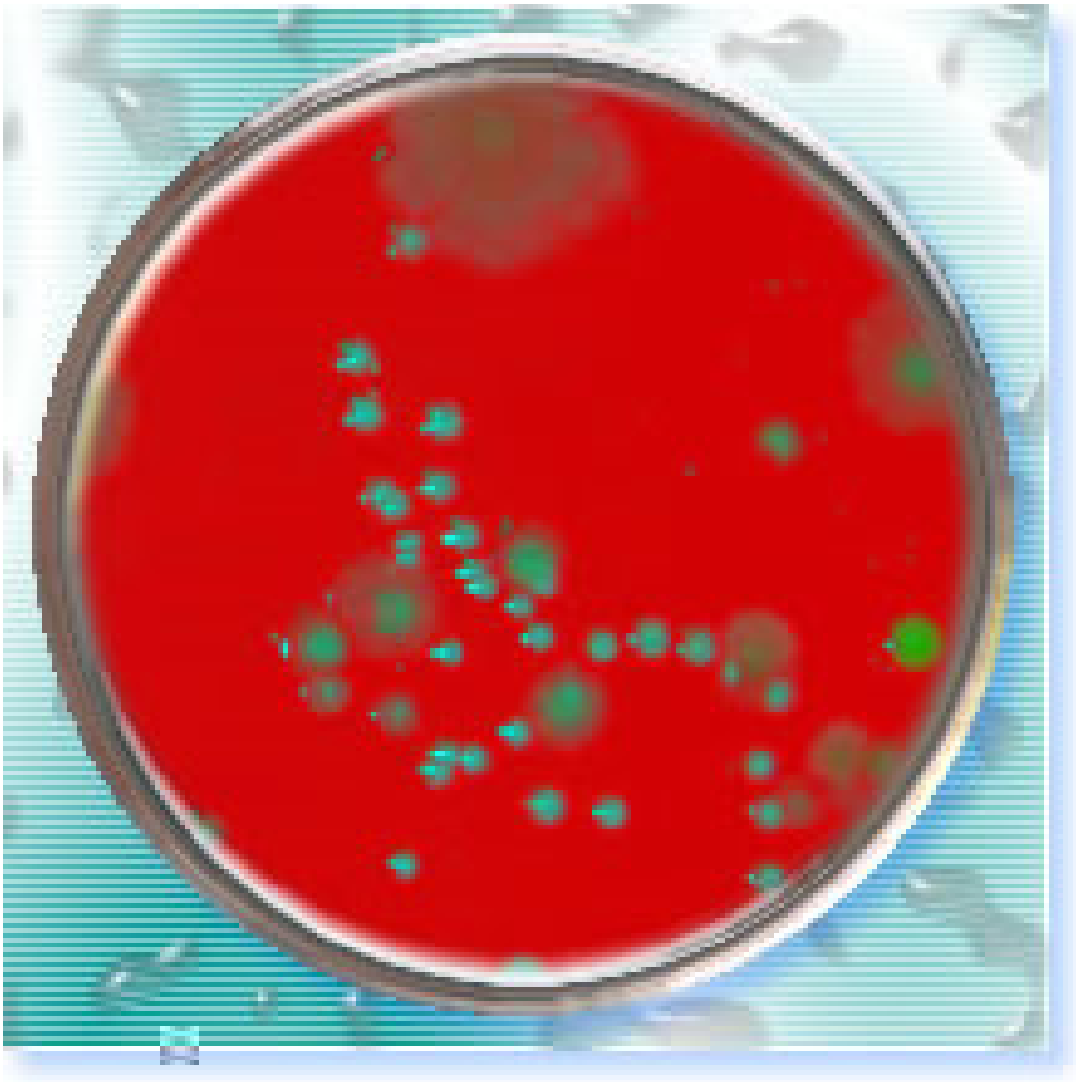
BACTERIOLOGICAL DISEASES

CONTINUING EDUCATION
PROFESSIONAL DEVELOPMENT COURSE



Technical Learning College

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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 Do not solely trust our list for it may be outdated.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.

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http://www.abctlc.com/downloads/PDF/CEU%20State%20Approvals.pdf

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

You can obtain a printed version from TLC for an additional $169.95 plus shipping charges.

All downloads are electronically tracked and monitored for security purposes.

IDEXX's SimPlate for HPC method is used for the quantification of heterotrophic plate count (HPC) in water. It is based on the Multiple Enzyme Technology which detects viable bacteria in water by testing for the presence of key enzymes known to be present in these little organisms. This technique uses enzyme substrates that produce a blue fluorescence when metabolized by waterborne bacteria.

The sample and media are added to a SimPlate Plate, incubated and then examined for fluorescing wells. The number of wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample. The MPN values generated by the SimPlate for HPC method correlate with the Pour Plate method using the Total Plate Count Agar incubated at 35°C for 48 hours as described in *Standard Methods for the Examination of Water and Wastewater, 19th Edition.*

We will go more into detail in the Water Monitoring Section.

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.

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Dr. Pete Greer S.M.E., Retired biology instructor, chemistry and biological review.

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.

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.)

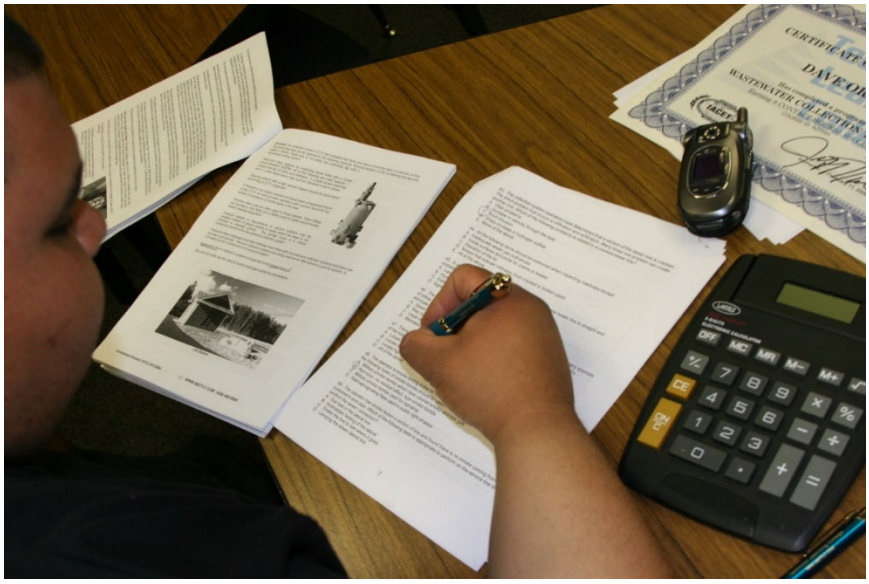
Students have direct contact with their instructor—primarily by e-mail or telephone. TLC's CEU courses may use such technologies as the World Wide Web, e-mail, CD-ROMs, videotapes and hard copies. (See the course description.) Make sure you have access to the necessary equipment before enrolling; i.e., 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

CEU Course Introduction

Bacteriological Diseases CEU Training Course

This short CEU Course will review commonly found water and wastewater diseases, symptoms, and identification techniques. This course will cover water and wastewater sampling techniques, waterborne disease identification ND control, general water quality operations and definitions. It will also cover disease symptoms, disease diagnosis, history of the disease, susceptibility, and disease sources of contamination.

This course will apply to all categories of water treatment/distribution and wastewater treatment/collection. As water professionals, it is our responsibility to identify, stop, and control all waterborne diseases.

This course was designed for the enhancement of laboratory technical abilities. This course was intended for Water Laboratory Analysts, but can be utilized by Wastewater Treatment, Collections, Water Distribution, Well Drillers, Pump Installers, and Water Treatment Operators.

This course is also an excellent introduction for a person interested in working in the water quality field, water/wastewater treatment or distribution or a collections facility and wishing to maintain CEUs for a certification license or to learn how to do the job safely and effectively, and/or to meet education needs for promotion. Every operator or customer service person that has contact with the public should have this booklet accessible to help answer water quality and waterborne disease related questions.

Course Procedures for Registration and Support

All of Technical Learning College's distance learning courses have complete registration and support services offered. Delivery of services will include e-mail, web site, telephone, fax and mail support. TLC will attempt immediate and prompt service.

When a student registers for a distance or correspondence course, he/she is assigned a start date and an end date. It is the student's responsibility to note dates for assignments and keep up with the course work. If a student falls behind, he/she must contact TLC and request an end date extension in order to complete the course. All students will be tracked by a unique number assigned to the student.

Course Assignment Material

Most of the EPA questions will come from the EPA summary, waterborne disease section identification, MCL listing, water sampling and laboratory procedures. Other detailed information will come from the Center of Disease Control. The EPA rules and laboratory procedures are also available online at the EPA Web site: **www.epa.gov**. You are expected to read and understand all these rules and laboratory procedures.

Instructions for Written Assignments

The ***BACTERIOLOGICAL DISEASES*** distance learning course uses a multiple-choice style answer key. You can find the answer key in the rear of the assignment. You may have the option of completion of the laboratory assignment, either the heterotrophic plate count, Method 1623 - Cryptosporidium and Giardia, or most EPA's data gathering and monitoring programs under the Unregulated Contaminant Monitoring Rule.

To receive alternate credit for the course, please contact TLC to receive permission from your Instructor.

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 ***BACTERIOLOGICAL DISEASES*** **CEU** 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. We will send the required information to Texas, Indiana, and Pennsylvania for your certificate renewals.

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.

Educational Mission

The educational mission of TLC is:

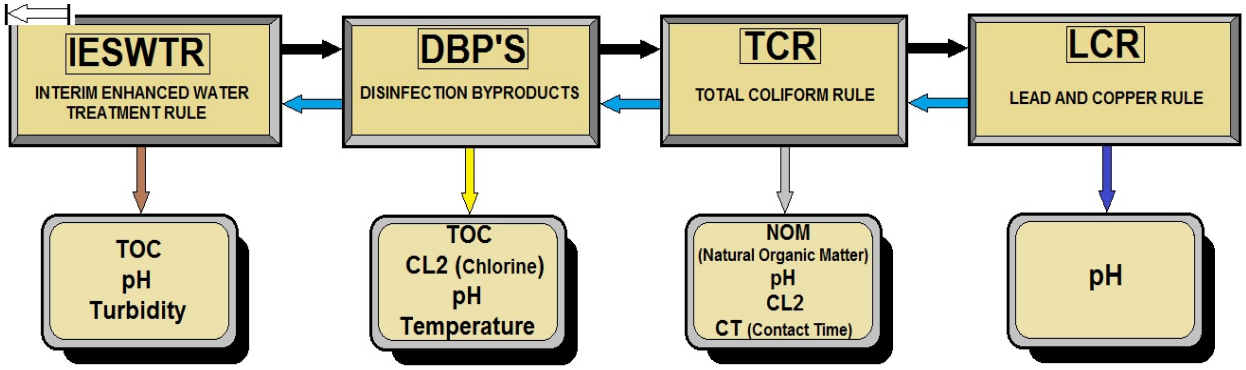
To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

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.



PARAMETERS THAT AFFECT SURFACE WATER TREATMENT RULES

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.

TABLE OF CONTENTS (Learning Objective Legend on Page 16)

Topic Legend

This CEU course covers several different educational topics/functions/purposes/objectives of conventional water treatment, filtration processes, bacteriological monitoring and regulatory compliance. The topics listed below are to assist in determining which educational objective or goal is covered for a specific topic area:

CRAO - Compliance and Regulatory Affairs: The regulatory and compliance component of your need to know. May be a requirement of the SDWA act or State Regulations, i.e. Compliance, non-compliance, process control related sampling or other drinking water related requirement. This EPA information is to satisfy the regulatory portion of your operator training. Part of O&M or laboratory training requirement for many operators.

DISN - Disinfection: This area covers plant disinfection procedures. Part of O&M training for many operators. May include alternative disinfection procedures, i.e. Ozone and Ultraviolet

GP - GROUNDWATER MINING OR PRODUCTION: This may be considered O&M training for many operators or credit for pump engineers or well drillers.

M/O - Microorganisms: The biological component. The microorganisms that are specifically found in drinking water. This section may be part of required sampling, i.e. Total Coliform Rule or other biological related sampling. Part of O&M or laboratory training requirement for many operators.

O&M - Operations and Maintenance: This area is for normal Operation and/or Maintenance of the plant. Part of O&M training requirement for many operators.

SAFETY: This area describes process safety procedures. It may be part of O&M training requirement for many operators.

TECH - TECHNICAL: The mechanical or physical treatment process/component. The conventional or microfiltration process including pretreatment processes/ applications/ engineering/ theories. Part of O&M training for many operators.

WQ – Water Quality: Having to do with Water Quality or pollutants, i.e., hard water to primary water standards. May be a requirement of the SDWA and/or water chemistry concerns. This along with the EPA information is to satisfy the regulatory portion of your operator training.

Common Water Related 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.

Nephelometric 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.

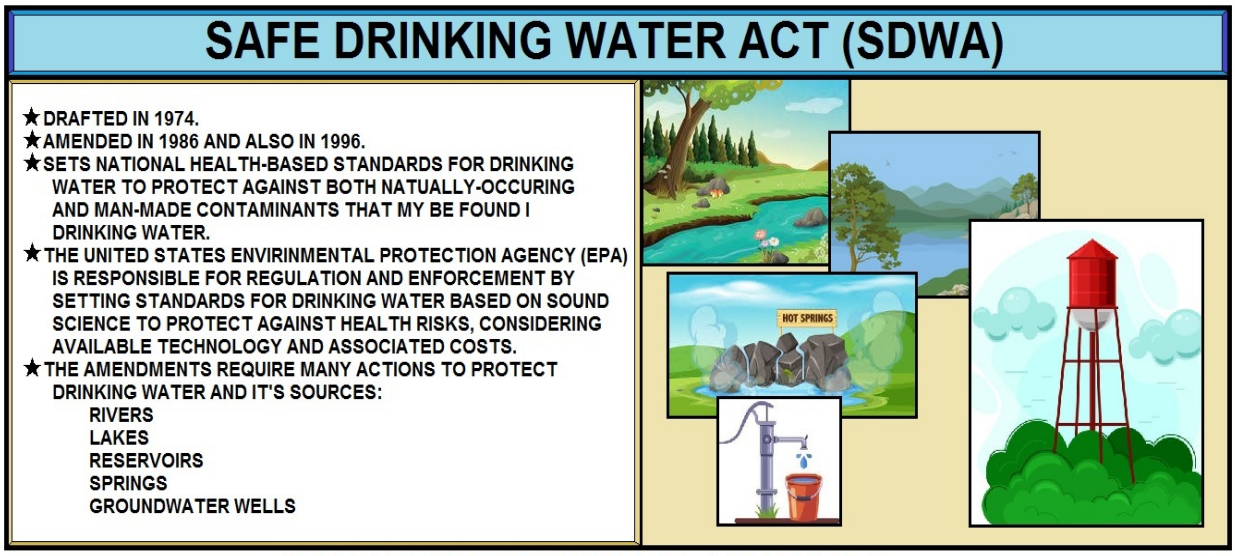
Watershed Area. A topographic area that is within a line drawn connecting the highest points uphill of a drinking water intake, from which overland flow drains to the intake.

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 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.

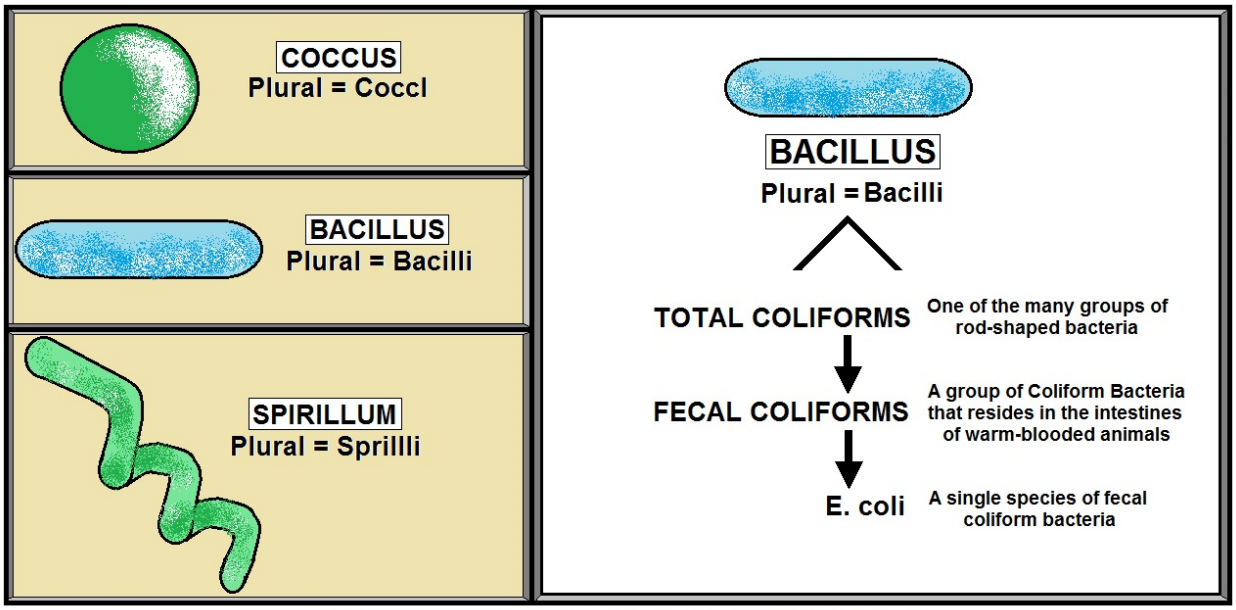
The 1996 Amendments authorize the EPA to set a standard at other than the feasible level if the feasible level would lead to an increase in health risks by increasing the concentration of other contaminants or by interfering with the treatment processes used to comply with other SDWA regulations. In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

In addition, 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.

Water Microbiology - Chapter 1

Section Focus: You will learn the basics of bacteria and the history for bacteriological discoveries. At the end of this section, you will be able to describe simple microbiology. 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 bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, (bacteria, viruses, cysts, single-celled organisms) particularly those that cause E. coli, gastroenteritis, cryptosporidiosis or giardia (primary domestic concerns).



COLIFORM BACTERIA EXAMPLE

Technical Learning College

Facilities that serve water to the public sometimes have elevated chlorine and bacteria levels in their treated drinking water, and additional actions are needed. Consumption of or contact with water contaminated with feces of warm-blooded animals can cause a variety of illnesses.

Minor gastrointestinal discomfort is probably the most common symptom; however, pathogens that may cause only minor sickness in some people may cause serious conditions or death in others, especially in the very young, old, or those with weakened immunological systems.

Bacteria and viruses can also be listed as “indicators,” which at a level outside of identified limits, may reflect “a problem in the treatment process or in the integrity of distribution system,” according to the EPA.

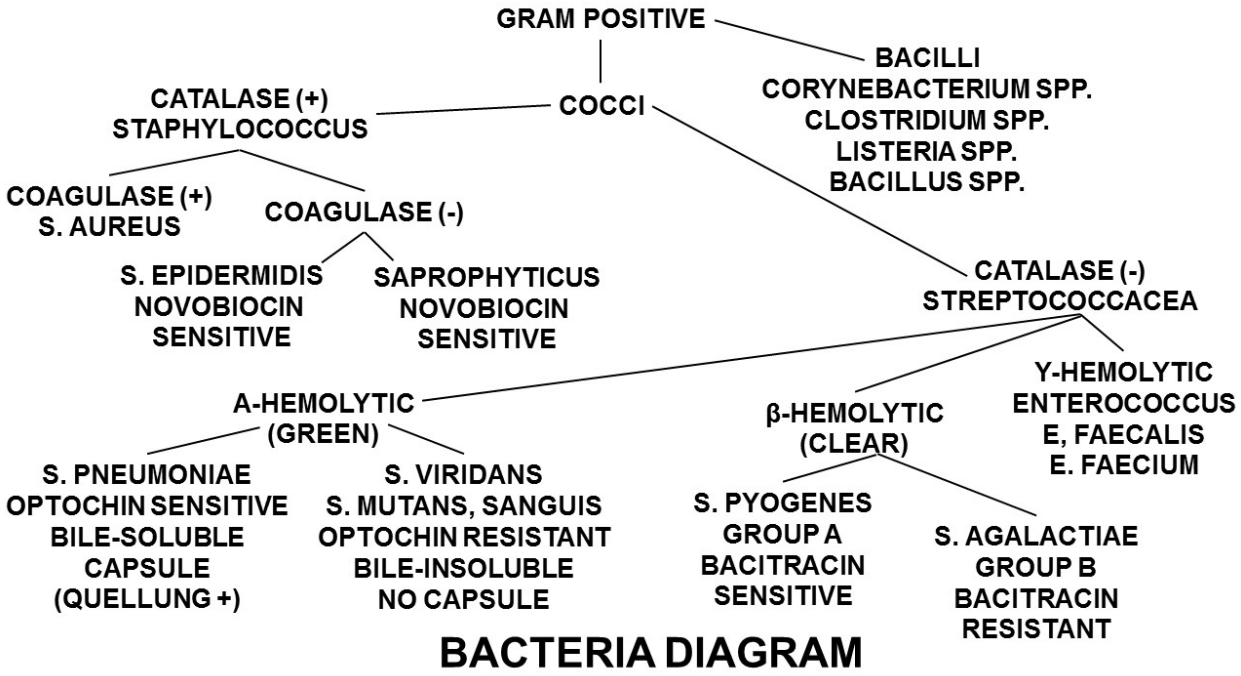
Here is a list of EPA regulated viral/bacterial indicators and their potential problems:

- Turbidity refers to the cloudiness of water and, although not a bacterium/virus, can hinder disinfection, providing an environment for microbial growth and can indicate the presence of bacteria/viruses as well as other disease-causing organisms that can produce symptoms such as nausea, diarrhea, cramps and headaches.

- Coliforms are bacteria naturally present in the environment and used as indicators that other possibly harmful bacteria may be present (a warning sign is if coliforms are found in more samples than allowed).

- Fecal indicators, *Enterococci* or coliphage, are microbes that can indicate human or animal wastes in water; they can cause short-term health effects, including: Cramps, nausea, diarrhea, headaches and more, and may pose a greater risk for people with severely weak immune systems, elderly, young children and infants (*Enterococci* are bacterial indicators of fecal contamination and coliphage are viruses that infect *E. coli*).

- *E. coli* and fecal coliform are bacteria whose presence can indicate water contaminated by human or animal wastes, causing short-term health effects, including: Cramps, nausea, diarrhea, headaches and more; they may also pose a greater risk for people with severely weak immune systems, elderly, young children and infants.



BACTERIA DIAGRAM

History of Water Microbiology Research

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.

LOUIS PASTEUR

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.

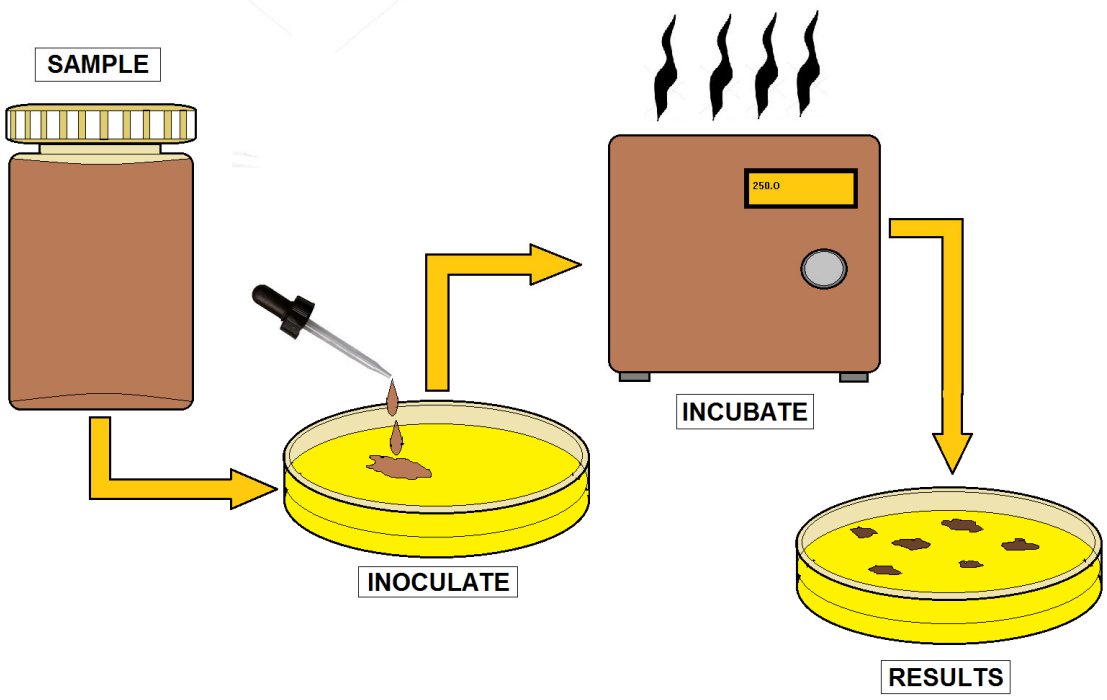
Rudolph Virchow completed the cell theory with the idea that all cells must arise from pre-existing cells.

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.

Cell 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.

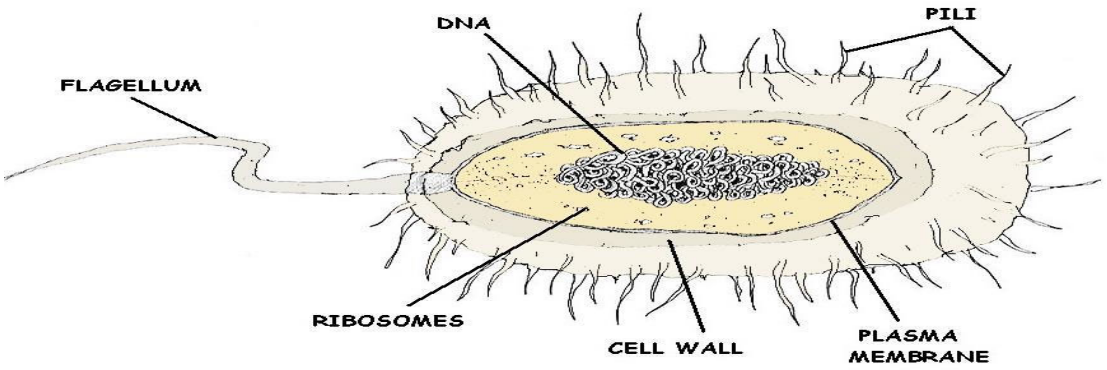


CULTURING MICROBAL SAMPLES

Credit EPA for the information in this Section.

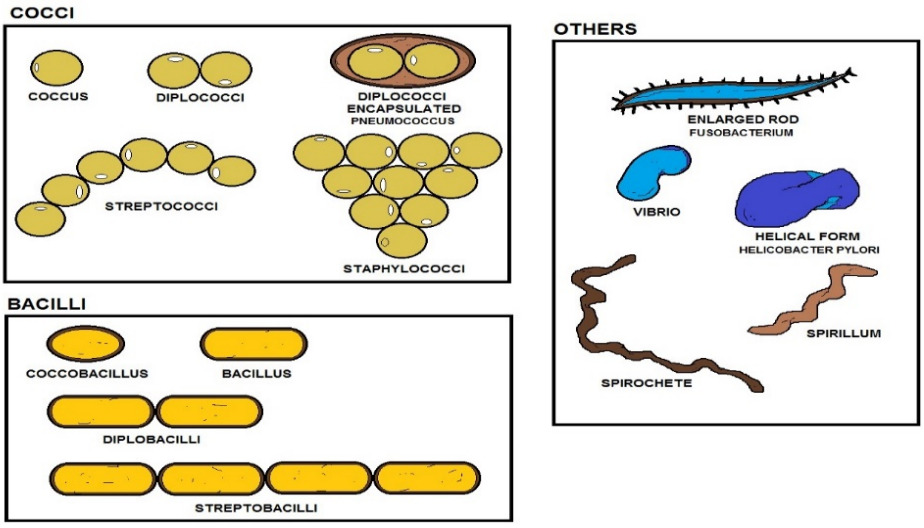
More on Bacteria

Bacteria consist of only a single cell, a highly designed and complex organism. More complex than a Boeing 747 or even a modern computer. We have been wrongly taught that bacteria and other single-celled organisms are “simple creatures”. Bacteria are 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.

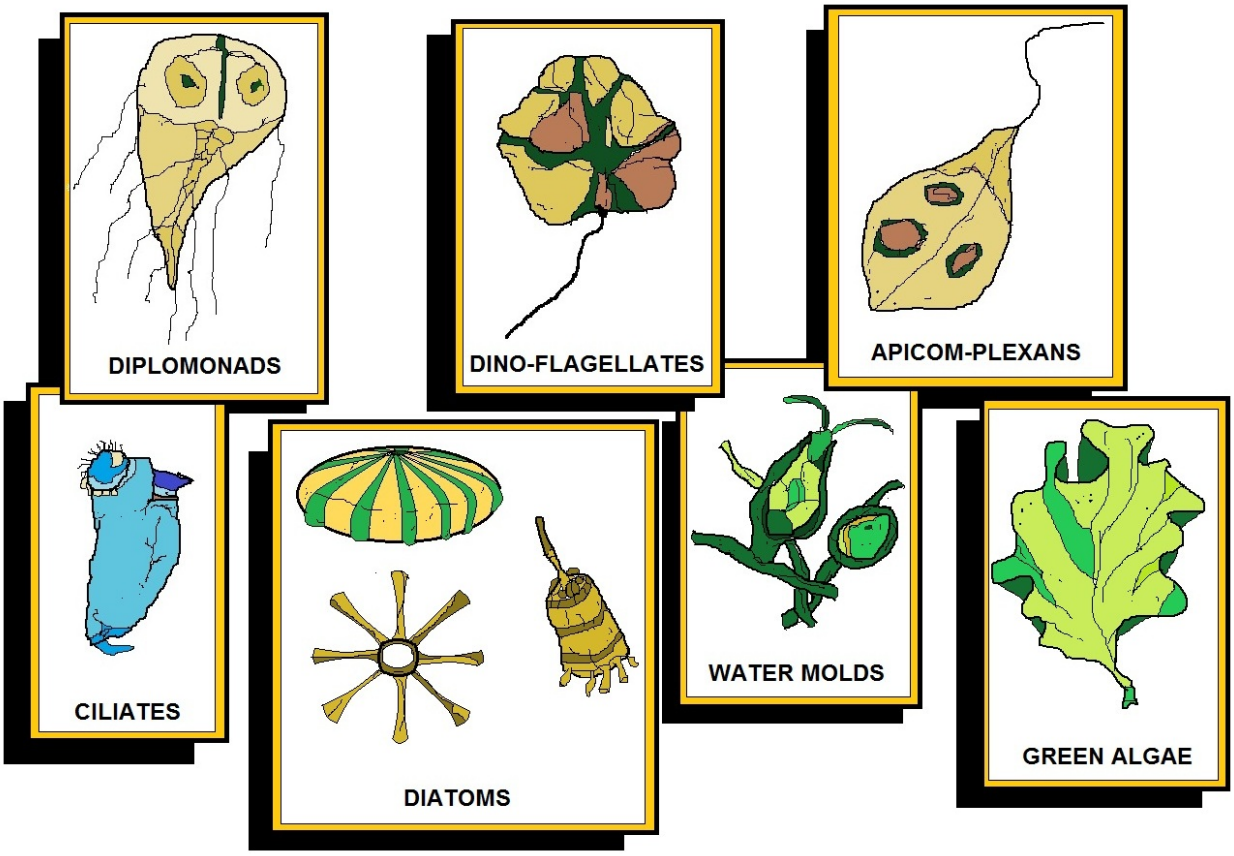


Schematic of a typical rod-type bacteria.

“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.

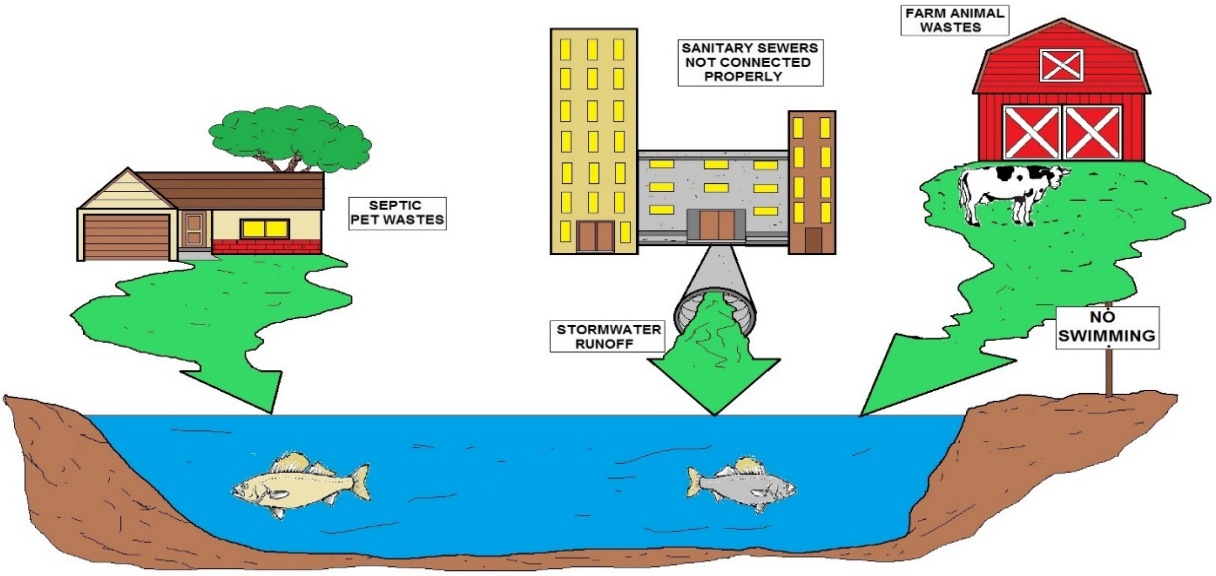


THE DIFFERENT SHAPES OF BACTERIA



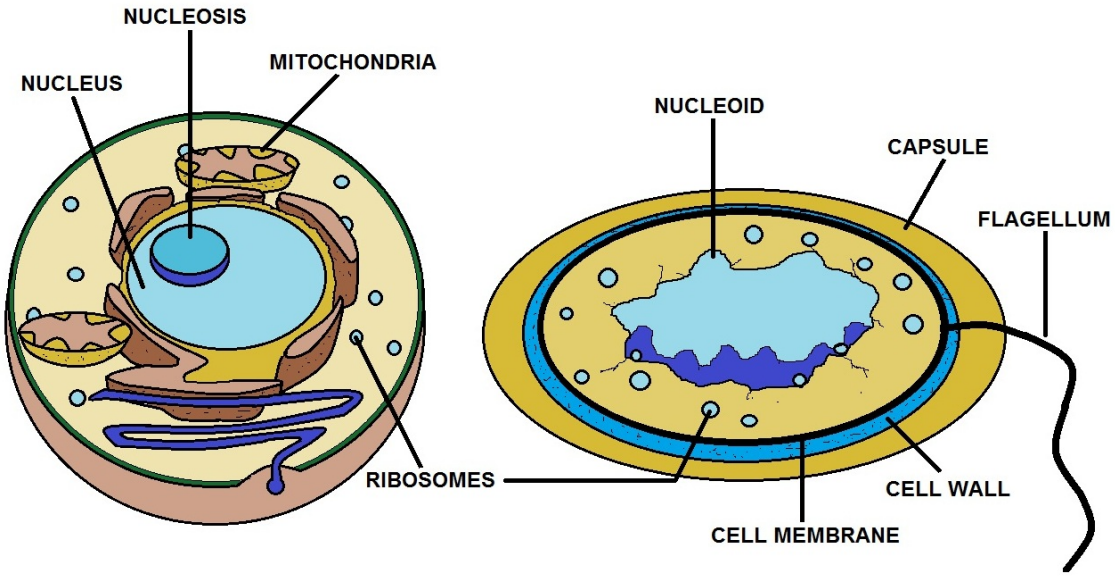
KINGDOM PROTISTA

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.



SOURCES OF FECAL COLIFORM BACTERIA

Prokaryotes



EUKARYOTE **PROKARYOTE**

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.)

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 that we call a cell wall.

However, bacterial cell walls are a very 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.

Bacteria Growth Terms

Lag-phase
During the lag-phase, bacteria are becoming acclimated to their new environment. They are digesting food and are developing the enzymes need to break down the types of nutrients that the bacteria have detected. Growth does not occur during this phase.

Accelerated Growth-phase
Bacteria begin to grow at a rapid rate because of the excess amount of food that is available. The cells are mostly dispersed and active. They are not sticking together to form floc.

Declining Growth-phase
Reproduction slows down at this phase because there is no long an excess amount of food. There are a large number of bacteria that have to compete for the remaining food. The bacteria begin to lose their flagella.

Stationary-phase
Because of the lack of food, some bacteria are reproducing but an equal number are also dying. Therefore, the number of bacteria remains relatively constant. They have not lost their flagella and have formed a stick substance covering the outside of the cell wall which allows them to agglomerate into floc.

Death-phase
In this phase the death rate increased with little or no growth occurring. The total number of bacteria keeps reducing.

Bacteria in the activated sludge system must be allowed to hang out in the aeration basin until they reach the stationary-phase. If they flow out of the basin too early, they will be active and motile and will not settle out as floc.

Food: Microorganism Ratio
The food to microorganism (F/M) ratio measures the amount of food that is available for the amount of microorganisms present in the aeration basin. The amount of food is determined by the biochemical oxygen demand (BOD) or chemical oxygen demand (COD) test. If there is too much food and not enough microorganism (high F/M ratio), settling problems may occur because in the presence of excess food bacteria are active and multiplying and will not develop into floc.

Factors Affecting Bacteria Growth
It is the responsibility of the operator to provide the best possible environment for the floc-forming bacteria to grow. The operator can control some of the conditions they require and there are some conditions they cannot control.

For instance, the operator has no control of the weather and very little control over the types and amount of nutrients entering the treatment plant. Therefore, it is important that the operator understand how the following factors affect the growth of the bacteria. Oxygen Utilization, Sludge Age, Dissolved Oxygen, Mixing, pH, Temperature, Nutrients

Oxygen Utilization
Actively growing bacteria eat food at a rapid rate therefore using oxygen at a rapid rate. The rate of oxygen use is normally termed the Oxygen Uptake Rate and is measured in mg O2/hr/gm of MLSS. Generally, a higher Uptake Rate is associated with a higher F:M ratio and younger sludge ages. A lower Uptake Rate is associated with a lower F:M ratio and older sludge ages.

