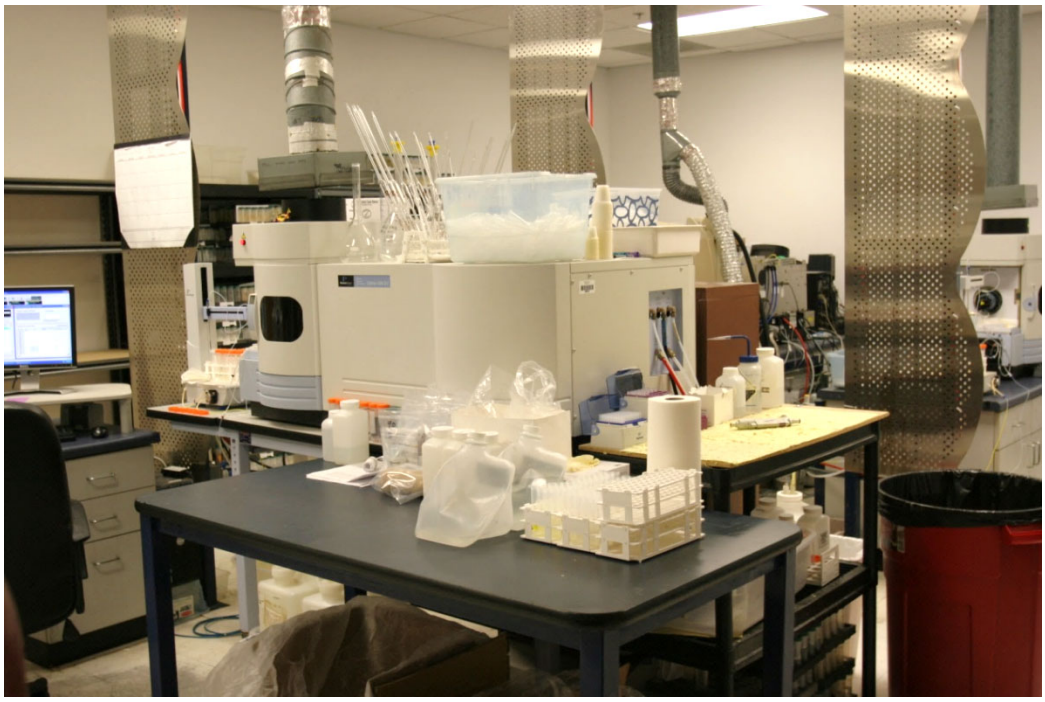
Should—This action, activity, or procedural step is suggested but not required.

Common Water Quality Lab Procedures

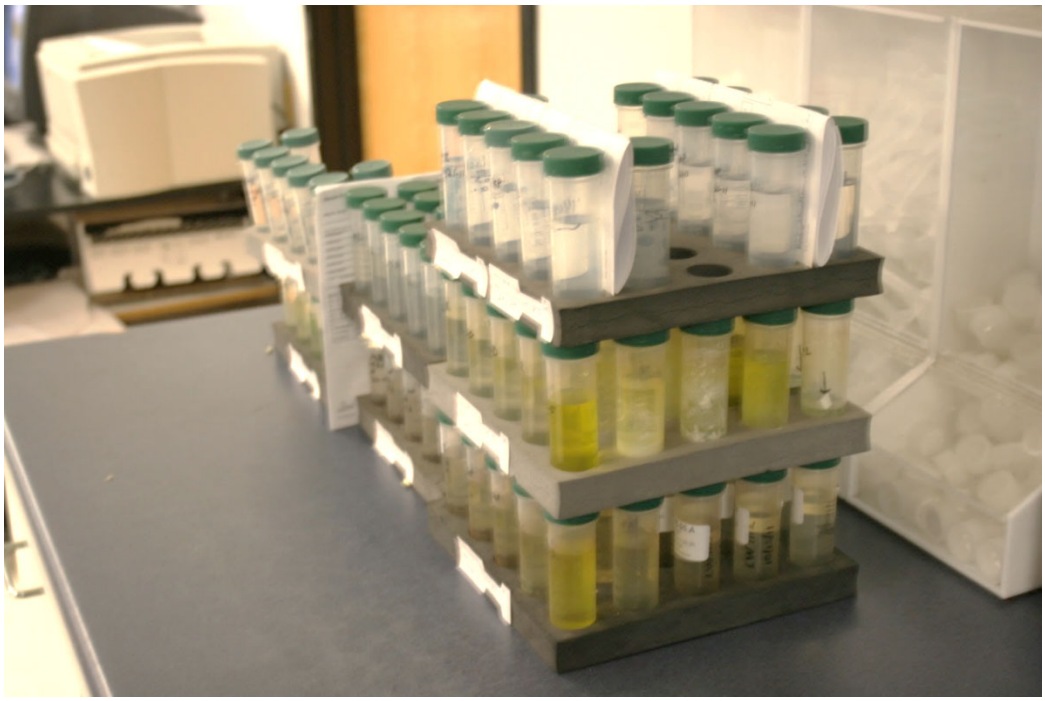
This lab equipment is used for testing parameters in water such as Metals, Pesticides and Hydrocarbons. Often geotechnical water testing that is performed to support characterization regarding the fate and transport of contaminants in soils and sediments. Information such as the grain size distribution provides insight into the absorption, accumulation and movement of contaminants as it may relate to various particle sizes.

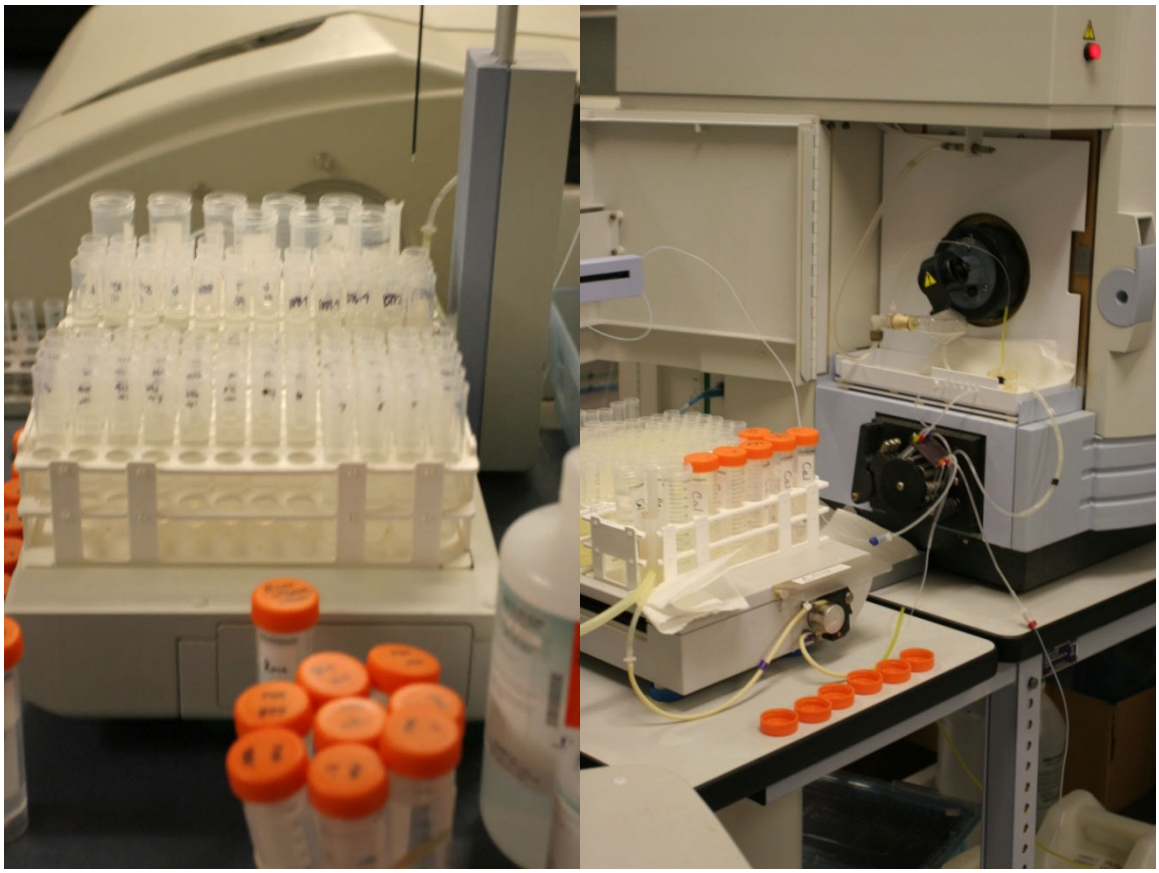
Top Photo: Extraction is a process used to concentrate trace levels of materials, in this case metals. Some metals are able to be complexed with an organic chelating agent. The organic chelating agent, Ammonium pyrrolidine dithiocarbamate (APDC), chemically binds the metals in the water. APDC is easily dissolved in the nonpolar organic solvent, methyl isobutyl ketone (MIBK). Because MIBK is nonpolar, it will not dissolve in water and will separate from the water like oil and water.

Bottom Photo: Atomic absorption requires the presence of ground state metal atoms (Me^{o}). The initial process which takes place in an atomic absorption spectrophotometer is to create a population of ground state atoms. This is accomplished in a variety of ways, usually classified as flame and non-flame. The simplest and most common method uses a flame to produce these atoms.



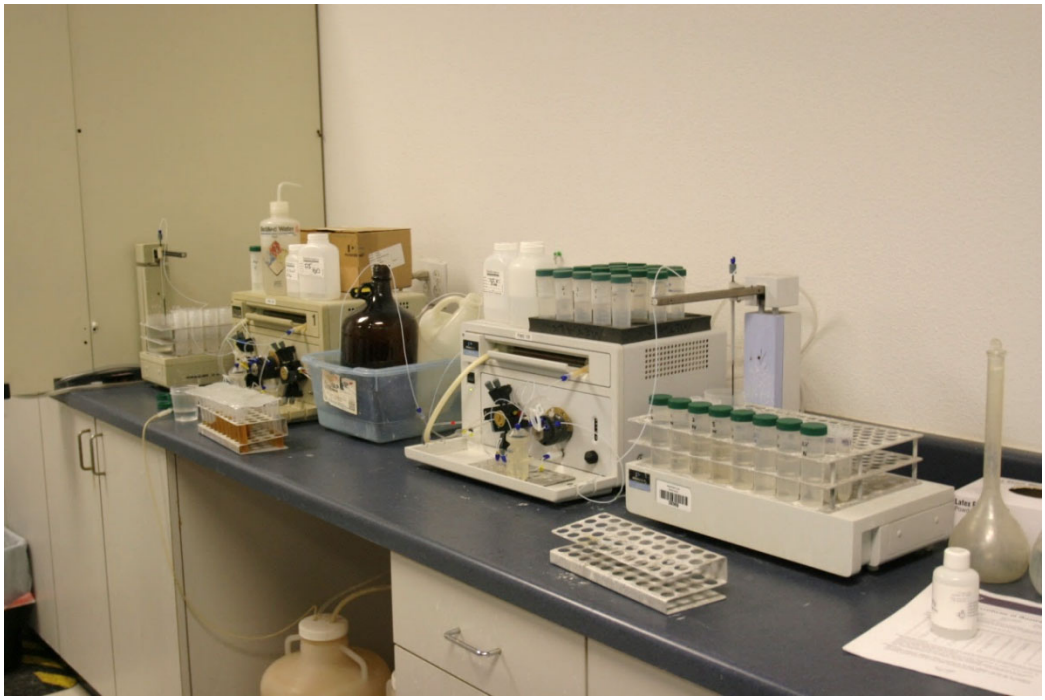
Vapor atomic fluorescence spectroscopy (CVAFS) analytical techniques were designed by the EPA for the analysis of Mercury at low and sub parts per trillion (ppt) concentrations. Low detection limits were developed to support risk assessment, Mercury bioaccumulation in fish is a heightened concern. Thirty-nine states have issued fish consumption advisories due to mercury contamination. Mercury is a toxic pollutant across most regulatory programs (air, water, hazardous waste & pollution prevention). It is persistent and harmful to human health and the environment at relatively low levels.

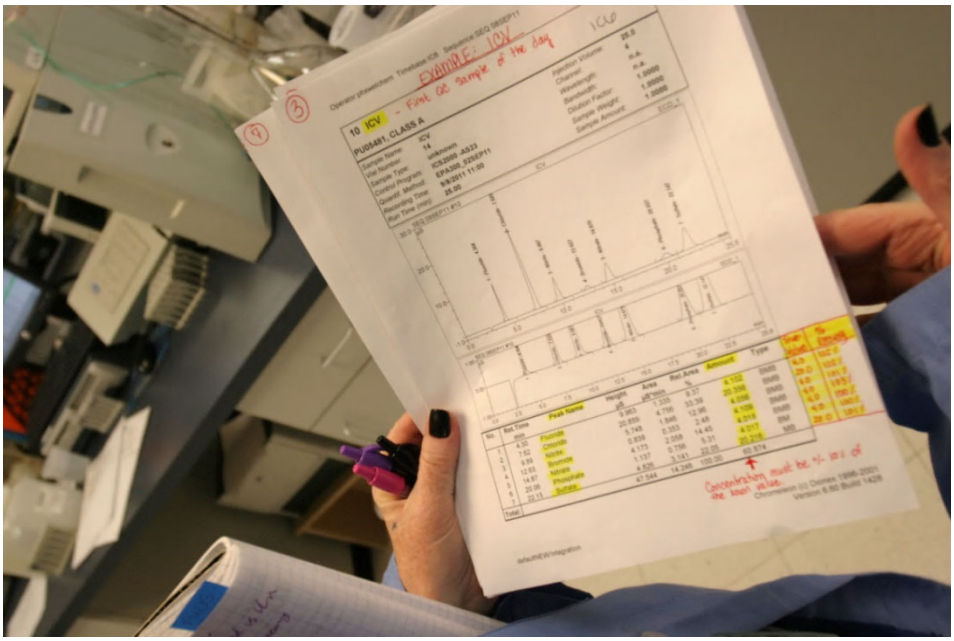




This lab's Quality Assurance Quality Control (QAQC) methods includes spikes, performance samples, replicates, detection limits and blanks.