Sludge Age
As bacteria first begin to develop in the system they grow singularly, in small clumps and chains. They are very active with flagella and do not have a well-developed slime layer. The bacteria are disperse and do not settle well. As the sludge is allowed to age, bacteria lose their flagella and accumulate more slime. The small clumps and chains begin to stick together and form floc large enough to settle.

Dissolved Oxygen
Aerobic bacteria require at least 0.1 - 0.3 mg/L of oxygen to survive. At least 2 mg/L of oxygen must be maintained in the bulk fluid in order for the bacteria in the center of the floc to get 0.1-0.3 mg/L of oxygen. If not, the bacteria in the center will die and the floc will begin to break up.

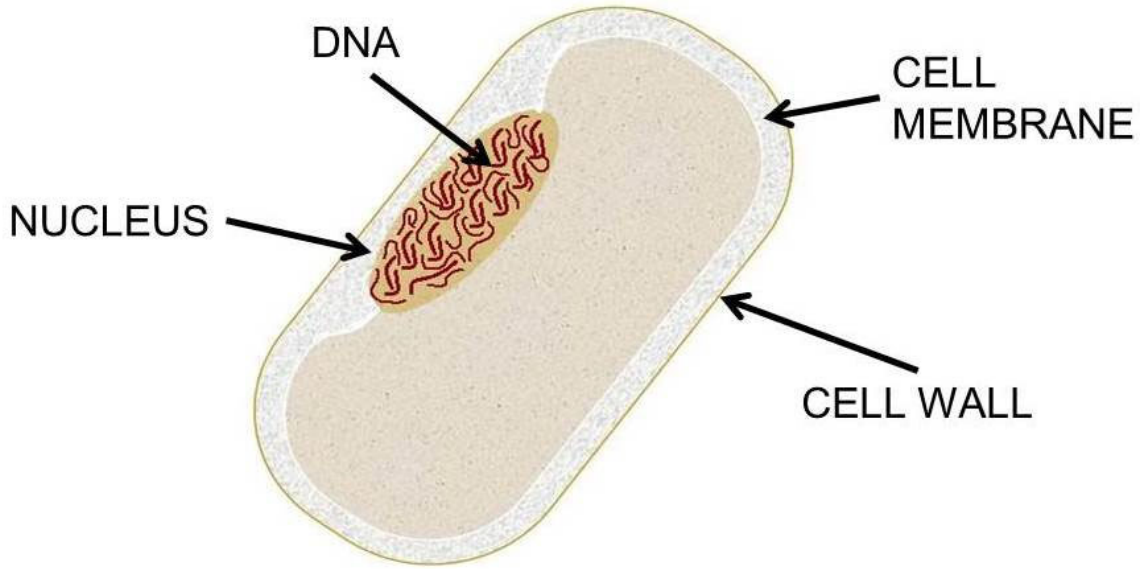
Mixing
Mixing is required to bring the bacteria, oxygen and nutrients in contact with each other. Remember, once food is limited the bacteria lose their flagella and can no longer swim. Without sufficient mixing, the bacteria will not bump into each other to form flow and proper treatment will not take place.

pH
It is the bacterial enzymes that are very pH dependent. Their optimal pH is between 7.0 and 7.5. Rapid pH changes should be avoided.

Temperature
Biochemical reactions are temperature dependent. Reactions are slower in colder temperatures so the system will require more organisms to do the work. Reactions are faster in warmer temperatures therefore fewer bacteria are required to do the same job during the summer months.

Nutrients
Bacteria require basic nutrients for growth (carbon, nitrogen, phosphorus as well as trace amounts of sodium, potassium, magnesium and iron. All these are present in normal domestic sewage. Generally, industrial wastes do not contain sufficient nutrients and must be supplemented.

Structure of a Eukaryotic Cell



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 Prokaryotic Cell
All bacteria are prokaryotes 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.

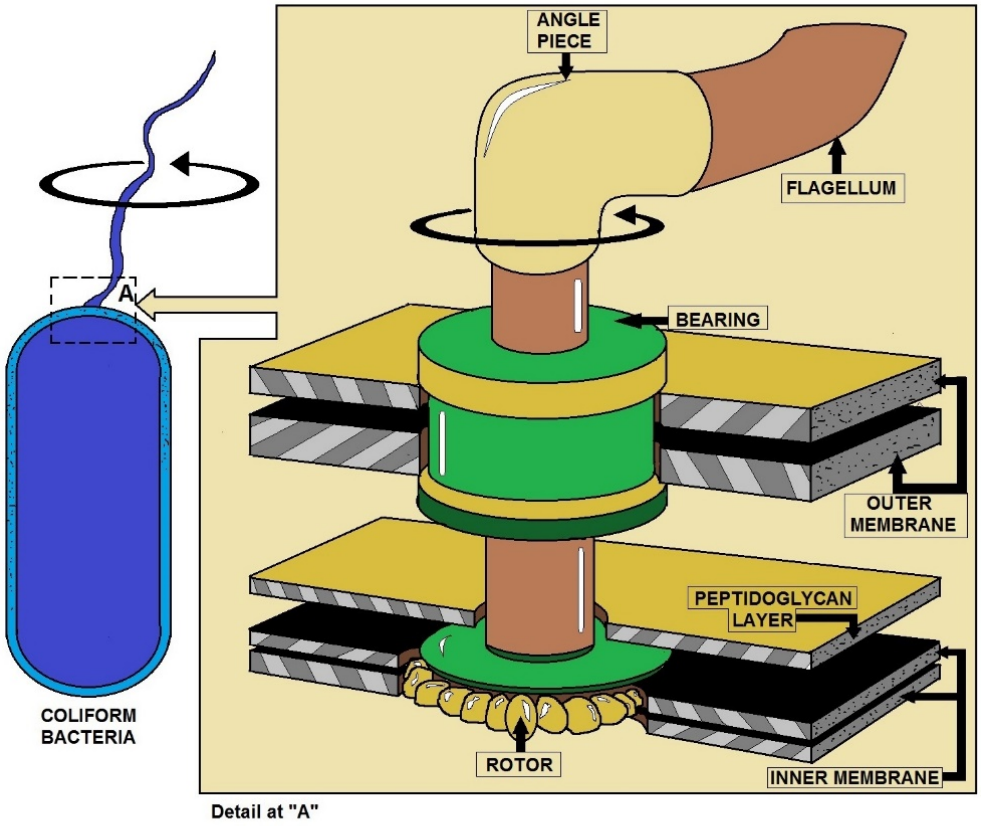
The composition of the capsule is useful in differentiating between different types of bacteria. Capsules are usually detected by negative staining, where the bacterial cell and the background become stained but the capsule remains unstained.

Encapsulated bacteria produce colonies on nutrient agar that are smooth, mucoid and glistening, whereas the non-capsulated 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.

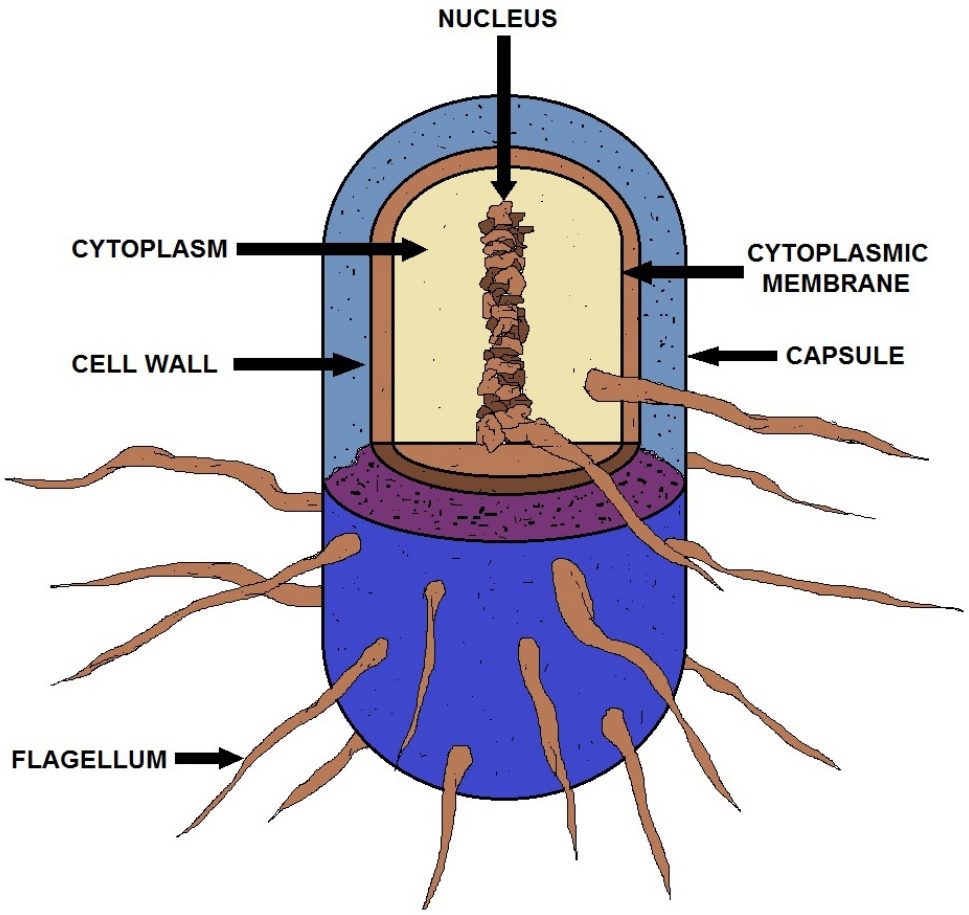


FLAGELLUM DIAGRAM

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 STRUCTURE

Bacterial Nutrition Sub-Section

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.

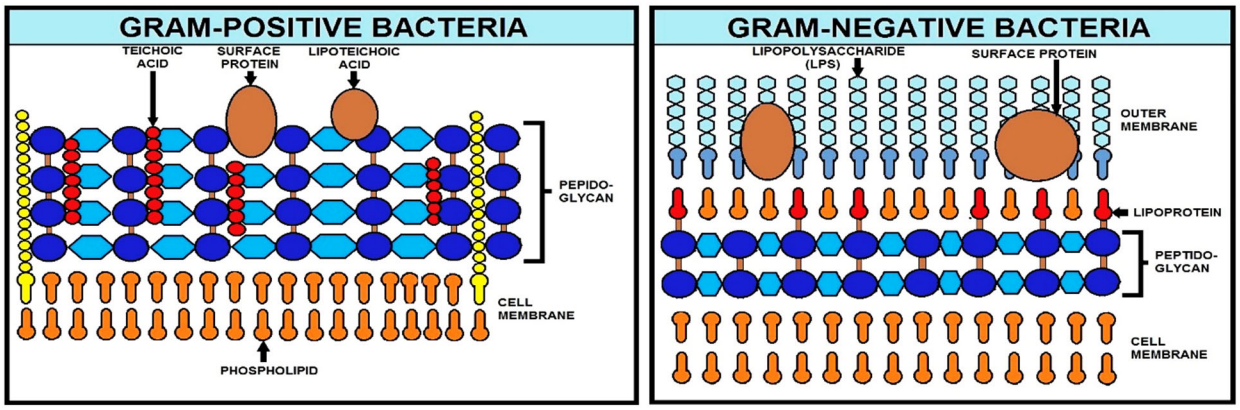
For example, some bacteria that are found in milk hardly make any of their own basic organic molecules; that is, they let the cow (or more to the point the number of microbes that live in the cow's gut) make these things for them. A simple rule of thumb is "***if humans can use something for food, many 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			

Gram Stain - Introduction

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.



GRAM STAIN DIFFERENCE DIAGRAM

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 $^{-}$.

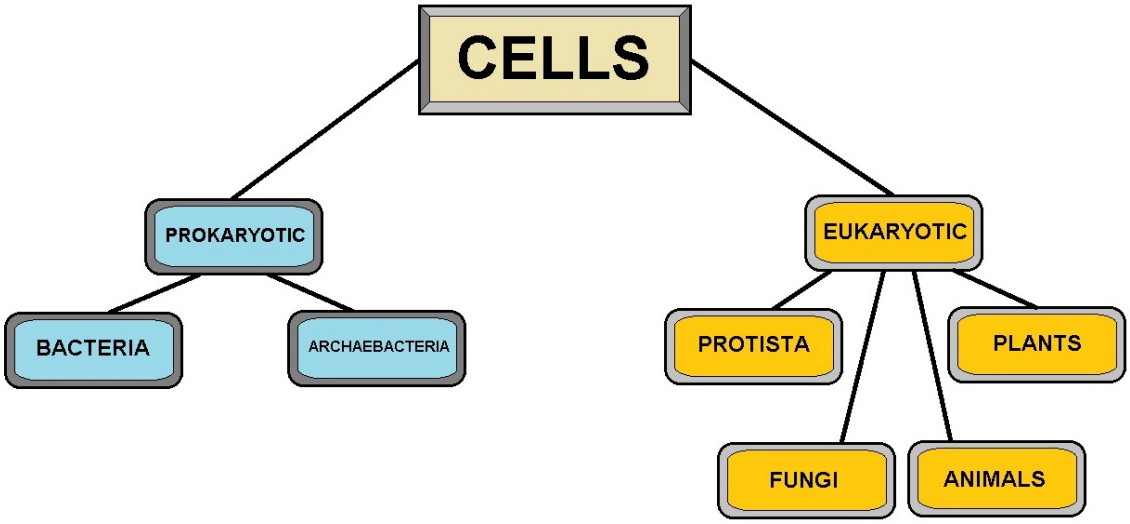
Gram^{+} bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color. Gram^{-} bacteria have more complex cell walls with less peptidoglycan, thus absorb less of the purple dye used and stain a pinkish color instead.

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 diagram.

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



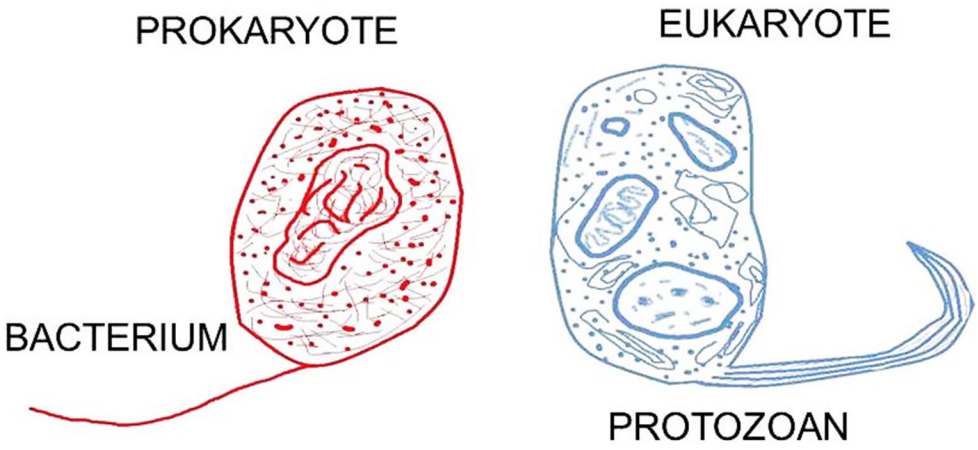
SINGLE CELL DIAGRAM

Two Types of Cells- Prokaryotes and Eukaryotes

A Prokaryotic 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 membrane bound organelles.
Example: Protozoa, fungi, algae, all plants and animal cells.

Eukaryote Defined

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.



PROKARYOTES ARE SIMPLER THAN EUKARYOTES

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.

Golgi Bodies or Dictyosomes
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.

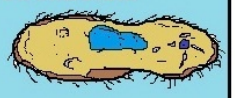
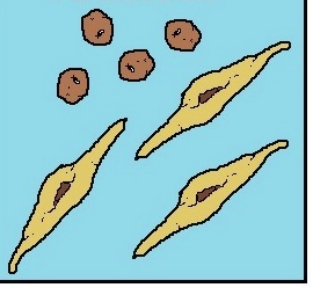
These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division.

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.

Paramecium Sub-Section

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. Because 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.

PHYLUM	COMMON NAME	LOCOMOTION	EXAMPLES
SARCODINA	SARCODINES	PSEUDOPODIA	AMOEBA 
CILIOPHORA	CILIATES	CILIA	PARAMECIUM 
SARCO-MASTIGOPHORA (ZOOMASTIGINA)	ZOOFLAGELLATES	FLAGELLA	TRYPANOSMA  GIARDIA
APICOMPLEXA (SPOROZOA)	SPOROZOANS	NONE IN ADULT FORM	PLASMODIUM 

PROTOZOA CLASSIFICATION

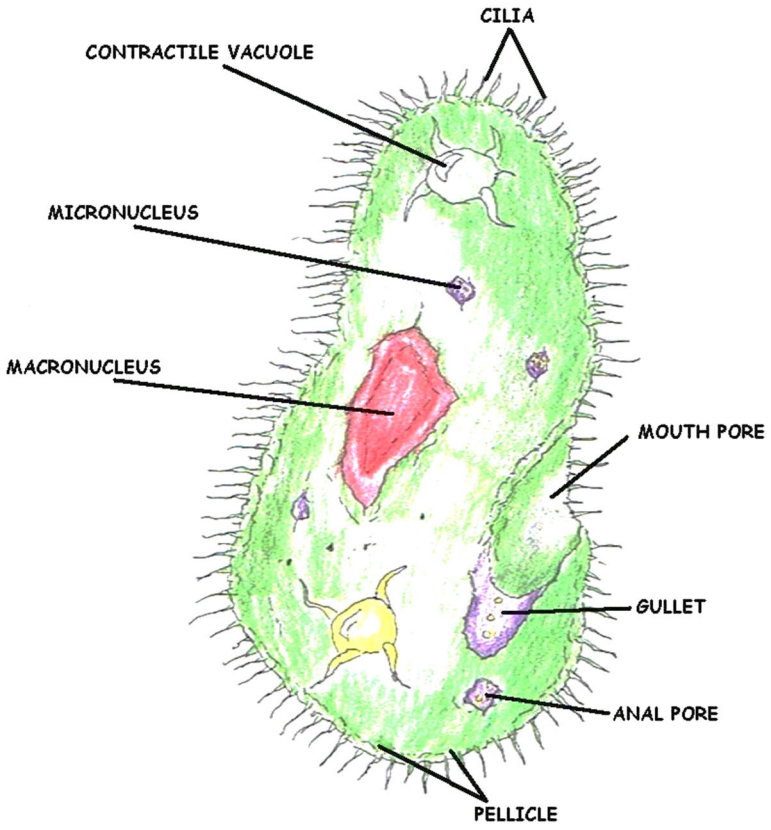
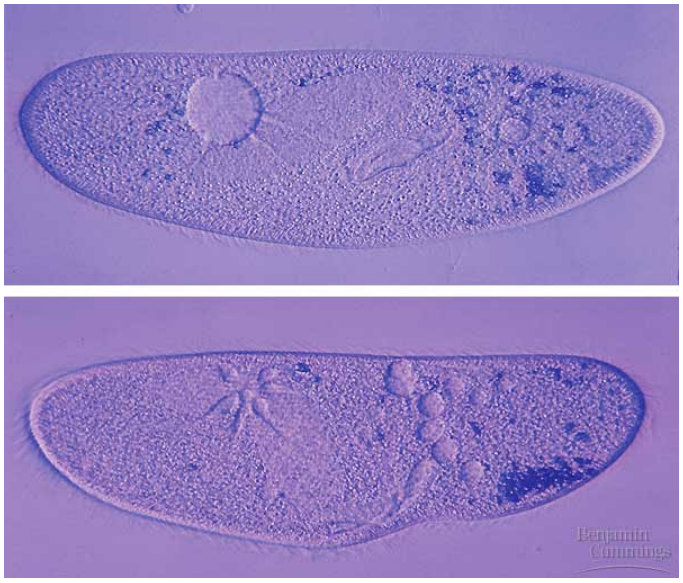


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.



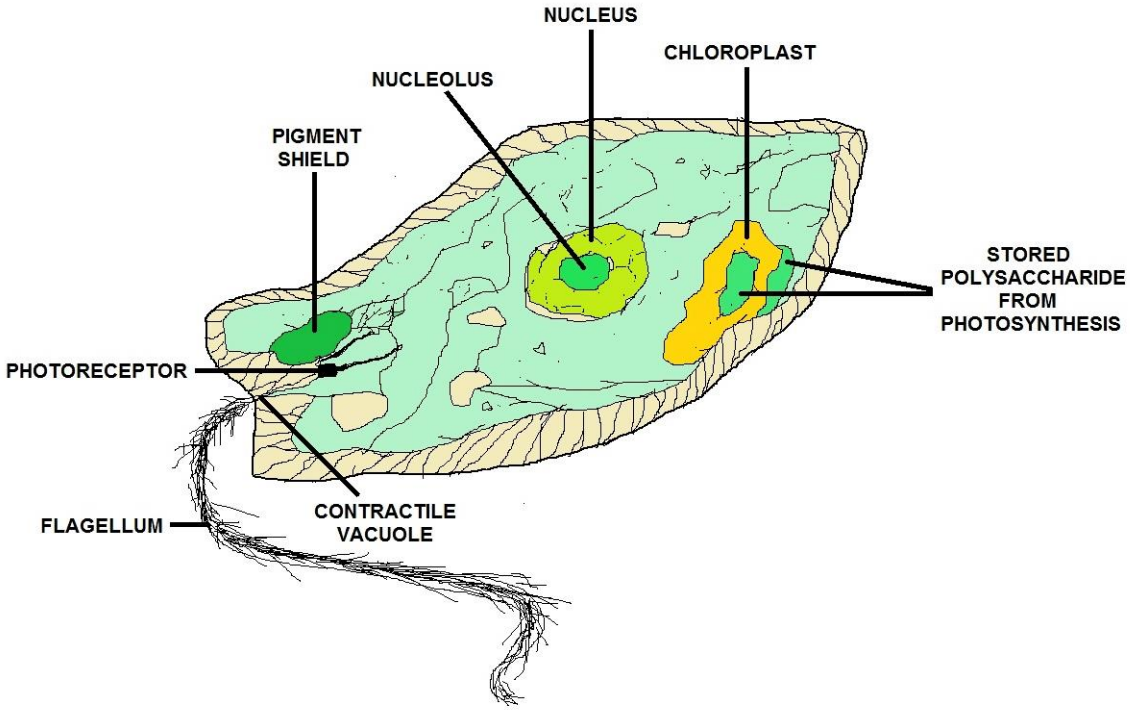
Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

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).

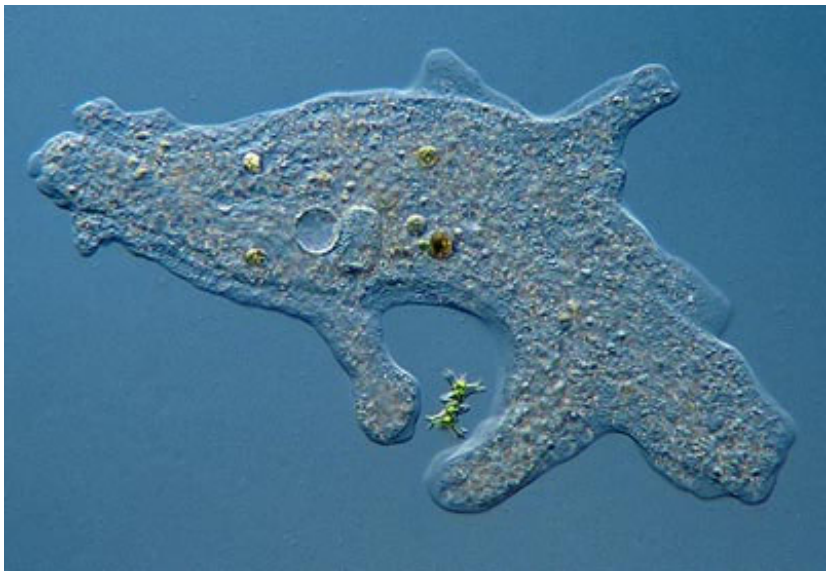


EUGLENA EXAMPLE

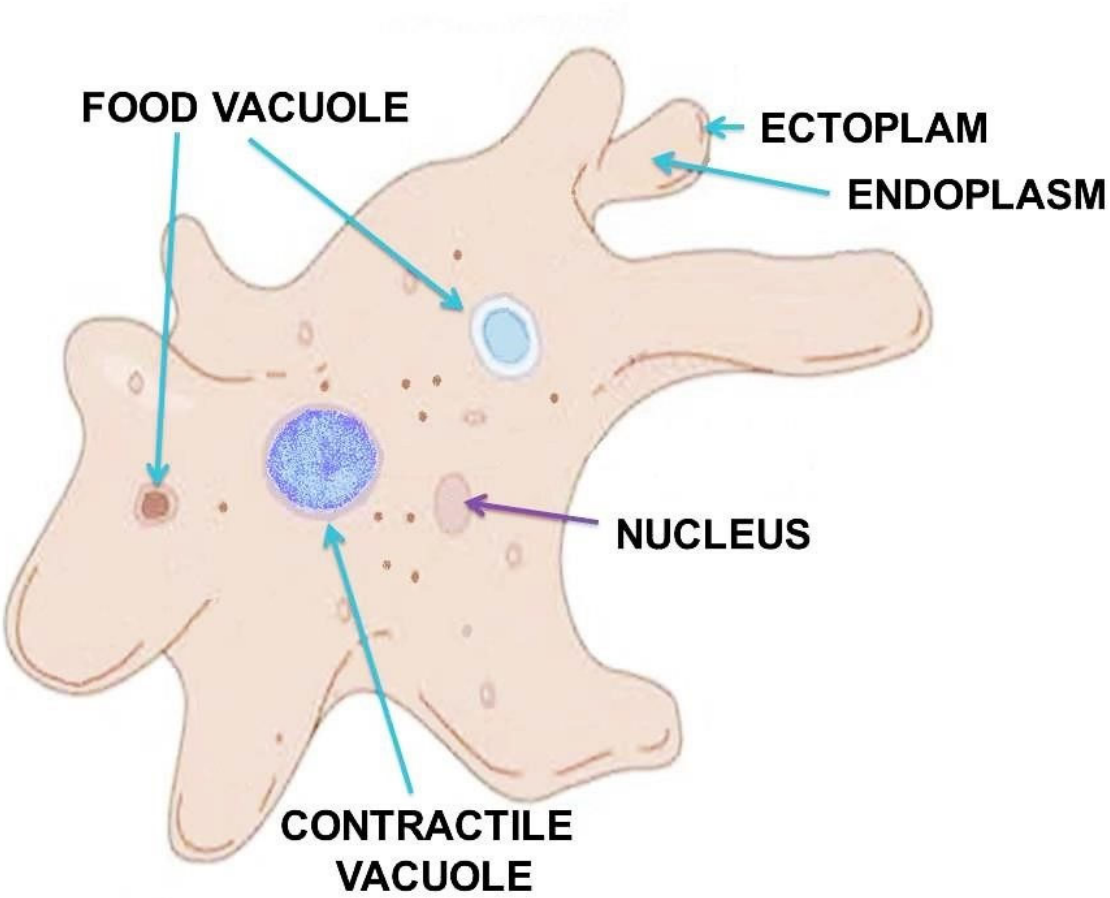
Free-living Protozoa

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.*



AMOEBA DIAGRAM #1

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/m^2 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 Protozoans

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.

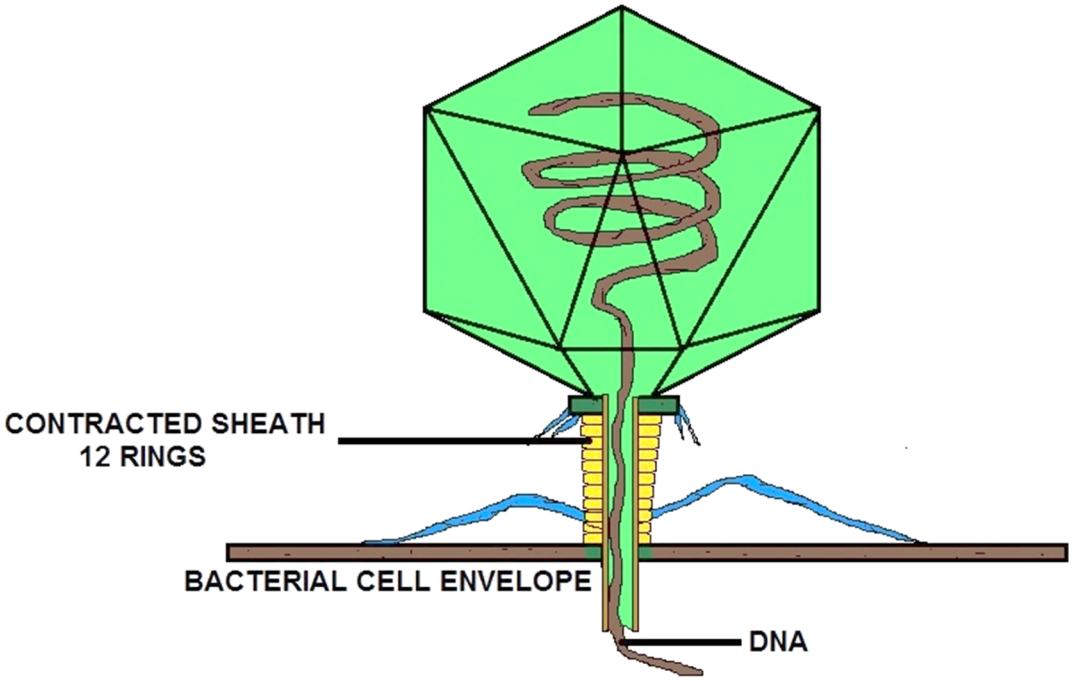
Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in 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).

Bacteriophage



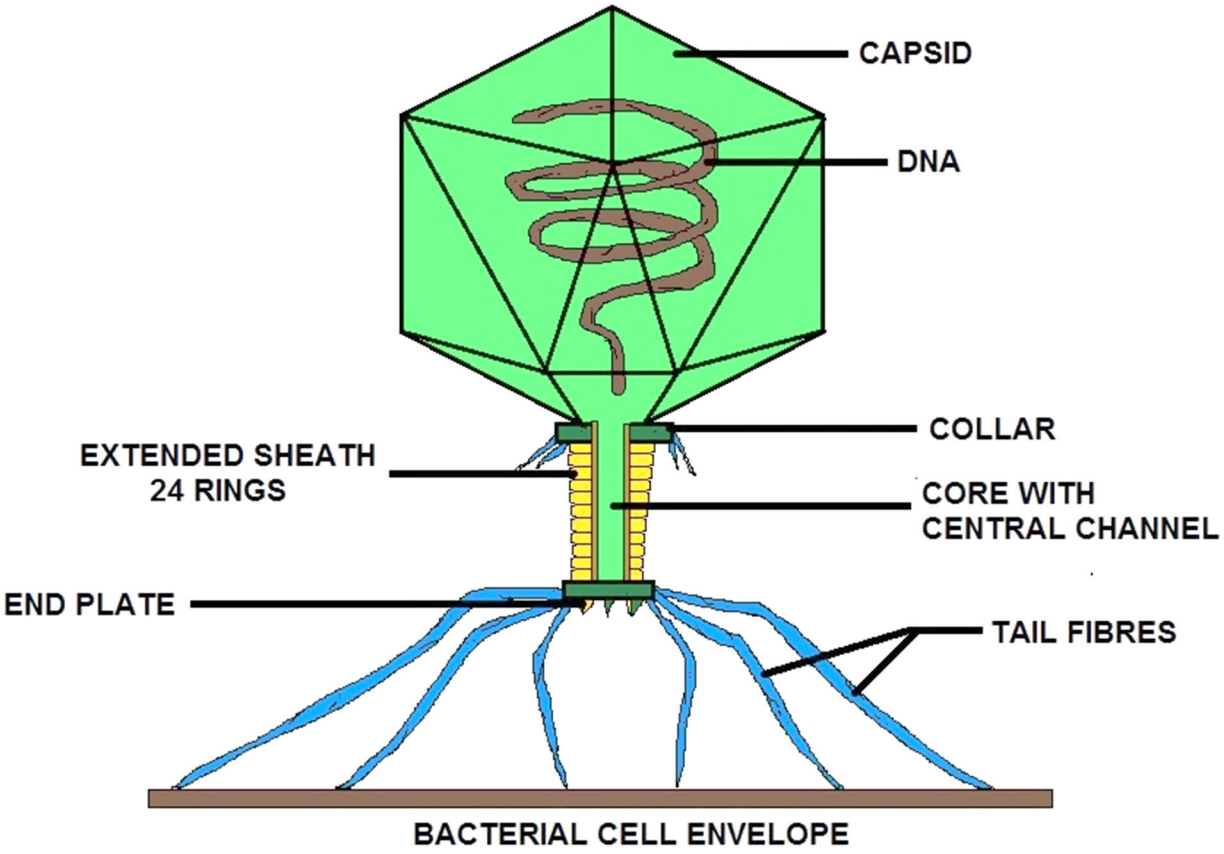
BACTERIOPHAGE (VIRUS CAPSID) #1

A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage.

Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestine of animals.

One of the densest natural sources for phages and other viruses is sea water, where up to 9×108 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.



BACTERIOPHAGE (VIRUS CAPSID) #2

Release of Virions

Phages may be released via cell lysis or by host cell secretion. In the case of the T4 phage, in just over twenty minutes after injection upwards of three hundred phages will be released via lysis within a certain timescale. This is achieved by an enzyme called endolysin which attacks and breaks down the peptidoglycan.

In contrast, "lysogenic" phages do not kill the host but rather become long-term parasites and make the host cell continually secrete more new virus particles. The new virions bud off the plasma membrane, taking a portion of it with them to become enveloped viruses possessing a viral envelope. All released virions are capable of infecting a new bacterium.

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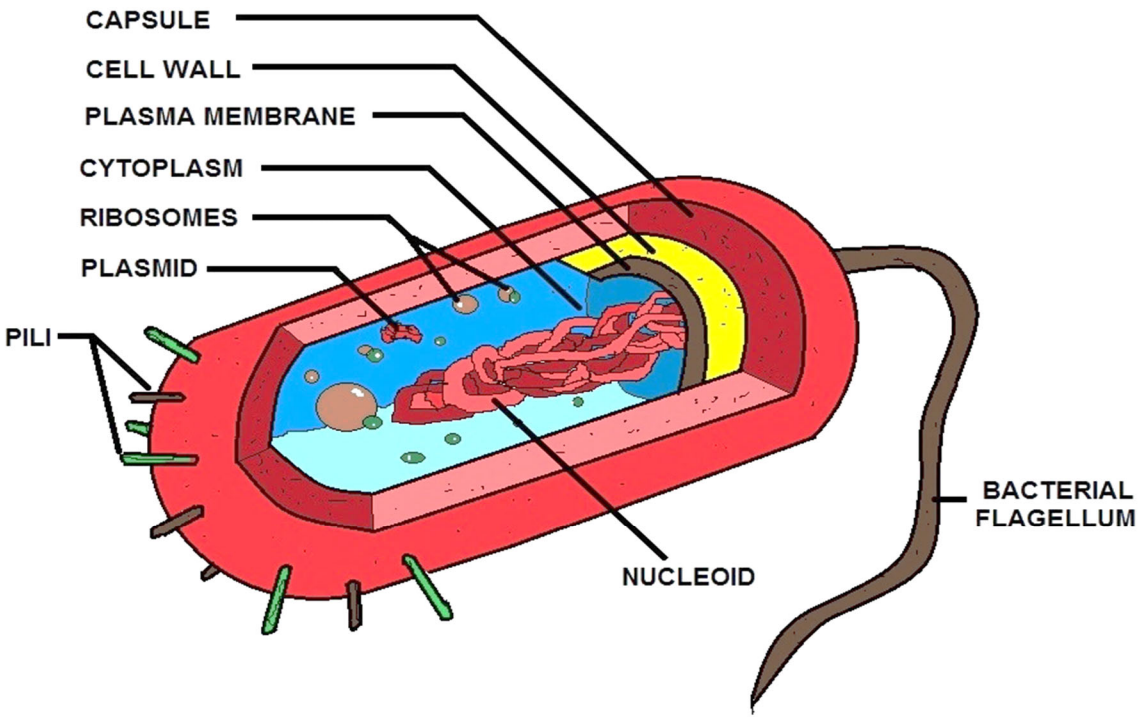
Bacteria Glossary

Type	Characteristics
Acetic acid	Rod-shaped, gram-negative, aerobic; highly tolerant of acidic conditions; generate organic acids
Actinomycete	Rod-shaped or filamentous, gram-positive, aerobic; common in soils; essential to growth of many plants; source of much of original antibiotic production in pharmaceutical industry
Coccoid	Spherical, sometimes in clusters or strings, gram-positive, aerobic and anaerobic; resistant to drying and high-salt conditions; *Staphylococcus* species common on human skin, certain strains associated with toxic shock syndrome
Coryneform	Rod-shaped, form club or V shapes, gram-positive, aerobic; found in wide variety of habitats, particularly soils; highly resistant to drying; include *Arthrobacter,* among most common forms of life on earth
Endospore-forming	Usually rod-shaped, can be gram-positive or gram-negative; have highly adaptable, heat-resistant spores that can go dormant for long periods, possibly thousands of years; include *Clostridium* (anaerobic) and *Bacillus* (aerobic)
Enteric	Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include *Escherichia coli, Salmonella* (over 1000 types), and *Shigella*
Gliding	Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures
Lactic acid	Gram-positive, anaerobic; produce lactic acid through fermentation; include *Lactobacillus*, essential in dairy product formation, and *Streptococcus*, common in humans
Mycobacterium	Pleomorphic, spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include *Mycobacterium tuberculosis,* cause of tuberculosis
Mycoplasma	Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (*myco-* means 'fungus')
Nitrogen-fixing	Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include *Azotobacter,* a common genus
Propionic acid	Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide
Pseudomonad	Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy
Rickettsia	Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to *Agrobacterium,* a common gall-causing plant bacterium

Sheathed	Filamentous, gram-negative, aerobic; 'swarmer' (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment
Spirillum	Spiral-shaped, gram-negative, aerobic; include *Bdellovibrio,* predatory on other bacteria
Spirochete	Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis
Sulfate- and Sulfur-reducing	Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes
Sulfur- and iron-oxidizing	Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment
Vibrio	Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids



PROKARYOTIC CELL (BACTERIA)

Water Microbiology Post Quiz

Internet Link to Assignment…

http://www.abctlc.com/downloads/PDF/Bacterilogical DiseasesAss.pdf

Prokaryotes

1. The only prokaryotes are Bacteria and archaea all other life forms are _____creatures whose cells have nuclei.

2. A single teaspoon of topsoil may contain more than a billion (1,000,000,000) bacteria.
A. True B. False

3. If a person stops an antibiotic, any living bacteria could start making _____, grow, and reproduce.

Gram Stain

4. What type of bacteria have more complex cell walls with less peptidoglycan, thus absorb less of the purple dye used and stain a pinkish color?

5. What exhibits all the characteristics of life but it lacks the complex system of membranes and organelles?

6. Cytoplasm is comprised of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, _____, microtubules, lysosomes and vacoules.

7. Some bacteria have a layer of material outside the?

8. What is motile while non-flagellated bacteria are generally non-motile?

9. Amphitrichous bacteria-bacteria with _____.

Bacterial Nutrition

10. What is needed in substantial quantities, but some seem to need it in trace amounts?

11. Whatterms represents animals, plants, and fungi, which are mostly multicellular, as well as various other groups called protists, many of which are unicellular?

12. Many cells ingest food and other materials through a process of osmosis, where the outer membrane invaginates and then pinches off to form a Flagella.
A. True B. False

13. What represents the causative organism of Legionnaires' disease?

14. Protozoa are the natural habitat for certain pathogenic bacteria.
A. True B. False

15. What terms represents an organism of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source?

Answers
1. Eukaryotes, 2.T, 3. Peptidoglycan, 4. Gram -, 5. Prokaryotic cell, 6. Centrioles, 7. Cell wall, 8. Flagellated bacteria, 9. One flagellum at each end, 10. Calcium, 11. Eukaryote(s), 12. F, 13. Bacterium Legionella pneumophila, 14. T, 15. Parasitic protozoa,

Bacteriological Monitoring Section – Chapter 2

Section Focus: You will learn the basics of the EPA's Total Coliform Rule and bacteriological sampling. At the end of this section, you will be able to describe the Total Coliform Rule. 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.

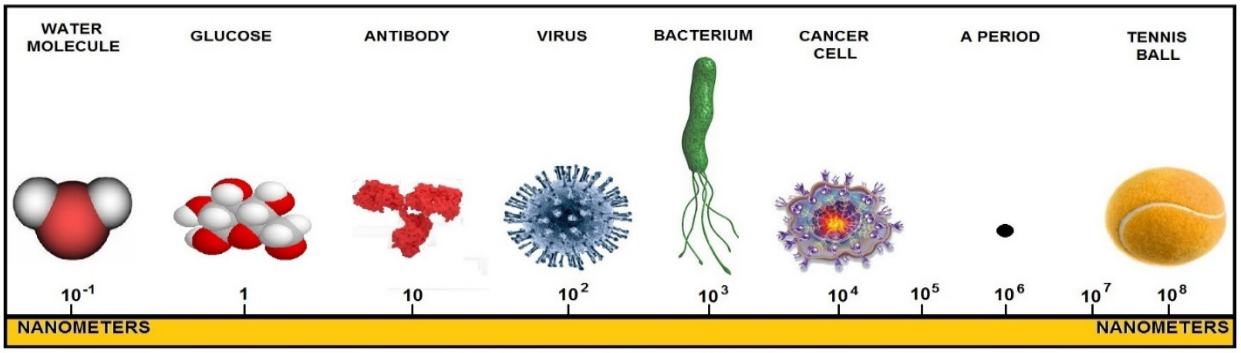
Scope/Background: The Environmental Protection Agency (EPA) published the Revised Total Coliform Rule (RTCR) in the Federal Register (FR) on February 13, 2013 (78 FR 10269) and minor corrections on February 26, 2014 (79 FR 10665). The RTCR is the revision to the 1989 Total Coliform Rule (TCR) and is intended to improve public health protection. The RTCR applies to all PWSs.

Microbiology Introduction

Microorganisms of greatest significance to water professionals can be classified into four groups:

1. Bacteria - Prokaryotes
2. Protozoans
3. Metazoans
4. Viruses

Each of these groups plays a key role in the complex world of wastewater biology.



SIZE COMPARISON
HOW SMALL IS SMALL ?

Bacteria Introduction

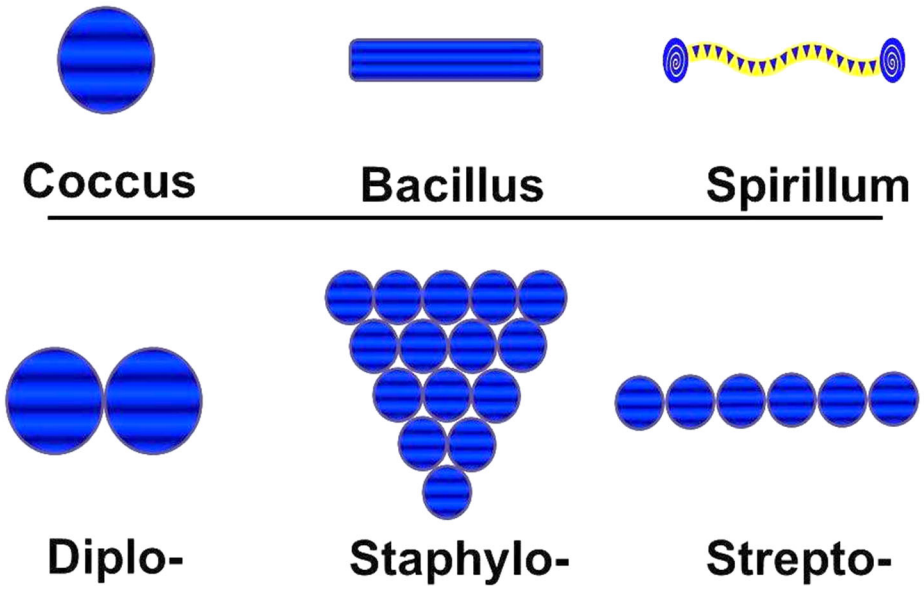
Bacteria are highly designed creatures formed in a variety of shapes. The simplest shape is a round sphere or ball.

Bacteria formed like this are called cocci (singular coccus). The next simplest shape is cylindrical.

Cylindrical bacteria are called rods (singular rod). Some bacteria are basically rods but instead of being straight they are twisted, bent or curved, sometimes in a spiral. These bacteria are called spirilla (singular spirillum). Spirochaetes are tightly coiled up bacteria.

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

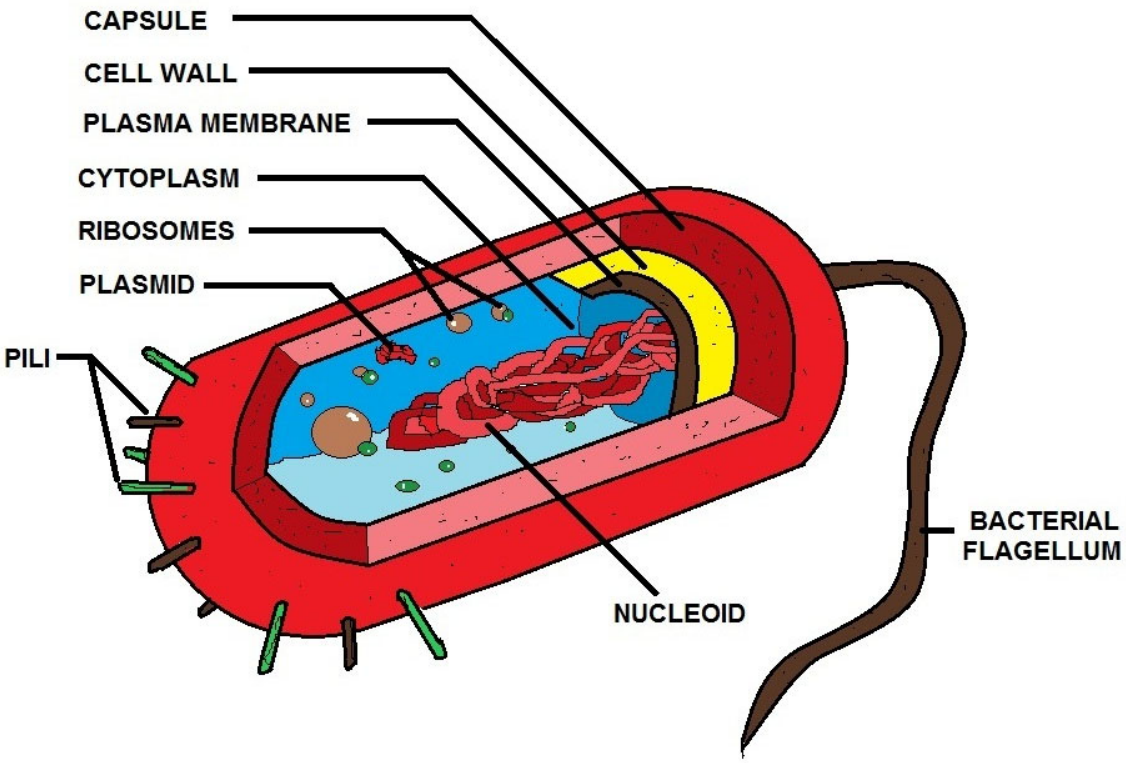


BASIC BACTERIA SHAPES DIAGRAM

Bacteria Biofilm or Colonies

Bacteria tend to live together in clumps, chains or planes. When they live in chains, one after the other, they are called filamentous bacteria - these often have long thin cells. When they tend to collect in a plane or a thin layer over the surface of an object, they are called a biofilm. Many bacteria exist as a biofilm and the study of biofilms is very important. Biofilm bacteria secrete sticky substances that form a sort of gel in which they live. The plaque on your teeth that causes tooth decay is a biofilm.

Bacteria Diagram



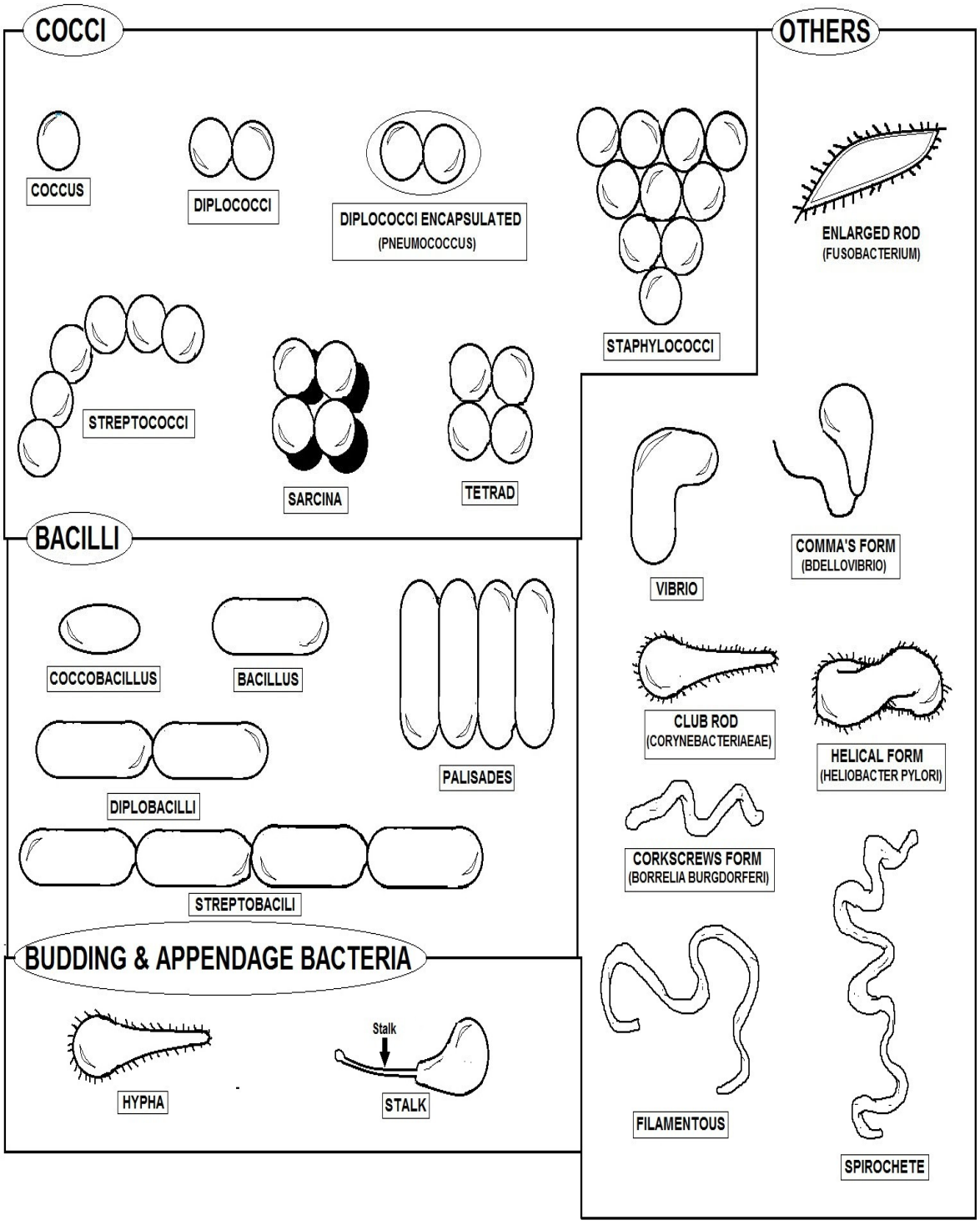
PROKARYOTIC CELL

Above is a typical bacterial cell has a rigid outer coating that gives them structure and maintains their shape. This is the cell wall. Bacteria also have an inner, flexible membrane called the *periplasmic membrane* or *cell membrane*. This dual-layered covering has been compared to a balloon inside a box.

The cell membrane is very important because it controls the intake of food and other nutrients and discharge of waste products. It keeps “in” what needs to be inside (e.g., enzymes, nutrients, and food) and keeps “out” what should be outside (e.g., excess water). The box is the cell wall. The cell wall provides the structural support and maintains the shape of the cell.

Much of the cellular contents are large protein molecules, known as enzymes, which are manufactured by the cell. Other cellular contents may include granules of polyphosphate, sulfur, or stored organic material.

Bacteria are somewhat predictable and, at a basic level, can be compared to miniature combustion engines. For an engine to function, it requires both a fuel and oxygen source. The oxygen sources is used to chemically burn fuel to release energy. The technically correct term for this process is oxidation. The byproducts of combustion when burning organic fuel with oxygen are carbon dioxide (CO_2) and water (H_2O).

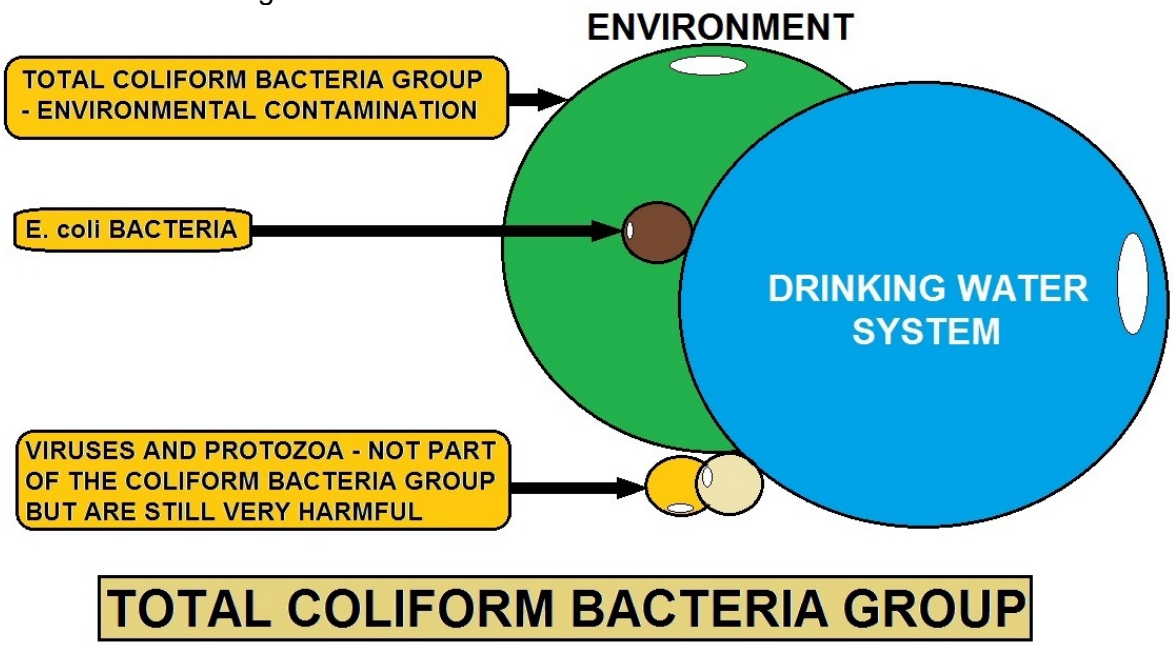


BACTERIA SHAPES

Microbiological Contaminant Information

The sources of drinking water include rivers, lakes, streams, ponds, reservoirs, springs, and wells. As water travels over the surface of the land or through the ground, it dissolves naturally occurring minerals and in some cases, radioactive material, and can pick up substances resulting from the presence of animals or human activity.

Contaminants that may be present in sources of drinking water include:
Microbial contaminants, such as viruses and bacteria, which may come from sewage treatment plants, septic systems, agricultural livestock operations and wildlife;
Inorganic contaminants, such as salts and metals, which can be naturally occurring or result from urban stormwater runoff, industrial or domestic wastewater discharges, oil and gas production, mining or farming;
Pesticides and herbicides, which may come from a variety of sources such as agriculture, urban stormwater run-off, and residential uses;
Organic chemical contaminants, including synthetic and volatile organic chemicals, which are by-products of industrial processes and petroleum production, and can also come from gas stations, urban stormwater run-off, and septic systems;
Radioactive contaminants, which can be naturally occurring or be the result of oil and gas production and mining activities.



Background
Coliform bacteria and chlorine residual are the only routine sampling and monitoring requirements for small ground water systems with chlorination. The coliform bacteriological sampling is governed by the Total Coliform Rule (TCR) of the SDWA. Although there is presently no requirement for chlorination of groundwater systems under the SDWA, State regulations require chlorine residual monitoring of those systems that do chlorinate the water.

TCR
The TCR requires all Public Water Systems (PWS) to monitor their distribution system for coliform bacteria according to the written sample sitting plan for that system. The sample sitting plan identifies sampling frequency and locations throughout the distribution system that are selected to be representative of conditions in the entire 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. A copy of the sample sitting plan for the system should be kept on file and accessible to all who are involved in the sampling for the water system.

Number of Monthly Samples
The number of samples to be collected monthly depends on the size of the system. The TCR specifies the minimum number of coliform samples collected, but it may be necessary to take more than the minimum number in order to provide adequate monitoring.

This is especially true if the system consists of multiple sources, pressure zones, booster pumps, long transmission lines, or extensive distribution system piping. Since timely detection of coliform contamination is the purpose of the sample-sitting plan, sample sites should be selected to represent the varying conditions that exist in the distribution system. The sample sitting plan should be updated as changes are made in the water system, especially the distribution system.

Sampling Procedures
The sample-sitting plan must be followed and all operating staff must be clear on how to follow the sampling plan. In order to properly implement the sample-sitting 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. In addition, proper procedures must be followed for repeat sampling whenever a routine sample result is positive for total coliform.

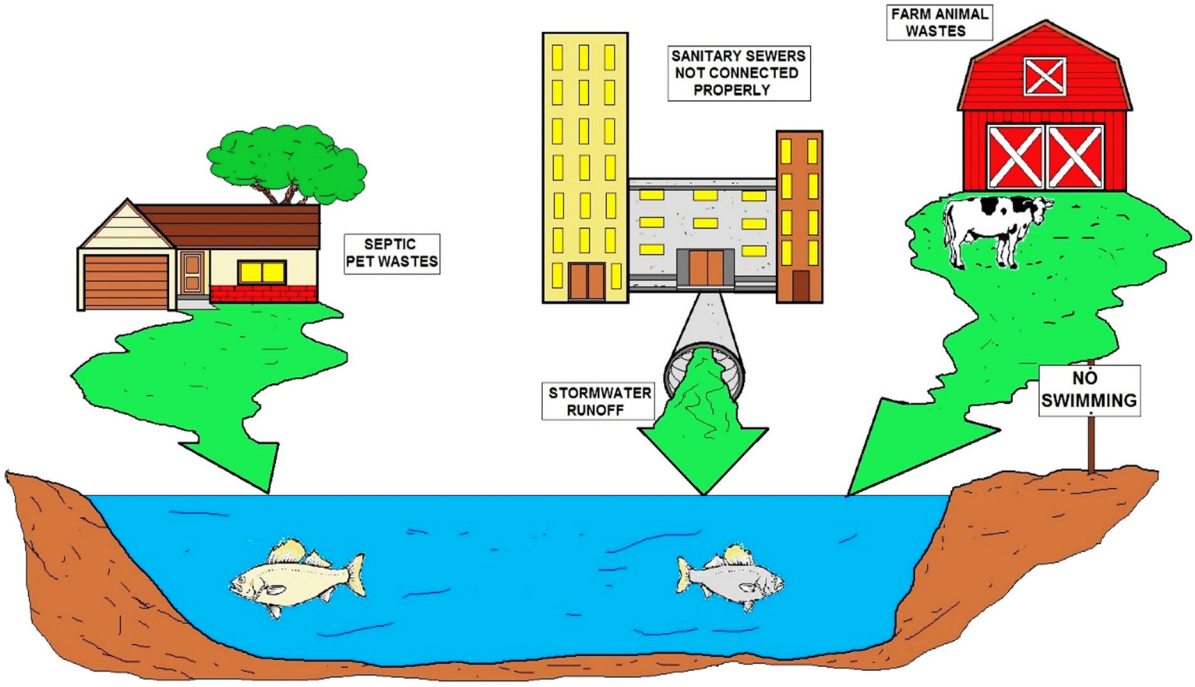
Routine Sampling Requirements
Total coliform samples must be collected by PWSs at sites which are representative of water quality throughout the distribution system according to a written sample siting plan subject to state review and revision.

For PWSs collecting more than one sample per month, collect total coliform samples at regular intervals throughout the month, except that ground water systems serving 4,900 or fewer people may collect all required samples on a single day if the samples are taken from different sites.

Each total coliform-positive (TC+) routine sample must be tested for the presence of E. coli.
► If any TC+ sample is also E. coli-positive (EC+), then the EC+ sample result must be reported to the state by the end of the day that the PWS is notified.
► If any routine sample is TC+, repeat samples are required. – PWSs on quarterly or annual monitoring must take a minimum of three additional routine samples (known as additional routine monitoring) the month following a TC+ routine or repeat sample.
► Reduced monitoring may be available for PWSs using only ground water and serving 1,000 or fewer persons that meet certain additional PWS criteria.

Coliform Bacteria Introduction

Total coliforms are a group of related bacteria that are (with few exceptions) not harmful to humans. A variety of bacteria, parasites, and viruses, known as pathogens, can potentially cause health problems if humans ingest them. EPA considers total coliforms a useful indicator of other pathogens for drinking water because they are easier to measure and associate with water contamination.



SOURCES OF FECAL COLIFORM BACTERIA

Total coliforms are used to determine the adequacy of water treatment and the integrity of the distribution system.

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.

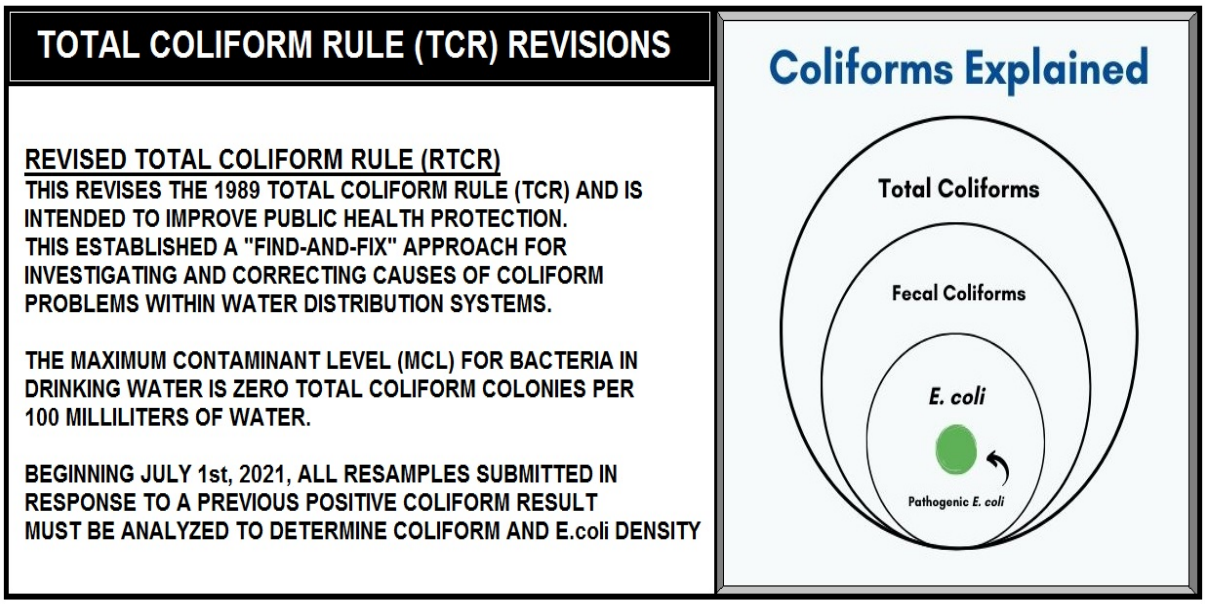
Key provisions of the RTCR include:

- Setting a maximum contaminant level goal (MCLG) and maximum contaminant level (MCL) for E. coli for protection against potential fecal contamination.
- Setting a total coliform treatment technique (TT) requirement.
- Requirements for monitoring total coliforms and E. coli according to a sample siting plan and schedule specific to the PWS.
- Provisions allowing PWSs to transition to the RTCR using their existing Total Coliform Rule (TCR) monitoring frequency, including PWSs on reduced monitoring under the existing TCR.
- Requirements for seasonal systems (such as Non-Community Water Systems not operated on a year-round basis) to monitor and certify the completion of a state-approved start-up procedures.

- Requirements for assessments and corrective action when monitoring results show that PWSs may be vulnerable to contamination.
- Public notification (PN) requirements for violations.
- Specific language for CWSs to include in their Consumer Confidence Reports (CCRs) when they must conduct an assessment or if they incur an E. coli MCL violation.

TCR Key Provisions

- To comply with the monthly MCL for total coliforms (TC), PWSs must not find coliforms in more than five percent of the samples they take each month to meet EPA's standards. If more than five percent of the samples contain coliforms, PWS operators must report this violation to the state and the public.
- If a sample tests positive for TC, the system must collect a set of repeat samples located within 5 or fewer sampling sites adjacent to the location of the routine positive sample within 24 hours.
- When a routine or repeat sample tests positive for total coliforms, it must also be analyzed for fecal coliforms or E. coli, which are types of coliform bacteria that are directly associated with feces. A positive result for fecal coliforms or E. coli can signify an acute MCL violation, which necessitates rapid state and public notification because it represents a direct health risk.
- At times, an acute violation due to the presence of fecal coliform or E. coli may result in a "boil water" notice. The system must also take at least 5 routine samples the next month of operation if any sample tests positive for total coliforms.



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TOTAL COLIFORM RULE (TCR) REVISIONS

All public water systems (PWSs), except aircraft PWSs subject to the Aircraft Drinking Water Rule (ADWR) (40 CFR 141 Subpart X), must comply with the RTCR starting April 1, 2016, or an earlier state effective date. Until then, PWSs must continue complying with the 1989 TCR.

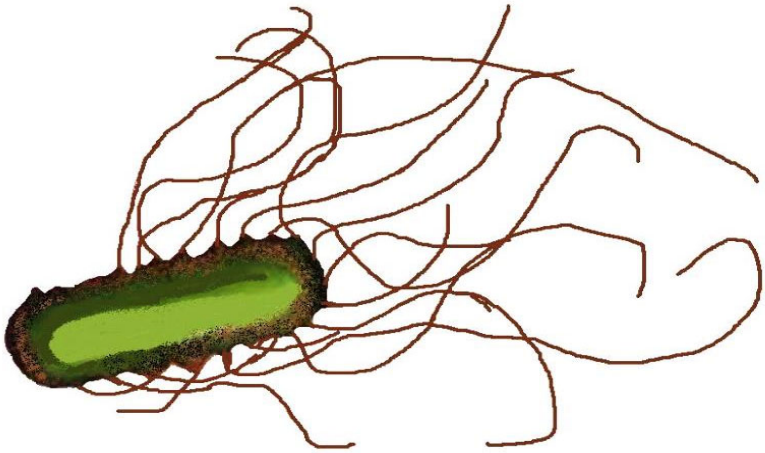
Related (Dangerous Waterborne) Microbes

Coliform Bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water are 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.

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 CDC have prepared advice for those with severely compromised immune systems who are concerned about *Cryptosporidium*.

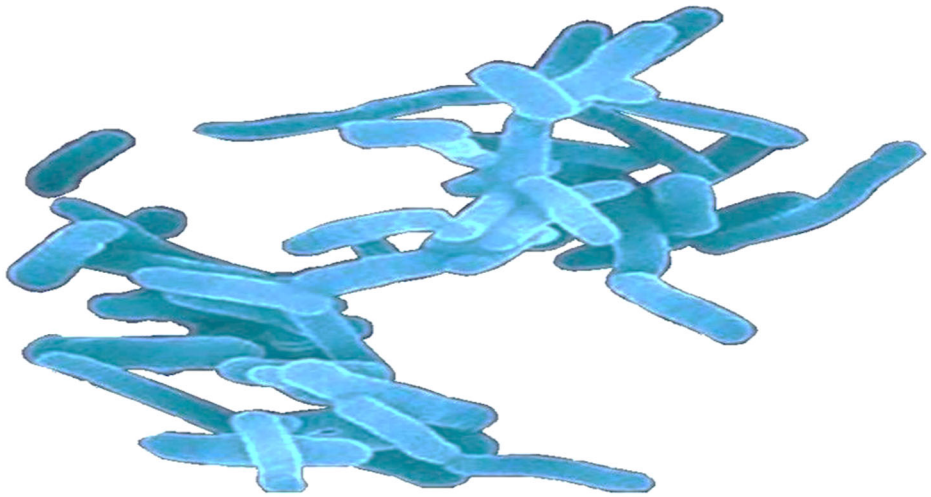
Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhea, vomiting, and cramps).



PERITRICHOUS SHAPED BACTERIA EXAMPLE

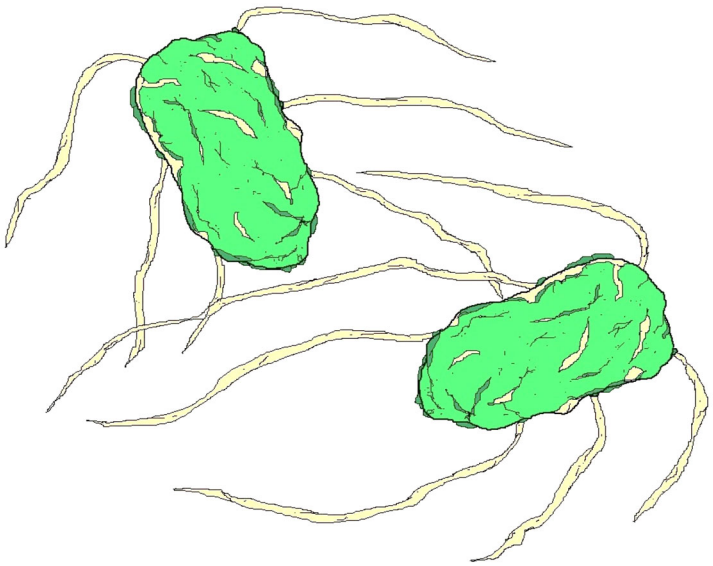
Microbiologists broadly classify bacteria according to their shape: spherical, rod-shaped, and spiral-shaped. Pleomorphic bacteria can assume a variety of shapes. Bacteria may be further classified according to whether they require oxygen (aerobic or anaerobic) and how they react to a test with Gram's stain.

Bacteria in which alcohol washes away Gram's stain are called gram-negative, while bacteria in which alcohol causes the bacteria's walls to absorb the stain are called gram-positive.



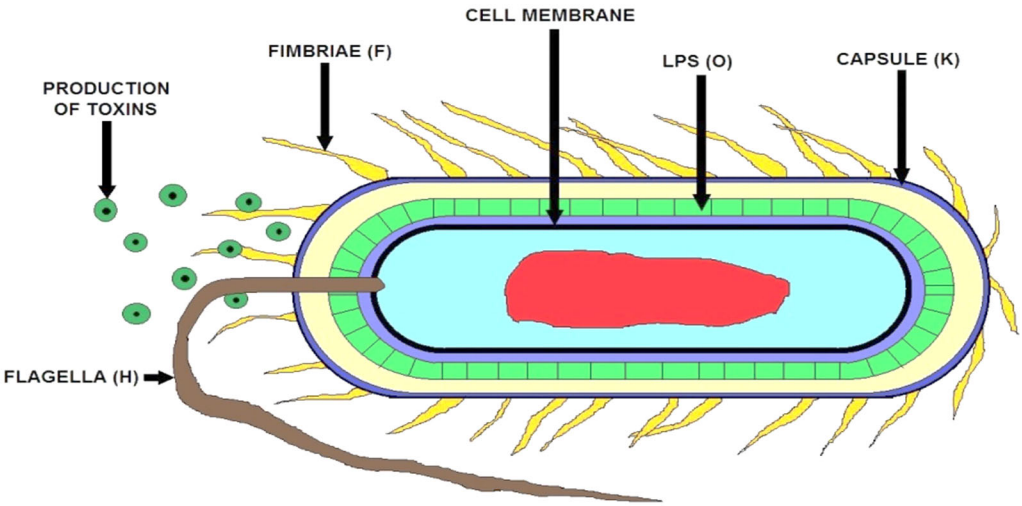
SHIGELLA DYSENTARIAE EXAMPLE

Shigella dysenteriae is a species of the rod-shaped bacterial genus Shigella. Shigella can cause shigellosis (bacillary dysentery). Shigellae are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.



SALMONELLA EXAMPLE

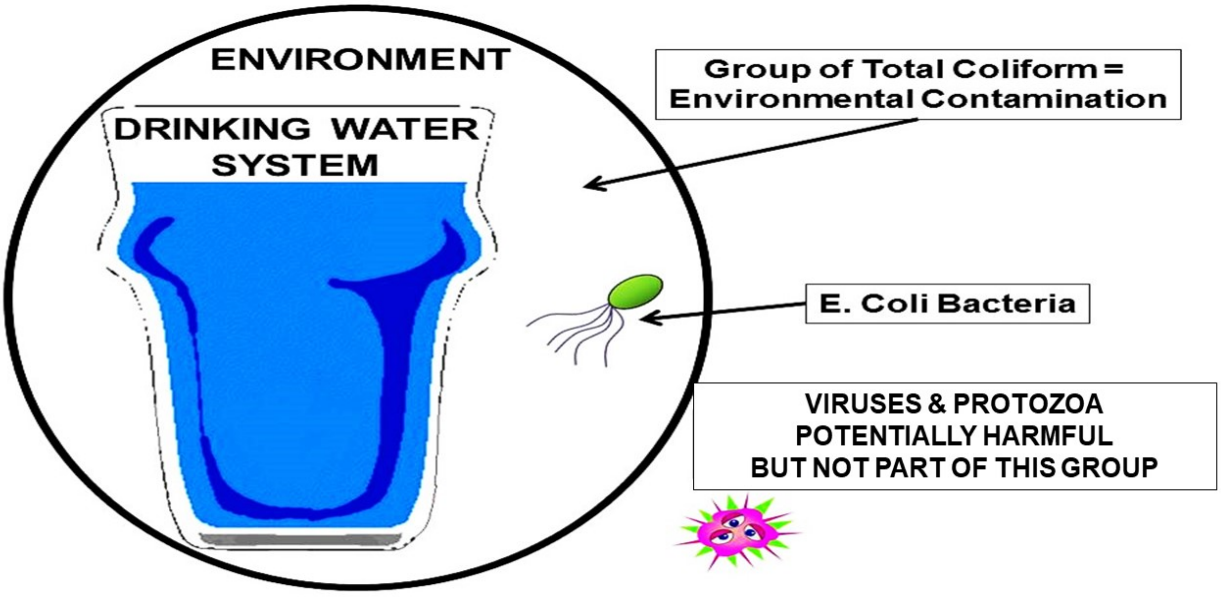
Salmonellae usually do not ferment lactose; most of them produce hydrogen sulfide that in media containing ferric ammonium citrate reacts to form a black spot in the center of the creamy colonies.



E. COLI

Fecal Coliform Bacteria

Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.