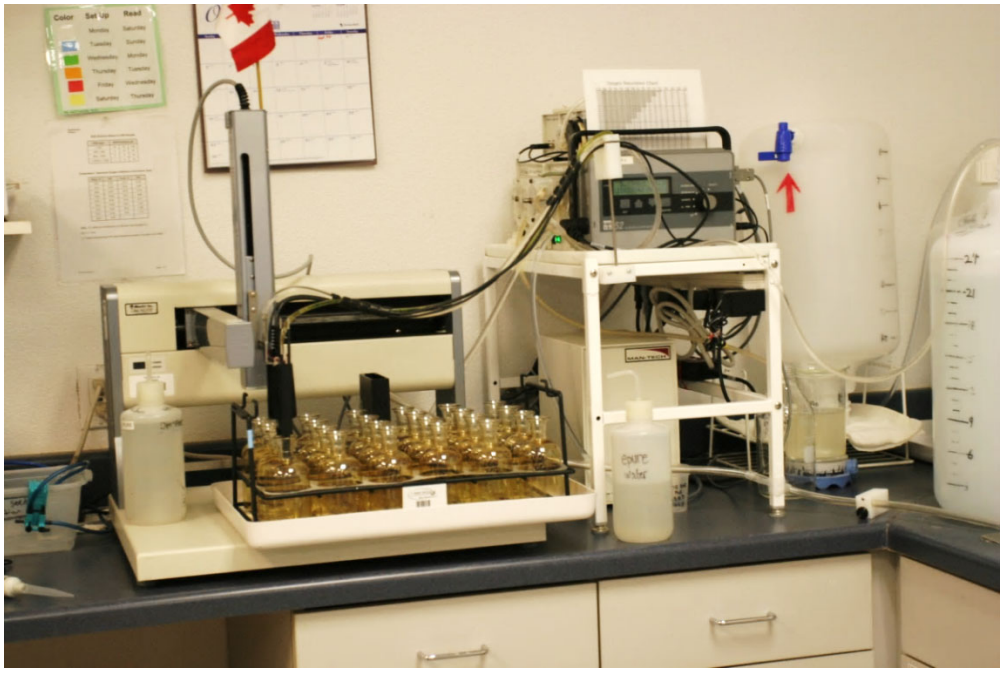
Prepared samples stored for metal analysis.

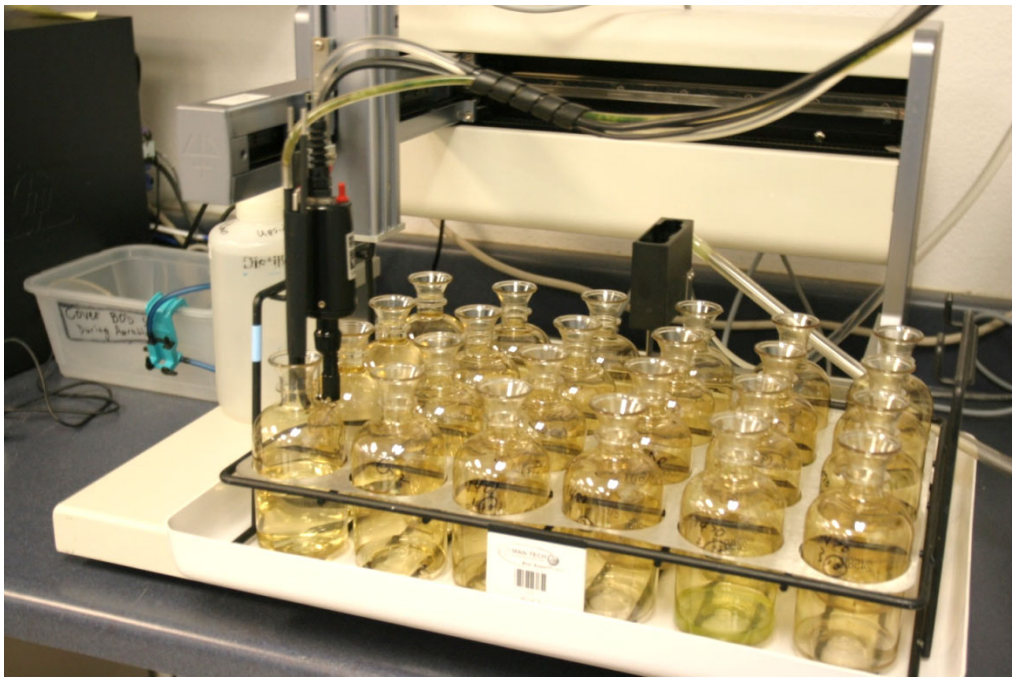




Top Photo: This form shows a typical ion chromatography run will have a standard curve consisting of 4 or 5 points for each ion of interest. A combined ion stock standard is used. The correlation coefficient of the standard curve for each ion should be >.998. The coefficient is calculated by plotting the peak area against the standard concentration using a linear fit.

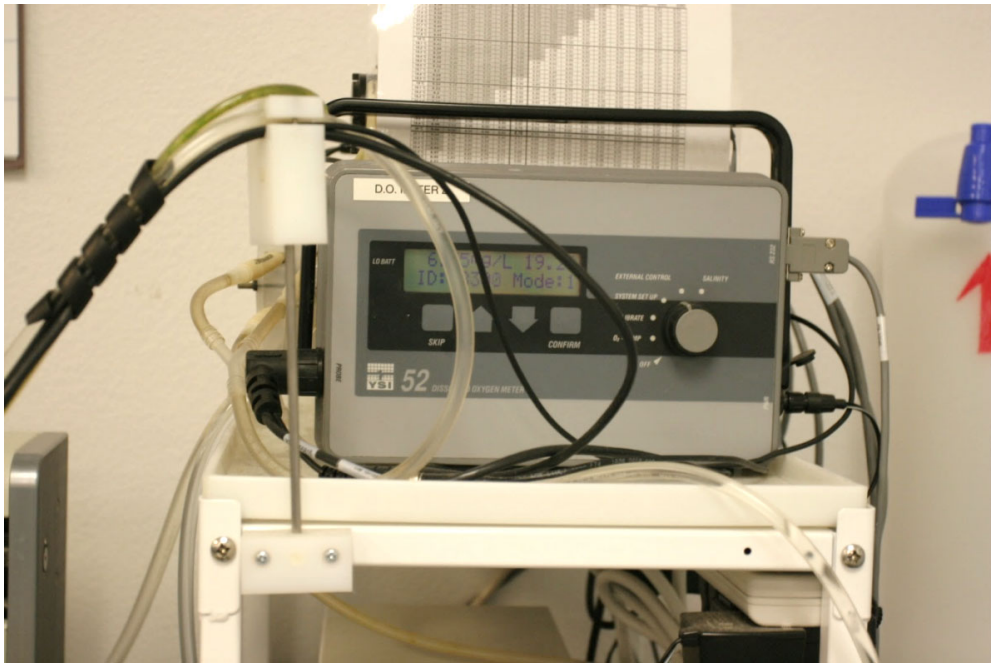
Bottom Photo: Some labs have the luxury of automatic filling dilution water for BOD bottles. If the BOD bottle is filled too rapidly, excessive agitation and bubbling may result in supersaturated water. This device also has the capability of measuring the oxygen level.

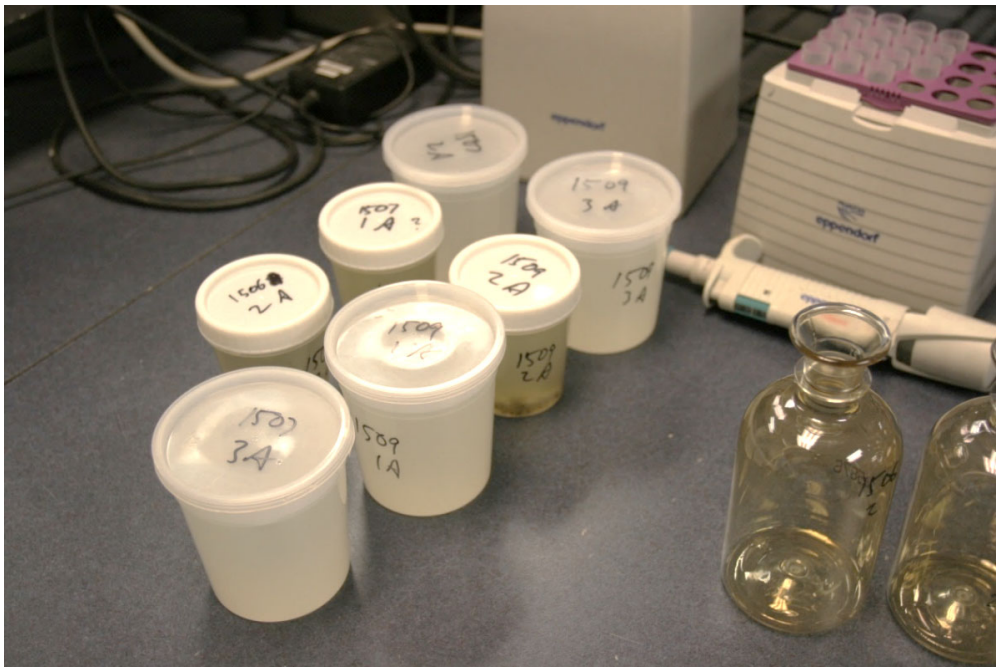




Top Photo: Running a BOD analysis on a sample consists of placing a portion of a sample (along with prepared dilution water) into an air-tight bottle (300 ml volume) and incubating the bottle at 20 +/- 1 deg C for (usually) 5 days.

Bottom Photo: Dissolved oxygen is often measured to determine the oxygen level in receiving streams, in the aeration basin, and in the BOD test. The DO meter and electrode work in similar fashion to other electronic meters but unlike the others, this meter must be continually "on" to remain polarized.



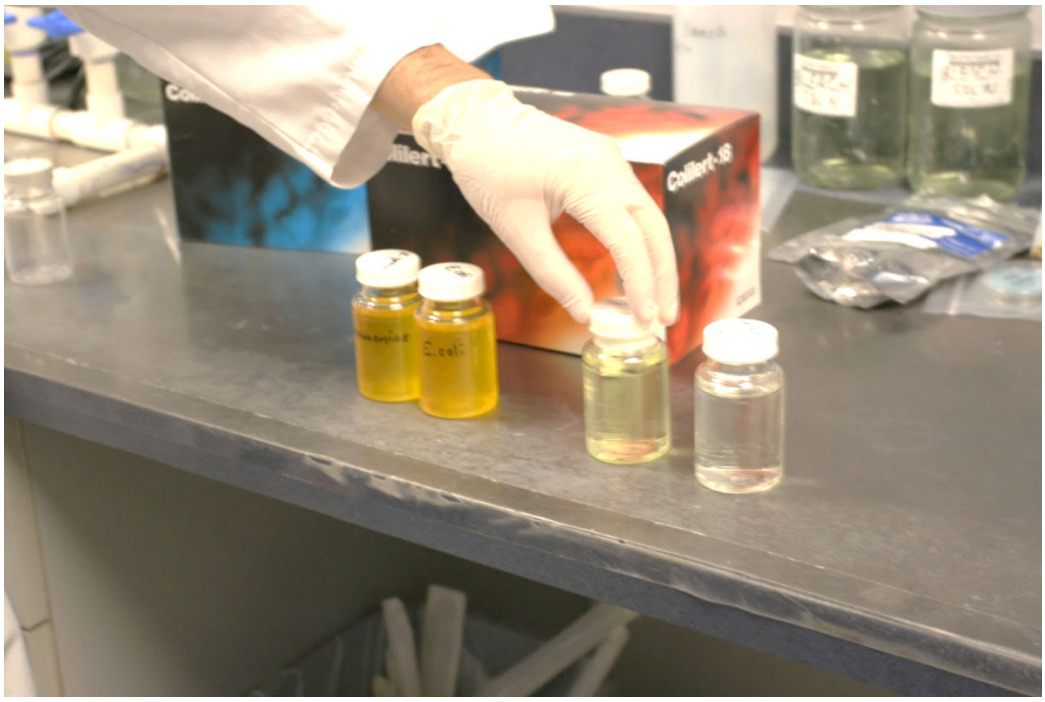


Top Photo: Collecting the seed in a 500 ml bottle and let settle at least 1 hour and up to 36 hours. This will allow settleable solids to settle and help assure the seed is homogeneous.

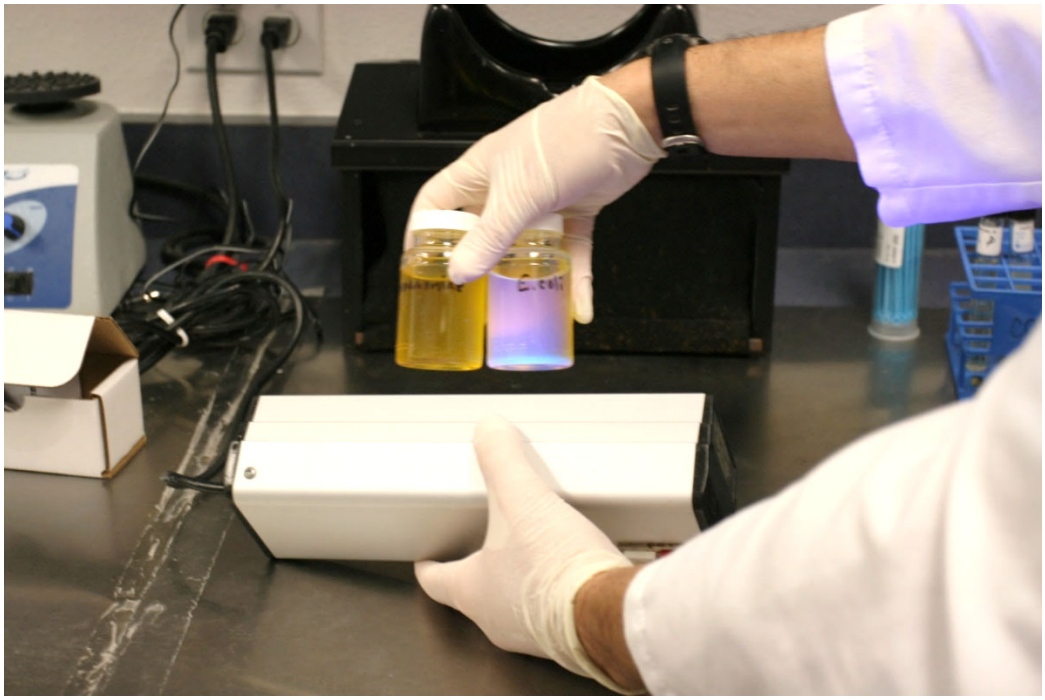
Bottom Photo: In order to validate the BOD test conditions, reagents, and procedure, it is necessary to measure a solution with a known amount of organics. A solution that has a known value is called a standard.