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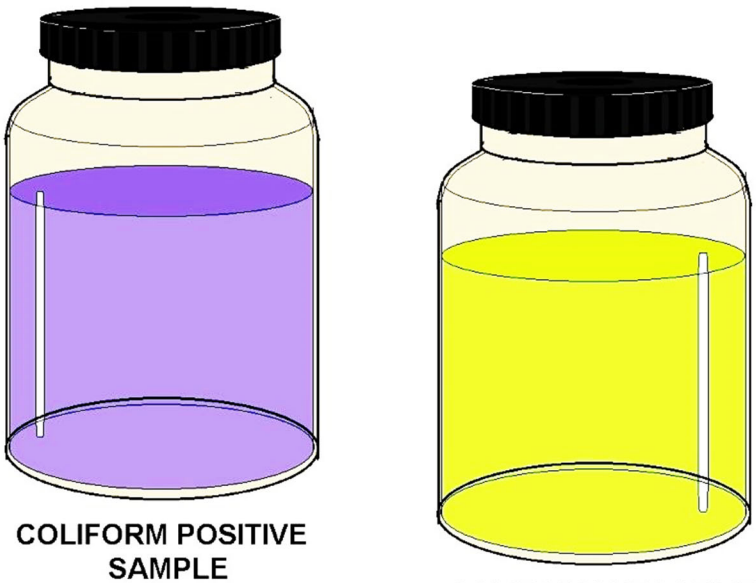
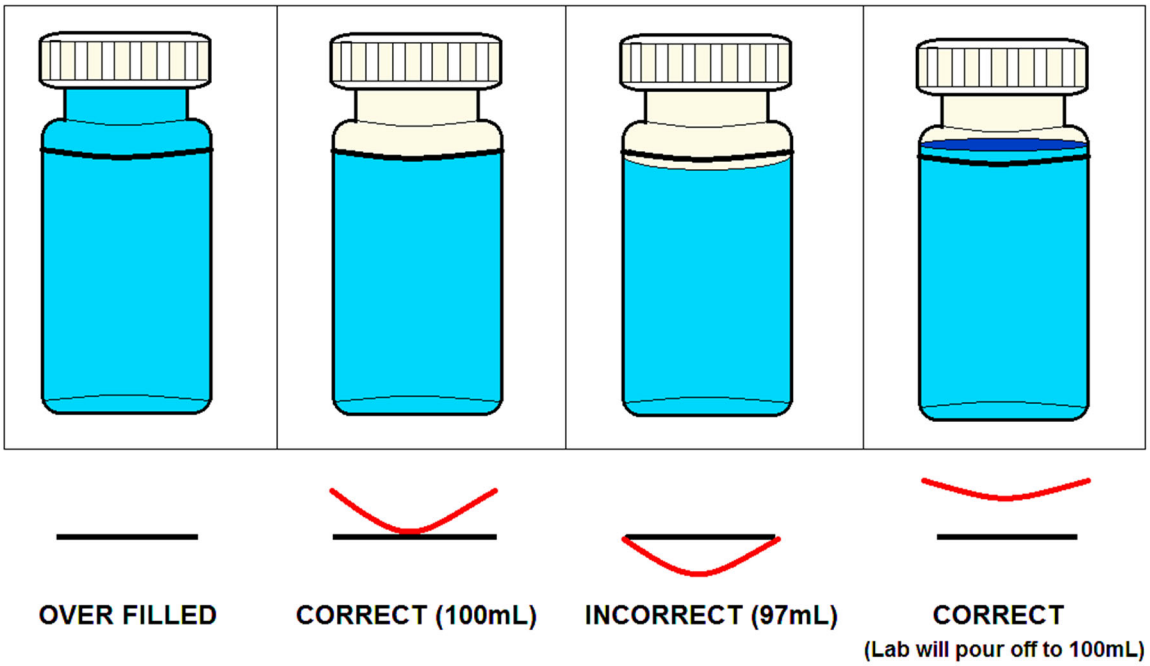
GROUP OF TOTAL COLIFORM BACTERIA



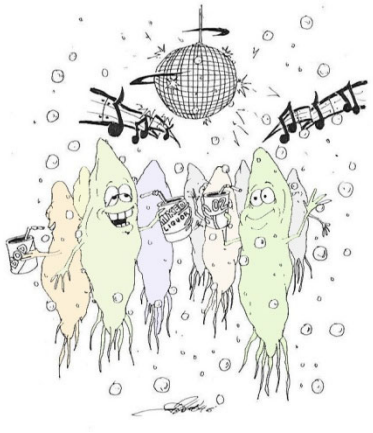
COLIFORM BACTERIA PRESENCE TEST EXAMPLE



BAC-T SAMPLE BOTTLE DIAGRAM

Bacteriological Monitoring Introduction

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 - 1 Example
Water samples for bacteria tests must always be collected in a sterile container. Take the sample from an outside faucet with the aerator removed. Sterilize by spraying a 5% Household beach or alcohol solution or flaming the end of the tap with a 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.

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 that distribute water, and indicates that the water may be contaminated with germs that can cause disease.

Laboratory Procedures
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:

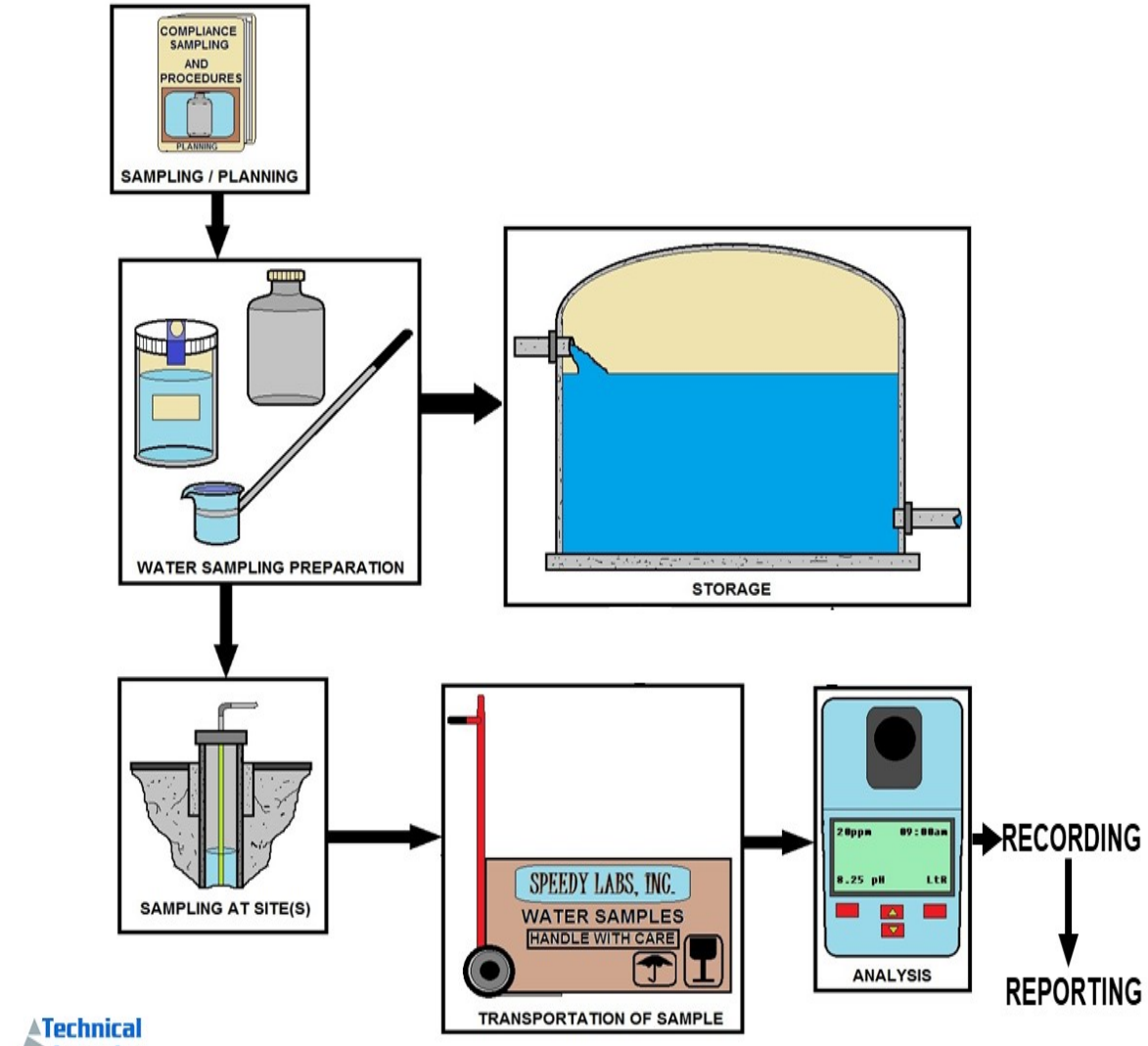
Methods

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.

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.



Basic 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.

No. 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 Introduction

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 has total coliform or fecal coliform present, a set of repeat samples must be collected within 24 hours after being notified by the laboratory. 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.

Positive or Coliform Present Results

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.

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 on 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.

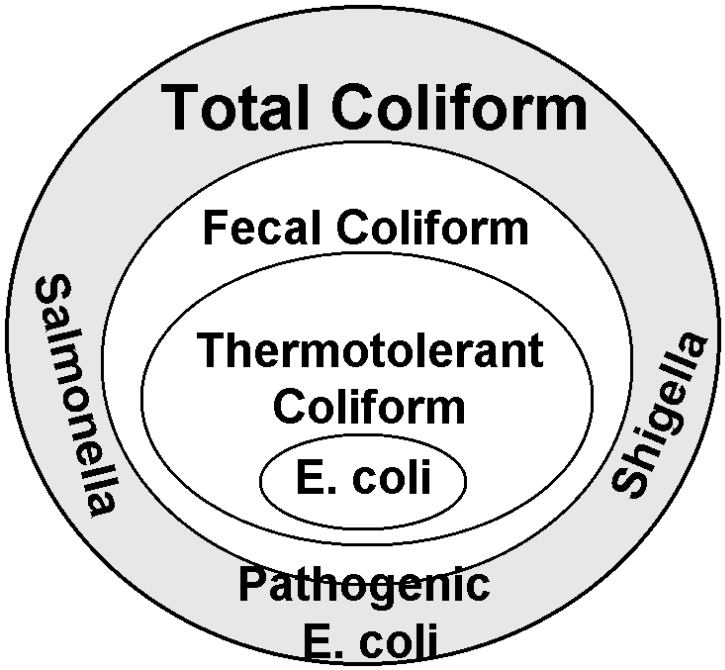
There are two types of MCL violations for coliform bacteria. The first is for total coliform; the second is an acute risk to health violation characterized by the confirmed presence of fecal coliform or E. coli.

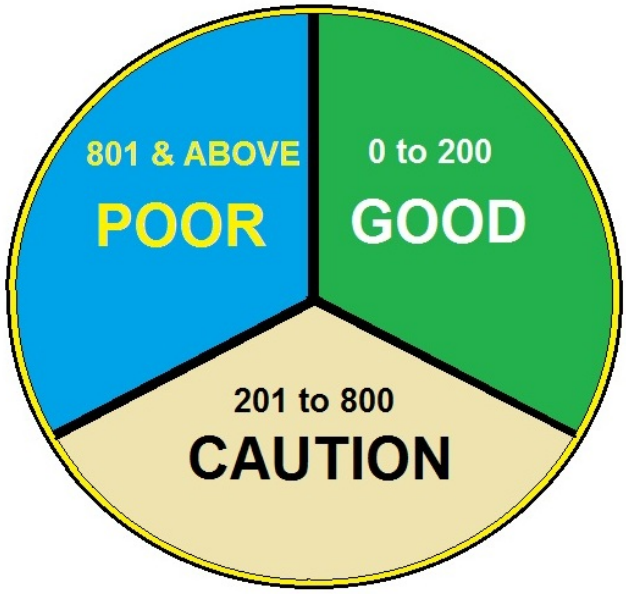
Looking under a black light to identify the presence of E. coli.

Colilert tests simultaneously detect and confirms coliform and E. coli in water samples in 24 hours or less.

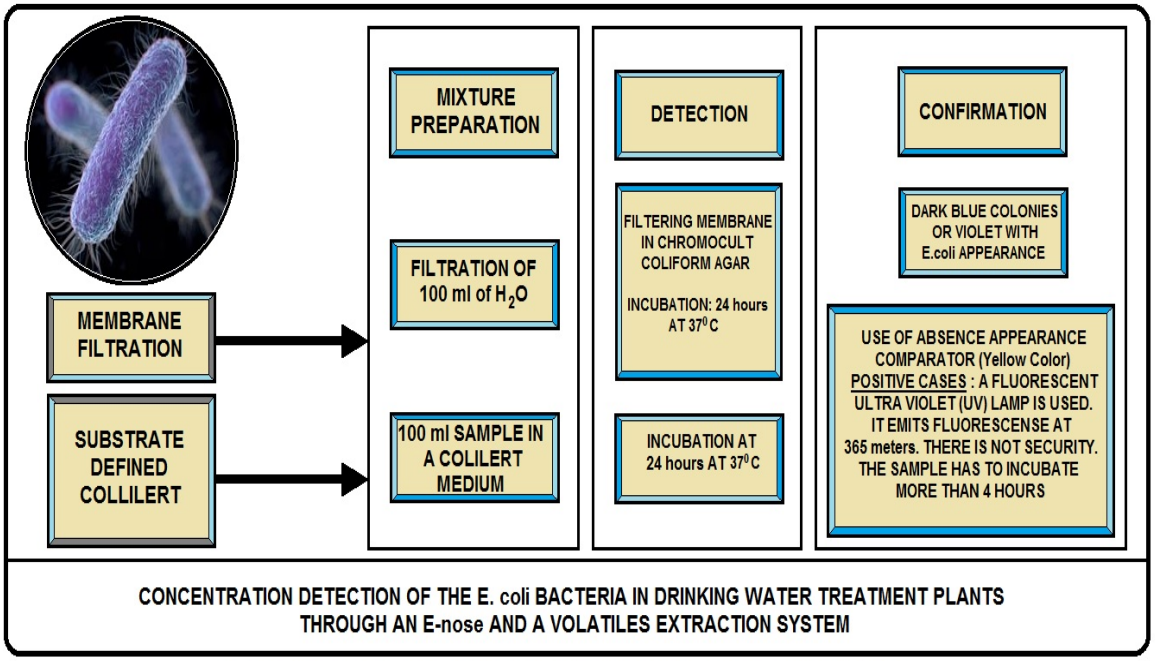
Simply add the Colilert reagent to the sample, incubate for 24 hours, and read results.

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

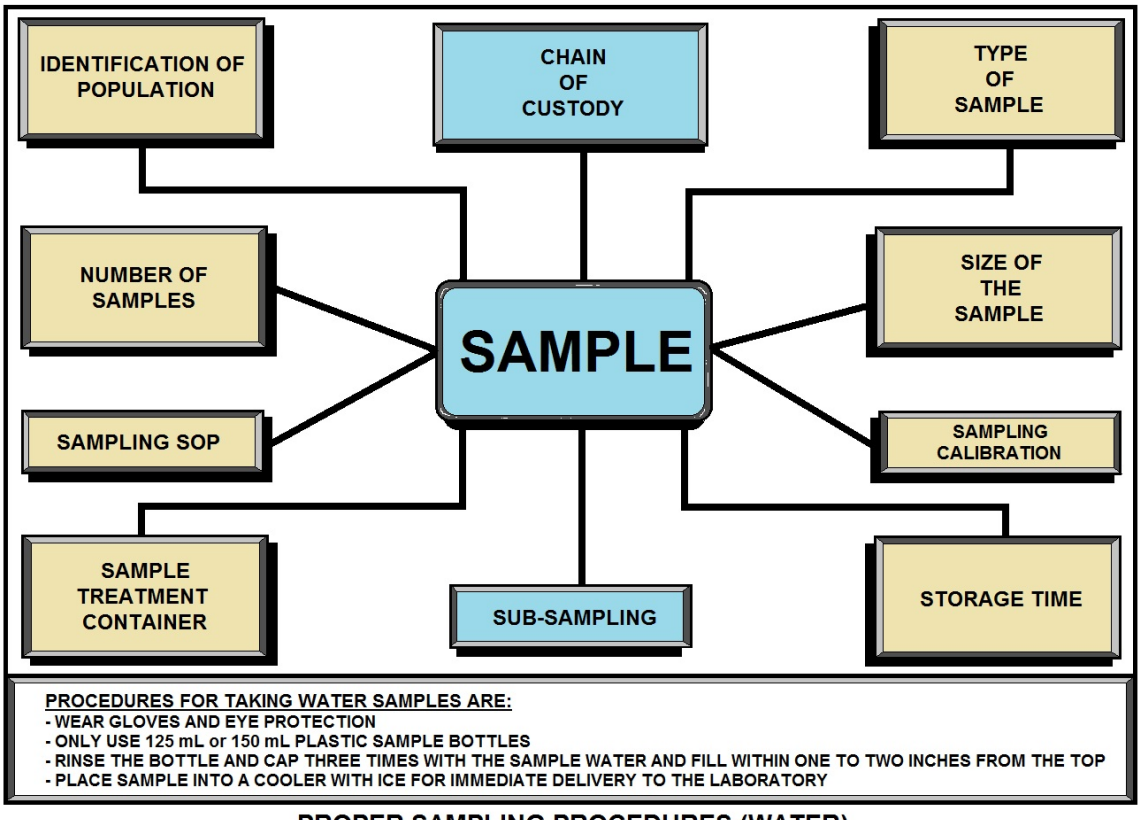




FECAL COLIFORM BACTERIA COLONIES
(PER 100 MILLILITERS)



CONVENTIONAL BACTERIOLOGICAL MONITORING



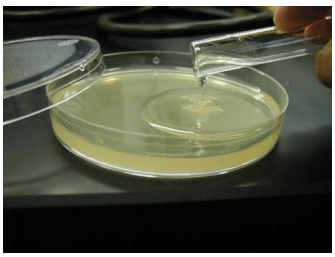
PROPER SAMPLING PROCEDURES (WATER)

Heterotrophic Plate Count - Introduction

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.

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 can be easily discerned and compared to published descriptions. See next page
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

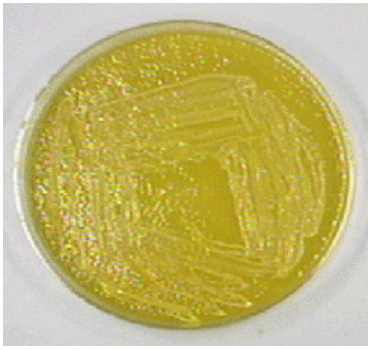
ii) Reagent and sample
- Reagent-grade water
- Nutrient agar
- Sample

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. Pipette 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°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.
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)



Laboratory Equipment Needed
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°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 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 a hand tally counter to maintain count. Count all colonies on the plate, regardless of size. Compute bacterial count per milliliter by the following equation:

$$\text{CFU} \Big/ \text{mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish, mL}}$$

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.

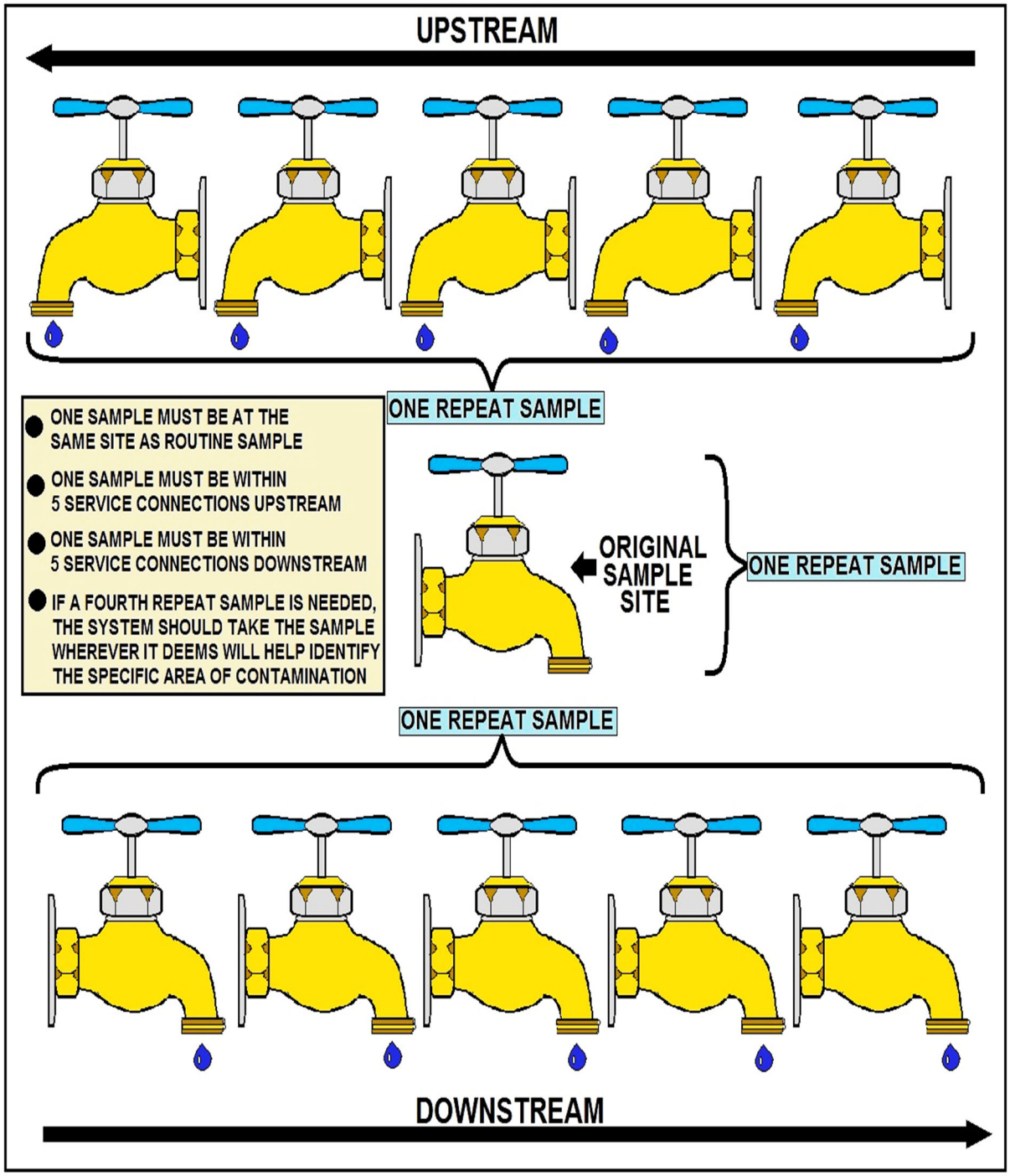
Assignment

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



EXAMPLE OF WHAT HAS TO BE DONE IF A PRESENCE OF COLIFORMS ARE DETECTED WHEN CONDUCTING ROUTINE SAMPLES AT DESIGNATED SAMPLE SITES

Total Coliforms

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.

Sim Plate Method

IDEXX's SimPlate for HPC method is used for the quantification of heterotrophic plate count (HPC) in water.

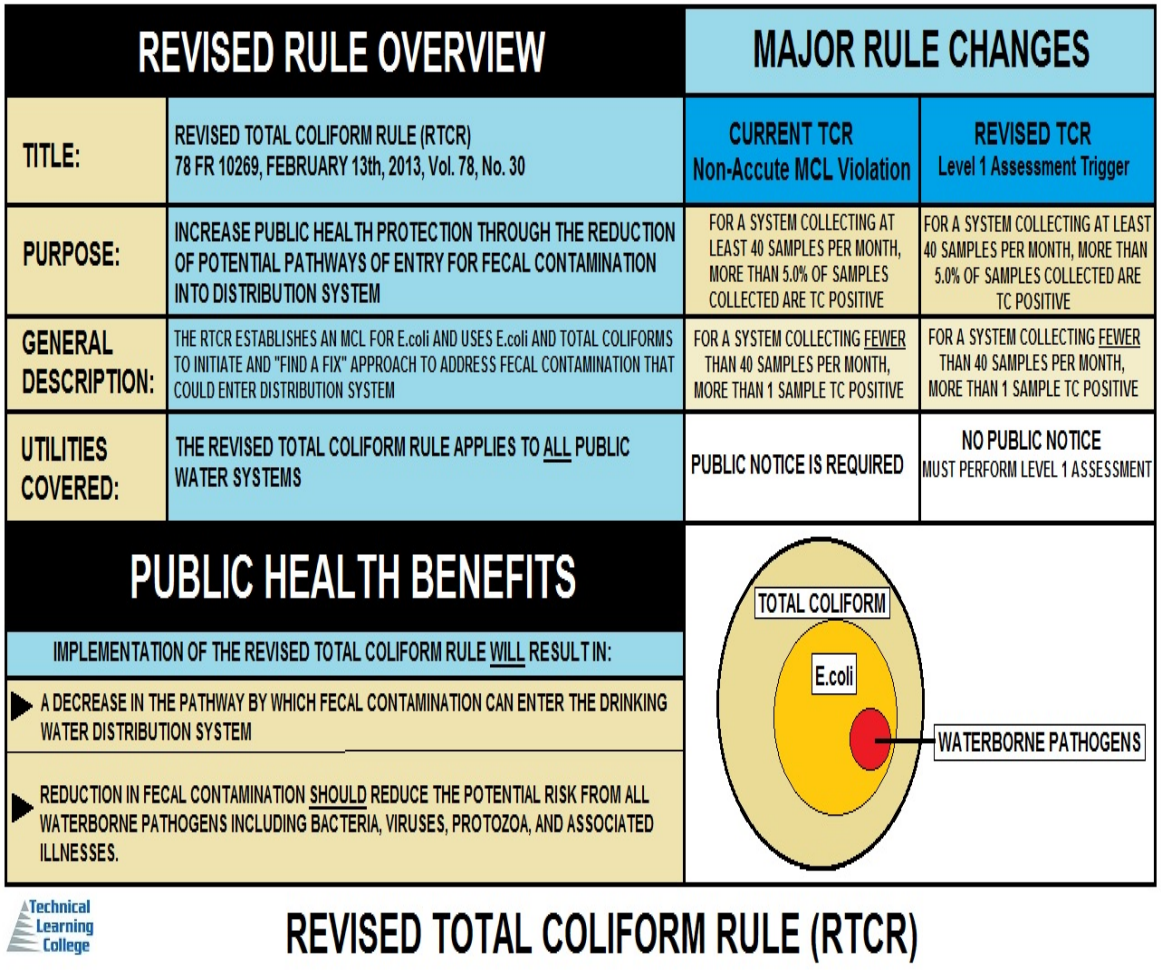
It is based on the Multiple Enzyme Technology which detects viable bacteria in water by testing for the presence of key enzymes known to be present in these little organisms.

This technique uses enzyme substrates that produce a blue fluorescence when metabolized by waterborne bacteria. The sample and media are added to a SimPlate Plate, incubated and then examined for fluorescing wells.

The number of wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample.

The MPN values generated by the SimPlate for HPC method correlate with the Pour Plate method using the Total Plate Count Agar, incubated at 35°C for 48 hours as described in *Standard Methods for the Examination of Water and Wastewater, 19th Edition.*

Revised Total Coliform Rule (RTCR) Summary



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).

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).

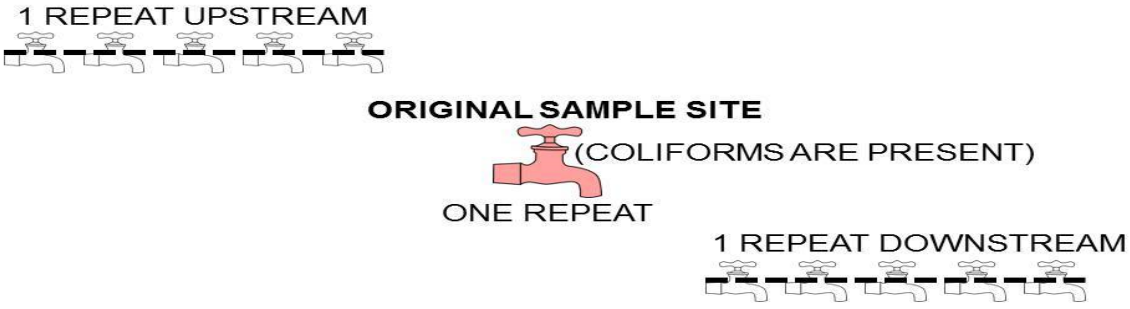
Minor Corrections to the Revised Total Coliform Rule (RTCR)
Minor corrections to the final RTCR became effective on April 28, 2014. No comments were received on the Direct Final Rule published on February 26, 2014 and the corrections therefore became effective without further notice. See the **Direct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



REPEAT SAMPLING PROCEDURES

RTCR Key Provisions *Most of this section comes from the USEPA.*

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. 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 occurrences. An MCL violation or failure to take repeat samples following a routine total coliform-positive sample will trigger a Level 1 or Level 2 assessment. Any sanitary defect identified during a Level 1 or Level 2 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. This includes location of routine and repeat water samples. Collect routine water samples on a regular basis (monthly, quarterly, annually). Have samples 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.

RTCR Key Provisions *Most of this section comes from the USEPA.*	
Level 1 and Level 2 Assessments and Corrective Actions	PWSs are required to conduct a Level 1 or Level 2 assessment if conditions indicate they might be vulnerable to contamination. PWSs must 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. The addition to the Requirements is the 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.

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

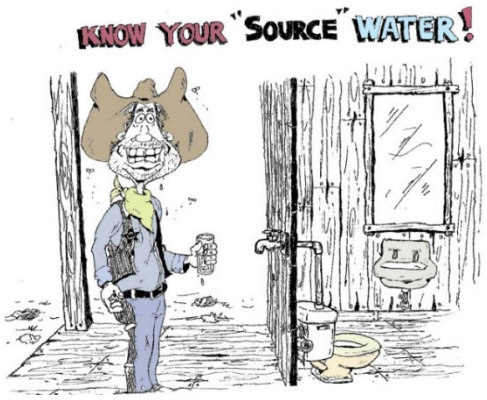
CT = Concentration of disinfectant x contact time

- The chlorine residual leaving the plant must be = or > 0.2 mg/L and measurable throughout the system

Troubleshooting Table for Bacteriological 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.

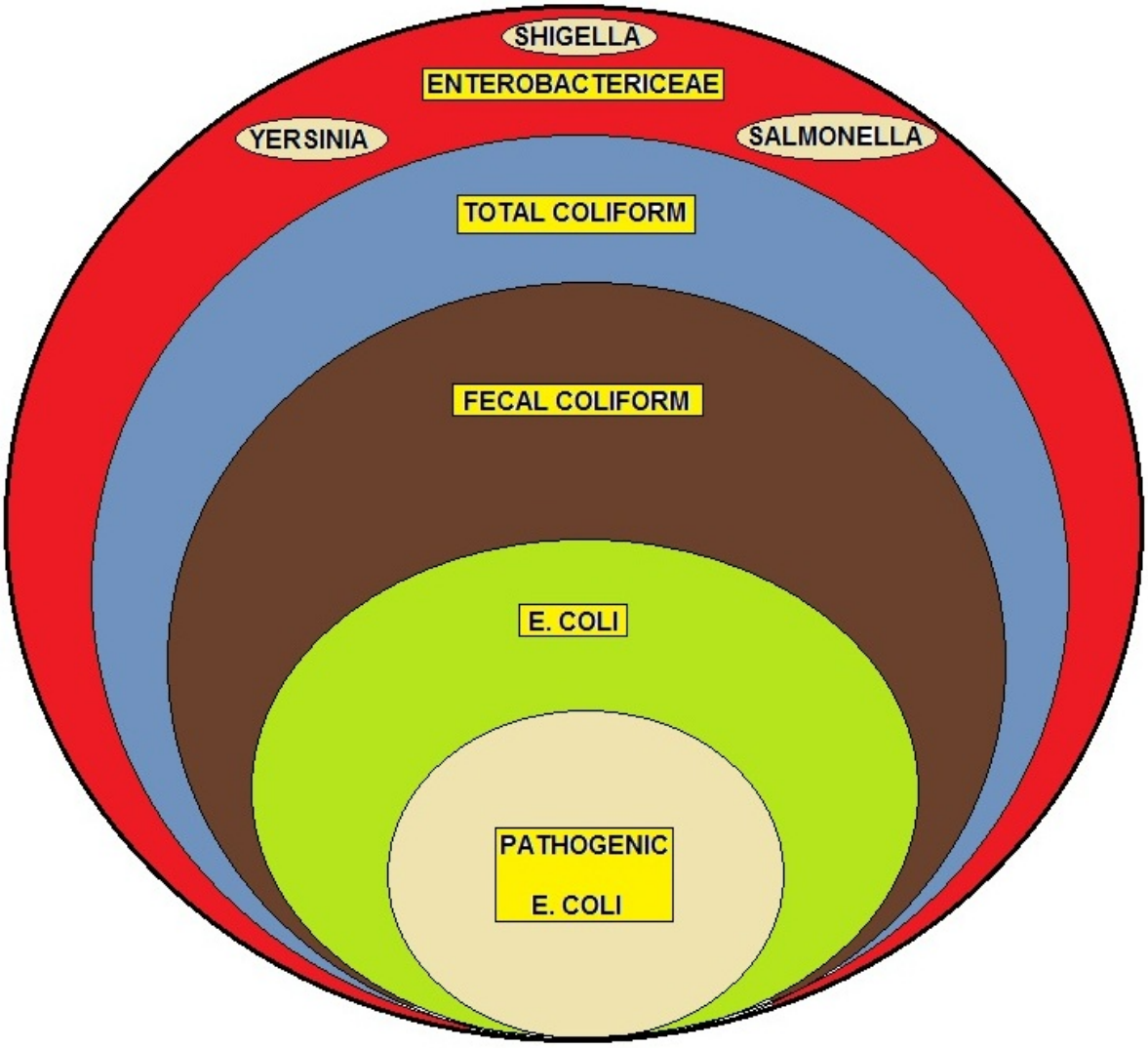


Possible Causes

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 Solutions

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.



COLIFORM BACTERIA SUB-SET #1
INDICATOR ORGANISMS

Waterborne Pathogen Section - Introduction

Bacteria, viruses, and protozoans that cause disease are known as pathogens. Most waterborne 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.

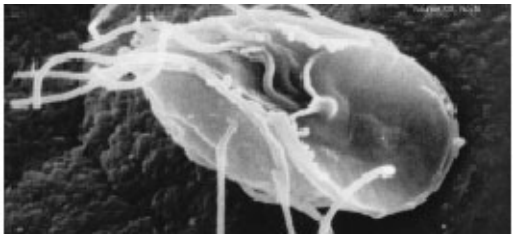
Pathogens that may cause waterborne outbreaks through drinking water have one thing in common: they are spread by the fecal-oral (or feces-to-mouth) route. Pathogens may get into water and spread when infected humans or animals pass the bacteria, viruses, and protozoa in their stool. For another person 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.

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 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 and a safe distribution system can ensure eliminating the spread of waterborne 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. You will usually be asked to sample for Giardia at these facilities.



Giardia

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 have digested within the past few days.

Chain of Transmission
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. 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 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; then illness (disease) will occur.

Emerging Waterborne Pathogens
Emerging waterborne pathogens constitute a major health hazard in both developed and developing nations. A new dimension to the global epidemiology of cholera-an ancient scourge-was provided by the emergence of Vibrio cholerae O139. Also, waterborne enterohemorrhagic Escherichia coli (E. coli O157:H7), although regarded as a problem of the industrialized west, has recently caused outbreaks in Africa.

Outbreaks of chlorine-resistant Cryptosporidium in the US have motivated water authorities to reassess the adequacy of current water-quality regulations. Of late, a host of other organisms, such as hepatitis viruses (including hepatitis E virus), Campylobacter jejuni, microsporidia, cyclospora, Yersinia enterocolitica, calciviruses and environmental bacteria like Mycobacterium spp, aeromonads, Legionella pneumophila and multidrug-resistant Pseudomonas aeruginosa have been associated with water-borne illnesses.

The protection and enhancement of our nation's water quality remains a chief concern of the U.S. Environmental Protection Agency. The Office of Research and Development is committed, through the extensive waterborne disease research efforts earlier described, to ensure that the most effective and efficient methods are developed to identify, detect, and inactivate/remove pathogens that may be present in our drinking water supplies.

Life cycles, mechanisms of infection, protective or dormant states, emergence of disinfection resistant variants, optimal pathogen removal techniques, regrowth in distribution lines…all are areas that must be investigated and understood to afford the water quality safeguards that are so often taken for granted. The successes and failures of these research efforts, relayed to the public and appropriate federal, state, and local agencies, have helped to ensure safe drinking water.

More on this subject in the Microorganism Appendix. **Hyperlink to the Glossary and Appendix** *http://www.abctlc.com/downloads/PDF/WTGlossary.pdf*

Primary Waterborne Diseases Section - Alphabetical Order

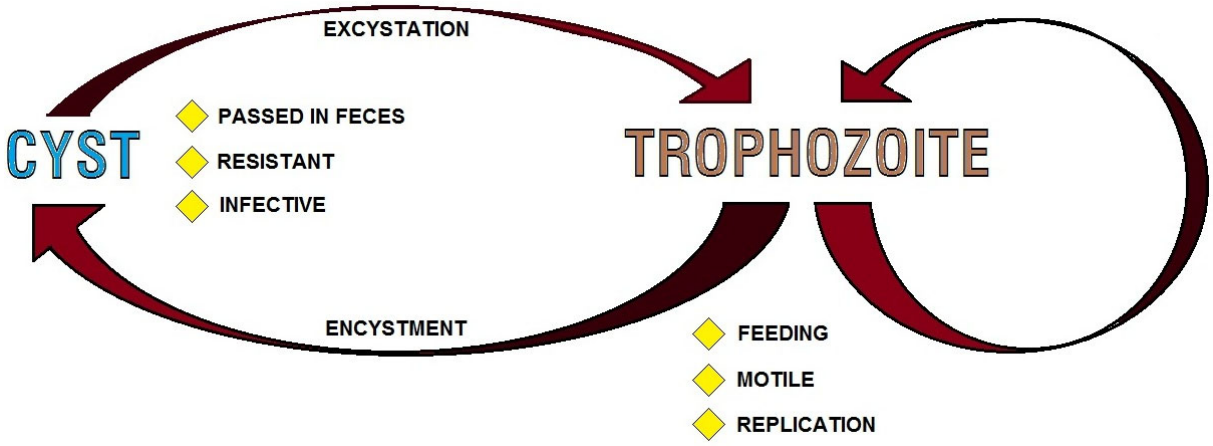
Campylobacter
Campylobacter, the basics. It is a bacterium. It causes diarrheal illness. Campylobacter is primarily associated with poultry, animals, and humans.

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.

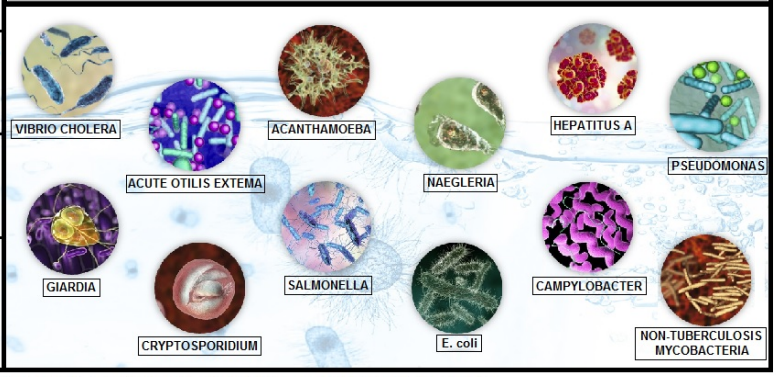
Shigella species prevention: Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

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.

PRESENCE OF PATHOGENS IN WATER
THE PRESENCE OF COLIFORM BACTERIA CAN INDICATE THERE MAY BE HARMFUL BACTERIA PATHOGENS IN THE WATER
THE PRINCIPAL REMOVAL PROCESSES ARE THOSE LIKELY USED TO REMOVE THE MAJORITY OF THE MICROBES IN WATER BEING TREATED
THE REMOVAL PROCESSES BEING UTILIZED ARE SEDIMENTATION, FLOTATION AND THE USE OF HIGH RATE GRANULAR MEDIA FILTRATION
DISINFECTION WITH IODINE OR CHLORINE IS THE MOST EFFECTIVE AT KILLING VIRUSES FOUND IN WATER.

TYPES OF PATHOGENS FOUND IN WATER



Technical Learning College

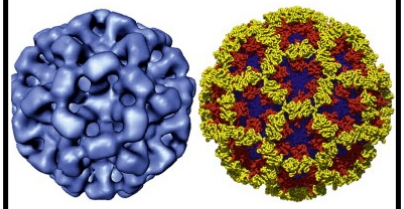
PATHOGENS FOUND IN WATER SUPPLIES

MICROBIOLOGICAL CONTAMINANTS FOUND IN WATER

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

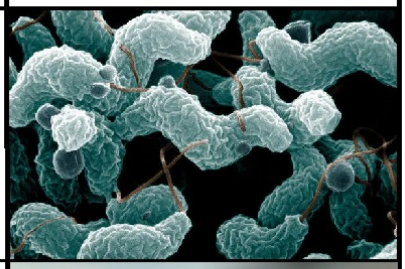
CALIVIVIRUS:

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



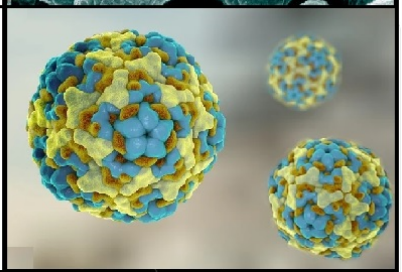
CAMPYLOBACTOR JEJUNI:

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

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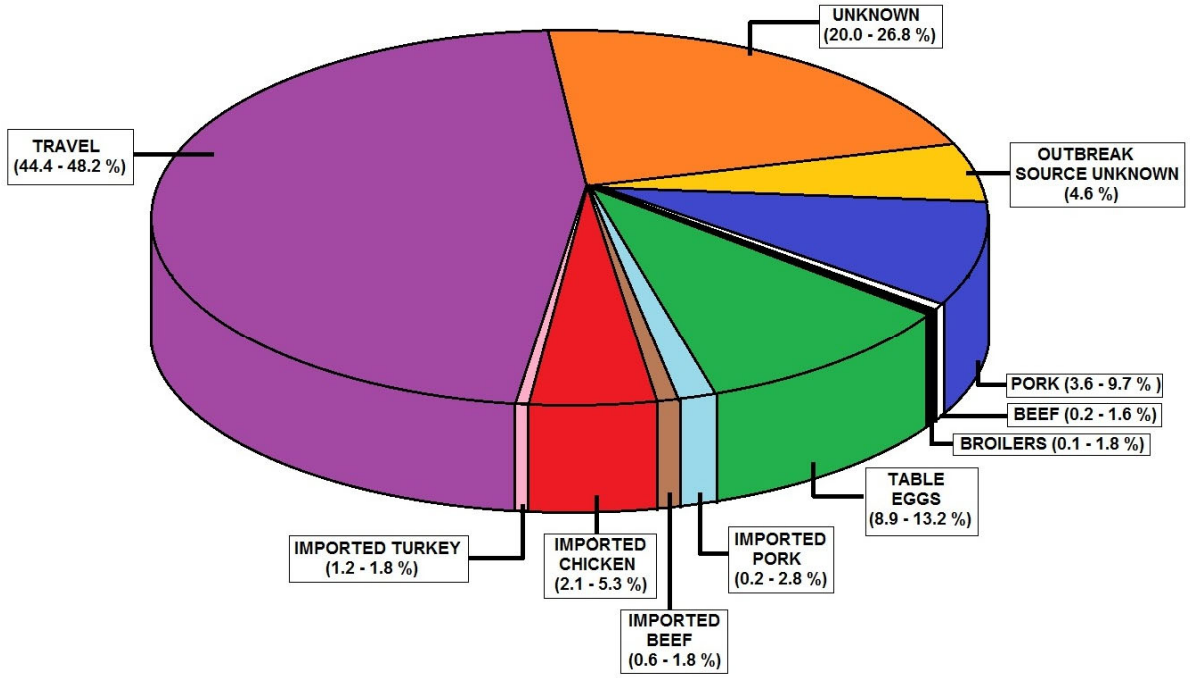
MICROBIOLOGICAL CONTAMINANTS

Waterborne Bacterial Diseases



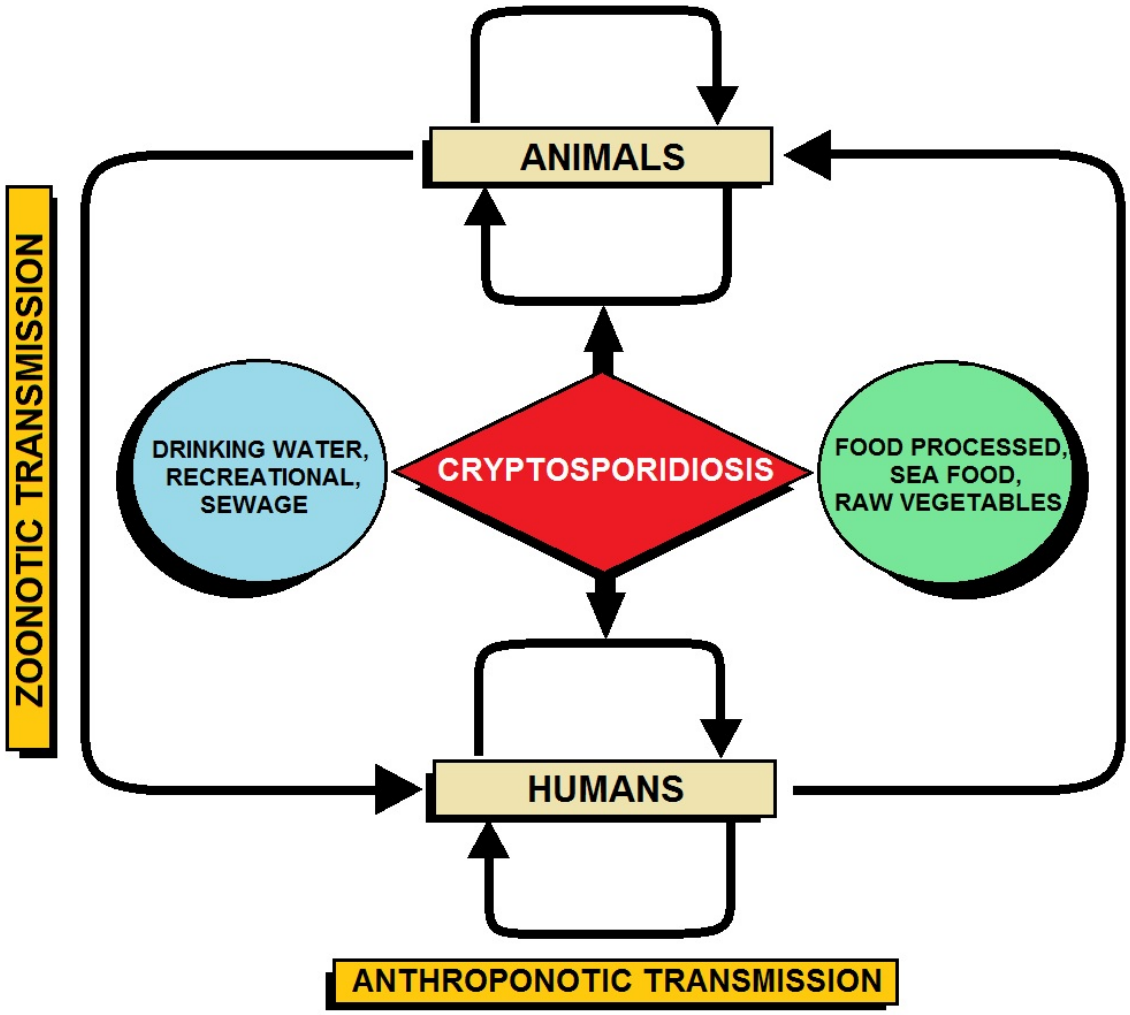
COURSES OF SAMONELLA PIE CHART

Campylobacteriosis is the most common diarrheal illness caused by bacteria. Other symptoms include abdominal pain, malaise, fever, nausea and vomiting; and 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 un-pasteurized 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, 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.

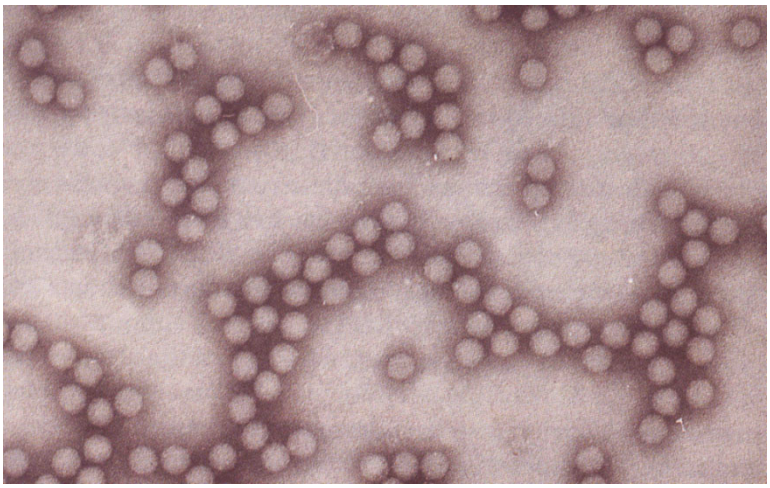
Gastroenteritis is an intestinal infection marked by watery diarrhea, abdominal cramps, nausea or vomiting, and sometimes fever. The most common way to develop viral gastroenteritis — often called stomach flu —is through contact with an infected person or by ingesting contaminated food or water. Because the symptoms are similar, it's easy to confuse viral diarrhea with diarrhea caused by bacteria, such as Clostridium difficile, salmonella and E. coli, or parasites, such as giardia.



TRANSMISSION OF CRYPTOSPORIDIOSIS

Waterborne Viral Diseases

- Drinking water must be free from viruses.
- Sometime viruses from intestinal tract of infected person get access to water along with feces.
- Some intestinal pathogenic viruses that are transmitted through contaminated water are- Rotavirus, Poliovirus, Hepatitis A and E, etc.



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). The incubation period is 15-50 days and averages 28-30 days.

Hepatitis A outbreaks have been related to fecally contaminated water; food contaminated by infected food handlers, including sandwiches and salads that are not cooked or are handled after cooking, and raw or undercooked mollusks harvested from contaminated waters. Aseptic meningitis, polio and viral gastroenteritis (Norwalk agent) are other viral diseases that can be transmitted through water. Most viruses in drinking water can be inactivated by chlorine or other disinfectants.

Norovirus

Norovirus, sometimes referred to as the winter vomiting bug, is the most common cause of gastroenteritis. Infection is characterized by non-bloody diarrhea, vomiting, and stomach pain. Fever or headaches may also occur. Symptoms usually develop 12 to 48 hours after being exposed, and recovery typically occurs within 1 to 3 days. Complications are uncommon, but may include dehydration, especially in the young, the old, and those with other health problems.

The virus is usually spread by the fecal–oral route. This may be through contaminated food or water or person-to-person contact. It may also spread via contaminated surfaces or through air from the vomit of an infected person. Risk factors include unsanitary food preparation and sharing close quarters. Diagnosis is generally based on symptoms. Confirmatory testing is not usually available but may be performed during outbreaks by public health agencies.

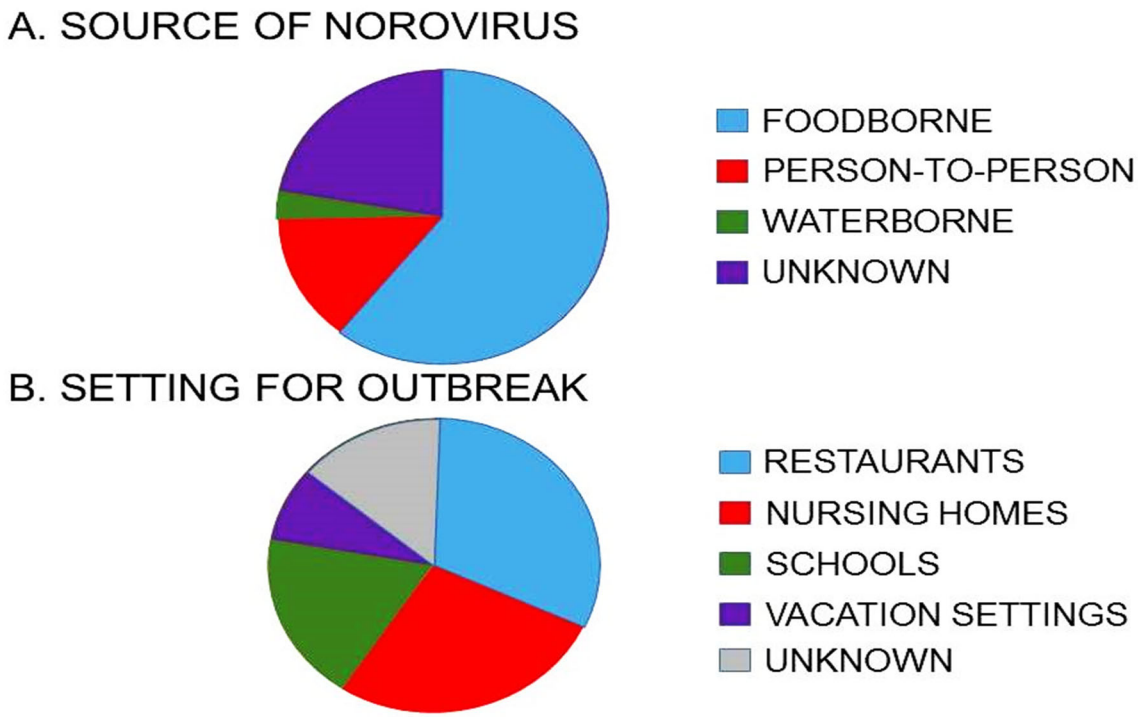
Norovirus results in about 685 million cases of disease and 200,000 deaths globally a year. It is common both in the developed and developing world. Those under the age of five are most often affected, and in this group it results in about 50,000 deaths in the developing world. Norovirus infections occur more commonly during winter months. It often occurs in outbreaks, especially among those living in close quarters. In the United States, it is the cause of about half of all foodborne disease outbreaks. The virus is named after the city of Norwalk, Ohio, where an outbreak occurred in 1968.

Coronavirus

It looks like the COVID-19 coronavirus may be able to live in water for a few days, potentially even a few weeks. Consider what is known about the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in water. Indeed studies have suggested that the SARS-CoV2 could actually hang out in the wet stuff for a little while.

SARS Virus

For example, a study published in the journal Water Research in 2009 found that two viruses that have similarities to the original SARS virus, the transmissible gastroenteritis (TGEV) and mouse hepatitis (MHV) viruses, could survive up to days and even weeks in water. The University of North Carolina team (Lisa Casanova, William A. Rutal, David J. Weber, and Mark D. Sobsey) that conducted the study concluded “coronaviruses can remain infectious for long periods in water and pasteurized settled sewage, suggesting contaminated water is a potential vehicle for human exposure if aerosols are generated.”



Waterborne Protozoan Diseases

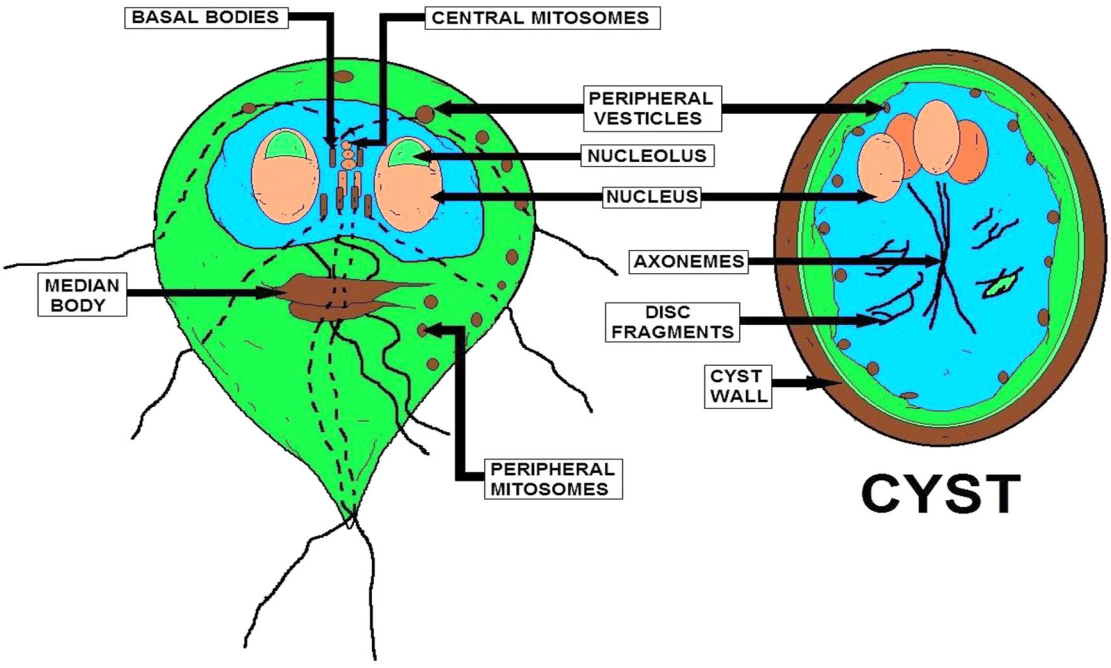
Protozoan pathogens are larger than bacteria and viruses, but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall called a “cyst.” The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis 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.

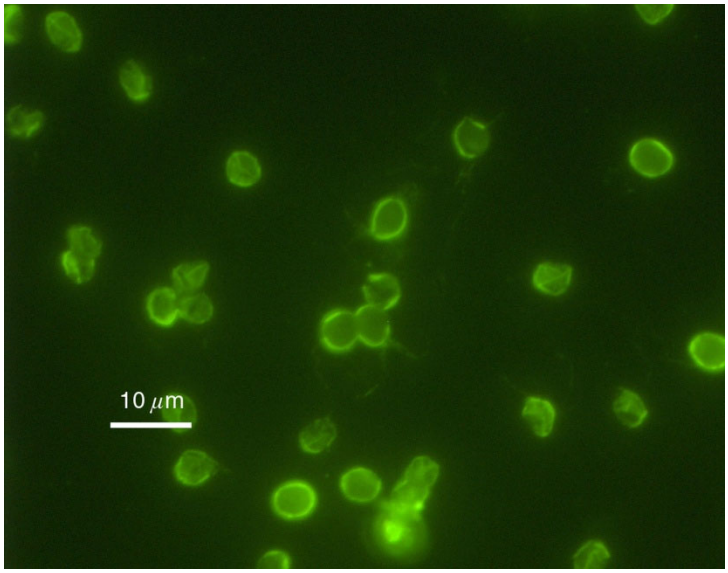
Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system.

- Drinking water should be free from disease causing parasites.
- Many species of protozoa and helminthes that causes water borne disease contaminates water through feces of infected patients.



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 these 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 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.

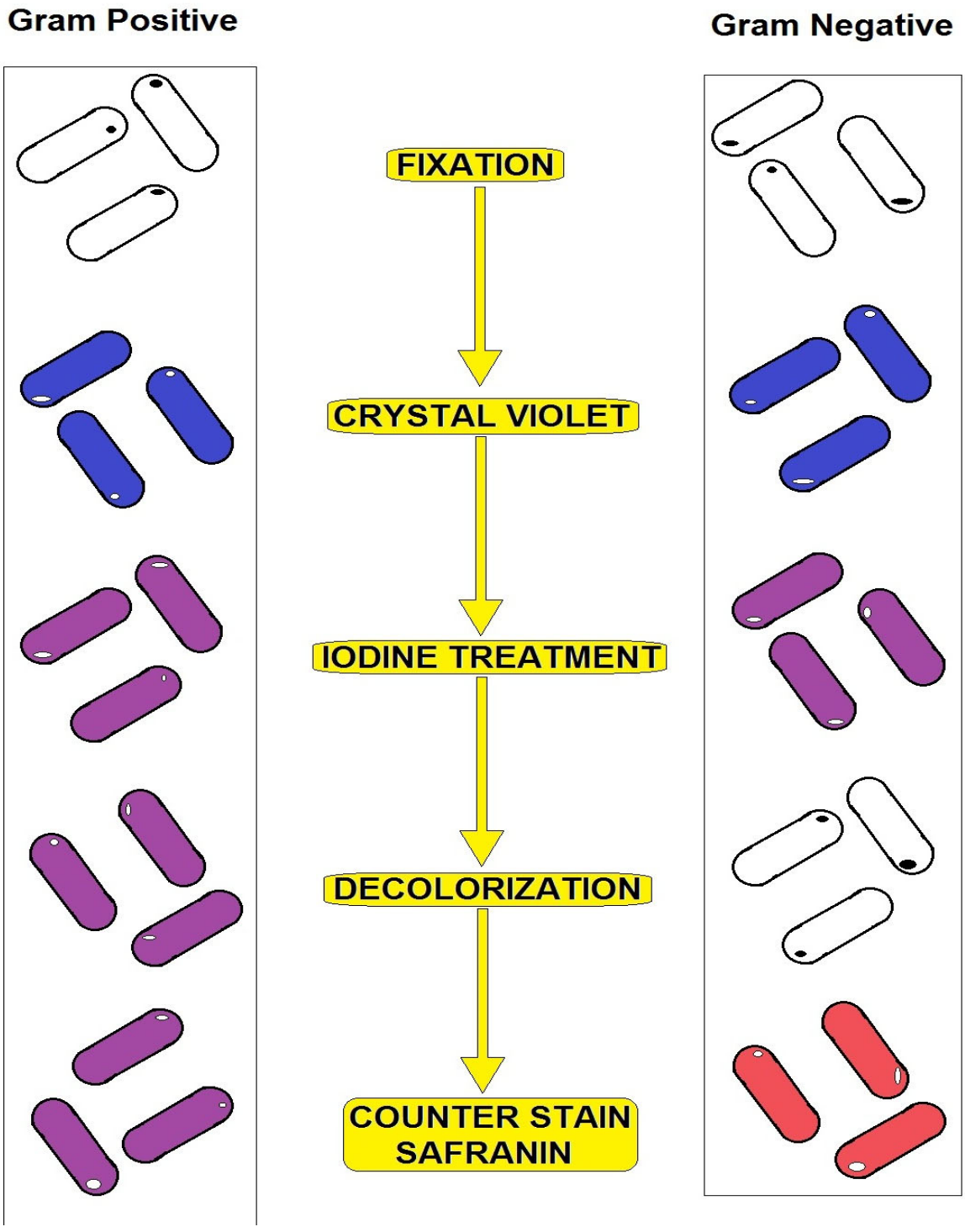
For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.

Common Waterborne Diseases Chart

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-sometime 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, enlarge liver, fever, vomiting, weight loss, abdomina pain-low mortality, last 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 pai — lasts (protozoan) days to weeks

Notes:

*1 http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm



GRAM STAINING DIAGRAM

Sampling Procedures –Sub-Section

The sample siting plan must be followed and all operating staff must be clear on how to follow the sampling plan. 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.

In addition, proper procedures must be followed for repeat sampling whenever a routine sample result is positive for total coliform.

What is a Sample Siting Plan?
A written sample siting plan specifies the routine sampling schedule and the locations (i.e., routine and repeat) in the distribution system where TC samples are collected. The locations selected must be representative of the finished water supplied to consumers. The purpose of sampling is to identify any coliform contamination so it can be dealt with quickly. Sample siting plans are subject to primacy agency review and revision. A sample siting plan must include the:
• PWS's sample sites (i.e., the location) where routine and repeat samples are collected: if approved by the primacy agency, also include sample sites for dual purpose samples that are used to meet the requirements for the RTCR repeat sampling and the Ground Water Rule (GWR) triggered source water monitoring.
• PWS's schedule for collecting the routine samples: For example, "[PWS_ID] will collect one routine TC sample every first Tuesday of the calendar month." The sample siting plan is a living document and should be updated to reflect changes to the PWS such as: major changes in population; new or additional water sources; infrastructure changes, such as a change in the distribution system (i.e., extended/ abandoned lines or pressure zones); or changes in disinfection or other treatment.

Most everyone can learn and master many of the basic lab procedures. Don't be intimidated, learn to take samples and analysis; it is an excellent career.

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 procedure outlined in this course manual is only a guideline. Consult your project manager or state agency for specific requirements.

If you have physical possession of a sample, have it in view, or have physically secured it 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 the chain-of-custody 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.

Using alcohol to disinfect a special sample tap before obtaining a sample.

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

PRIORITY:

RELINQUISHED BY:

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Chain of Custody Example.

Various water sample bottles and chain-of-custody form.

Collection of Surface Water Samples- 1 Example

Most of this section comes from the USEPA.

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.
7. Use appropriate gloves when collecting the sample.

Streamflow Measurement

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 per cent of the total discharge within it.

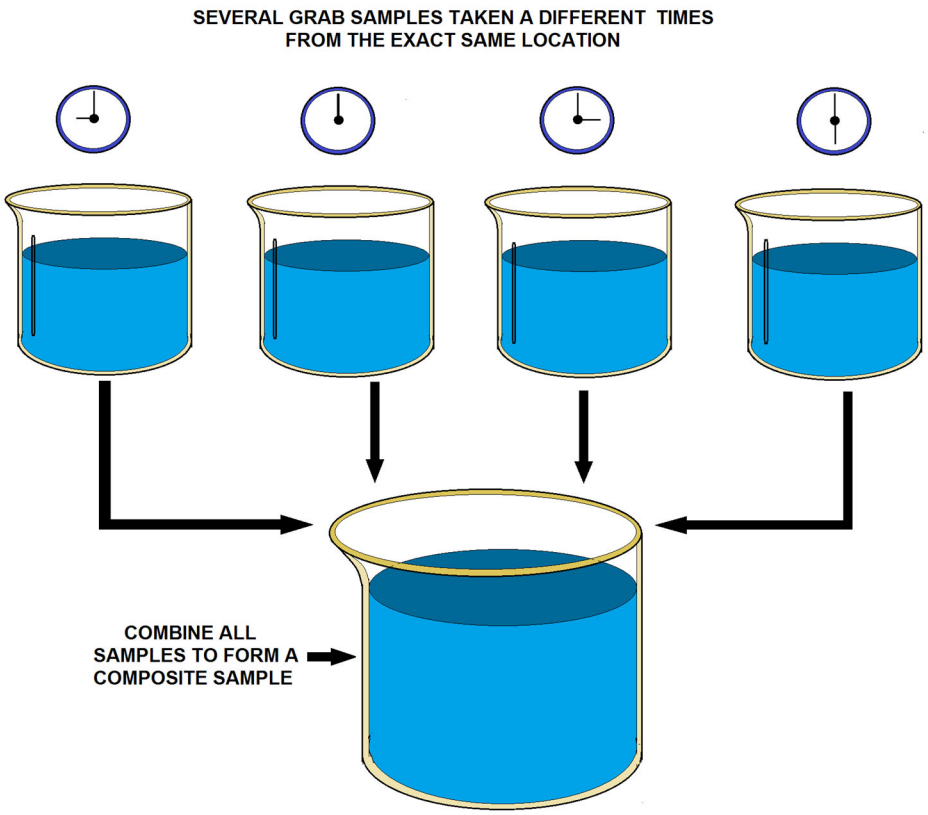
At the first vertical, face upstream and lower the velocity meter to the channel bottom, record its depth, then raise the meter to 0.8 and 0.2 of the distance from the stream surface, measure the water velocities at each level, and average them. Move to the next vertical and repeat the procedure until 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 the stream’s discharge rate. Calculate the discharge in each increment by multiplying the averaged velocity in each increment by the increment width and averaged depth.

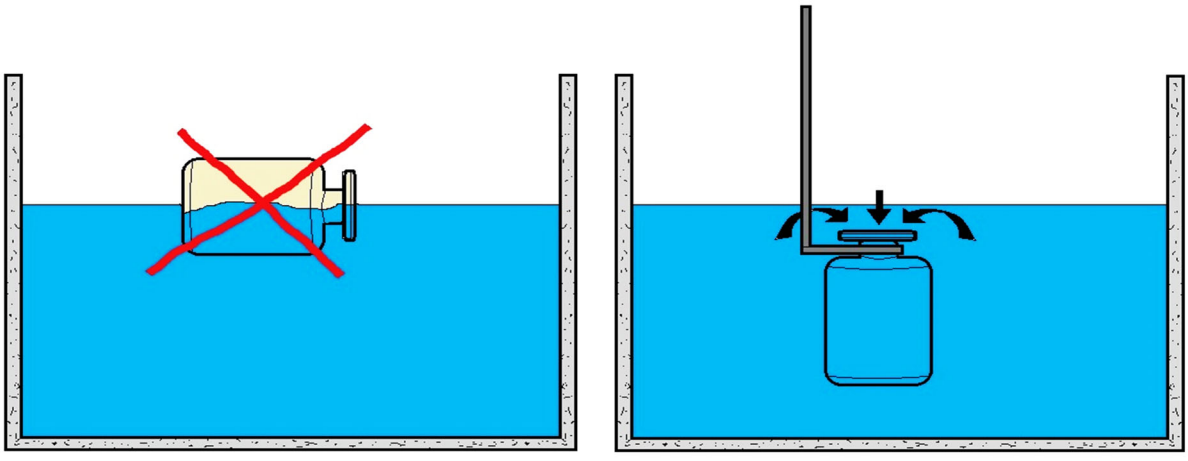
(Note that the first and last stations are located at the edge of the waterway and have a depth and velocity of zero.) Add up the discharges for each increment to calculate the total stream discharge rate. Record the flow in liters (or cubic feet) per second in your field book.

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, ideally located mid-stream.



MAKING A COMPOSITE SAMPLE FROM GRAB SAMPLES DIAGRAM



PROPER MATHOD OF TAKING IMMERSE TYPE WATER SAMPLES.

Note: Both of these sampling methods are not correct for taking Bac-T or disinfection byproduct sampling.

Summary

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature.

The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Detection and investigation of waterborne disease outbreaks is the primary responsibility of local, state and territorial public health departments, with voluntary reporting to the CDC. The CDC and the U.S. Environmental Protection Agency (EPA) collaborate to track waterborne disease outbreaks of both microbial and chemical origins. Data on drinking water and recreational water outbreaks and contamination events have been collected and summarized since 1971.

While useful, statistics derived from surveillance systems do not reflect the true incidence of waterborne disease outbreaks because many people who fall ill from such diseases do not consult medical professionals.

For those who do seek medical attention, attending physicians and laboratory and hospital personnel are required to report diagnosed cases of waterborne illness to state health departments. Further reporting of these illness cases by state health departments to the CDC is voluntary, and statistically more likely to occur for large outbreaks than small ones.

Despite these limitations, surveillance data may be used to evaluate the relative degrees of risk associated with different types of source water and systems, problems in current technologies and operating conditions, and the adequacy of current regulations. (Craun, Nwachuku, Calderon, and Craun, 2002).

Understanding Cryptosporidiosis

Cryptosporidium is an emerging parasitic protozoan pathogen because its transmission has increased dramatically over the past two decades. Evidence suggests it is newly spread in increasingly popular day-care centers and possibly in widely distributed water supplies, public pools and institutions such as hospitals and extended-care facilities for the elderly.

Recognized in humans largely since 1982 and the start of the AIDS epidemic, Cryptosporidium is able to cause potentially life-threatening disease in the growing number of immunocompromised patients.

Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April 1993. More than 100 deaths are attributed to this outbreak. Cryptosporidium remains a major threat to the U.S. water supply (Ibid.).

The EPA is developing new drinking water regulations to reduce Cryptosporidium and other resistant parasitic pathogens.

Key provisions of the Long Term 2 Enhanced Surface Water Treatment Rule include source water monitoring for Cryptosporidium; inactivation by all unfiltered systems; and additional treatment for filtered systems based on source water

Cryptosporidium concentrations. EPA will provide a range of treatment options to achieve the inactivation requirements. Systems with high concentrations of Cryptosporidium in their source water may adopt alternative disinfection methods (e.g., ozone, UV, or chlorine dioxide).

However, most water systems are expected to meet EPA requirements while continuing to use chlorination. Regardless of the primary disinfection method used, water systems must continue to maintain residual levels of chlorine-based disinfectants in their distribution systems.

Understanding Giardia lamblia

Giardia lamblia, discovered approximately 20 years ago, is another emerging waterborne pathogen. This parasitic microorganism can be transmitted to humans through drinking water that might otherwise be considered pristine. In the past, remote water sources that were not affected by human activity were thought to be pure, warranting minimal treatment. However, it is known now that all warm-blooded animals may carry Giardia and that beaver are prime vectors for its transmission to water supplies.

There is a distinct pattern to the emergence of new pathogens. First, there is a general recognition of the effects of the pathogen in highly susceptible populations such as children, cancer patients and the immunocompromised.

Next, practitioners begin to recognize the disease and its causative agent in their own patients, with varied accuracy. At this point, some may doubt the proposed agent is the causative agent, or insist that the disease is restricted to certain types of patients.

Finally, a single or series of large outbreaks result in improved attention to preventive efforts. From the 1960’s to the 1980’s this sequence of events culminated in the recognition of Giardia lamblia as a cause of gastroenteritis (Lindquist, 1999).

Bacteriological Monitoring Section –Post Quiz

Contaminants that may be present in sources of drinking water include:

1. Which of the following can be synthetic and volatile organic chemicals, which are by-products of industrial processes and petroleum production, and can come from gas stations, urban stormwater run-off, and septic systems?

A. Inorganic contaminants C. Organic chemical contaminants
B. Pesticides and herbicides D. Microbial contaminants

2. Which of the following can be naturally occurring or be the result of oil and gas production and mining activities?

A. Radioactive contaminants C. Inorganic contaminants
B. Pesticides and herbicides D. Microbial contaminants

Background

3. Coliform bacteria and chlorine residual are the only routine sampling and monitoring requirements for small ground water systems with chlorination.
True or False

TCR

4. The TCR sets a maximum contaminant level goal (MCLG) and maximum contaminant level (MCL) for E. coli for protection against potential fecal contamination.
True or False

5. The TCR sets a total coliform treatment technique (TT) requirement.
True or False

6. The TCR sets requirements for monitoring total coliforms and E. coli according to a sample siting plan and schedule specific to the PWS.
True or False

Routine Sampling Requirements

7. If any TC+ sample is also E. coli-positive (EC+), then the EC+ sample result must be reported to the state by the end of the month that the PWS is notified.
True or False

8. Reduced monitoring is general available for PWSs using only surface water and serving 1,000 or fewer persons that meet certain additional PWS criteria.
True or False

Dangerous Waterborne Microbes

9. Which of the following is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhea, vomiting, and cramps)?

A. Coliform Bacteria C. Protozoa
B. Cryptosporidium D. None of the above

10. Which of the following is a species of the rod-shaped bacterial genus Shigella?

A. Shigella dysenteriae C. Fecal coliform bacteria
B. Cryptosporidium D. None of the above

11. Which of the following are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria?
A. Fecal coliform bacteria C. Shigellae
B. Cryptosporidium D. None of the above

12. Which of the following are usually harmless, occur in high densities in their natural environment and are easily cultured in relatively simple bacteriological media?
A. Indicator bacteria C. Viruses
B. Amoebas D. None of the above

13. Water samples for _____must always be collected in a sterile container.
A. Amoebas C. Viruses
B. Bacteria tests D. None of the above

Basic Types of Water Samples
14. It is important to properly identify the type of sample you are collecting.
True or False

The three (3) primary types of samples are:
15. Samples collected following a coliform present routine sample. The number of repeat samples to be collected is based on the number of _____ samples you normally collect.
A. Repeat C. Routine
B. Special D. None of the above

16. 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. Trigger: Level 1 Assessment C. All of the above
B. Trigger: Level 2 Assessment D. None of the above

17. A PWS has a second Level 1 Assessment within a rolling 12-month period.
A. Trigger: Level 1 Assessment C. All of the above
B. Trigger: Level 2 Assessment D. None of the above

18. A PWS fails to take every required repeat sample after any single TC+ sample
A. Trigger: Level 1 Assessment C. All of the above
B. Trigger: Level 2 Assessment D. None of the above

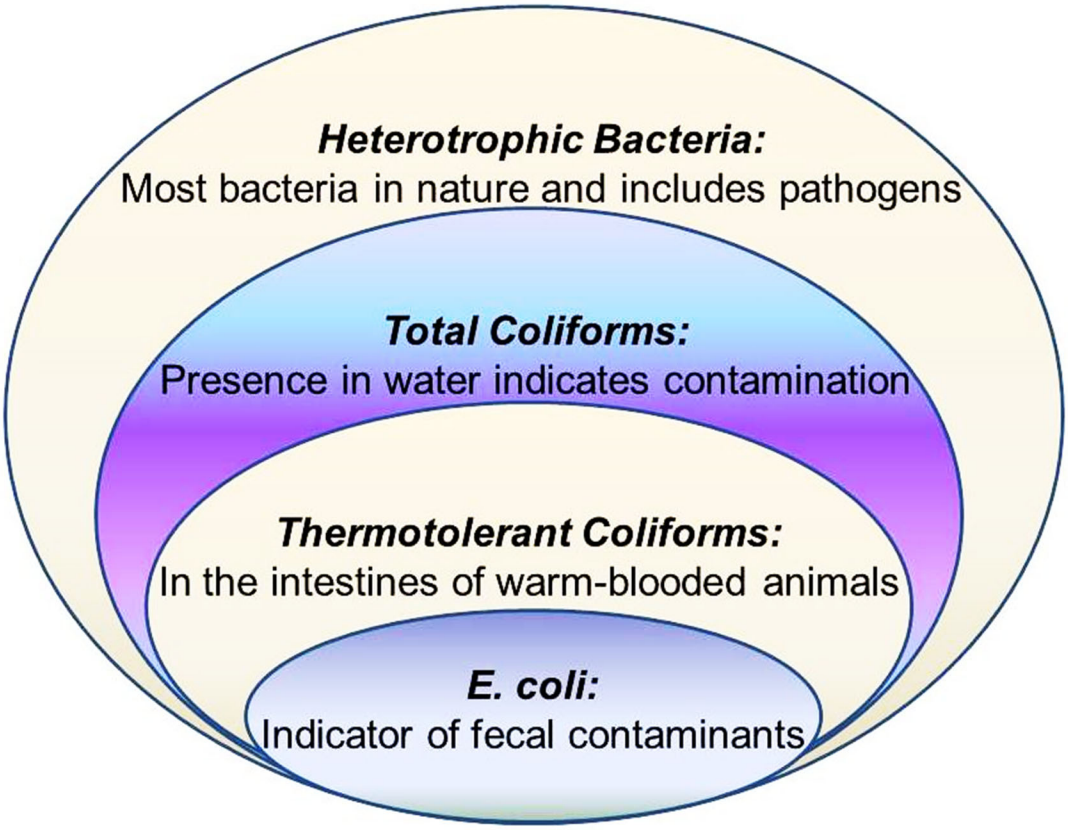
19. A PWS incurs an E. coli MCL violation.
A. Trigger: Level 1 Assessment C. All of the above
B. Trigger: Level 2 Assessment D. None of the above

Answers
1.C, 2. A, 3. T, 4. T, 5. T, 6. T, 7. F,8. F, 9.B, 10.A, 11.C, 12.A, 13.B, 14.T, 15.C, 16.A, 17.B, 18.A, 19. B

Escherichia Coli - Chapter 3

Section Focus: You will learn the basics of Escherichia coli (e. 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: The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, (bacteria, viruses, cysts, single-celled organisms) particularly those that cause E. coli, gastroenteritis, cryptosporidiosis or giardia (primary domestic concerns).