Top Photo: This area is used for Fecal coliform which the most common dilution is prepared by transferring 11 ml of sample to 99 ml of sterile phosphate dilution water using a sterile serological pipet.

Bottom Photo: Some labs perform a variation of coliform testing this technician is making sure his area follows QAQC standards.

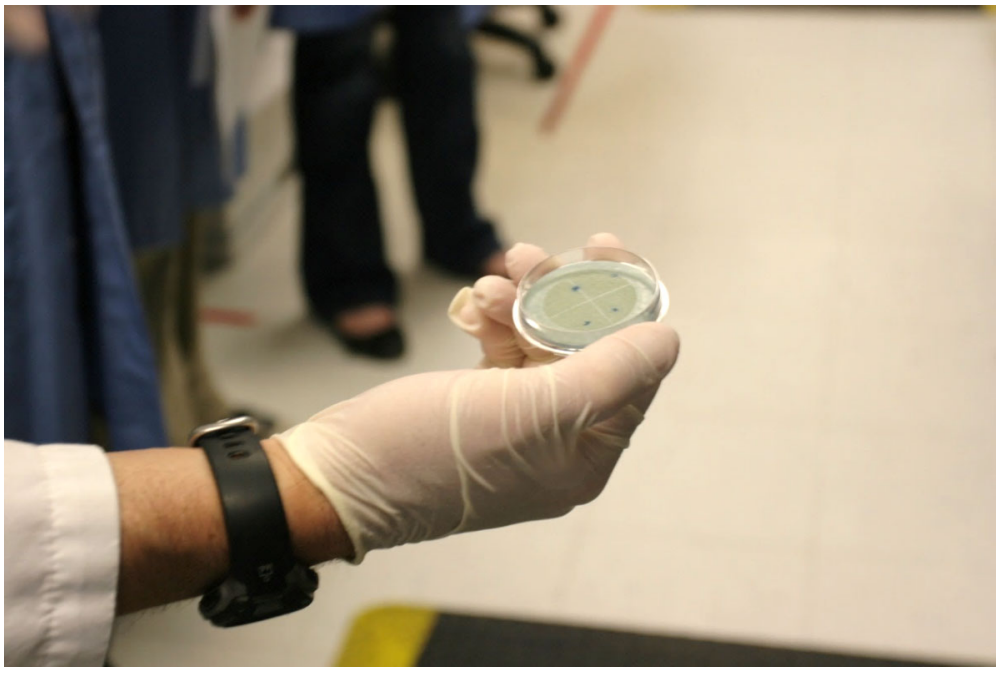


IDEXX bottles are clear plastic disposable containers that are supplied by the manufacturer with the colilert testing kits. Many labs that use the colilert method also issue the IDEXX bottles. Positive identification of total coliform is indicated by fluorescent under the UV light. E-coli are indicated by fluorescent blue colonies.



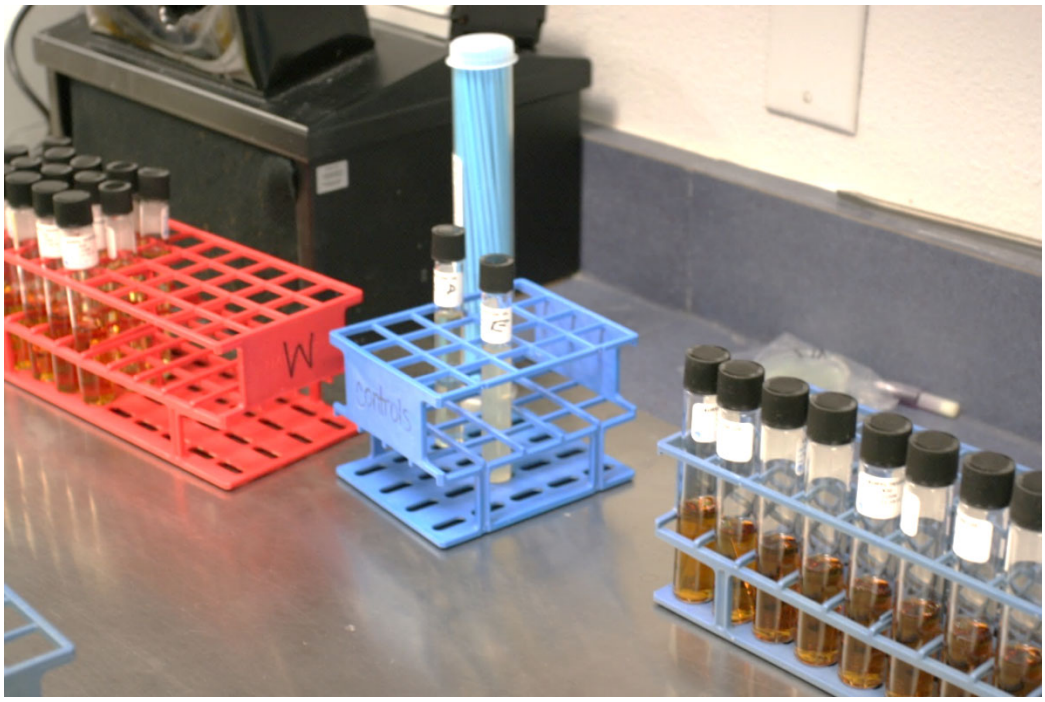
Top Photo: This technician is using Colilert which is a commercially available enzyme-substrate liquid-broth medium (IDEXX Laboratories, Inc.) that allows the simultaneous detection of total coliforms and *Escherichia coli* (*E. coli*). It is available in the most-probable number (MPN) or the presence/absence (PA) format. The MPN method is facilitated by use of a specially designed disposable incubation tray called the Quanti-Tray®.

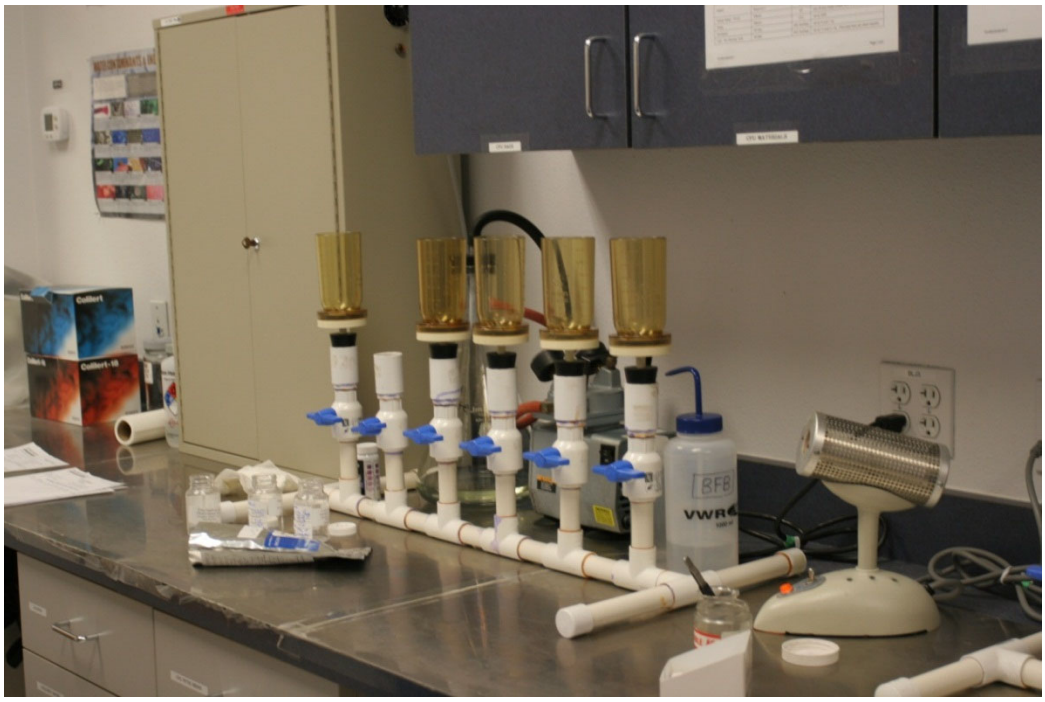
Bottom Photo: Another method is using a petri dish with a filter membrane. The broth and membrane used vary depending on the sample type for water or wastewater.



The MPN method (also called Multiple Tube Fermentation Technique) for fecal coliform detection is often used when the sample has excessive turbidity. Excessive turbidity in the sample will plug the membrane filter, causing poor bacteria recovery and slow filtration times.

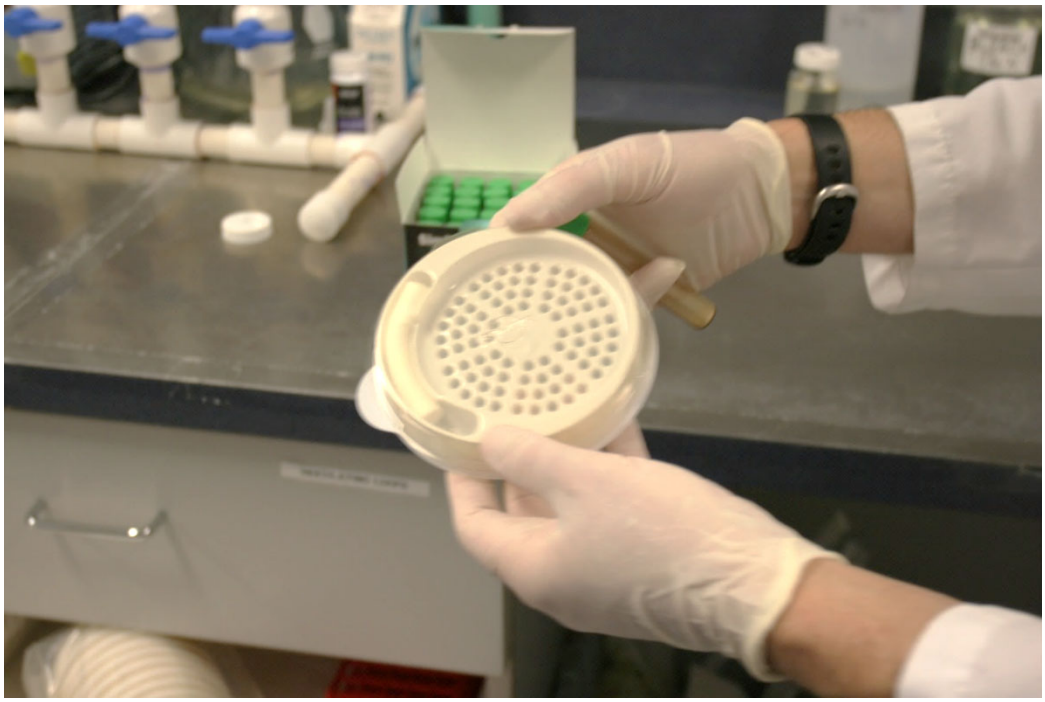
The MPN method involves adding the wastewater sample to a series of 5 sets of tubes, each of which contains either lactose broth or lauryl tryptose broth and an inverted tube. The tubes are then incubated at 35 + 0.5oC for 24 to 48 hours.





Top Photo: Analytical funnels are 100 ml filtration units that allow the membrane to be removed.

Bottom Photo: The heterotrophic plate count (HPC) is a procedure for estimating the number of live heterotrophic bacteria (requiring organic compounds of carbon and nitrogen for nourishment) in water. The technician will count the colored holes for the results.



River Sampling Procedure Photos

Top Photo: Technicians use several different devices to sample wells depending on the depth of the water table. Some use hand bailers or small gas powered submersible pumps.

Bottom Photo: Technicians are always concerned with Quality Assurance; here the containers have DI water for rinsing in the field. The containers that look like milk bottles are used for the equal width depth integrated sampler.

Top Photo: This multi probe device measures dissolved oxygen (DO), pH, conductivity and total dissolved solids (TDS), the device requires calibration for each parameter.

Bottom Photo: Collection of isokinetic, depth-integrated samples is done using either an equal-width-increment (EWI) or equal-discharge-increment (EDI) sampling method. The methods typically result in a composite sample that represents the streamflow-weighted concentrations of the stream cross section being sampled. That sample is poured into the bottles that resemble old fashion milk bottles.

The churn splitter was designed to facilitate the withdrawal of a representative subsample from a large composite sample of a water-sediment mixture. For example, samples from several verticals in a stream cross section, differing slightly from each other in chemical quality and sediment concentration, can be placed in the churn and be mixed into a relatively homogenous suspension, any subsample withdrawn from the churn should be equal in chemical quality and sediment concentration to any other subsample from the churn.

Ampullariidae, common name the apple snails, is a family of large freshwater snails, aquatic gastropod mollusks with a gill and an operculum. This family is in the superfamily Ampullarioidea and is the type family of that superfamily. The Ampullariidae are unusual because they have both a gill and a lung, the mantle cavity being divided in order to separate the two types of respiratory structures. This adaptation allows these snails to be amphibious. Bottom photo, snail eggs clusters on rocks.

Top Photo: When sampling in the river it is suggested that a minimum of two people participate. One person is holding the collection net while the other carefully disturbs the sediment for collection.

Bottom Photo: This river contained larvae of mayfly and stone flies along with leaches.

Sieving invertebrate samples reduces the volume of sediment that must be sorted through in the lab. A #60 sieve is recommended because the smaller invertebrates will be retained by the #60 sieve and should yield more complete invertebrate community data for a site. Any large debris should be cleaned (remove invertebrates and add them to the sample) and removed from the sample. The sample is then washed through the sieve over the side of the boat or in a tub with site water until no more fine sediment washes through the mesh.

Symbols and Characters

These symbols and characters are specific to water sampling methods but have been conformed to common usage as much as possible.

Units of weight and measure and their abbreviations

Symbols

°C degrees Celsius
µL microliter
< less than
> greater than
% percent

Alphabetical characters

ACUK: Acid chrome violet K
AECL: Alternate enhanced coagulant level
AOC: Assimilable organic carbon
ASDWA: Association of State Drinking Water Administrators
AWWA: American Water Works Association
AWWARF: AWWA Research Foundation
BAC: Biologically active carbon
BAF: Biologically active filtration
BAT: Best Available Technology
BCAA: Bromochloroacetic acid
BDOC: Biodegradable organic carbon
BMP: Best management practice
BOM: Biodegradable Organic Matter (=BDOC + AOC)
Br-: Bromide ion
BrO^{2}-: Bromite ion
BrO^{3}-: Bromate ion
CI: Confidence interval
Cl^{2}: Chlorine
ClO^{2}: Chlorine Dioxide
cm: centimeter
CT: Concentration-Time
CWS: Community Water System
D/DBP: Disinfectants/disinfection byproducts
DBP: Disinfection byproduct
DBPFP: Disinfection byproduct formation potential
DBPP: Disinfection byproduct precursors
DBPR: Disinfectants/disinfection byproducts rule
DBPRAM: DBP Regulatory Assessment Model
DBPs: Disinfection byproducts
DOC: Dissolved organic carbon
DPD: N,N-diethyl-p-phenylenediamine
DWEL: Drinking Water Equivalent Level
EBCT: Empty bed contact time
EMSL: EPA Environmental Monitoring and Support Laboratory (Cincinnati)
EPA: United States Environmental Protection Agency
ESWTR: Enhanced Surface Water Treatment Rule
FBR: Filter Backwash Rule

FY: Fiscal year
G: acceleration due to gravity
g: gram
GAC: Granular activated carbon
GWR: Ground Water Rule
GWSS: Ground Water Supply Survey
H2O2: Hydrogen Peroxide
HAA5: Haloacetic acids (five)
HOBr: Hypobromous acid
HOCl: Hypochlorous acid
hr: hour
IC: Ion chromotography
ICR: Information Collection Rule
ID: inside diameter
IESWTR: Interim Enhanced Surface Water Treatment Rule
in.: inch
IOA: International Ozone Association
IOC: Inorganic chemical
KMnO4: Potassium permanganate
L: liter
LOAEL: Lowest observed adverse effect level
LOQ: Limit of quantitation
LT1ESWTR: Long Term Stage 1 Enhanced Surface Water Treatment Rule
m: meter
MCL: Maximum Contaminant Level
MCLG: Maximum Contaminant Level Goal
M-DBP: Microbial and disinfection byproducts
MDL: Method Detection Limit
mg: milligram
mg/L: Milligrams per liter
mgd: Million gallons per day
MIB: Methylisoborneol
mL: milliliter
mm: millimeter
mM: millimolar
MRDL: Maximum Residual Disinfectant Level (as mg/l)
MRDLG: Maximum Residual Disinfectant Level Goal
MRL: Minimum Reporting Level
MX: 3-chloro-4-(dichloromethyl)-5-hydroxyl-2(5H)-furanone
N: normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
NaCl: Sodium chloride
NCI: National Cancer Institute
ND: Not detected
NH^2Cl: Monochloramine
NIOSH: National Institute for Occupational Safety and Health
NIPDWR: National Interim Primary Drinking Water Regulation
NOAEL: No Observed Adverse Effect Level
NOM: Natural Organic Matter
NOMS: National Organic Monitoring Survey
NORS: National Organics Reconnaissance Survey for Halogenated Organics

NPDWR: National Primary Drinking Water Regulation
NTNCWS: Nontransient noncommunity water system
NTP: Normal Temperature and Pressure
O^2: Oxygen
$O^{3:}$ Ozone
OBr-: Hypobromite ion
OCl-: Hypochlorite ion
PCE: Perchloroethylene
PE: Performance evaluation
POE: Point-of-Entry Technologies
POU: Point-of-Use Technologies
ppb: Parts per billion
ppm: Parts per million
PQL: Practical Quantitation Level
PTA: Packed Tower Aeration
PWS: Public water system
RIA: Regulatory Impact Analysis
RMCL: Recommended Maximum Contaminant Level
RNDB: Regulations Negotiation Data Base
RSC: Relative Source Contribution
RSD: relative standard deviation
SDWA: Safe Drinking Water Act, or the “Act,” as amended in 1996
SM: Standard Method
SMCL: Secondary Maximum Contaminant Level
SMR: Standardized mortality ratios
SOC: Synthetic Organic Chemical
s_r: standard deviation of recovery
SWTR: Surface Water Treatment Rule
TCE: Trichloroethylene
THM: Trihalomethane
THMFP: Trihalomethane formation potential
TMV: Tobacco mosaic virus
TOC: Total organic carbon
TTHM: Total trihalomethanes
TWG: Technologies Working Group
UV: Ultraviolet
VOC: Volatile Organic Chemical
WIDB: Water Industry Data Base
WS: Water supply
X: average percent recovery
XDBPs: Halogenated DBPs

Never keep food or drinks in your sample refrigerator. I know all of you have done this in the past and I know you've seen someone work without gloves, but you need to be strong and remind personnel that you had enough of tasting all the nastiness. If you are new to this industry, don't fret, you will get a free taste very soon, one way or another. My advice, ask for the hepatitis injections and prepare for a case of the runs that will last for about 1-2 days, after this, you should be good to go. All of us have suffered through this ordeal.

What are the Symptoms of Viral Gastroenteritis?
The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which virus causes the illness.

Lots of fun…

Common Water Quality and Sampling Statements

These statements will be more explained in the previous chapters.

1. What are the correct procedures to follow in collecting bacteriological samples?

Use a sterile plastic or glass bottle. Sodium thiosulfate should be added to neutralize the chorine residual. Refrigerate the sample to 4° C. The regulations call for a minimum of five samples for the month from any system that has positive sample results. Small systems that take only one sample per month have to take four (4) repeats when they get a total coliform positive test result. If any system has to take repeat samples, it must also take a minimum of five (5) routine samples the following month. Small systems that normally take less than 5 samples/month will have to increase the number to 5 samples. They can return to normal sampling schedules the following month if no repeats are required.

2. What are the proper sampling techniques for microbiological sampling?

Proper sampling techniques are extremely important in obtaining accurate water quality information. An improperly taken coliform sample may indicate bacteriological contamination of your water when the water is actually safe. You can avoid the cost of additional testing by using good sampling procedures. Carefully follow these steps in taking a sample for bacteriological testing:

1. Select the sampling point. The sampling point must be a faucet from which water is commonly taken for public use.

- ✓ The sampling point should be a non-swivel faucet.
- ✓ Remove any aerator or screen and flush.
- ✓ It should not be a faucet that leaks, permitting water to run over the outside of the faucet. Leaking faucets can promote bacterial growth.
- ✓ If an outside faucet must be used, disconnect any hoses or other attachments and be sure to flush the line thoroughly.
- ✓ Do not use fire hydrants as sampling points. Do not dip the bottle in reservoirs, spring boxes or storage tanks in order to collect the sample.

3. What do the following abbreviations stand for and what do they mean: gpm, MGD, TTHM, psi, HAA, NTU, and mg/L.

Gallons per minute- Million Gallons a Day - Total Trihalomethanes – Pounds Per Square Inch –Haloacetic acids - Nephelometric turbidity unit -Milligrams Per Liter

4. What is the relationship between mg/L and ppm; ug/L and ppb?

Milligram per liter: Milligram per liter of substance and part per million are equals amounts in water. While you can easily convert between micrograms/liter and milligrams/liter, and between PPM and PPB, its not so easy to convert between the different types of units such as milligrams/liter to PPM.

To convert micrograms per liter to milligrams per liter, divide by 1000.

To convert to PPM, you would first need to know the density of the substance, and the density of what the substance is in.

5. Ug/L: Represents the concentration of something in water or soil. One ppb represents one microgram of something per liter of water (ug/l), or one microgram of something per kilogram of soil (ug/kg).

Parts per million (ppm) or Milligrams per liter (mg/l) - one part per million corresponds to one minute in two years or a single penny in $10,000.

Parts per billion (ppb) or Micrograms per liter - one part per billion corresponds to one minute in 2,000 years, or a single penny in $10,000,000.

Parts per trillion (ppt) or Nanograms per liter (nanograms/l) - one part per trillion corresponds to one minute in 2,000,000 years, or a single penny in $10,000,000,000.

6. What do the following terms represent in reference to water quality.
Total coliform: The coliform family has been divided into two groups. Results may come back as either total coliform positive (TC positive) or fecal coliform positive, or (FC positive or *E. coli* positive.) Total coliform positive means that no human coliform are present.

7. Fecal Coliform: Fecal coliform positive indicates the presence of *E. coli*, which means there is a greater chance of pathogens being present. The laboratory tests for coliform include the MPN method, the Membrane Filter test, the Colilert test, and the presence-absence test.

8. Presence-absence Test: Presence-Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

9. Physical Characteristics of Water: A characteristic of water defined by the temperature, turbidity, color, taste, and odor of the water.

10. Point-of-entry sample (POE): A type of water sample taken after treatment and before reaching the first consumer.

11. Acute Health Effect: An immediate (i.e. within hours or days) effect that may result from exposure to certain drinking water contaminants (e.g., pathogens).

12. Non-acute violation: If the MCL is exceeded and none of the positive results indicated a presence of Fecal Coliform, a Tier 2 violation has occurred. This level of violation used to be called a non-acute violation.

13. Routine Sample: Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.

14. Repeat Sample: Short answer… Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect. Long Answer. Anytime a microbiological sample result comes back positive, indicating the presence of total or fecal coliform/ E.coli, repeat samples must be taken. Three repeats are usually required. One must be taken at the site of the positive sample. The two samples must be taken upstream and downstream of the original site (within five service connections). These repeat samples must be taken within 24 hours of notification of positive results. They must be identified as a Repeat Sample on the sample form. Repeat samples may be required to be sealed with a red evidentiary seal tape. The tape must cover the cap and extend down the sides of the bottle. The sample forms must also include the reference number for the positive sample.

There is an important exception to the three repeat samples rule. The regulations also state that when repeats are taken the minimum number of samples is raised to five for the month. A system that collects just one sample a month must collect four repeat samples, when the sample is positive, in order to have five samples as required.

Whenever a system has to take repeat samples, a minimum of five routine samples must also be submitted the following month. This is only an issue for systems that normally turn in four or fewer samples each month. If the five samples are negative the system can return to its normal sampling schedule the next month.

Small systems that have fewer than four sampling sites have a problem complying with the "upstream and downstream" aspects of the repeat sampling requirements. In this case, samples should be taken at as many separate sites as possible and then wait a minimum of 2 hours before resampling enough sites to get the required number of samples. Repeat sample with red seal tape.

15. Treatment technique: An enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

16. Action level: The level of lead or copper which, if exceeded, triggers treatment or other requirements that a water system must follow.

17. What does the membrane filter test analyze with regards to bacteriological sampling?

Membrane Filter Technique: A standard test used for measuring coliform numbers (quantity) in water is the membrane filter technique. This technique involves filtering a known volume of water through a special sterile filter. These filters are made of nitrocellulose acetate and polycarbonate, are 150 µm thick, and have 0.45 µm diameter pores. A grid pattern is printed on these filter disks in order to facilitate colony counting. When the water sample is filtered, bacteria (larger than 0.45 µm) in the sample are trapped on the surface of the filter. The filter is then carefully removed, placed in a sterile petri plate on a pad saturated with a liquid medium, and incubated for 20-24 hours at 37°C.

One assumes that each bacterium trapped on the filter will then grow into a separate colony. By counting the colonies one can directly determine the number of bacteria in the water sample that was filtered. The broth medium usually employed in detecting total coliforms is M-Endo Broth MF. Total coliform colonies will be pink to dark red in color and will appear to have a golden green color.

18. What do the following terms mean in relation to drinking water quality: disinfection, pathogenic, toxic, pH, aesthetic, culinary and potable.
Disinfection: The chemical process of killing or inactivating most microorganisms in water. See also Sterilization.

19. Pathogenic: Organisms or bugs that cause disease. These include bacteria, viruses, cysts and anything capable of causing disease in humans, like cryptosporidiosis, typhoid, cholera and so on. There are other organisms that do not create disease, these are called non-pathogenic organisms.

20. Toxic: Stuff that will kill you. A substance which is poisonous to living organisms.

21. pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on. Normal rain has a pH of **5.6** – slightly acidic because of the carbon dioxide picked up in the earth's atmosphere by the rain.

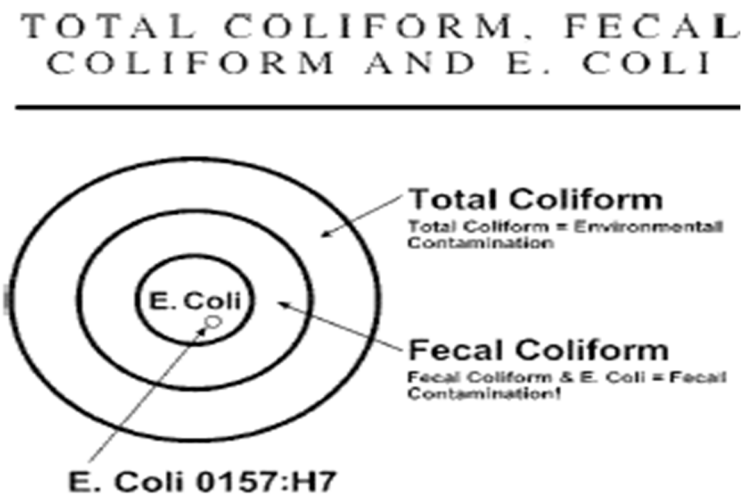
22. Aesthetic: Attractive or appealing water or things in water that will not make you sick but may appear to change the water's color or taste.