BACTERIAL ANALYSIS SUB-SET #1

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.

Understanding the Impact of E. coli and Enterococci
The presence of pathogens in a waterway can cause cloudy water, unpleasant odors, and decreased levels of dissolved oxygen. Enterococci levels should be measured in marine and fresh waters while E. coli should only be measured in fresh waters.

Acceptable levels of E. coli are measured in cfu (colony forming units) and commonly include both a 30-day mean (126 cfu/100mL) and a single sample number (235 cfu/100mL – 575 cfu/100mL). Suitable levels for enterococci in marine waters are 35 cfu/100mL for a 30-day mean and 104 – 501 cfu/100mL for a single sample, while levels in fresh water should be less than 33 cfu/100mL for a 30-day mean and 61 – 151 cfu/100 mL as a single sample reading. Be sure to compare your results with tribal, state, or federal standards when measuring for either enterococci or E. coli.

E. col i and enterococci levels are used as indicators of the presence of fecal material in drinking and recreational waters. Both indicate the possible presence of disease-causing bacteria, viruses, and protozoans. Such pathogens may pose health risks to people fishing and swimming in a water body. Sources of bacteria include improperly functioning wastewater treatment plants, leaking septic systems, storm water runoff, animal carcasses, and runoff from animal manure and manure storage areas

Symptoms
People infected with pathogenic *E. coli* can start to notice symptoms anywhere from a few days after consuming contaminated food or as much as nine days later. Generally, the symptoms include severe stomach cramps, diarrhea, fever, nausea, and/or vomiting.

The severity or presence of certain symptoms may depend on the type of pathogenic *E. coli* causing the infection. Some infections can cause severe bloody diarrhea and lead to life-threatening conditions, such as a type of kidney failure called hemolytic uremic syndrome (HUS), or the development of high blood pressure, chronic kidney disease, and neurologic problems. Other infections may have no symptoms or may resolve without medical treatment within five to seven days.

Due to the range in severity of illness, people should consult their health care provider if they suspect that they have developed symptoms that resemble a(n) *E. coli* infection.

At-Risk Groups
People of any age can become infected with pathogenic *E. coli*. Children under the age of 5 years, adults older than 65, and people with weakened immune systems are more likely to develop severe illness as a result of an *E. coli* infection. However, even healthy older children and young adults can become seriously ill.

Foods Linked to U.S. Outbreaks of *E. coli*?
Different types of *E. coli* tend to contaminate different types of foods and water. Previous U.S. outbreaks of pathogenic *E. coli* have included leafy greens, sprouts, raw milk and cheeses, and raw beef and poultry.

Shiga toxin-producing *E. coli* (STEC), including *E. coli* O157:H7, can be particularly dangerous. The primary sources of STEC outbreaks are raw or undercooked ground meat products, raw milk and cheeses, and contaminated vegetables and sprouts.

Credit EPA for the information in this Section.

Fecal Coliform Bacteria. *More information in the Laboratory Section.*

Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.

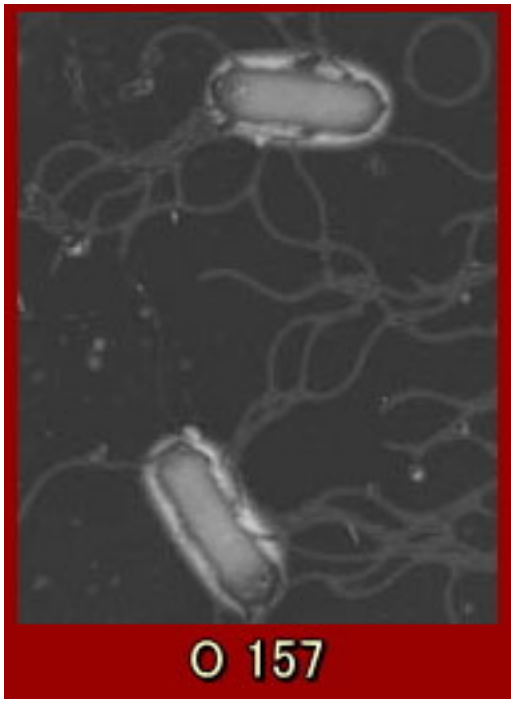
Reasons for Natural Variation
Unlike the other conventional water quality parameters, fecal coliform bacteria are living organisms. They do not simply mix with the water and float straight downstream. Instead they multiply quickly when conditions are favorable for growth, or die in large numbers when conditions are not. Because bacterial concentrations are dependent on specific conditions for growth, and these conditions change quickly, fecal coliform bacteria counts are not easy to predict. For example, although winter rains may wash more fecal matter from urban areas into a stream, cool water temperatures may cause a major die-off. Exposure to sunlight (with its ultraviolet disinfection properties) may have the same effect, even in the warmer water of summertime.

Expected Impact of Pollution
The primary sources of fecal coliform bacteria to fresh water are wastewater treatment plant discharges, failing septic systems, and animal waste.

Bacteria levels do not necessarily decrease as a watershed develops from rural to urban. Instead, urbanization usually generates new sources of bacteria.

Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in fecal coliform bacteria concentrations.

The presence of old, disintegrating storm and sanitary sewers, misplaced sewer pipes, and good breeding conditions are common explanations for the high levels measured.



Coliform Standards (in colonies/100ml)
Drinking water.....0FC
Total body contact (swimming).....200FC
Partial body contact (boating).....1000FC
Threatened sewage effluent .....not to exceed 200 FC
*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

Indicator Connection Varies
General coliforms, E. Coli, and Enterococcus bacteria are the "indicator" organisms generally measured to assess microbiological quality of water. However, these aren't generally what get people sick. Other bacteria, viruses, and parasites are what we are actually worried about.

Because it is so much more expensive and tedious to do so, actual pathogens are virtually never tested for. Over the course of a professional lifetime pouring over indicator tests, in a context where all standards are based on indicators, water workers tend to forget that the indicators are not the things we actually care about.

What are these indicators? More information in the Laboratory section.

- **General coliforms** indicate that the water has come in contact with plant or animal life. General coliforms are universally present, including in pristine spring water. They are of little concern at low levels, except to indicate the effectiveness of disinfection. Chlorinated water and water from perfectly sealed tube wells is the only water I've tested which had zero general coliforms. At very high levels they indicate there is what amounts to a lot of compost in the water, which could easily include pathogens (Ten thousand general coliform bacteria will get you a beach closure, compared to two or four hundred fecal coliforms, or fifty enterococcus).
- **Fecal coliforms**, particularly E. coli, indicate that there are mammal or bird feces in the water.
- **Enterococcus bacteria** also indicate that there are feces from warm blooded animals in the water. Enterococcus are a type of fecal streptococci. They are another valuable indicator for determining the amount of fecal contamination of water. According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater.

The more closely related the animal, the more likely pathogens excreted with their feces can infect us.

Human feces are the biggest concern, because anything which infects one human could infect another. There isn't currently a quantitative method for measuring specifically human fecal bacteria (expensive genetic studies can give a presence/absence result).

Ingesting a human stranger's feces via contaminated water supply is a classic means for infections to spread rapidly. The more pathogens an individual carries, the more hazardous their feces. Ingesting feces from someone who is not carrying any pathogens may gross you out, but it can't infect you. Infection rates are around 5% in the US, and approach 100% in areas with poor hygiene and contaminated water supplies.

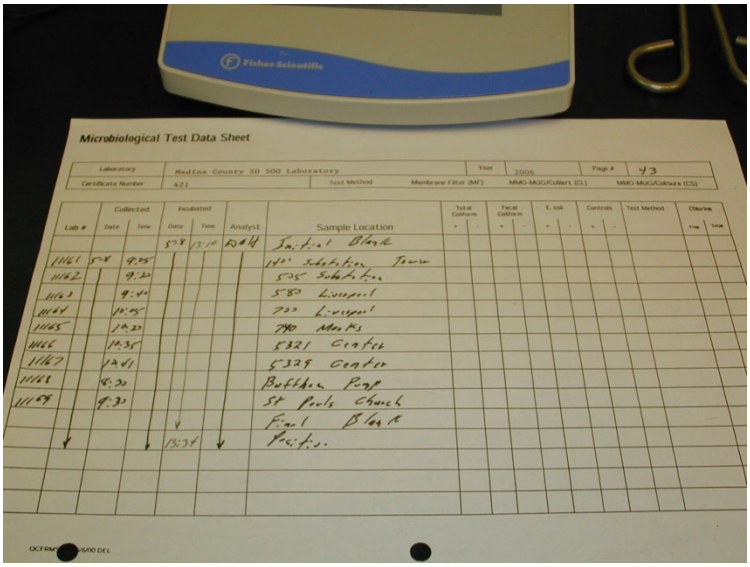
Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment factor for your best guess of the ratio between indicators and actual pathogens.

Membrane Filter Total Coliform Technique

The membrane filter total Coliform technique is used for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers.

The samples are taken in sterile 100 mL containers. These containers, when used for chlorinated water samples, have a sodium thiosulfate pill or solution to dechlorinate the sample.

The sample is placed in cold storage after proper sample taking procedures are followed. (See sample procedures below)



The samples are taken to the laboratory with a chain of custody to assure no tampering of samples can occur.

These samples are logged in at the laboratory.

No longer than 30 hours can lapse between the time of sampling and time of test incubation. (8 hours for heterotrophic, nonpotable 6 hours, others not longer than 24 hours)

All equipment is sterilized by oven and autoclave.

Glassware in oven at 170°C ± 10°C with foil (or other suitable wrap) loosely fitting and secured immediately after sterilization.

Filtration units in autoclave at 121°C for 30 minutes.

Use sterile petri dishes, grid, and pads bought from a reliable company – certified, quality assured - test for satisfactory known positive amounts.

Incubators – 35°C ± .5°C (60% relative humidity)

M-endo medium is prepared and heated to near boiling removed from heat cooled to 45°C pH adjusted to 7.2 ± .2 and immediately dispensed 8ml to plates. Keep refrigerated and discard after 2 weeks.

Plates can be stored in a dated box with expiration date and discarded if not used. No denatured alcohol should be used. Everclear or 95% proof alcohol or absolute methyl may be used for sterilizing forceps by flame.

Procedure
1. Counters are alcohol wiped.
2. Bench sheets are filled out.
3. Samples are removed from refrigeration.
4. Sterile wrapped utensils are placed on counters.
5. Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
6. Sterile petri dishes are labeled.
7. The samples closures are clipped.
8. The sample is shaken 25 times 1 foot in length within 7 seconds.
9. 100 mL is filtered and rinsed with sterile distilled water 3 times.
10. The membrane filter is aseptically removed from filter holder.
11. A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
12. The sterile labeled lid is placed on the petri dish.
13. 2 blanks and a known is run with each series of samples.
14. The samples are placed in the 35^{o}C $\pm$ .5^{o}C incubator stacked no higher than 3 for 22 – 24 hours (Humidity can be maintained by saturated paper towels placed under containers holding petri dishes.)
15. After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
16. Count all colonies that appear pink to dark red with a metallic surface sheen – the sheen may vary in size from a pin head to complete coverage.
17. Report as Total Coliform per 100 mL.
18. If no colonies are present report as <1 coliform/100mL.

Anything greater than 1 is over the limit for drinking water for 2 samples taken 24 hours apart. Further investigation may be necessary – follow Standard Methods accordingly.

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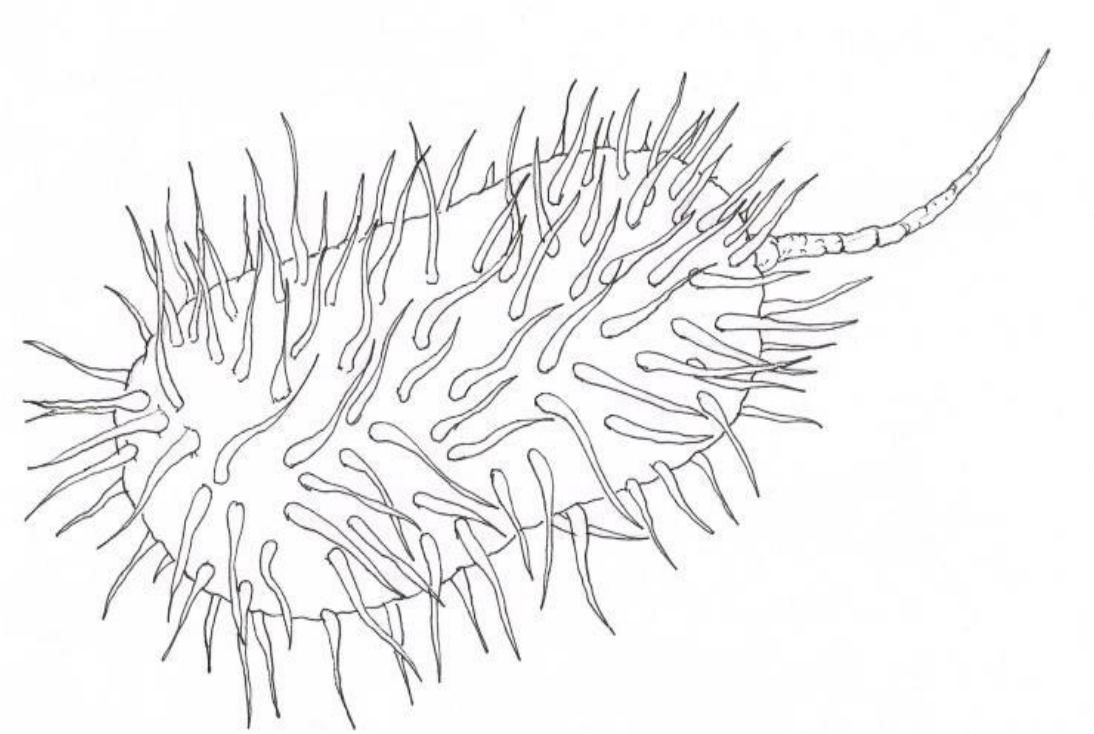
Aran^{TM} Aqua Analytical Laboratory Director.

Types of Escherichia Coli



Two primary 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.

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*.

Diarrheagenic Strains of E. coli

Escherichia coli is the predominant nonpathogenic facultative flora of the human intestine. Some E. coli strains, however, have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even the most robust human hosts.

Diarrheagenic strains of E. coli can be divided into at least six different categories with corresponding distinct pathogenic schemes. Taken together, these organisms probably represent the most common cause of pediatric diarrhea worldwide.

Several distinct clinical syndromes accompany infection with diarrheagenic E. coli categories, including traveler's diarrhea (enterotoxigenic E. coli), hemorrhagic colitis and hemolytic-uremic syndrome (enterohemorrhagic E. coli), persistent diarrhea (enteroaggregative E. coli), and watery diarrhea of infants (entero-pathogenic E. coli). This review discusses the current level of understanding of the pathogenesis of the diarrheagenic E. coli strains and describes how their pathogenic schemes underlie the clinical manifestations, diagnostic approach, and epidemiologic investigation of these important pathogens.

Common E. Coli Questions

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.

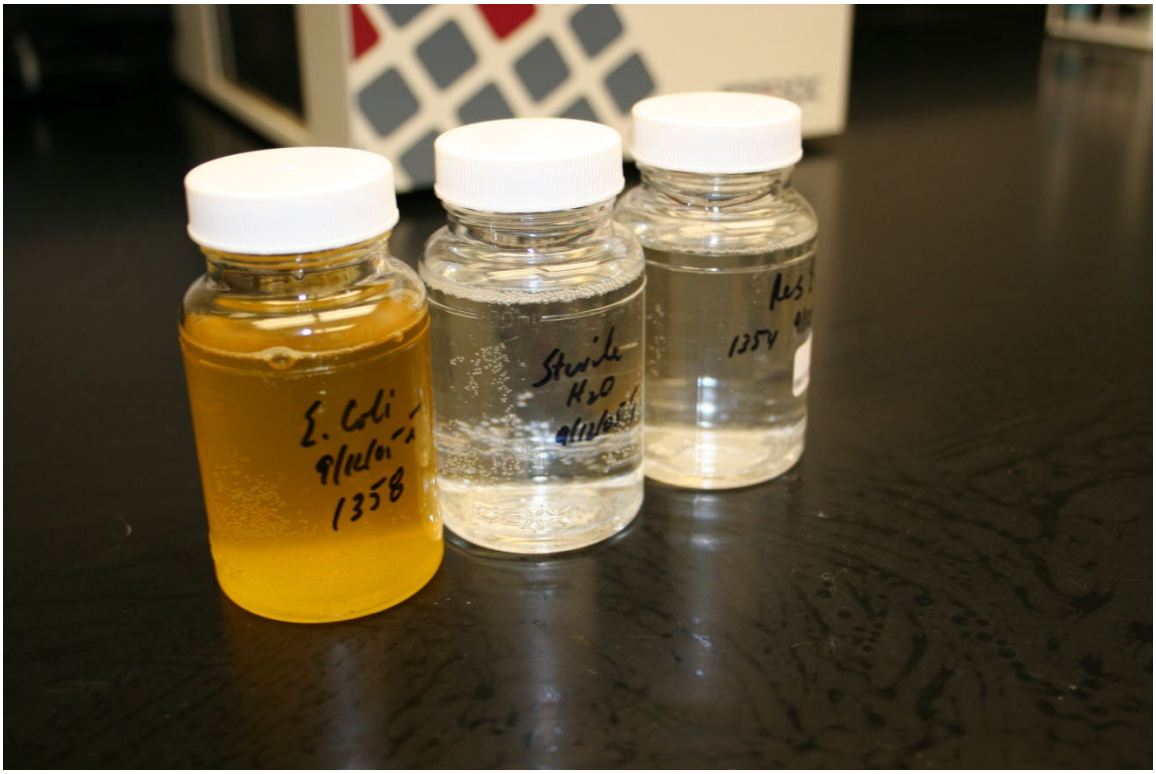
The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect 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.

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.

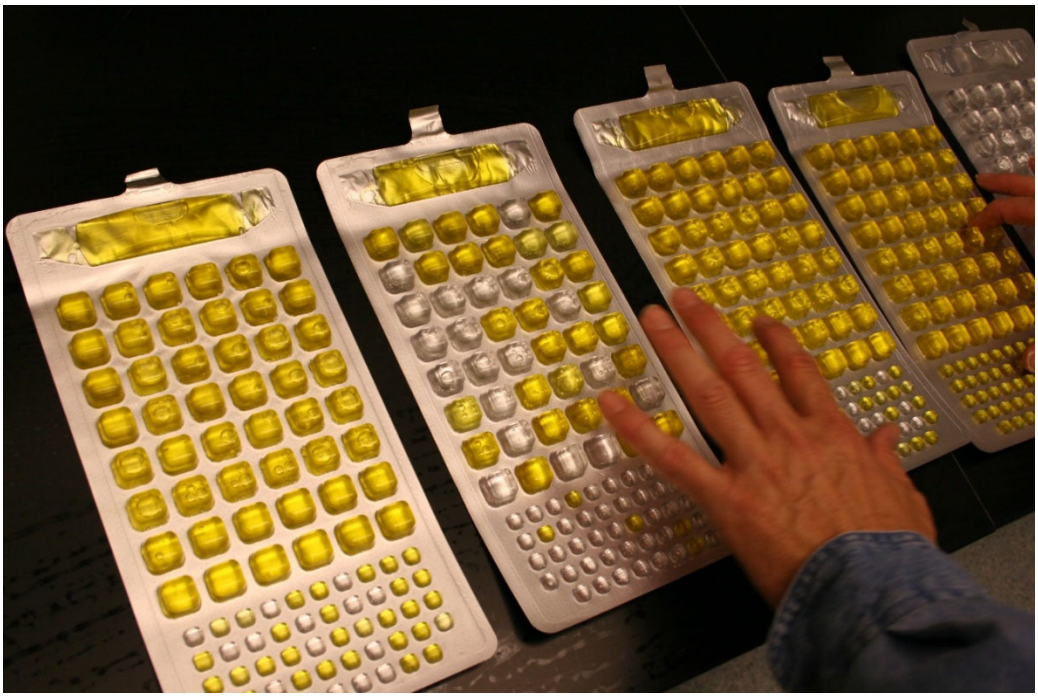


How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment 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 non-bloody 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.

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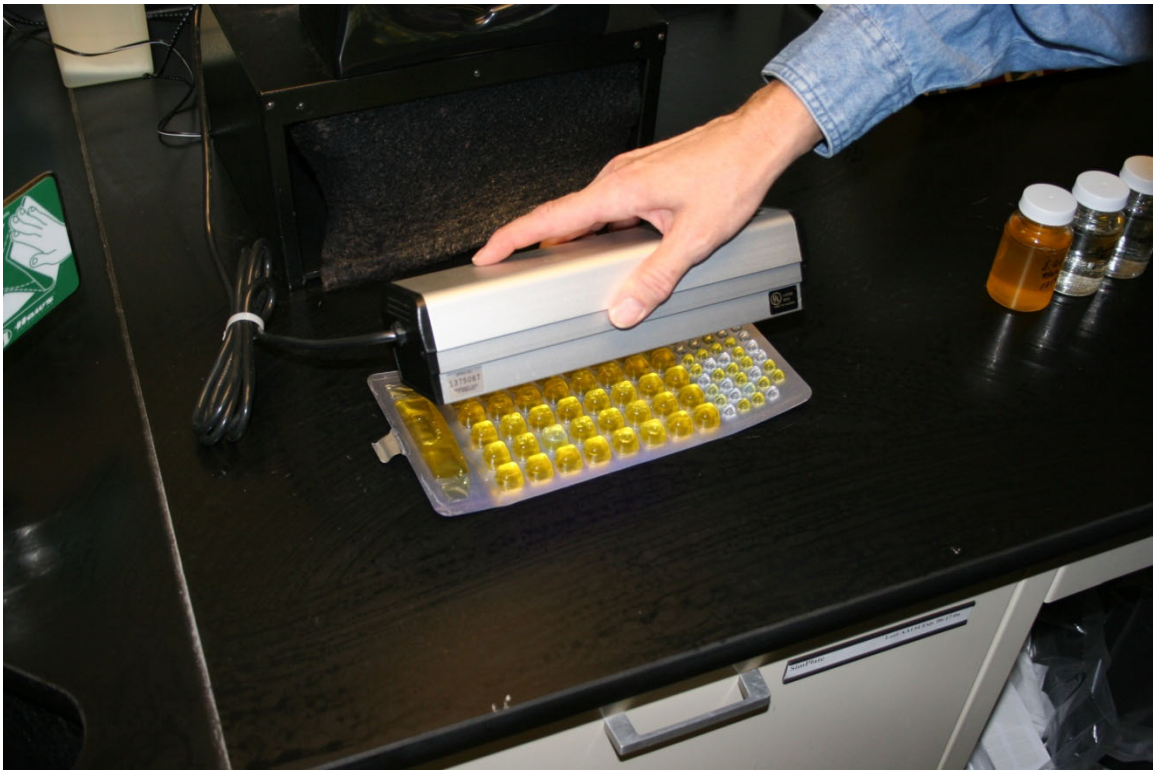
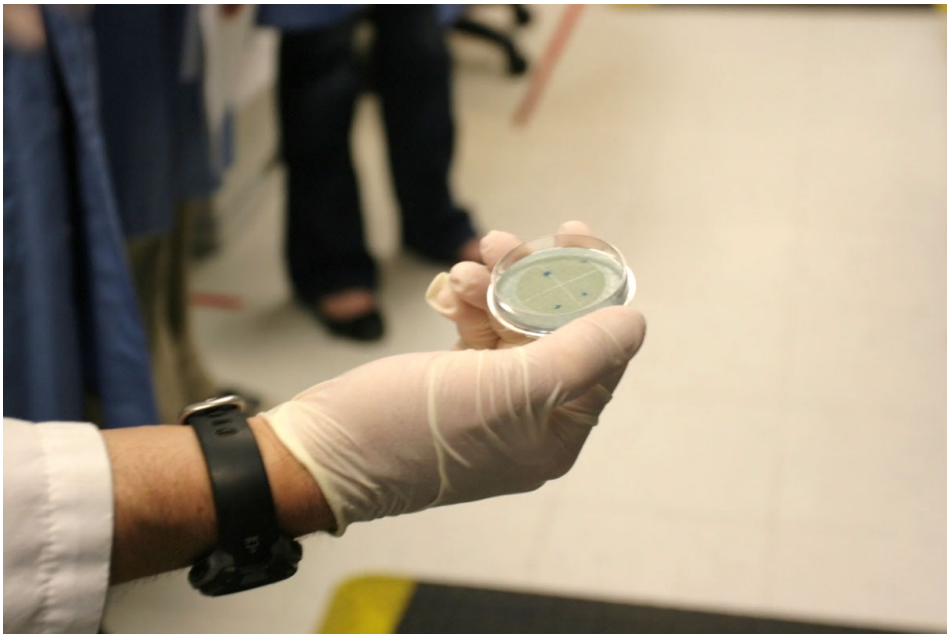


Top Photo: This technician is using Colilert which is a commercially available enzyme-substrate liquid-broth medium (IDEXX Laboratories, Inc.) that allows the simultaneous detection of total coliforms and *Escherichia coli* (*E. coli*). It is available in the most-probable number (MPN) or the presence/absence (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.



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

Summary
Escherichia coli (*E. coli*) is a gram-negative, rod-shaped flagellated bacterium. Although it is an essential component of the bacterial gut flora, disease may be caused by direct intake of a pathogenic *E. coli* subtype (e.g., in contaminated food) or spreading of the intestinal bacteria to another organ (cystitis, pneumonia). Enterohemorrhagic *E. coli* (EHEC), for instance, can lead to severe colitis and hemolytic-uremic syndrome (HUS), particularly in children and infants. In such cases, diarrhea should only be treated symptomatically, as antibiotics can lead to increased toxin secretions that exacerbate the course of disease.

Escherichia Coli - Post Quiz

1. Fecal Coliform Bacteria live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received _____ from one source or another.

2. Winter rains may wash more _____ from urban areas into a stream; cool water temperatures may cause a major die-off.

3. Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in?

E. coli O157:H7

4. Symptoms of E. coli O157:H7 (bacterium) vary with type caused ____.

5. What term is used to express that in families and childcare centers are an important mode of transmission and that infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water?

6. Consumers can prevent _____ infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully.

7. 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.
A. True B. False

8. 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.
A. True B. False

9. Larger types of systems can qualify for five samples a month.
A. True B. False

10. E. coli O157:H7 is one of hundreds of strains of the Enterococcus bacteria.
A. True B. False

11. 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.
A. True B. False

12. 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.
A. True B. False

13. Currently, there are four recognized classes of _____ (collectively referred to as the EEC group) that cause gastroenteritis in humans.

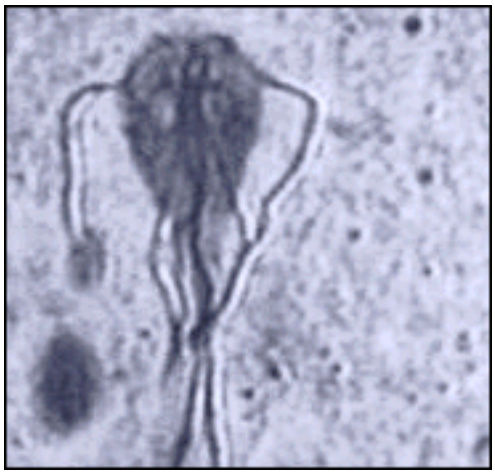
Answers

1. Fecal matter, 2. Fecal matter, 3. Fecal coliform bacteria concentrations, 4. E. coli, 5. Person-to-person contact, 6. E. coli O157:H7, 7.T, 8. T, 9.F, 10.F, 11.T, 12.T, 13. Enterovirulent E. coli

Giardiasis *Giardia lamblia* - Chapter 4

Section Focus: You will learn the basics of Giardia. At the end of this section, you will be able to describe *Giardia* (also known as *Giardia intestinalis*, *Giardia lamblia*, or *Giardia duodenalis*. 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 bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, (bacteria, viruses, cysts, single-celled organisms) particularly those that cause E. coli, gastroenteritis, cryptosporidiosis or giardia (primary domestic concerns).



GIARDIA

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.

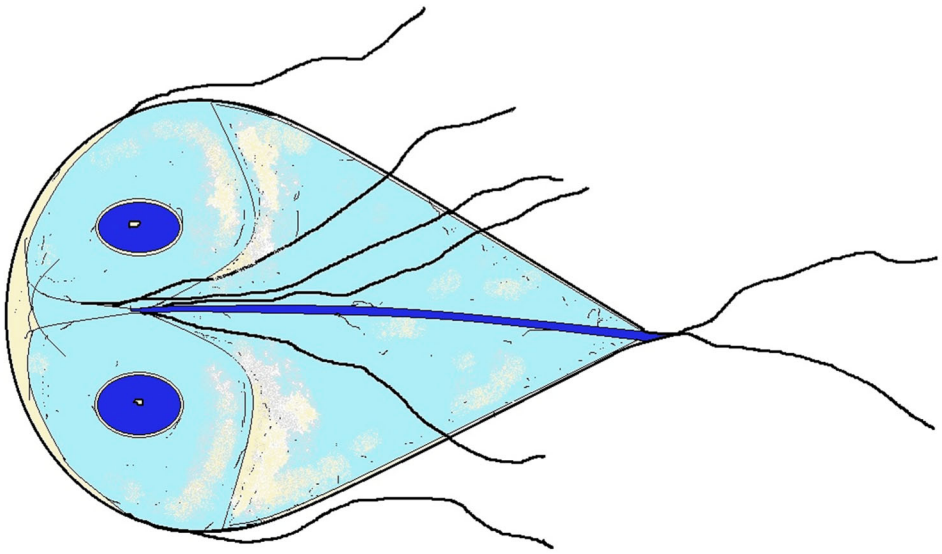
Giardiasis is the most frequent cause of non-bacterial diarrhea in North America. *Giardia duodenalis*, cause of giardiasis (GEE-are-DYE-uh-sis), is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become 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.



GIARDIA LAMBLIA

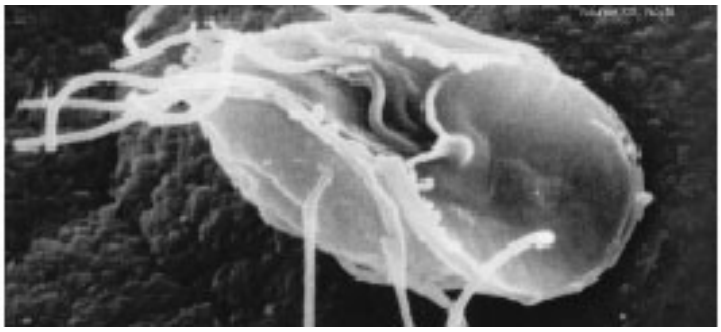
One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the parasite. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.

Giardia intestinalis*, Giardia lamblia, or* Giardia duodenalis
Giardia is a microscopic parasite that causes the diarrheal illness known as giardiasis. *Giardia* (also known as *Giardia intestinalis*, *Giardia lamblia*, or *Giardia duodenalis*) is found on surfaces or in soil, food, or water that has been contaminated with feces (poop) from infected humans or animals.

Giardia is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it tolerant to chlorine disinfection. While the parasite can be spread in different ways, water (drinking water and recreational water) is the most common mode of transmission.

Organism Description

While numerous species of Giardia have been described, there is no general agreement on the criteria which define species in this genus. Criteria that has been used include: host specificity; body size and shape, and the morphology of a microtubular organelle, the median body; and biochemical, molecular, and genetic techniques, such as the polymerase chain reaction (PCR) for DNA-based detection and identification.



The pyriform bodies of trophozoites of the genus Giardia range from 9 to 21 µm long, 5 to 15 µm wide, and 2 to 4 µm thick. Trophozoites are identified by the presence of two morphologically indistinguishable anterior nuclei, eight flagella, two central axonemes, microtubular median bodies, and a ventral adhesive disk. A pair of staining structures (median bodies) lie dorsal to the axonemes and are tipped dorsoventrally and anterioposteriorly so that the right tip is more dorsal and anterior. Median bodies consist of random arrangements of microtubules that lack an origin or insertion into any other structure and may play a supporting function in the posterior portion of the trophozoite behind the striated (ventral) disk.

In the Giardia life cycle, the trophozoites divide by binary fission, attach to the brush border of the small intestinal epithelium, detach for unknown reasons, then become rounded and elaborate a cyst wall. The viable, environmentally-resistant cyst is excreted in the feces, moves passively through the environment, primarily aquatic, and may be transmitted to another vertebrate host if it is ingested. Following ingestion of a viable cyst, the excystation process to a trophozoite is initiated by low pH conditions in the stomach.

Excystation is completed in the less acidic conditions of the small intestine where the trophozoites attach to the small intestinal epithelium. Encystment is initiated by exposure of the trophozoites to bile in the upper bowel and continues in the lower small intestine where the trophozoite rounds up and secretes cyst wall components. The wall provides protection when cysts pass out of the host with the feces. Unlike the trophozoites, cysts are not motile.

Giardia cysts are typically ovoid, and measure from 10 to 15 µm in length, and from 7 to 10 µm in width, with the cyst wall being approximately 0.3 µm thick. Newly formed cysts contain two morphologically indistinguishable nuclei. Each nucleus in the cyst undergoes a single further division, so that mature cysts contain four nuclei. Filice (1952) stated that the median bodies of the trophozoites were rarely, if ever, seen in cysts, but Sheffield and Bjorvatn (1977) found a group of randomly arranged microtubules near the flagellar axonemes in cysts that could be median bodies. They also observed that the microtubules in cysts were less compact than those observed in the trophozoite (Friend, 1966), possibly accounting for the apparent absence of median bodies in cysts when viewed with visible-light microscopy.

Gillin et al. (1989) reported that in a Type I cyst the median body is visible when viewed in relief with Nomarski differential interference contrast optics.

The viability and longevity of Giardia cysts in the environment is significantly affected by temperature; as the temperature increases, survivability decreases. A small fraction of cysts can withstand a single freeze-thaw cycle. Cysts may remain viable in water for long periods of time under typical environmental conditions. Survival in water is dependent primarily on decreased water temperature; no relationship was found between cyst survival and water pH, dissolved oxygen, turbidity, color, hardness, ammonia, nitrate or phosphorous.

After being stored in water for 77 days at 8/ C, G. lamblia cysts were found to be viable by dye exclusion testing (Bingham et al., 1979). Cysts survived about 26 days at 21/ C and about 6 days at 37/ C. Less than 1% of cysts survived freezing at -13/ C for 14 days. Raising the temperature to boiling immediately inactivated the cysts. G. muris cysts were found to be infective in mice after periods of 28 days storage in distilled water at 5/ to 7/ C; none were found infective after 56 days (deRegnier et al., 1989).

G. muris cysts remained viable for 2 to 3 months when stored in natural surface water at temperatures of less than 10/C. The thermal death point (i.e., the lowest temperature at which G. muris cysts are inactivated in 10 minutes) is 54/ C (Schaefer et al., 1984).

In marine waters in Hawaii, Johnson et al. (1997) found that the viability of G. muris cysts, as determined by excystation, was reduced by 99.9% in 3 hours during sunlight and 77 hours in conditions of darkness.

Using dye exclusion as an indicator of viability, Deng and Cliver (1992) found that a 90% reduction of viable G. lamblia cysts occurred in septic tank effluent within 18 days. In anaerobic digester sludge from municipal wastewater treatment plants, Van Praagh et al. (1993) found that 99.9% of G. muris cysts were inactivated in 15.1 days, 20.5 hours, and 10.7 minutes at temperatures of 21.5/, 37/, and 50/ C, respectively.

Direct Transmission Between Humans

Giardia cysts are highly infective for humans (Rendtorff, 1954a, b; 1979). In a controlled, clinical study of male volunteers who were fed human-source Giardia cysts contained in gelatin capsules, a dosage of ten cysts produced human infection. Infection was assessed by observing presence of Giardia in fecal smears. Eight dosage levels ranging from 1 to 1,000,000 cysts per capsule were studied.

Rendtorff (1954a, b;1979) found that the incubation period of giardiasis in human volunteers ranged from 9 to 22 days with a mean of 13.1 days. Benenson (1995) reported that the incubation period is usually 3 to 25 days or longer, with a median of 7 to 10 days. In a prospective epidemiological study, Jokipii et al. (1985) found that the incubation period for giardiasis was typically 12 to 19 days. In human volunteers, Nash et al. (1987) found that diarrhea or loose stools appeared within 7.25 (± 2.99) days of inoculation with G. lamblia trophozoites by intestinal intubation.

Nash et al. (1987) found evidence of possible strain differences between Giardia trophozoites that had been isolated from two infected persons. All five volunteers that were inoculated with 50,000 trophozoites of an isolate from one of the human hosts became infected, but none of five volunteers that received 50,000 trophozoites of an isolate from the other host became infected.