23. Culinary: Having to do with cooking food. Potable water is often called culinary water.

24. Potable: Water that is free of objectionable pollution, contamination, or infective agents. Generally speaking, we serve only potable water and not palatable water. Palatable is pleasant tasting water.

25. What is hardness in water and what chemicals cause it?
Hardness: Water that contains high amounts of dissolved minerals, specifically calcium and magnesium. **Ion Exchange:** A method of water softening where hardness causing ions are exchanged with sodium ions; also effective in removing many inorganic contaminants such as nitrates, copper, and lead; and treating aesthetic water problems.

26. What is Escherichia Coliform and what does it indicate in relation to drinking water?
E. coli is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination meaning there is a greater risk that pathogens are present.



Total coliform, fecal coliform, and *E. coli* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. *E. coli* is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or *E. coli*, depending on the lab testing method.

27. What problems are associated with Hydrogen Sulfide in the water?
Hydrogen sulfide is a gas which, when dissolved in water, gives it a “rotten egg” odor. Chlorination will remove this gas from the water but the effectiveness of the chlorine for disinfection is lessened.

28. When Hydrogen sulfide reacts with chlorine, it produces Sulfuric acid and elemental Sulfur: It is therefore recommended that aeration be applied prior to the addition of chlorine for the most effective disinfection.

29. Why is it important to know what the turbidity of the water is when using chlorine?
To be careful not to overdose with chlorine or properly dose with chlorine.

30. What is the log removal for Cryptosporidium?
The LT1ESWTR extends further this necessary protection from Cryptosporidium to communities of fewer than 10,000 persons. Today's rule for the first time establishes Cryptosporidium control requirements for systems serving less than 10,000 persons by requiring a minimum 2-log removal for Cryptosporidium. The rule also strengthens filter performance requirements to ensure 2-log Cryptosporidium removal, establishes individual filter monitoring to minimize poor performance in individual units, includes Cryptosporidium in the definition of GWUDI, and explicitly considers unfiltered system watershed control provisions. The rule also reflects a commitment to the importance of maintaining existing levels of microbial protection in public water systems as plants take steps to comply with newly applicable DBP standards.

31. What is the log removal?

This log-reduction terminology was developed by engineers as a way to express levels of decreased biological contamination in water by factors of 10 that could be easily converted to percent reduction. The most commonly used logarithmic base is 10 because it is compatible with our base-10 decimal system. The log of 10 in the base 10 logarithmic system is 1 and the log of 100 is 2, with the log of 1000 being 3, etc. A 1-log reduction is nine out of 10 and would be equivalent to a 90 percent reduction. A 2-log reduction would be 99 out of 100 or 99 percent reduction and a 3-log reduction would be 999 out of 1000 or 99.9 percent reduction. A 99.99 percent reduction would be called a 4-log reduction.

32. What are the turbidity requirements for Direct and Conventional filtration plants?

For conventional and direct filtration systems (including those systems utilizing in-line filtration), the turbidity level of representative samples of a system's filtered water (measured every four hours) must be less than or equal to ***0.3 NTU*** in at least 95 percent of the measurements taken each month. The turbidity level of representative samples of a system's filtered water must not exceed ***1 NTU*** at any time. Conventional filtration is defined as a series of processes including coagulation, flocculation, sedimentation, and filtration resulting in substantial particulate removal. Direct filtration is defined as a series of processes including coagulation and filtration but excluding sedimentation resulting in substantial particle removal.

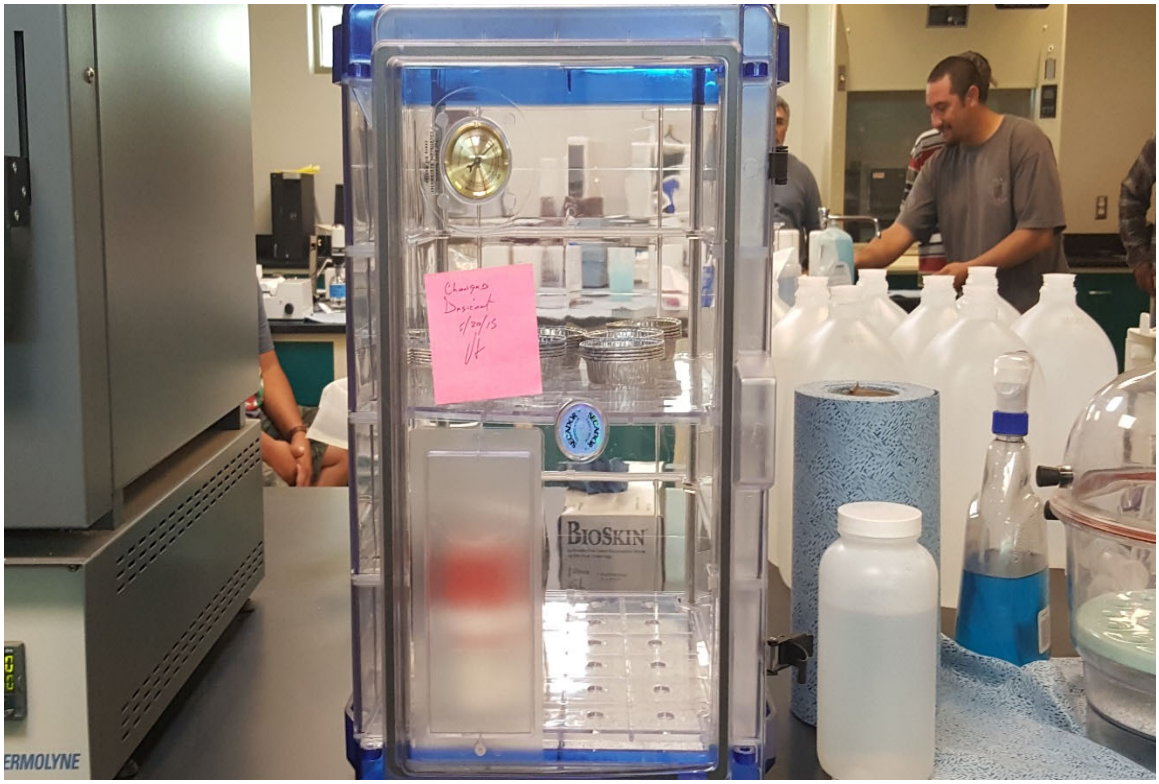
33. What are chloramines, how are they formed, and do they have any beneficial use?

Chloramines: Ammonia and Chlorine are combined. Cl_2NH_3 Yes, limited use and this chemical will create less THMS than chlorine alone. Chloramine is a disinfectant used to treat drinking water. It is formed by mixing chlorine with ammonia. Although it is a weaker disinfectant than chlorine, it is more stable and extends disinfectant benefits throughout a water utility's distribution system (a system of pipes water is delivered to homes through). Some water systems use chloramine as a secondary disinfectant to maintain a disinfectant residual throughout the distribution system so that drinking water remains safe as it travels from the treatment facility to the customer.

Chloramine has been used by water systems for almost 90 years, and its use is closely regulated. Since chloramine is not as reactive as chlorine, it forms fewer disinfection byproducts. Some disinfection byproducts, such as the trihalomethanes (THMs) and haloacetic acids (HAAs), may have adverse health effects and are closely regulated. Because a chloramine residual is more stable and longer lasting than free chlorine, it provides better protection against bacterial regrowth in systems with large storage tanks and dead-end water mains.

Chloramine, like chlorine, is effective in controlling biofilm, which is a coating in the pipe caused by bacteria. Controlling biofilm also tends to reduce coliform bacteria concentrations and biofilm-induced corrosion of pipes.

Wastewater Laboratory Analysis Section



Wastewater treatment operators run laboratory tests and analysis to monitor the treatment plant operation. These analyses are for testing the process control and indicate how well a particular process is working. Operators will analyze the results and if needed, will make operational adjustments.

In a typical wastewater treatment plant, there are several locations to sample. As wastewater flows through the treatment plant, including the collection system, its characteristics frequently change. By taking samples at different locations throughout the process, the operator has a better understanding of how to treat the flow.

Laboratory duties include some of the following:

- Collect and preserve samples
- Prepare samples for analysis
- Analyze samples and interpret results
- Operate and maintain equipment and instruments
- Handle chemicals and wastes (PPE)
- Quality assurance/quality control (Engineering and Administrative controls)
- Manage laboratory
- Laboratory safety (OSHA)

SAMPLING PLAN

Prior to laboratory analysis a sampling plan should consider the following:

1. Why is the sample being collected?
2. What tests need to be performed?
3. At what location will the sample be taken?
4. Will the sample be analyzed at the location?
5. When and how often must the sample be analyzed?
6. Is it a grab or composite sample?
7. Is it for process control or compliance?

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Grab Samples (Snapshot)
A grab sample consists of a single container or large bucket of wastewater analyzed at one specific time. Grab samples indicate the condition of the wastewater at that specific time and may or may not represent the normal conditions. Grab samples are required when the analysis change rapidly. For instance, grab samples are required for certain tests such as temperature, pH, D.O. (dissolved oxygen), and bacteriological analysis.

Composite Samples
A composite sample consists of several grab samples collected from the same spot over a specific period of time and merged into a single sample. A composite sample is more arduous, complicated and usually inconvenient than a simple grab sample. Collecting a sample every few minutes and adding it to a single bottle is tedious, boring, and costly. To help solve this problem, a 24-hour automatic sampler is often used. The automatic sampler consists of a battery pack, a programmable timer, a pump, and as many as 24 bottles.

The automatic sampler has the capability to be programmed to draw a certain volume of sample every few minutes and deposit each sample into one bottles that are preserved or refrigerated. At the end of the sampling period, the operator can retrieve the bottles, bring them back to the lab and create a single composite sample. Analysis can now be performed on a single composite sample that is more representative of the wastewater quality than a grab sample.

Unweighted Composite
An unweighted composite collects the same sample volume at a constant time interval. For example, the operator collects 100 ml every hour for 6 hours. At the end of the time period, there will be 12 individual bottles representing the wastewater quality over the 6 hour time period. The operator now composites the samples by pouring from each bottle into a large bottle and mixes the composite.

Flow Weighted Composite
A flow meter is connected to the composite sampler and the sampler is programed to draw at different flow intervals. As the flow increases so does the number of samples.

The refrigerated automatic WWT sampler will have a Data programmer that will allow you to set the time to collect the sample or samples. This machine can also measure the amount of the sample. These can devices also be used for the collection of composite samples. Sometimes you will see a pH probe with real-time readings sent to the Operator's Command Center. These are a common sight at most wastewater plants and SIUs.

METHOD DETECTION LIMIT OR MDL

The **Method Detection Level (MDL)** is the basic measure of whether a pollutant or parameter has been detected. It's the minimum concentration at which we can be confident that the effluent concentration is greater than zero. The **MDL** is dependent upon the analytical method used for the pollutant. A sensitive analytical method will typically have a lower **MDL** and can provide more accurate results. In general, if the reported pollutant concentration is less than three times the magnitude of the **MDL**, the accuracy or reliability of these results is questionable, and permit decisions using data in this range should be avoided if possible.

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COMPOSITE SAMPLE

To have significant meaning, samples for laboratory tests on wastewater should be representative of the wastewater. The best method of sampling is proportional composite sampling over several hours during the day. Composite samples are collected because the flow and characteristics of the wastewater are continually changing. A composite sample will give a representative analysis of the wastewater conditions.

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WASTEWATER BACTERIA

Bacteria are categorized by the way that they obtain oxygen. In wastewater treatment, there are **three types of bacteria** used to treat the waste that comes into the treatment plant: **aerobic**, **anaerobic** and **facultative**. Aerobic bacteria are used in most new treatment plants in an aerated environment.

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Dissolved Oxygen Sub-Section

Dissolved oxygen (**DO**) in water is not considered a contaminant. However, the (DO) level is important because too much or not enough dissolved oxygen can create unfavorable conditions. Generally, a lack of (DO) in natural waters creates anaerobic conditions. Anaerobic means without air. Certain bacteria thrive under these conditions and utilize the nutrients and chemicals available to exist. *Under anaerobic conditions the reaction is:*

Anaerobic:

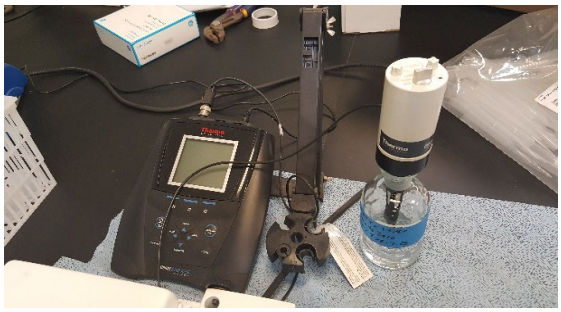
Organics → intermediates + CO_2 + H_2O + energy

Where the intermediates are butyric acid, mercaptans and hydrogen sulfide gas. At least two general forms of bacteria act in balance in a wastewater digester: Saprophytic organisms and Methane Fermenters. The saprophytes exist on dead or decaying materials. The methane fermenters live on the volatile acids produced by these saprophytes. The methane fermenting bacteria require a pH range of 6.6 to 7.6 to be able to live and reproduce. Aerobic conditions indicate that dissolved oxygen is present. Aerobic bacteria require oxygen to live and thrive. When aerobes decompose organics in the water, the result is carbon dioxide and water.

Aerobic:

Organics + Oxygen → CO_2 + H_2O + energy

Dissolved Oxygen in a water sample can be detrimental to metal pipes in high concentrations because oxygen helps accelerate corrosion. Oxygen is an important component in water plant operations. Its primary value is to oxidize iron and manganese into forms that will precipitate out of the water. It also removes excess carbon dioxide. The amount of dissolved oxygen in a water sample will affect the taste of drinking water also.



Methods of Determination

There are two methods that we will be using in the lab. The membrane electrode method procedure is based on the rate of diffusion of molecular oxygen across a membrane. The other is a titrimetric procedure (Winkler Method) based on the oxidizing property of the (DO). Many factors determine the solubility of oxygen in a water sample. Temperature, atmospheric pressure, salinity, biological activity and pH all have an effect on the (DO) content.

Iodometric Test

The iodometric (titration) test is very precise and reliable for (DO) analysis of samples free from particulate matter, color and chemical interferences. Reactions

take place with the addition of certain chemicals that liberate iodine equivalent to the original (DO) content. The iodine is then measured to the starch iodine endpoint. We then calculate the dissolved oxygen from how much titrate we use. Certain oxidizing agents can liberate iodine from iodides (positive interference), and some reducing agents reduce iodine to iodide (negative interferences). The alkaline Iodide-Azide reagent effectively removes interference caused by nitrates in the water sample, so a more accurate determination of (DO) can be made.

Methods of analysis are highly dependent on the source and characteristics of the sample. The membrane electrode method involves an oxygen permeable plastic membrane that serves as a diffusion barrier against impurities. Only molecular oxygen passes through the membrane and is measured by the meter. This method is excellent for field testing and continuous monitoring. Membrane electrodes provide an excellent method for (DO) analysis in polluted, highly colored turbid waters and strong waste effluents.

These interferences could cause serious errors in other procedures. Prolonged usage in waters containing such gases as H_2S tends to lower cell sensitivity. Frequent changing and calibrating of the electrode will eliminate this interference.

Samples are taken in BOD bottles where agitation or contact with air is at a minimum. Either condition can cause a change in the gaseous content. Samples must be determined immediately for accurate results.

The dissolved oxygen test is the one of the most important analyses in determining the quality of natural waters. The effect of oxidation wastes on streams, the suitability of water for fish and other organisms and the progress of self-purification can all be measured or estimated from the dissolved oxygen content. In aerobic sewage treatment units, the minimum objectionable odor potential, maximum treatment efficiency and stabilization of wastewater are dependent on maintenance of adequate dissolved oxygen. Frequent dissolved oxygen measurement is essential for adequate process control.

Terms Review

Aerobic (AIR-O-bick) - a condition in which free or dissolved oxygen is present in the aquatic environment.

Aerobic Bacteria (aerobes) – bacteria which will live and reproduce only in an environment containing oxygen. Oxygen combined chemically, such as in water molecules (H_2O), cannot be used for respiration by aerobes.

Anaerobic (AN-air O-bick) - a condition in which ***"free"*** or dissolved oxygen is not present in the aquatic environment.

Procedure for Dissolved Oxygen Determination

METER-PROBE METHOD

1. Collect a water sample in the clean 300-ml glass stoppered BOD bottle for two or three minutes to make sure there are no air bubbles trapped in the bottle. Do one Tap water sample and one DI water sample. Mark the BOD bottles.

2. Insert the DO probe from the meter into your BOD bottles. Record the DO for Tap and DI water. Now continue with the Winkler Burette method.

PROCEDURES FOR WINKLER BURET METHOD

1. Add the contents of one MANGANESE SULFATE powder pillow and one ALKALINE IODIDE-AZIDE reagent powder pillow to each of your BOD bottles (TAP and DI)
2. Immediately insert the stoppers so that no air is trapped in the bottles and invert several times to mix. A flocculent precipitate will form. It will be brownish-orange if dissolved oxygen is present or white if oxygen is absent.
3. Allow the samples to stand until the floc has settled and leaves the solution clear (about 10 minutes). Again invert the bottles several times to mix and let stand until the solution is clear.
4. Remove the stoppers and add the contents of one SULFAMIC ACID powder pillow to each bottle. Replace the stoppers, being careful not to trap any air bubbles in the bottles, and invert several times to mix. The floc will dissolve and leave a yellow color if dissolved oxygen is present.
5. Measure 200 ml of the prepared solution by filling a clean 250-ml graduated cylinder to the 200-ml mark. Pour the solutions into clean 250-ml Erlenmeyer flasks. Save the last 100 mls for a duplicate.
6. Titrate the prepared solutions with PAO Titrant, 0.025N, to a pale yellow color. Use a white paper under the flask.
7. Add two droppers full of Starch Indicator Solution and swirl to mix. A dark blue color will develop.
8. Continue the titration until the solution changes from dark blue to colorless (end point). Go Slow- drop by drop. Record the burette reading to the nearest 0.01mls.
9. The total number of ml of PAO Titrant used is equal to the mg/L dissolved oxygen.

Dissolved Oxygen Results

Meter Results

1. De-ionized water _____mg/L

2. Tap water _____mg/L

3. What is the meter procedure measuring?

4. What factors would determine which the best method to use is?

5. What are two forms of bacteria present in a wastewater digester?

Wrinkler Method Results

1. **De-ionized Water**

200ml Sample final Burette reading- initial Burette reading- -_____ = _____mg/L

100ml duplicate final Burette reading- initial Burette reading- -_____dup=_____mg/L
mls x 2

2. **Tap water**

200ml Sample final Burette reading- initial Burette reading- -_____=_____mg/L
mls

100ml Sample final Burette reading initial Burette reading- -_____=_____mg/L
mls x 2

3. What are some factors that can alter the (DO) content prior to testing?

4. Were your samples anaerobic or aerobic?

5. Why is it important to monitor the (DO) content of water and wastewater?

Be specific and give a detailed explanation.

Total Dissolved Solids Sub-Section

Water is a good solvent and picks up impurities easily. Pure water is tasteless, colorless, and odorless and is often called the universal solvent. Dissolved solids refer to any minerals, salts, metals, cations or anions dissolved in water. Total dissolved solids (TDS) comprise inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulfates) and some small amounts of organic matter that are dissolved in water.

TDS in drinking-water originate from natural sources, sewage, urban run-off, industrial wastewater, and chemicals used in the water treatment process, and the nature of the piping or hardware used to convey the water, i.e., the plumbing. In the United States, elevated TDS has been due to natural environmental features such as: mineral springs, carbonate deposits, salt deposits, and sea water intrusion, but other sources may include: salts used for road de-icing, anti-skid materials, drinking water treatment chemicals, stormwater and agricultural runoff, and point/non-point wastewater discharges.

In general, the total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) ions in the water. Therefore, the total dissolved solids test provides a qualitative measure of the amount of dissolved ions, but does not tell us the nature or ion relationships.

In addition, the test does not provide us insight into the specific water quality issues, such as: Elevated Hardness, Salty Taste, or Corrosiveness. Therefore, the total dissolved solids test is used as an indicator test to determine the general quality of the water.

Total Solids

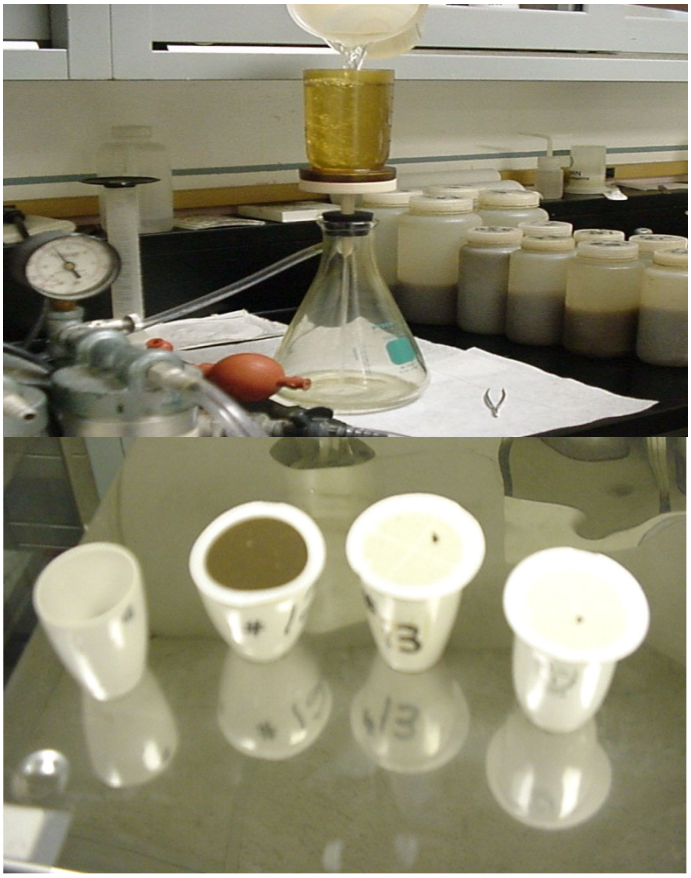
The term "total solids" refers to matter suspended or dissolved in water or wastewater, and is related to both specific conductance and turbidity.

Total solids (also referred to as total residue) are the term used for material left in a container after evaporation and drying of a water sample.

Total Solids includes both total suspended solids, the portion of total solids retained by a filter and total dissolved solids, the portion that passes through a filter (American Public Health Association, 1998).

Total solids can be measured by evaporating a water sample in a weighed dish, and then drying the residue in an oven at 103 to 105° C.

The increase in weight of the dish represents the total solids. Instead of total solids, laboratories often measure total suspended solids and/or total dissolved solids.



Lab tech removing filter for TSS analysis.

Total Suspended Solids (TSS)
Total Suspended Solids (TSS) are solids in water that can be trapped by a filter. TSS can include a wide variety of material, such as silt, decaying plant and animal matter, industrial wastes, and sewage. High concentrations of suspended solids can cause many problems for stream health and aquatic life.

High TSS can block light from reaching submerged vegetation. As the amount of light passing through the water is reduced, photosynthesis slows down. Reduced rates of photosynthesis causes less dissolved oxygen to be released into the water by plants. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. As the plants are decomposed, bacteria will use up even more oxygen from the water. Low dissolved oxygen can lead to fish kills.

Sampling downstream from a wastewater plant's discharge point.

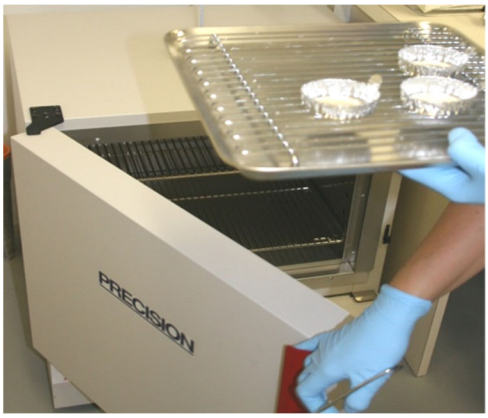
High TSS can also cause an increase in surface water temperature, because the suspended particles absorb heat from sunlight. This can cause dissolved oxygen levels to fall even further (because warmer waters can hold less DO), and can harm aquatic life in many other ways, as discussed in the temperature section. (The decrease in water clarity caused by TSS can affect the ability of fish to see and catch food.

Suspended sediment can also clog fish gills, reduce growth rates, decrease resistance to disease, and prevent egg and larval development. When suspended solids settle to the bottom of a water body, they can smother the eggs of fish and aquatic insects, as well as suffocate newly hatched insect larvae. Settling sediments can fill in spaces between rocks which could have been used by aquatic organisms for homes.

Dead fish in lake using reclaimed water.►

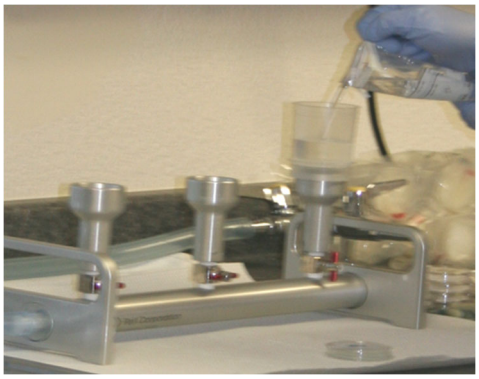
High TSS in a water body can often mean higher concentrations of bacteria, nutrients, pesticides, and metals in the water. These pollutants may attach to sediment particles on the land and be carried into water bodies with storm water. In the water, the pollutants may be released from the sediment or travel farther downstream. High TSS can cause problems for industrial use, because the solids may clog or scour pipes and machinery.

Measurement of Total Suspended Solids
To measure TSS, the water sample is filtered through a pre-weighed filter. The residue retained on the filter is dried in an oven at 103 to 105° C until the weight of the filter no longer changes. The increase in weight of the filter represents the total suspended solids. TSS can also be measured by analyzing for total solids and subtracting total dissolved solids.



Total Dissolved Solids (TDS) are solids in water that can pass through a filter (usually with a pore size of 0.45 micrometers). TDS is a measure of the amount of material dissolved in water.

This material can include carbonate, bicarbonate, chloride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions. A certain level of these ions in water is necessary for aquatic life. Changes in TDS concentrations can be harmful because the density of the water determines the flow of water into and out of an organism's cells (Mitchell and Stapp, 1992). However, if TDS concentrations are too high or too low, the growth of many aquatic lives can be limited, and death may occur.



Similar to TSS, high concentrations of TDS may also reduce water clarity, contribute to a decrease in photosynthesis, combine with toxic compounds and heavy metals, and lead to an increase in water temperature. TDS is used to estimate the quality of drinking water, because it represents the amount of ions in the water. Water with high TDS often has a bad taste and/or high water hardness, and could result in a laxative effect.

The TDS concentration of a water sample can be estimated from specific conductance if a linear correlation between the two parameters is first established. Depending on the chemistry of the water, TDS (mg/l) can be estimated by multiplying specific conductance (micromhos/cm) by a factor between 0.55 and 0.75. TDS can also be determined by measuring individual ions and adding them up.

Conductivity Meter

Suspended Matter for Mixed Liquor and Return Sludge (MLSS)

Suspended matter in mixed liquor and return sludge can be used to determine process status, estimate the quantity of biomass, and evaluate the results of process adjustments.

Apparatus

- Buchner funnel and adaptor
- Filter flask
- Filter paper 110 mm diam., Whatman 1-4
- 103^0 drying oven
- Desiccator
- Balance
- Graduated Cylinder

Procedure

1. Dry the filter papers in oven at 103^0 c to remove all traces of moisture.

2. Remove papers from oven and desiccate to cool for approximately 5 minutes.

3. Weigh to the nearest 0.01g and record the mass (W_1)

4. Place the paper in the bottom of the Buchner funnel and carefully arrange so that the outer edges lay snugly along the side. Careful not to touch it with your finger. Use a glass rod. Wet the paper, turn on the vacuum and make a good seal, make a pocket covering the bottom of the funnel.