Nature of Giardiasis 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.

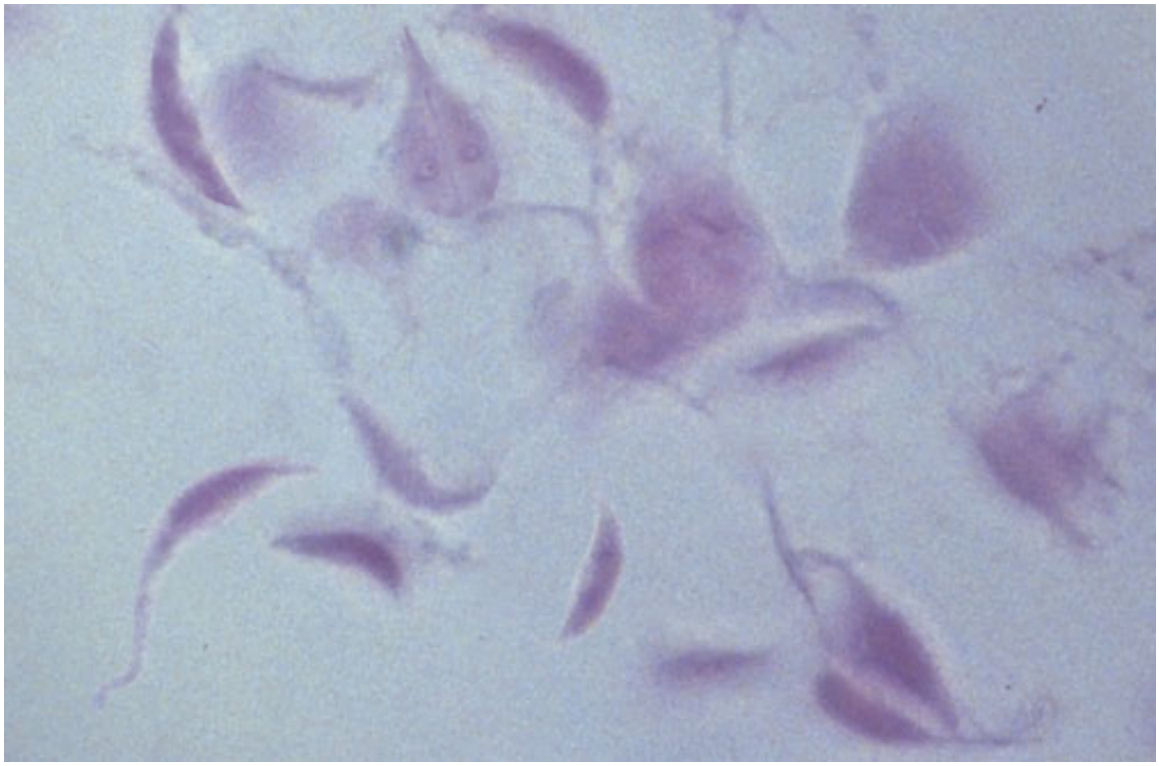
A commercial fluorescent antibody kit is available to stain the organism. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample.

An enzyme linked immunosorbent 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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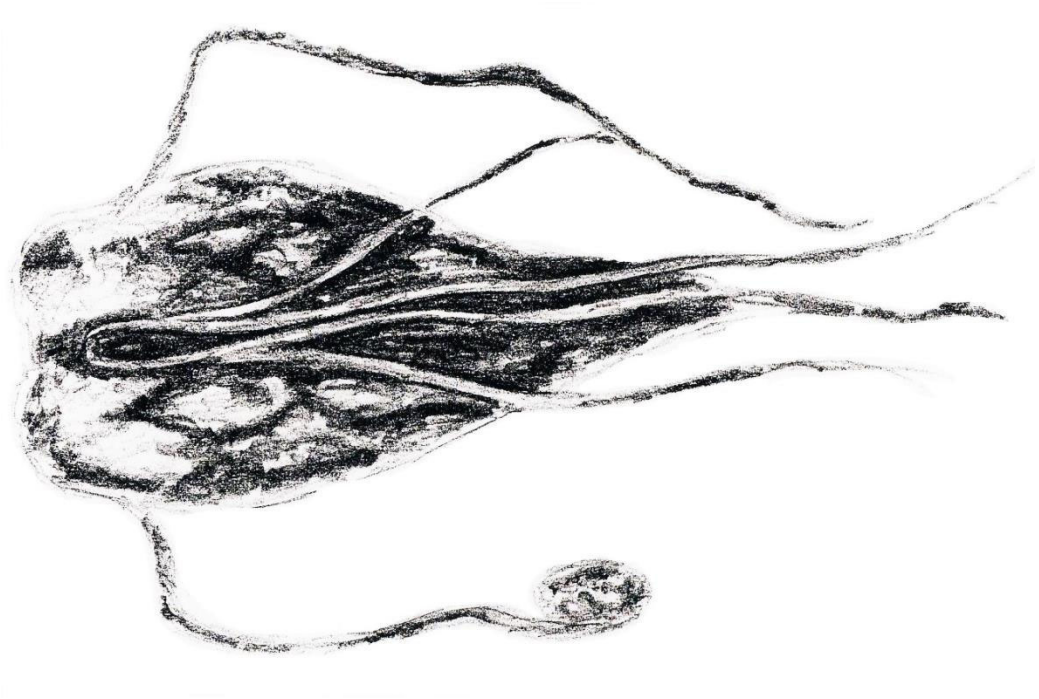
- Tovar J, Levila G, Shez LB, Sutak R, Tachezy J, Van Der Giezen Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. Nature 2003;426:172-176



Giardia

Giardia Images

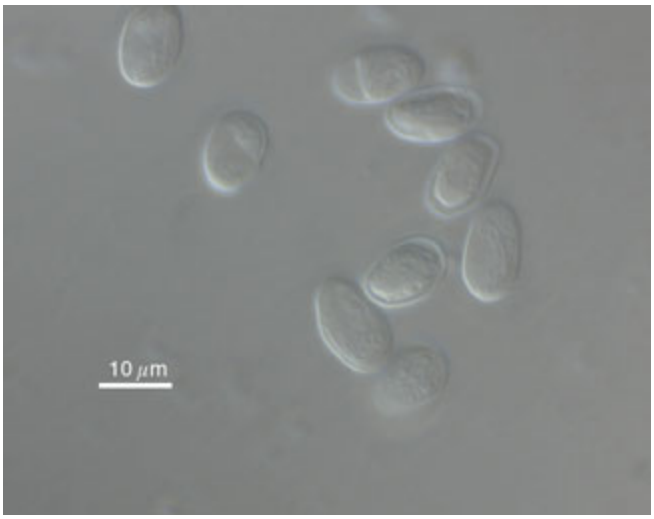


Photo Credit: H.D.A Lindquist, U.S. EPA

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.

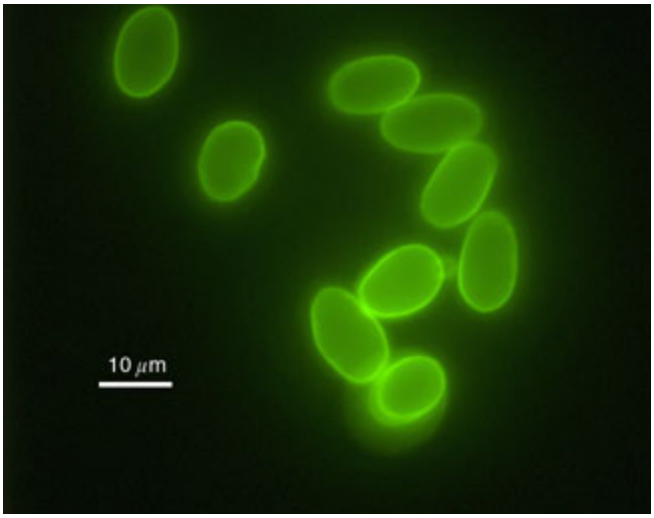


Photo Credit: H.D.A Lindquist, U.S. EPA

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.

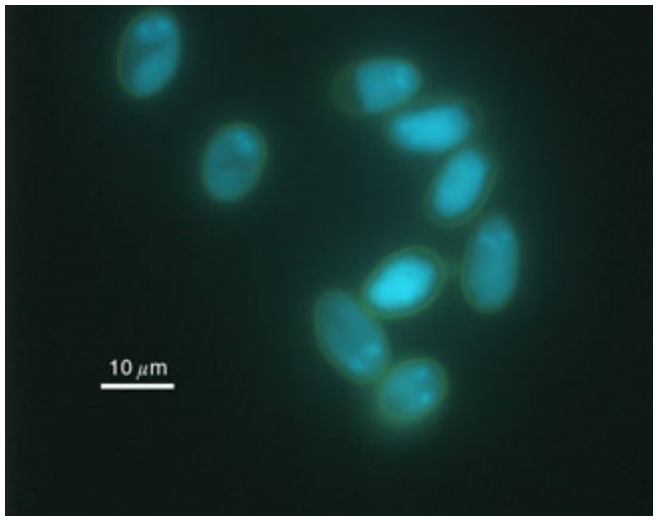
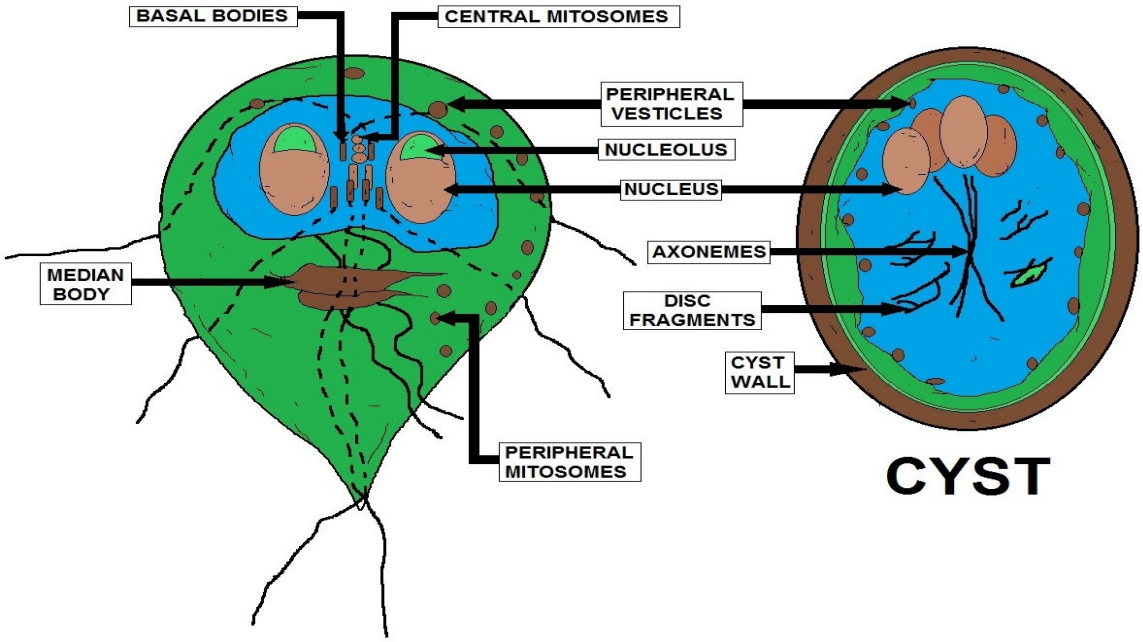


Photo Credit: H.D.A Lindquist, U.S. EPA

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* - Post Quiz

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.
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.
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 _____.
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.
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. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

13. An _____linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available.
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

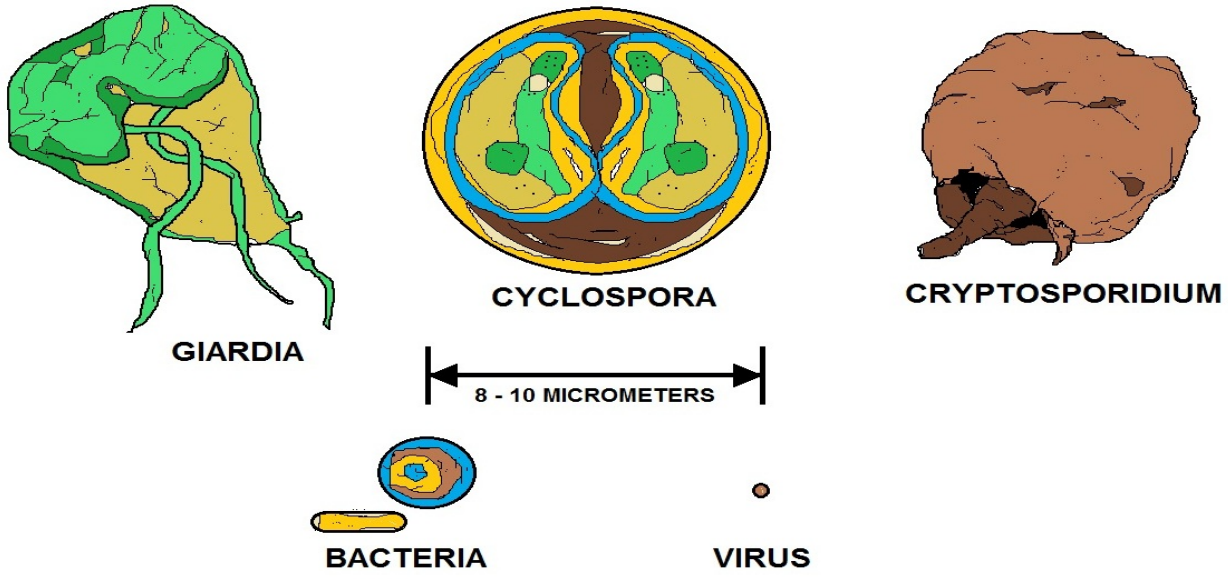
20. Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.
A. True
B. False

Answers
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

Cryptosporidiosis - *Cryptosporidium* - Chapter 5

Section Focus: You will learn the basics of cryptosporidiosis. At the end of this section, you will be able to describe Cryptosporidium infection (cryptosporidiosis) is an illness caused by tiny cryptosporidium parasites. 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 bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, (bacteria, viruses, cysts, single-celled organisms) particularly those that cause E. coli, gastroenteritis, cryptosporidiosis or giardia (primary domestic concerns).



COMPARATIVE SIZES OF PROTOZOAN PARASITES

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.

Cryptosporidium infection (cryptosporidiosis) is an illness caused by tiny, one-celled cryptosporidium parasites. When cryptosporidia (krip-toe-spoe-RID-e-uh) enter your body, they travel to your small intestine and then burrow into the walls. Later, they are shed in your feces. In most healthy people, a cryptosporidium infection produces a bout of watery diarrhea. The infection usually goes away within a week or two. If you have a compromised immune system, a cryptosporidium infection can become life-threatening without treatment.

Surface Waters
Cryptosporidium may be more common in surface water than ground water because surface waters are more vulnerable to direct contamination from sewage discharges and runoff. Lisle and Rose (1995) reported that between 5.6% and 87.1% of source waters (i.e., surface, spring, and groundwater samples not impacted by domestic and/or agricultural waste) tested contained 0.003 to 4.74 Cryptosporidium oocysts/L.

In another major study, LeChevallier and Norton (1995) reported finding oocysts in 60.2% of surface waters tested in the U.S. and Canada. However, all surface waters are subject to a complex set of watershed processes and characteristics that may lead to the presence of Cryptosporidium oocysts (Crockett and Haas, 1997; States et al., 1997; LeChevallier et al., 1997).

Cryptosporidium oocysts have also been found in more than 50% of raw sewage samples (Bukhari et al., 1997; Zuckerman et al., 1997), 4.5% of raw water samples, and 3.5% of treated water samples (Wallis et al., 1996). Ong et al. (1996a, 1996b) found that water from rivers flowing through cattle pastures in British Columbia exhibited higher Cryptosporidium counts than did water in a protected watershed.

Groundwater
Cryptosporidium oocysts are found less frequently in ground water than in surface water, although new data contradict previous assumptions that ground water is inherently free of parasites such as Cryptosporidium. For example, Hancock et al. (1998) recently reported a study of 199 ground water samples tested for Cryptosporidium. They found that 5% of vertical wells, 20% of springs, 50% of infiltration galleries, and 45% of horizontal wells tested contained Cryptosporidium oocysts.

Cryptosporidium oocysts are common and widespread in ambient water and can persist for months in this environment. The dose that can infect humans is low, and a number of waterborne disease outbreaks caused by this protozoan have occurred in the United States. Otherwise healthy people recover within several weeks after becoming ill, but illness may persist and contribute to death in those whose immune systems have been seriously weakened (e.g., AIDS patients).

No Treatment Available
Drugs effective in preventing or controlling this disease are not yet available. The public health concern is worsened by the resistance of Cryptosporidium to commonly used water disinfection practices such as chlorination.

However, a well-operated water filtration system is capable of removing at least 99 of 100 Cryptosporidium oocysts in the water. Monitoring for this organism in water is currently difficult and expensive. EPA believes that there is sufficient information to conclude that Cryptosporidium may cause a health problem and occurs in public water supplies at levels that may pose a risk to human health.

Cryptosporidiosis Parvum

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.

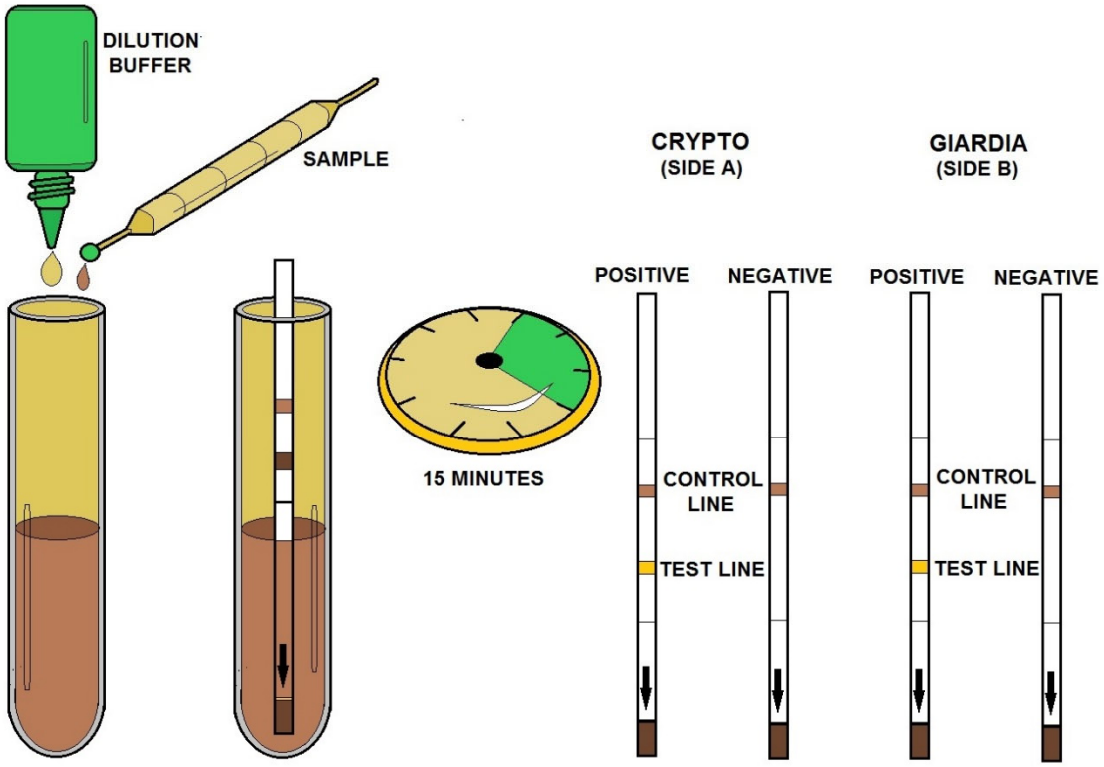
AIDS
Patients with AIDS can have a large number of stools per day for months or even years. There is currently no cure for cryptosporidiosis, though drug research is continuing. Patients who suspect they may have cryptosporidiosis should drink extra fluids and may wish to drink oral rehydration therapy 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.

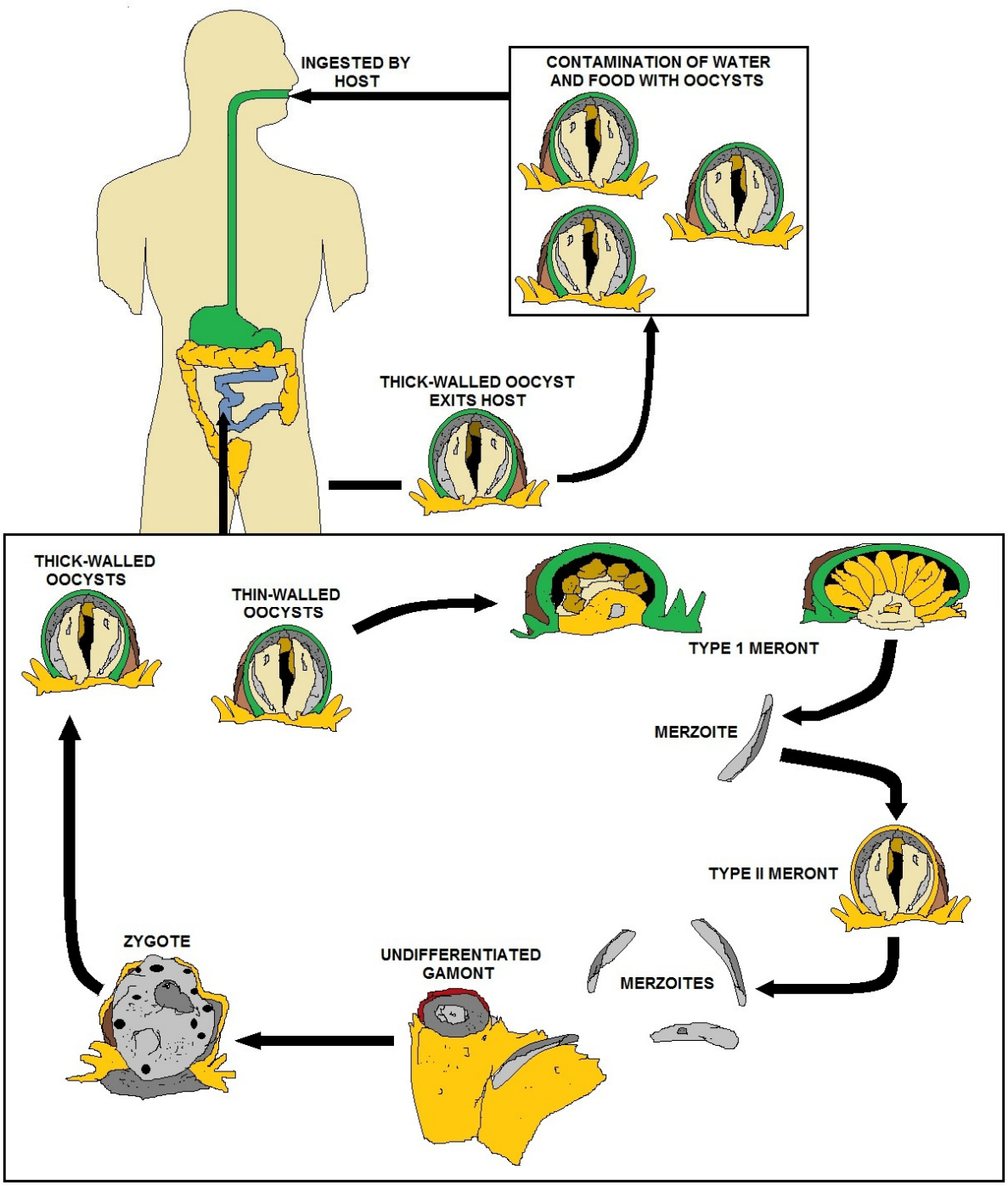
They should also avoid close contact with anyone who has a weakened immune system. Individuals with diarrhea should not swim in public bathing areas while they have diarrhea and for at least 2 weeks after each attack of diarrhea.

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.



CRYPTO-GIARDIA DUO-STRIP



LIFE CYCLE OF CRYPTOSPORIDIOSIS

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.

Cryptosporidiosis Oocysts

Cryptosporidium oocysts

Genus *Cryptosporidium*

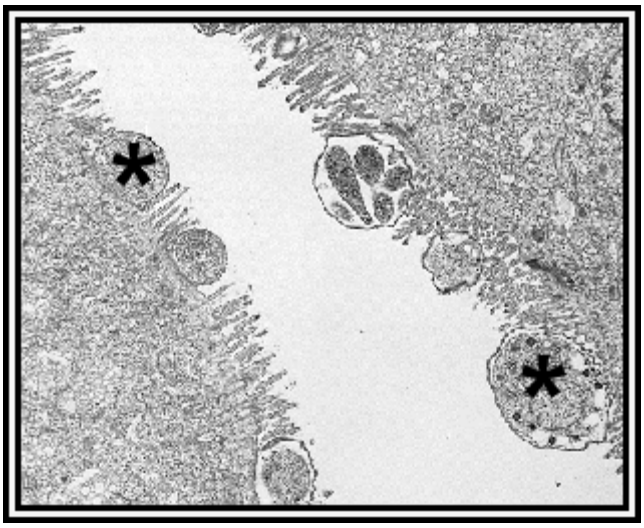
Members of the genus *Cryptosporidium* are parasites of the intestinal tracts of fishes, reptiles, birds, and mammals. It seems that members of this genus do not display a high degree of host specificity, so the number of species in this genus remains a matter of some discussion.

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.

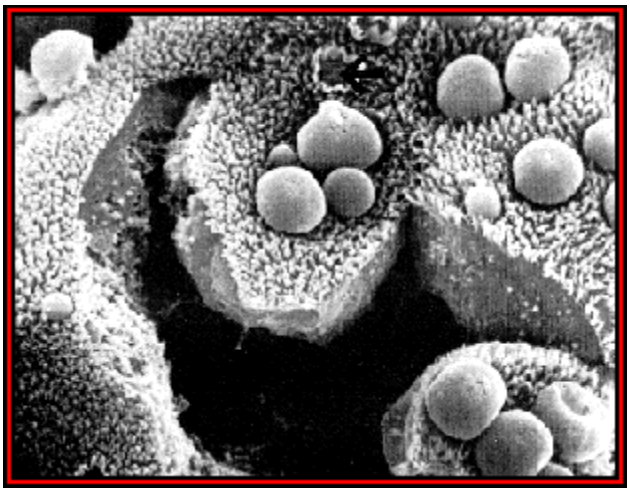
Cryptosporidium is a small parasite, measuring about 3-5 µm. It lives on (or just under) the surface of the cells lining the small intestine, reproduces asexually, and oocysts are passed in the feces. Transmission of the infection occurs via the oocysts. Many human infections have been traced to the 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.

Cryptosporidium Taxonomy

Cryptosporidium is one of several protozoan genera in the phylum Apicomplexa, which develop within the gastrointestinal tract of vertebrates throughout their entire life cycles (Fayer et al., 2000). Apicomplexans are obligate intracellular parasites. They are characterized by the presence of special organelles located at the tips (apexes) of cells that contain materials used to penetrate the host cells and establish successful infections. Examples of Apicomplexa other than Cryptosporidium include Plasmodium (the causative agent of malaria) (Tortora et al., 1994).

The taxonomy of the genus Cryptosporidium is uncertain and changing. The current classification scheme entails ten species of Cryptosporidium (Fayer et al., 2000).

Table 1 lists these ten Cryptosporidium species and the host organism(s) in which each parasite was originally found; some of these species have since been shown to occur in additional hosts (Fayer et al., 2000; Fayer et al., 1997a).

Cryptosporidium has been observed in over 150 mammalian species (Fayer et al., 2000); however, illness in humans is confined primarily to infections associated with C. parvum (O'Donoghue, 1995).

Table 1. Valid Cryptosporidium Species Cryptosporidium Species Initially Described Host Species

C. andersoni Bos taurus (cattle)
C. baileyi Gallus (domestic chicken)
C. felis catis (domestic cat)
C. meleagridis Meleagris gallopavo (turkey)
C. muris Mus musculus (house mouse)
C. nasorum Naso literatus (fish)
C. parvum Mus musculus (house mouse)
C. saurophilum Eumeces schneideri (skink)
C. serpentis Elaphe guttata (corn snake) E. subocularis (rat snake) Sanzinia madagasarensus (Madagascar boa)
C. wrairi Cavia porcellus (guinea pig)
Source: Adapted from Fayer et al. (2000) and Fayer et al. (1997a)

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.

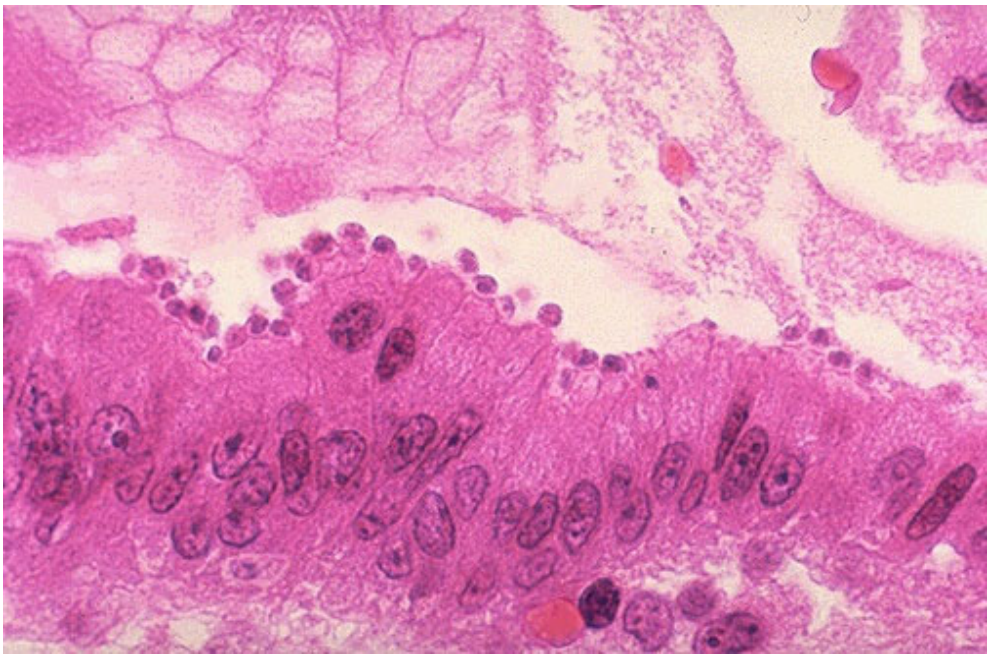
1. Wash and/or peel all raw vegetables and fruits before eating.
2. Use safe, uncontaminated water to wash all food that is to be eaten raw.
3. Avoid eating uncooked foods when traveling in countries with minimal water treatment and sanitation systems.

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.

Cryptosporidiosis - Post Quiz

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

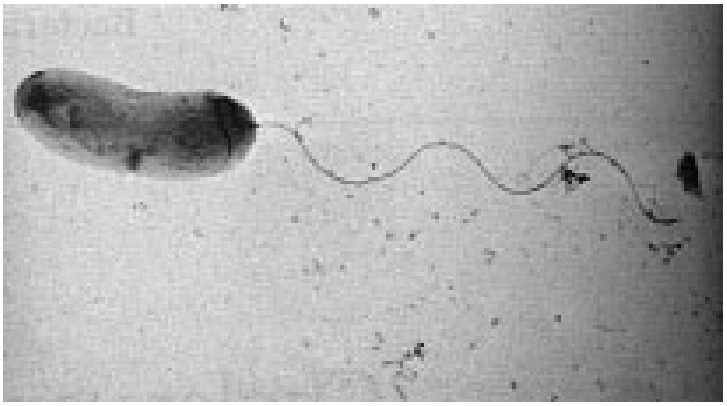
7. Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool.
A. True
B. False

Answers 1.A, 2.A, 3.A, 4.A, 5.A, 6.A, 7.A

Cholera -*Vibrio cholerae* - Chapter 6

Section Focus: You will learn the basics of cholera. At the end of this section, you will be able to describe the infection of small intestine caused by bacteria vibrio cholerae - cholera. This is a bacterial infection which is caused due to intake of contaminated water. There are many types of bacteria which are held responsible for causing cholera and this infection is occurs in varied degrees of severity. 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 bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, (bacteria, viruses, cysts, single-celled organisms) particularly those that cause E. coli, gastroenteritis, cryptosporidiosis or giardia (primary domestic concerns).



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.

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.

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.

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.

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.

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.

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***."

Isolation and Identification
Isolation and identification of *Vibrio cholerae* serogroup O1 or O139 by culture of a stool specimen remains the gold standard for the laboratory diagnosis of cholera.

Cary Blair media is ideal for transport, and the selective thiosulfate–citrate–bile salts agar (TCBS) is ideal for isolation and identification. Reagents for serogrouping *Vibrio cholerae* isolates are available in all state health department laboratories in the U.S. Commercially available rapid test kits are useful in epidemic settings but do not yield an isolate for antimicrobial susceptibility testing and subtyping, and should not be used for routine diagnosis.

In many countries where cholera is not uncommon, but access to diagnostic laboratory testing is difficult, WHO recommends the following clinical definition be used for suspected cholera cases.

Suspected cholera case

- In areas where a cholera outbreak has not been declared: Any patient 2 years old or older presenting with acute watery diarrhea and severe dehydration or dying from acute watery diarrhea.
- In areas where a cholera outbreak is declared: any person presenting with or dying from acute watery diarrhea.

Rapid Tests
In areas with limited or no laboratory testing, the Crystal® VC dipstick rapid test can provide an early warning to public health officials that an outbreak of cholera is occurring. However, the sensitivity and specificity of this test is not optimal. Therefore, it is recommended that fecal specimens that test positive for *V. cholerae* O1 and/or O139 by the Crystal® VC dipstick always be confirmed using traditional culture-based methods suitable for the isolation and identification of *V. cholerae.*

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.

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.

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.

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.

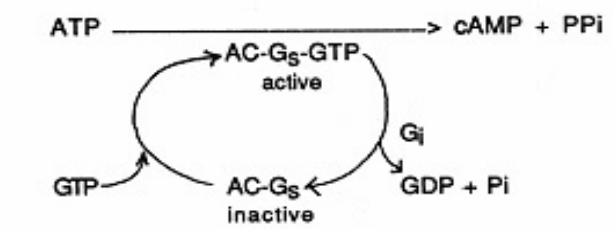
Cholera's Toxin

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.

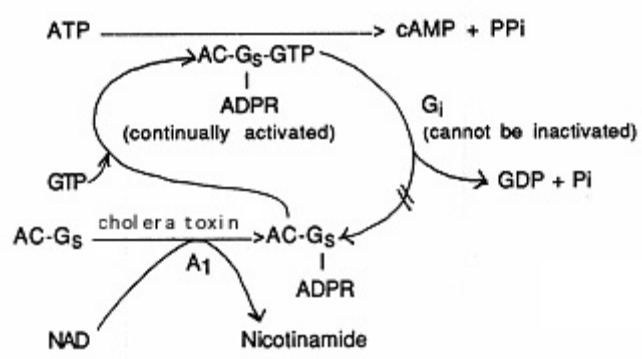
The toxin has been characterized and contains **5 binding (B) subunits** of 11,500 daltons, an active **(A1) subunit** of 23,500 daltons, and a **bridging piece (A2)** of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from 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

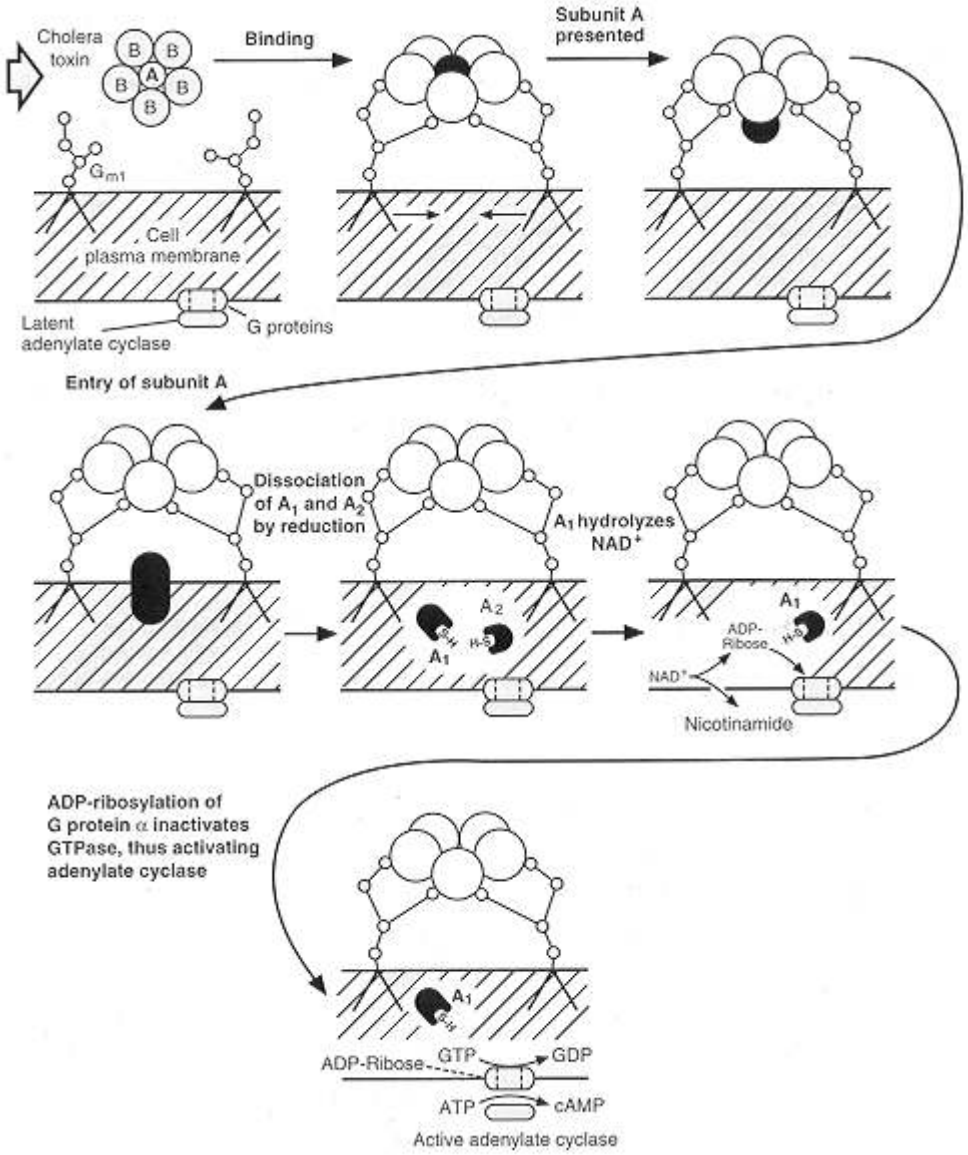
E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT toxin is on a plasmid that can be transferred to other *E. coli* strains and probably to other enteric bacteria, as well.