5. Add 20 to 100 mls of sample at a sufficient rate to keep the bottom of the funnel covered, but not fast enough to overflow the pocket made by the filter paper. Record the Volume used.

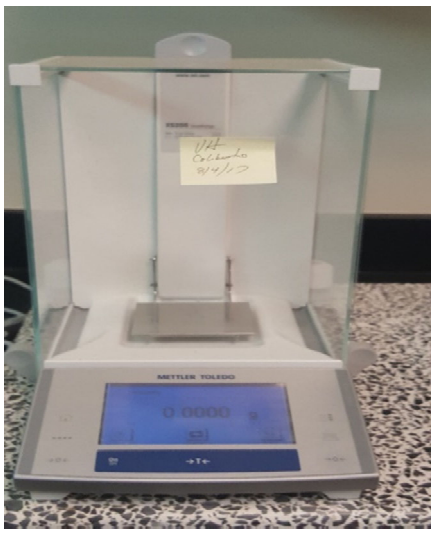
6. Remove the filter paper with tweezers. Dry in a 103^0 c oven for 30 minutes. Remove and desiccate. Reweigh the filter paper (W_2) to the nearest 0.01g.

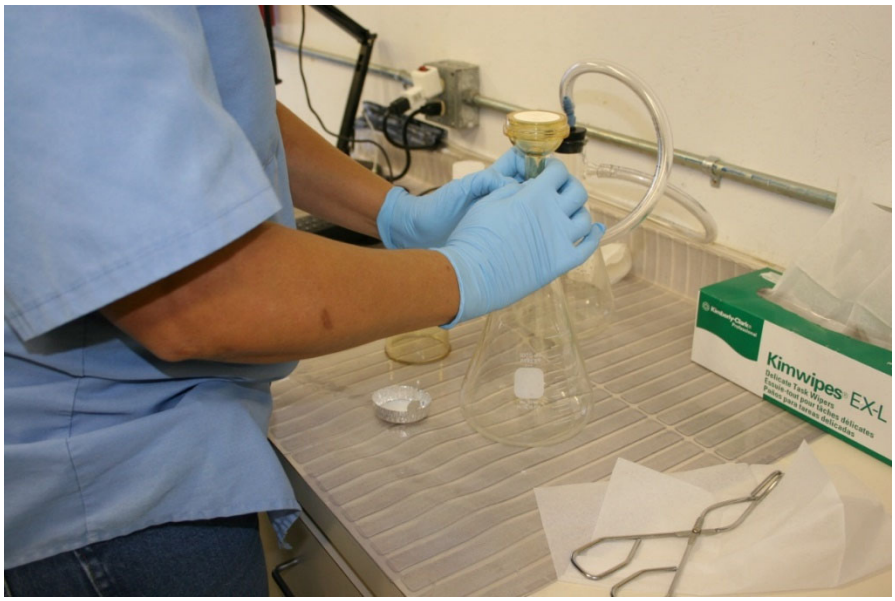
Calculation:

mg/L Suspended Matter

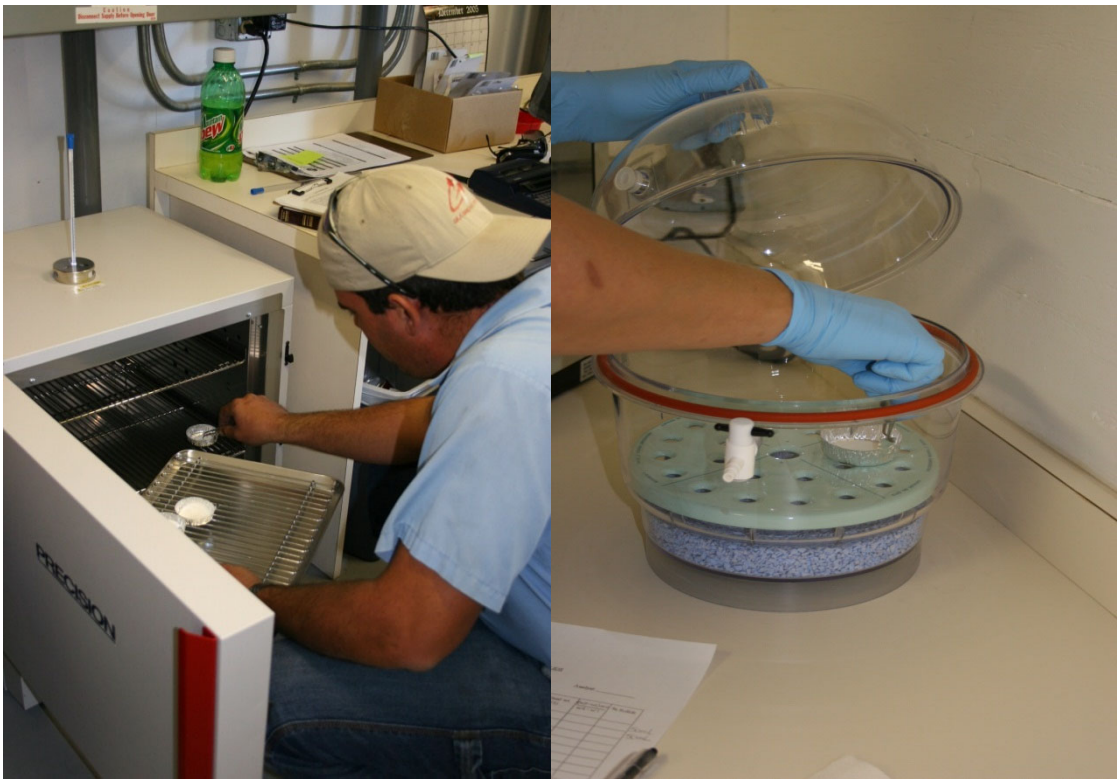
$$\frac{(W_2) - (W_1) \times 1000 \text{ ML/L}}{\text{ML Sample}}$$

Where:
(W_1) and (W_2) are expressed in mg.
(W_1) = mass of the prepared filter
(W_2) = mass of the filter and sample after the filtration step.





Total dissolved solids - The weight per unit volume of all volatile and non-volatile solids dissolved in a water or wastewater after a sample has been filtered to remove colloidal and suspended solids.



Top left, filters being baked at 105°C. Right photograph, filters in desiccant.

SETTLEABILITY TEST

The settleability test is an analysis of the settling characteristics of the activated sludge mixed liquor suspended solids (MLSS). This analysis is often referred to as "running a settleometer." The analysis is normally done within the treatment plant rather than a certified laboratory.

This analysis includes five basic items:

1. A clear container to hold the MLSS
2. A timing device or clock to track elapsed time
3. A paddle or other mixing device
4. A clip board, or place to record the readings
5. Operator patience, attentiveness and diligence

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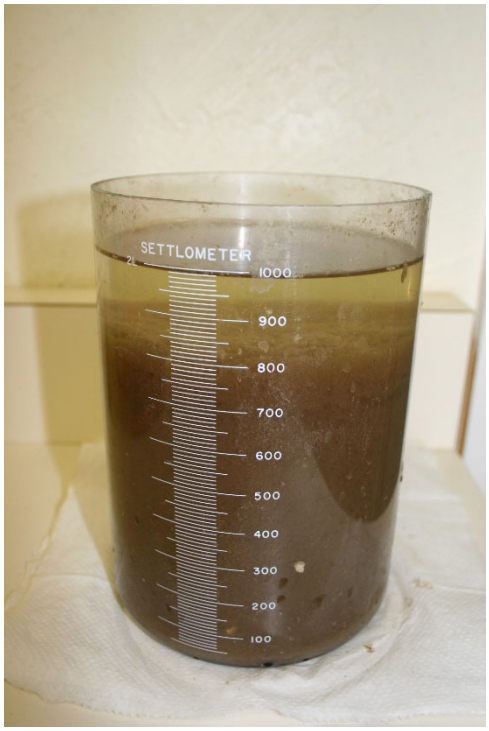
Sludge Volume Index (SVI)



1. Pour sample of mixed liquor from the process into a 2 liter settlometer.

2. Allow it to settle for 30 minutes

3. After the time period, read the marking to determine the volume occupied by the settled sludge and the reading is expressed in terms of mL/L and this is figure is known as the sludge volume SV value.

4. Next, for MLSS, there are actually two approaches to get the value. A conventional standard approach is by filtering the sludge, drying it and then weigh the second portion of the mixed liquid. However, this can be time consuming and a faster way is by using MLSS meter.

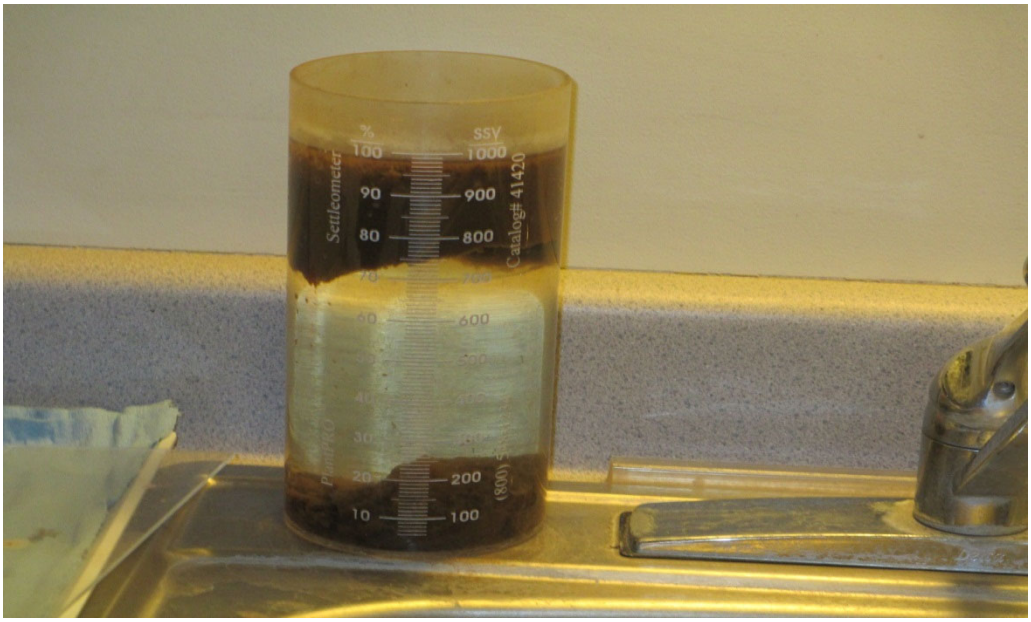
.

Calculation:
The results obtained from the suspended matter test and settleability test on aerated mixed liquor are used to obtain the SVI.

Calculation:

$$\textbf{SVI} = \frac{\text{sludge volume SV}}{\text{MLSS}} \times 1000$$

The settleometer is a great tool for operators. It indicates how the solids will settle in the clarifier and the density of the sludge.



During the settleometer test, operators not only check how the solids settle out they can also determine the rate of denitrification in the clarifier.

Sludge Volume Index Lab Report Worksheet

Suspended Mater Calculations:

(W_1) = _____ mg Duplicate (W_1) = _____ mg

(W_2) = _____ mg (W_2) = _____ mg

mls Sample = _____ mls Sample = _____ .

mg/L suspended matter = _____ dup. _____ .

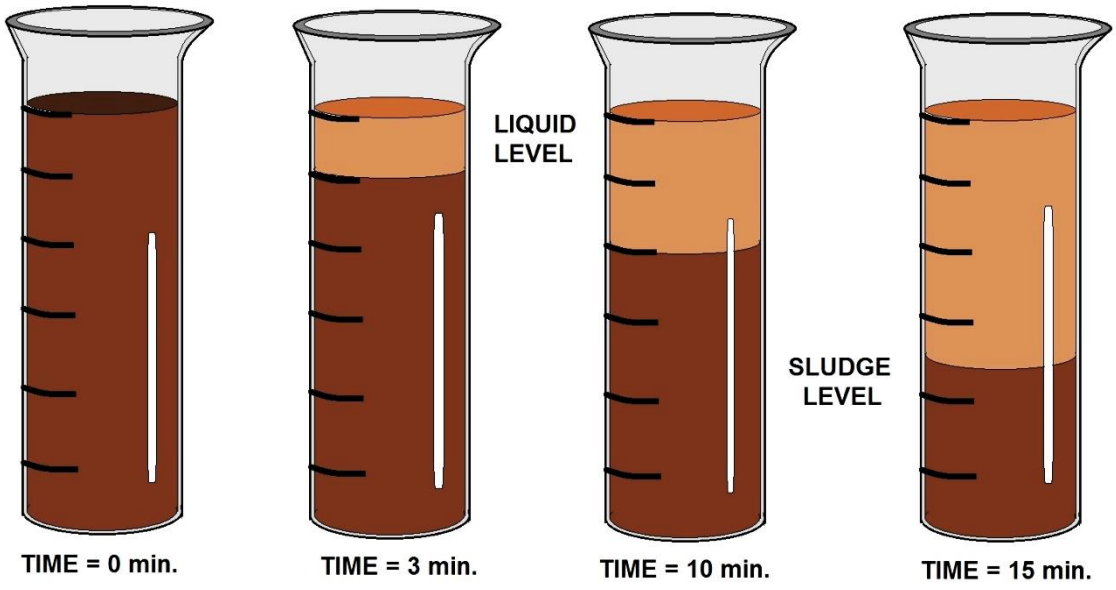
Settleability Calculations:

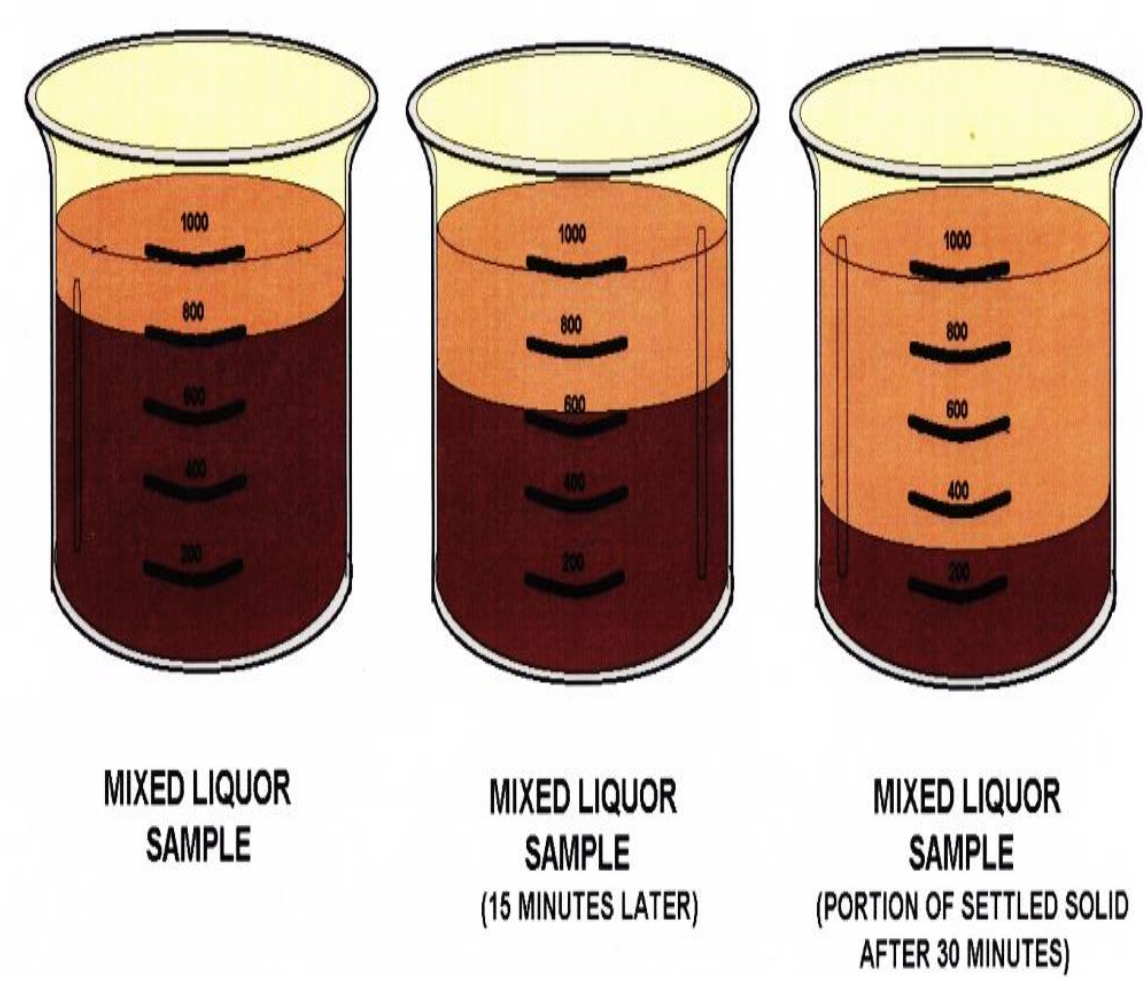
% settled sludge = _____

$$\frac{\text{(ml of sludge in settled mixed liquor or returned sludge x 100)}}{1000}$$

Sludge Volume Index Calculations:

$$\frac{\text{(ml of sludge in settled mixed liquor in 30 minutes x 1000 mg/g)}}{\text{mg/L of suspended matter in mixed liquor}}$$





Activated sludge process control calculations may include determination of the thirty and sixty minute settled sludge volume (SSV30 and SSV60), sludge volume index (SVI) and pounds of waste activated sludge removed from the process. The sample jars used are 1,000 milliliters or 2,000 milliliters.

Some plants are designed in steps for nitrification/denitrification as shown in the picture above.

Nitrification is a microbial process by which ammonia is sequentially oxidized to nitrite and then to nitrate. The nitrification process is accomplished primarily by two groups of autotrophic nitrifying bacteria that can build organic molecules by using energy obtained from inorganic sources—in this case, ammonia or nitrite.

Denitrification is the process by which nitrates are reduced to gaseous nitrogen by facultative anaerobes. Facultative anaerobes, such as fungi, can flourish in anoxic conditions because they break down oxygen containing compounds to obtain oxygen.

MIXED LIQUOR DEFINITION

Mixed liquor suspended solids (MLSS) is the concentration of suspended solids, in an aeration tank during the activated sludge process, which occurs during the treatment of wastewater. The units MLSS is primarily measured in are milligrams per liter (mg/L). Mixed liquor is a combination of raw or unsettled wastewater and activated sludge within an aeration tank.

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MLSS

Mixed Liquor Suspended Solids (**MLSS**) is a test for the total suspended solids in a sample of mixed liquor. This test is essentially the same as the test you performed for **TSS** in the last lab, except for the use of mixed liquor as the water sample. In addition, the concentration of suspended solids found in the mixed liquor is typically much greater than that found in the raw or treated water. **MLSS** concentrations are often greater than 1,000 mg/L, but should not exceed 4,000 mg/L.

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MLVSS

Mixed Liquor Volatile Suspended Solids is generally defined as the microbiological suspension in the aeration tank of an activated-sludge biological wastewater treatment plant.

The biomass solids in a biological waste water reactor are usually indicated as **total suspended solids (TSS)** and **volatile suspended solids (VSS).** The mixture of solids resulting from combining recycled sludge with influent wastewater in the bioreactor is termed **mixed liquor suspended solids (MLSS)** and **mixed liquor volatile suspended solids (MLVSS).** The solids are comprised of biomass, **nonbiodegradable volatile suspended solids (nbVSS),** and inert **inorganic total suspended solids (iTSS).**

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MIXED LIQUOR CALCULATION

MLSS (g/L) = SV [mL/L]/SVI [mL/g]

Where:
SVI = sludge volume index (mL/g)
SV = Volume of settled solids per 1 litre after 30 minutes
SVI is a calculation from two analyses: SV30 and MLSS.
$0=(Q+Q_r)(X')-(Q_rX'_r+Q_wX'_r)$

Where:
Q = wastewater flow rate (m^3/d)
Q_r = return sludge flow rate (m^3/d)
X' = MLSS (kg/m^3)
X'_r = return sludge concentration (kg/m^3)
Q_w = sludge wasting flow rate (m^3/d)

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MIXED LIQUOR ADJUSTMENT

If content is too high
1. The process is prone to bulking of solids and the treatment system can become overloaded.
2. This can cause the dissolved oxygen content to drop; this may reduce the efficiency of nitrification and the settleability of the sludge.
3. Excessive aeration will be required, which wastes electricity.
4. It will create thick foam on upper layer.

If content is too low
1. The process may not remove sufficient organic matter from the wastewater.
2. The sludge age may be too low to enable nitrification.

The typical control band for the concentration of MLSS is 2 to 4 g/L for conventional activated sludge, or up to 15 g/l for membrane bioreactors.

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Fecal Coliform Analysis Sub-Section

FECAL TESTING CONCEPT

A sample is collected and analyzed using aseptic (sterile) technique. A measured volume of sample is filtered through a sterile 0.45µ membrane filter, transferred to an absorbent pad containing m-FC broth, then incubated at 44.5°C for 24 hours. Blue/blue gray colonies are counted and reported as colony forming units (cfu) per 100 ml of sample. The method is limited by turbidity in the sample. Excessive turbidity will reduce fecal coliform recovery, requiring the MPN method to be used instead of the membrane filter method.

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Sample Collection

Fecal coliform must be collected in a clean, sterile borosilicate glass or plastic bottle containing sodium thiosulfate. Pre-sterilized bags or bottles containing sodium thiosulfate can also be used. Sodium thiosulfate is added to remove residual chlorine which will kill fecal coliforms during transit. 0.1 ml of 10% sodium thiosulfate is added to a 120 ml sample bottle prior to sterilization. The minimum bottle size should be 120 ml to allow enough head space (1") for proper sample mixing.

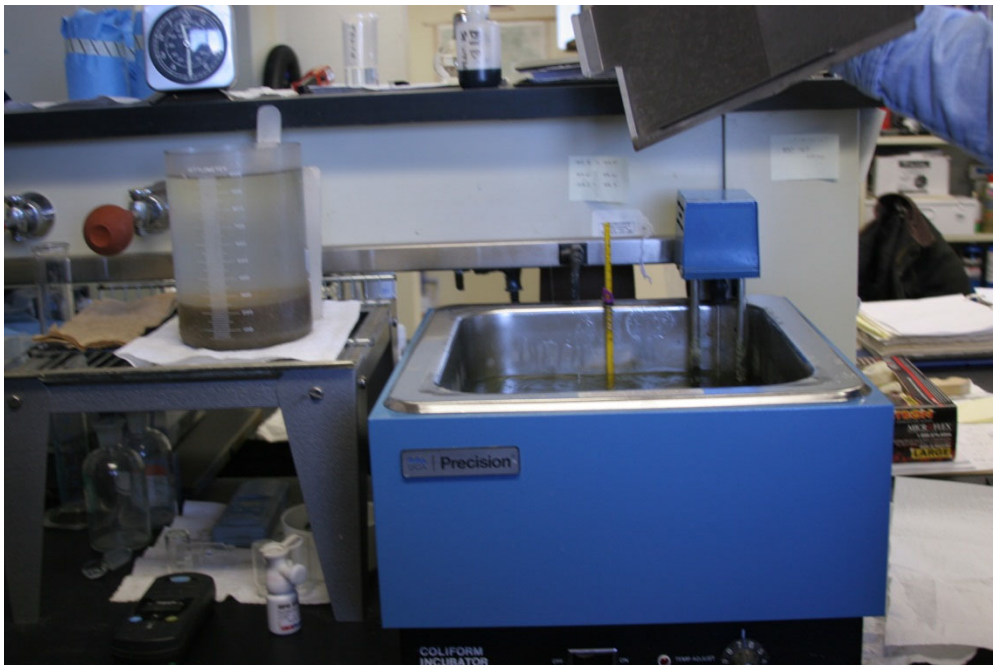
Collection Procedure

Select a site that will provide a representative sample. Fecal coliform samples are always grab samples and should be drawn directly from the flow stream without using collection other devices. We do not want to cross contaminate the sample. Keep the sample bottle lid closed tightly until it is to be filled.

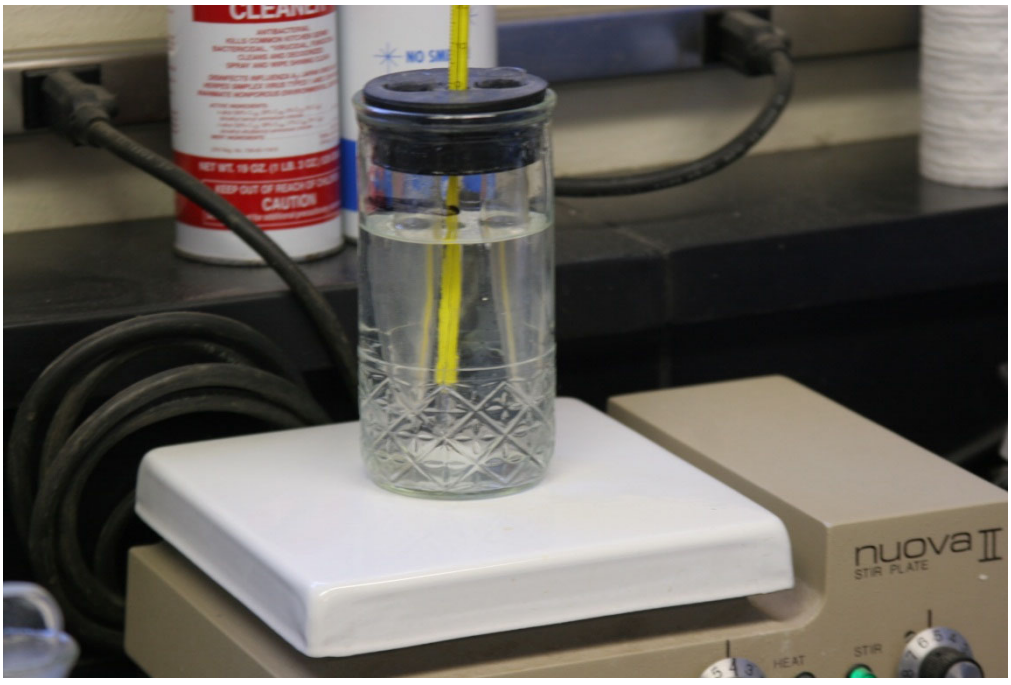
Remove the cap and do not contaminate the inner surface of the bottle, neck, threads or cap. Fill the container without rinsing, being sure to leave ample air space to allow mixing. Rinsing will remove the dechlorinating agent. All samples should be labeled properly with date and time of collection, sampler's name, and sample collection location. Leaking sample bottles allow for contamination of the sample and should be discarded and the sampling repeated.

Preservation

Fecal coliform samples should be analyzed as soon as possible after collection to prevent changes to the microorganism population. Fecal coliforms must be transported on ice, if they cannot be analyzed within 1 hour of collection. Fecal coliforms transported at ambient temperature may reproduce and higher bias to the numbers than desired or they may be killed off resulting in lower numbers, if handled poorly such as transport in sunlight. Fecal coliform samples should be stored by the laboratory in a refrigerator until time of analysis. The maximum holding time for state or federal permit reporting purposes is 6 hours.



An incubator for the coliform test. The operator will place the sample in this device for 24 to 48 hours depending on the desired results. There are several different methods to calculate coliform bacteria. This is an older true and tested method.



This glass bottle is used for quality control (QA/QC) for bacteria samples tubes.

This operator is wearing the proper safety measures for preparation of the fecal test.

This operator is splitting the sample for bacteriological analysis. Always wear gloves for your and others' safety. We have all seen the operator holds a sandwich in one hand while working in the lab, or the operator does not wear gloves at all.

Phase microscopes are used to see indicator bugs and other MO's microorganisms. This examination is used so that the operator knows how well the process is working.

This is a filter used for the coliform test.

Course Credits

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