Close relationships between the genetic code for LT toxin and the cholera toxin un-doubtedly exist but have not been documented as yet.

The genetic information for the toxin in *V. cholerae* is located on the bacterial chromo-some. 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.

Cholera - Post Quiz

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

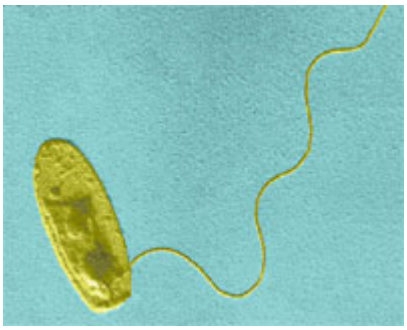
15. The resulting diarrhea allows the _____ to spread to other people under unsanitary conditions.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

Answers 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

Legionnaires' Disease - *Legionella* –Chapter 7

Section Focus: You will learn the basics of Legionella bacteria. At the end of this section, you will be able to describe Legionella bacteria and its effects - Legionnaires' disease. This is a bacterial infection which is caused due to intake of contaminated water. There are many types of bacteria which are held responsible for causing cholera and this infection is occurs in varied degrees of severity. 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: *Legionella* bacteria can be found throughout the world, mostly in aquatic and moist environments (e.g., lakes, rivers, ground water and soil). *Legionella* can adversely impact public health. CDC estimates that 8,000 to 18,000 people are hospitalized with Legionnaires' disease each year in the U.S. EPA has developed the Surface Water Treatment Rules (SWTRs) to improve drinking water quality. The regulations provide protection from disease-causing pathogens, such as Giardia lamblia, Legionella, and Cryptosporidium. The regulations also protect against contaminants that can form during drinking water treatment.



Legionella bacteria is the bacteria known to cause legionellosis or Legionnaires' Disease. Legionella was first discovered in 1976 after an outbreak among people who became ill after an American Legion convention held in a hotel in Philadelphia. The people who were affected suffered from a type of severe pneumonia (lung infection) that eventually became known as Legionnaires' disease.

Legionnaires' Disease Introduction

Legionnaires' disease is still a very big concern today. According to the Centers for Disease Control and Prevention (CDC), there are approximately 5,000 cases of Legionnaires' disease reported in the United States each year. It is estimated that one out of every 10 people who get sick from Legionnaires' Disease will die. Recently, during the summer of 2015, New York City had its worst outbreak in recorded history in which 16 people died and 133 people were sickened. New York City alleges that the source of the outbreak was an infected cooling tower located in the South Bronx.

In reaction to this tragedy, New York City and State have passed some of the most comprehensive cooling tower maintenance legislation in the United States, which has also shined a light on the liability associated with not having a plan for controlling Legionella in publicly accessed water systems. Soon property owners, investors and managers, as well as commercial, hospitality, healthcare and residential real estate developers may no longer be able to claim ignorance on the dangers of Legionella. For these groups, being proactive and having a plan is the first step in legionella prevention.

Where has Legionella been Found?
Legionella bacteria are found naturally in the environment worldwide, usually in aquatic environments. The bacteria also occur in distribution systems and premise plumbing.

How are People Exposed to *Legionella*?
People are exposed to *Legionella* when they inhale water droplets containing the bacteria.

Legionella can grow in water systems in the premise plumbing of:
- large buildings (consisting of hot water heaters, storage tanks and pipes)
- cooling towers
- decorative fountains
- hot tubs

What are the Health Effects from Exposure to *Legionella* ?
Legionellosis is a respiratory disease caused by Legionella bacteria. Sometimes the bacteria infects the lungs and can cause a severe pneumonia called Legionnaires' disease. The bacteria can also cause a less serious infection that seems like a mild case of the flu called Pontiac fever. For more information, please visit: http://www.cdc.gov/legionella/about/index.html.

Microbiology, Morphology, and Ecology
Legionella bacteria are small gram-negative rods. They are un-encapsulated and non-spore-forming, with physical dimensions from 0.3 to 0.9 m in width and from 2 to more than 20 m in length (Winn 1988).

Most species exhibit motility through one or more polar or lateral flagella. Legionella cell walls are unique from other gram-negative bacteria in that they contain significant amounts of branched-chain cellular fatty acids and also ubiquinones with side chains of more than 10 isoprene units (Brenner et al. 1984).

Legionella are aerobic, microaerophillic, and have a respirative metabolism that is nonfermentative and is based on the catabolism of amino acids for energy and carbon sources (Brenner et al. 1984).

Ubiquitously found in nature, Legionella species exist primarily in aquatic environments, although some have been isolated from potting soils and moist soil samples (Fields 1996). Legionella can survive in varied water conditions, in temperatures of 0-63 oC, a pH range of 5.0-8.5, and a dissolved oxygen concentration in water of 0.2-15 ppm (Nguyen et al. 1991).

Introduction Genus: *Legionella* Species: *pneumophila*

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.

Symbiosis in Microorganisms

Legionella proliferation is dependent on their symbiotic relationships with other microorganisms. Experiments have demonstrated that Legionella in sterile tap water show long-term survival but do not multiply, whereas Legionella in non-sterile tap water have been shown to survive and multiply (Surman et al. 1994). Furthermore, Legionella viability is maintained when they are combined with algae in culture, whereas Legionella viability decreases once the algae are removed (Winn 1988).

Currently, Legionella are known to infect a total of 13 species of amoebae and two species of ciliated protozoa (Fields 1996). Legionella also can multiply intracellularly within protozoan hosts (Vandenesch et al. 1990).

Legionella strains that multiply in protozoa have been shown to be more virulent, possibly due to increased bacterial numbers (Kramer and Ford 1994). The ability to proliferate within these symbiont hosts provides Legionella with protection from otherwise harmful environmental conditions. Thus, Legionella are able to survive in habitats with a greater temperature range, are more resistant to water treatment with chlorine, biocides and other disinfectants, and survive in dry conditions if encapsulated in cysts. Their enhanced resistance to water treatment has major implications for both disease transmittance and water treatment procedures.

Legionella also grow symbiotically with the aquatic bacteria attached to the surface of biofilms (Kramer and Ford 1994). Biofilms provide the bacteria with nutrients for growth and also offer protection from adverse environmental conditions (including during water disinfection). The concentration of Legionella in biofilms depends upon water temperature; at higher temperatures, they can more effectively compete with other bacteria. Because biofilms colonize drinking water distribution systems, they provide a habitat suitable for Legionella growth in potable water, which can lead to human exposure.

Occurrence

Because routine environmental monitoring for Legionella is not a common practice, the occurrence of these bacteria is often indicated by outbreaks or sporadic cases of legionellosis (i.e., any disease caused by Legionella).

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.

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.

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.

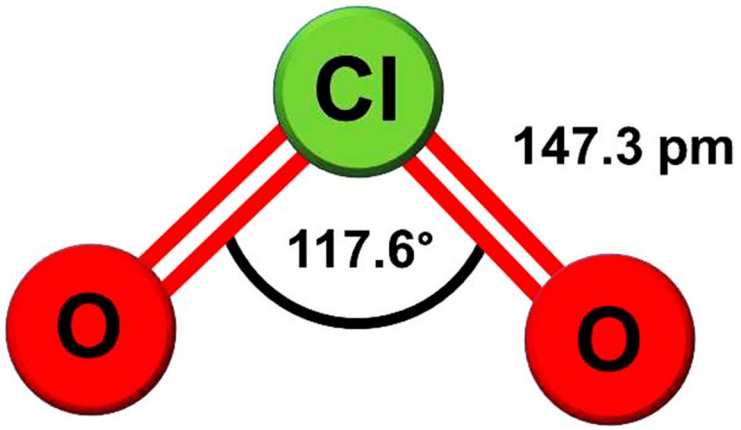
The flu-like symptoms are still seen in Pontiac fever patients, but pneumonia does not develop and infection does not spread beyond the lungs. Most *L. pneomophila* infections are easily treated with erythromycin.

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 MOLECULE

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 NH_3 or NH_4^+;
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 NH_3 or NH_4+;
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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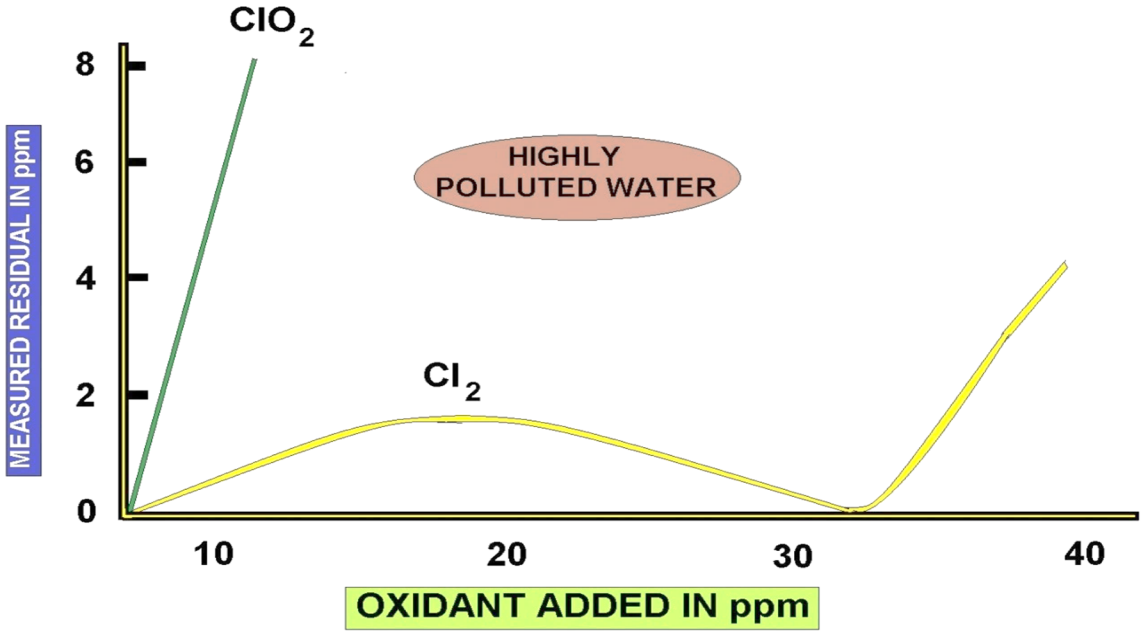
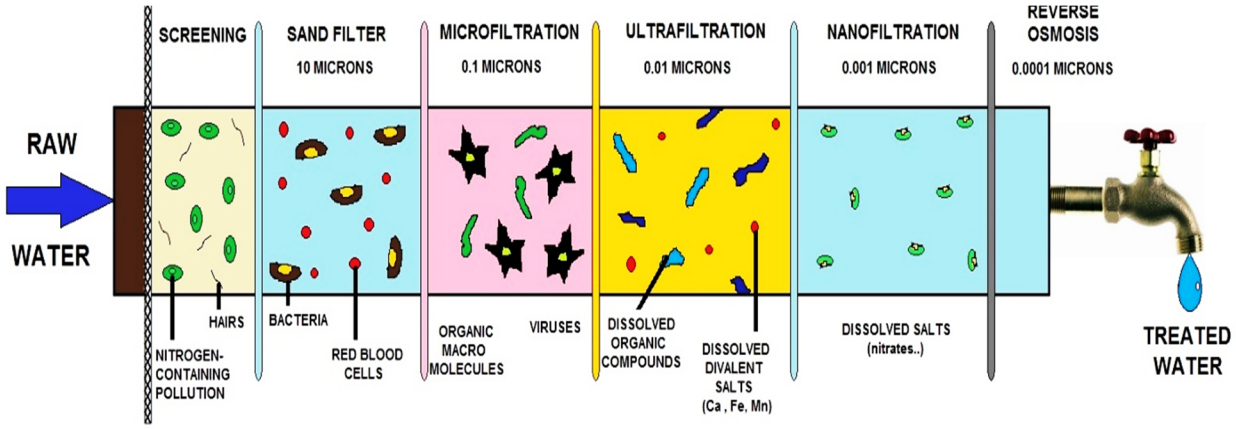


CHLORINE DIOXIDE VS CHLORINE DIAGRAM



FILTRATION METHODS AND REMOVAL SIZES

Legionella - Post Quiz

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. 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 _____.
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 colonize 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

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

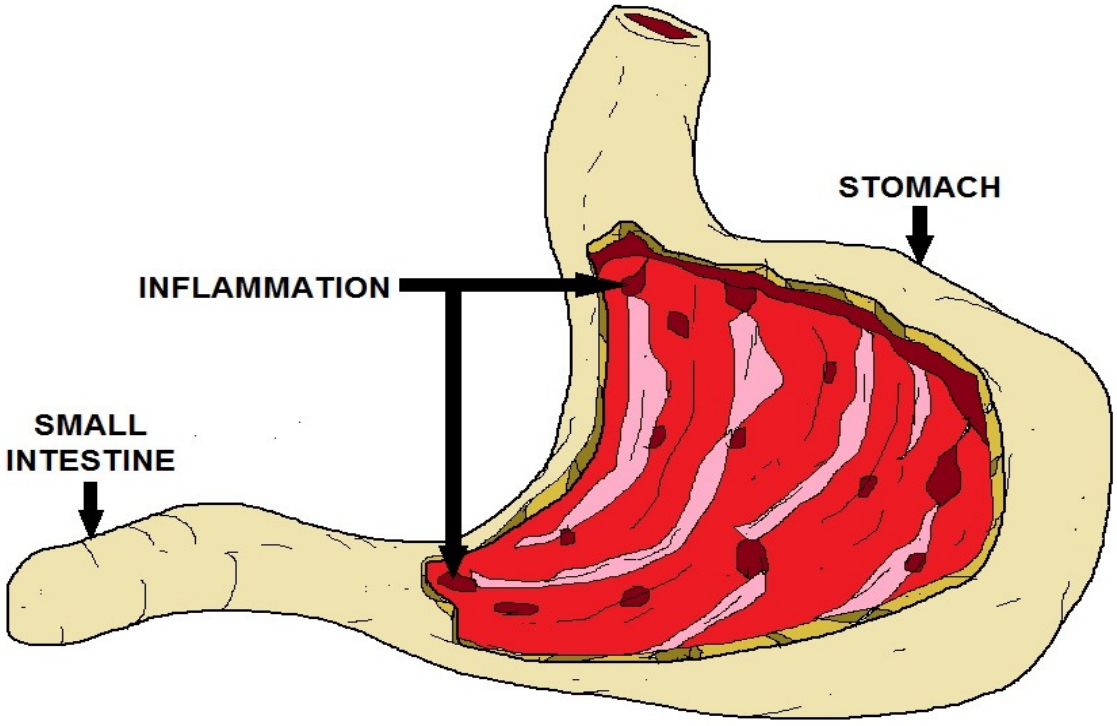
15. Most labs will provide a _____ microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative epifluorescence

Answers
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

Related Diseases and Associated Illnesses - Chapter 8

Section Focus: You will learn the basics of secondary waterborne diseases. At the end of this section, you will be able to describe various secondary waterborne diseases and related concerns. This is a bacterial infection which is caused due to intake of contaminated water. There are many types of bacteria which are held responsible for causing cholera and this infection is occurs in varied degrees of severity. 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 bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply (potable) of water. The Laborotory examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage (re-use effluent) or improperly functioning sewage treatment systems.



GASTROENTERITIS

Types of Waterborne Diseases

According to the Environmental Protection Agency (EPA), only about 22.5 outbreaks occur per year, with an average of about 4,640 to 9,331 people infected annually. Those cases occurred mostly as a result of water coming from non-community systems (as opposed to community or individual systems). Fortunately, the majority of waterborne disease cases are not fatal.

The EPA reports that an average of 6 people per year die of a waterborne disease in the US.

- **Amebiasis**: caused by protozoa. Symptoms include fatigue, diarrhea, flatulence, abdominal discomfort and weight loss.
- **Campylobacteriosis**: caused by bacteria. Symptoms include diarrhea, abdominal pain and fever.
- **Cholera**: caused by bacteria. Symptoms include muscle cramps, vomiting and diarrhea.
- **Cryptosporidiosis**: caused by protozoa. Symptoms include diarrhea and abdominal discomfort.
- **Giardiasis**: caused by protozoa. Symptoms include diarrhea and abdominal discomfort.
- **Hepatitis**: caused by a virus. Symptoms include fever, chills, jaundice, dark urine and abdominal discomfort.
- **Shigellosis**: caused by bacteria. Symptoms include bloody stool, diarrhea and fever.
- **Typhoid fever**: caused by bacteria. Symptoms include fever, headache, constipation, diarrhea, nausea, vomiting, loss of appetite and an abdominal rash.
- **Viral gastroenteritis**: caused by a virus. Symptoms include gastrointestinal discomfort, diarrhea, vomiting, fever and headache.

Although these are all potential waterborne disease threats within the U.S., some are more common than others. For example, cholera and typhoid fever were the most common of the waterborne diseases in the U.S. during the late 19th and 20th centuries, but have dropped dramatically in the decades since.

Today, the most waterborne disease outbreaks in the U.S. have resulted from either giardiasis or cryptosporidiosis. These two waterborne diseases are marked by an ability to survive in cold water, a low dose required for infection and a resistance to some of the water treatment practices commonly used today. Fortunately, researchers are using information about the outbreaks of these diseases to further update and improve water treatment practices and standards all over the country.

Coronavirus

Coronavirus has been found in feces, which can wind up in the waterways from untreated wastewater or after a large storm, when rainwater carries it in. Researchers have found that bacteria in the water could cause more than 90 million cases of gastrointestinal, respiratory, ear, eye, and skin-related ailments very year in the U.S. So it’s conceivable there might be coronavirus in ocean water near sewage runoffs, says Charles Gerba, PhD, professor of microbiology and immunology at the University of Arizona.

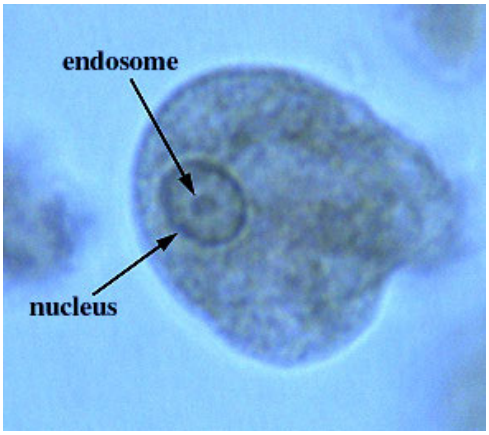
He has studied coronaviruses in water and found they can survive for 2 to 3 days. Nevertheless, it remains unclear whether the coronavirus in fecal matter is infectious. “I’d be more worried about hepatitis, swimming in raw sewage discharge, or many of the other pathogens,”" he says. “They present a far greater magnitude of risk, even if it turns out that coronavirus is transmitted by water.”

Amebiasis - *Entamoeba histolytica* Section

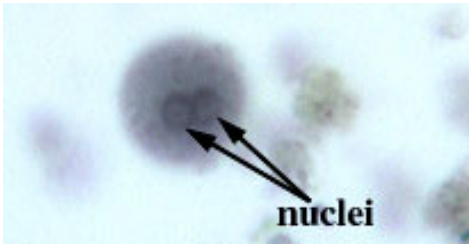
Amebiasis is a parasitic infection of the intestines caused by the protozoan *Entamoeba histolytica,* or *E. histolytica*. The symptoms of amebiasis include loose stool, abdominal cramping, and stomach pain. However, most people with amebiasis will not experience significant symptoms.

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.

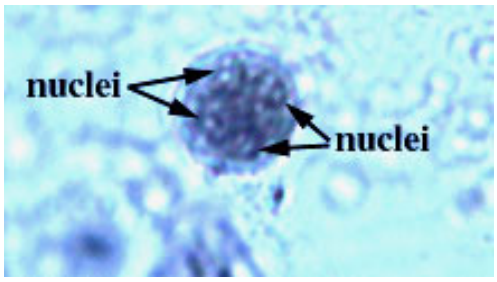
Life Cycle of *Entamoeba histolytica*
The life cycle of *Entamoeba histolytica* involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and cysts that are passed in the host's feces. Humans are infected by ingesting cysts, most often via food or water contaminated 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*

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.

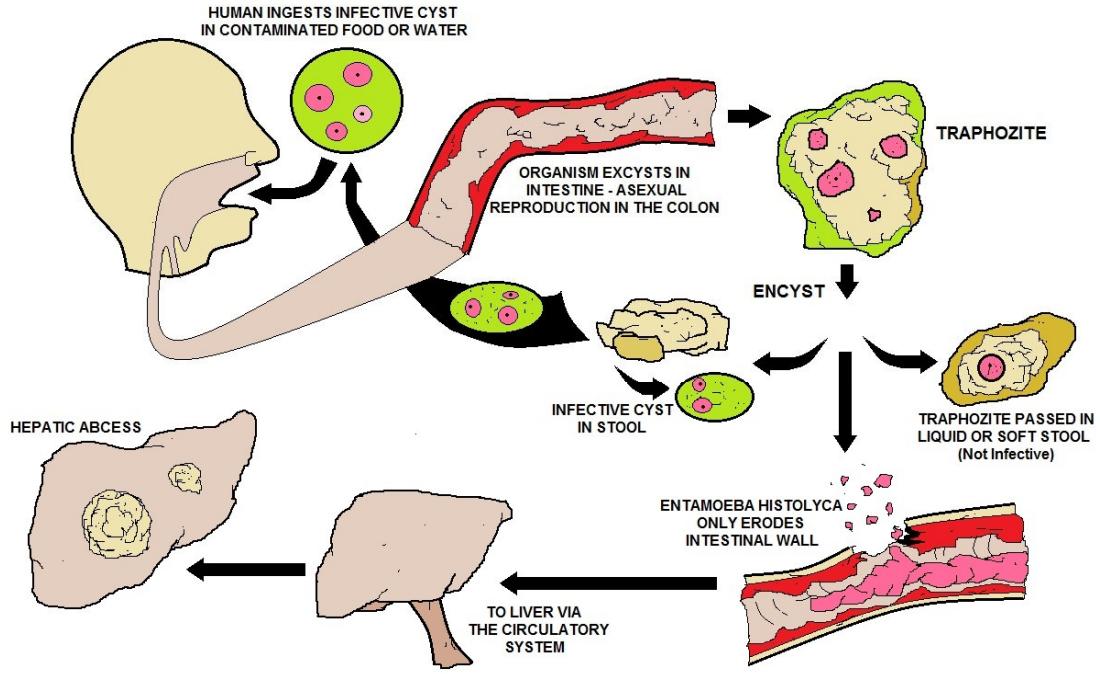
Trophoziotes in diarrheic feces are not stimulated to encyst because the feces are not dehydrating. If they are not encysted they cannot long survive in the external environment. Secondary bacterial infection can complicate an already severe pathology.

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.

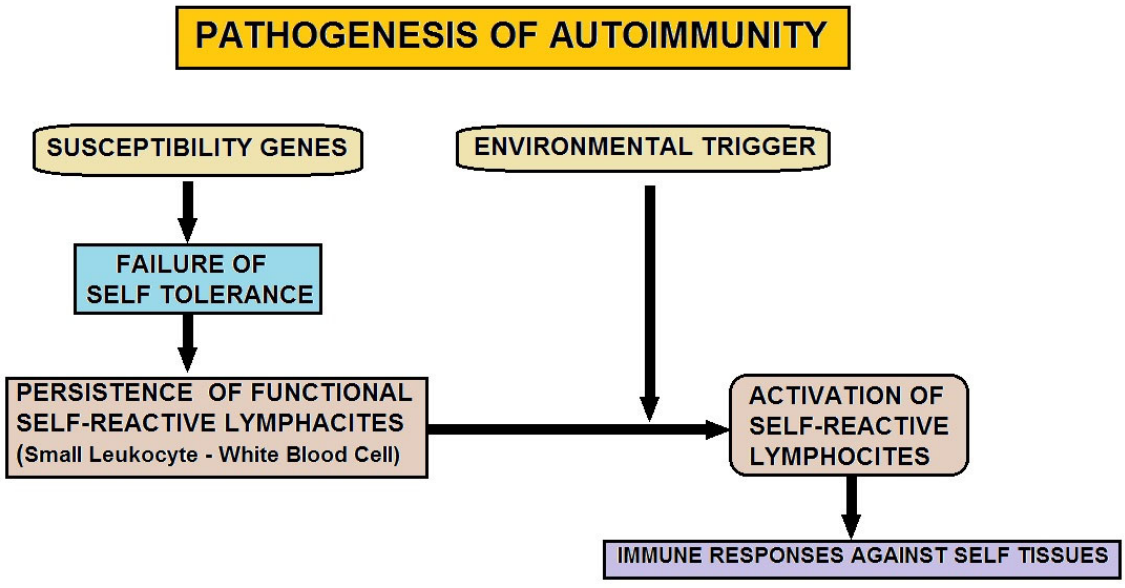
In some cases, the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal.

As with most of the amoebae, infections of *E. histolytica* are often diagnosed by demonstrating cysts or trophozoites in a stool sample.



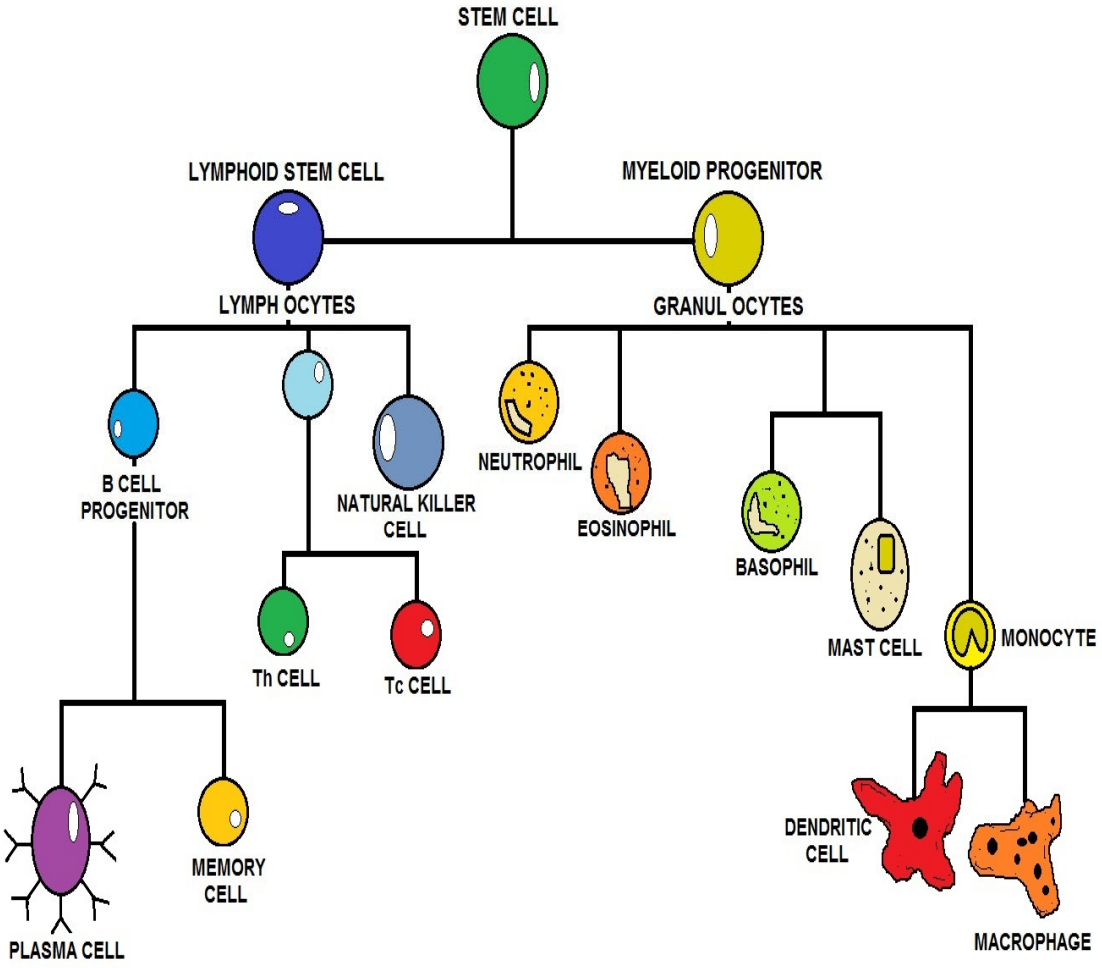
AMEBIASIS

(INFECTIOUS DISEASE BY CAUSED ONE-CELLED PARASITIC ORGANISM: ALSO KNOWN AS DYSENTARY)



PATHOGENESIS

(The Manner in Which a Disease Develops)

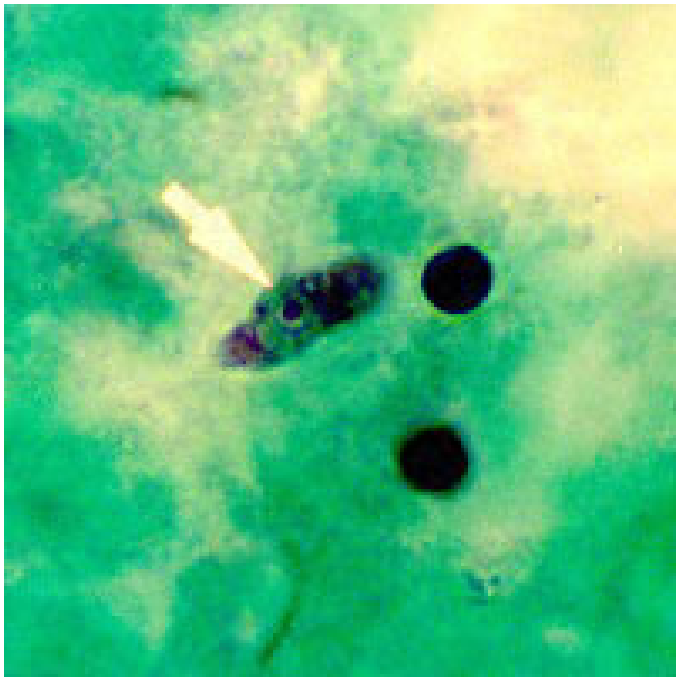


THE DIFFERENT CELLS OF THE IMMUNE SYSTEM

Amebic Meningoencephalitis -PAM - *Naegleria fowleri*

Naegleria fowleri (commonly referred to as the “brain-eating amoeba” or “brain-eating ameba”), is a free-living microscopic ameba, (single-celled living organism). It can cause a rare and devastating infection of the brain called primary amebic meningoencephalitis (PAM). The ameba is commonly found in warm freshwater (e.g. lakes, rivers, and hot springs) and soil. *Naegleria fowleri* usually infects people when contaminated water enters the body through the nose. Once the ameba enters the nose, it travels to the brain where it causes PAM, which is usually fatal. Infection typically occurs when people go swimming or diving in warm freshwater places, like lakes and rivers. In very rare instances, *Naegleria* infections may also occur when contaminated water from other sources (such as inadequately chlorinated swimming pool water or heated and contaminated tap water) enters the nose. You cannot become infected from swallowing water contaminated with *Naegleria*.

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.

Recent 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 hyper-chlorinate swimming pools.

Supply to the affected area was switched to a chlorinated surface water source, and a flushing program with hyper-chlorinated 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.

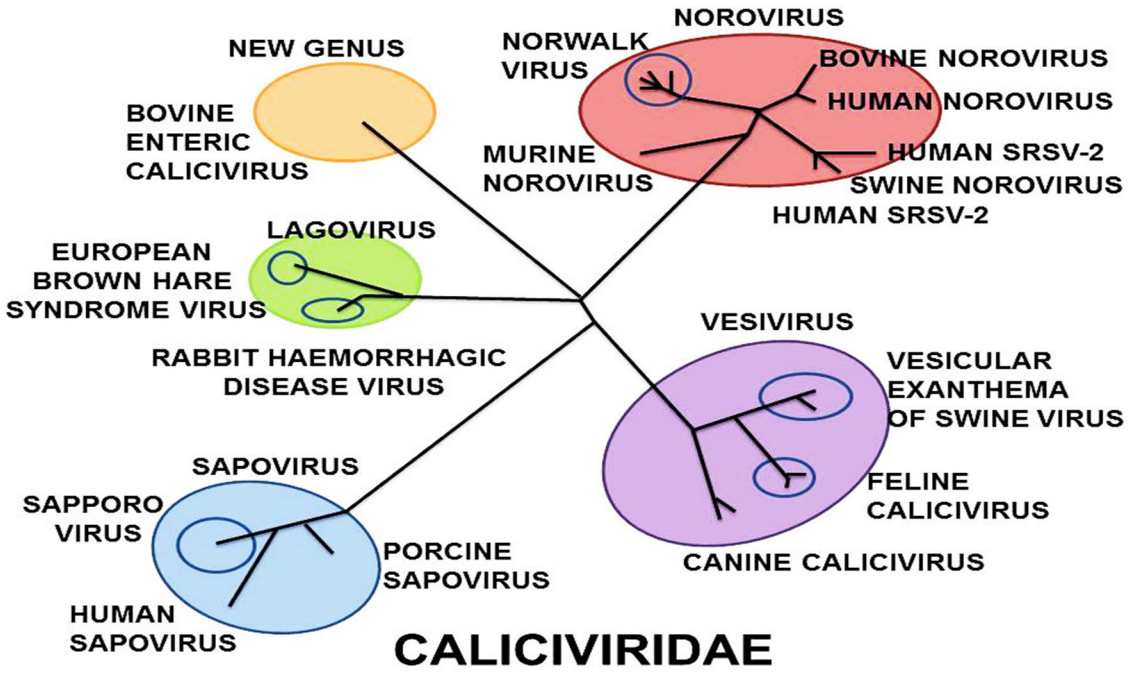
Investigators found *N. fowleri* in the water supply pipelines, and concluded that the high water temperatures reached in summer provided a suitable environment for growth of the organism. Tap water may also have been the primary source of infections attributed to swimming pools in these towns. The incidence 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.

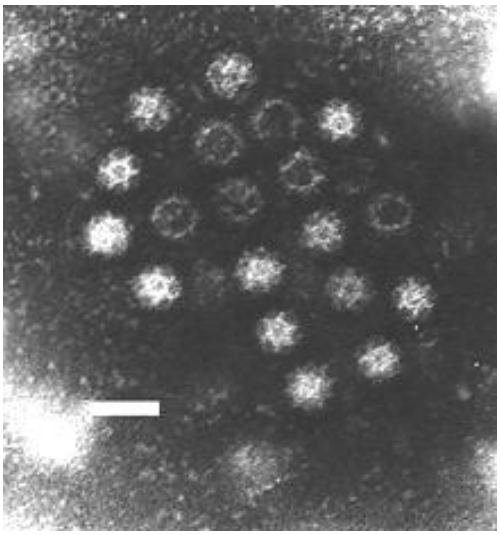
Calicivirus - Caliciviral Infections

See Gastroenteritis section, Norovirus Infection (aka Norwalk virus, calicivirus, viral gastroenteritis)

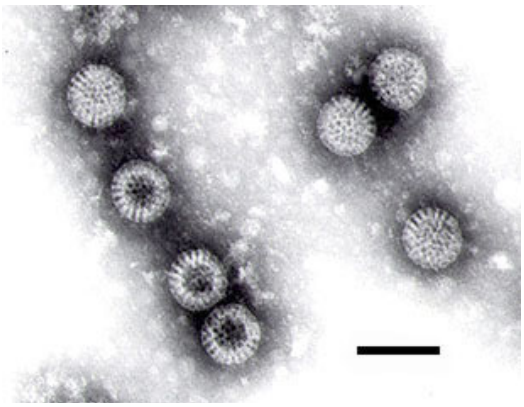
Caliciviral infections in humans, among the most common causes of viral-induced vomiting and diarrhea, are caused by the Norwalk group of small round structured viruses, the Sapporo caliciviruses, and the hepatitis E agent. Human caliciviruses have been resistant to in vitro cultivation, and direct study of their origins and reservoirs outside infected humans or water and foods (such as shellfish contaminated with human sewage) has been difficult. Modes of transmission, other than direct fecal-oral routes, are not well understood. In contrast, animal viruses found in ocean reservoirs, which make up a second calicivirus group, can be cultivated in vitro. These viruses can emerge and infect terrestrial hosts, including humans. This article reviews the history of animal caliciviruses, their eventual recognition as zoonotic agents, and their potential usefulness as a predictive model for noncultivatable human and other animal caliciviruses (e.g., those seen in association with rabbit hemorrhagic disease).



Only one of the five known calicivirus groups can be grown in vitro and subjected to the full range of host-parasite tests and conditions necessary to more fully define a virus in nature. Therefore, extrapolations developed from this group, the cultivatable marine caliciviruses, should provide insights as a predictive model to help answer questions for the noncultivatable caliciviruses such as small round structured virus, Sapporo virus, hepatitis E virus, and rabbit caliciviruses. From the replicative strategy of the *Caliciviridae* (as RNA viruses), one would predict considerable diversity. In vitro cultivation has shown that caliciviruses exhibit survivability and plasticity in nature. Many of the factors regarding host spectrum, zoonotic potential, disease conditions, transport, intermediate hosts, and abrupt appearance or disappearance, which may be unknown in newly emerging calicivirus diseases (e.g., RHD), may be more reliably predicted with an established model such as the cultivatable marine caliciviruses.



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



Rotavirus
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

New and better biologic tools for diagnostic and epidemiologic assessments must be developed. This should be augmented by recognizing the zoonotic potential of the cultivatable caliciviruses of ocean origin and then examining them as possible models to help solve many unanswered questions for pathogenic *Caliciviridae.*

Gastroenteritis - *Primary Customer Complaint*

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, calciviruses, 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.

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.

How is Viral Gastroenteritis Diagnosed?
Generally, viral gastroenteritis is diagnosed by a physician on the basis of the symptoms and medical examination of the patient. Rotavirus infection can be diagnosed by laboratory testing of a stool specimen. Tests to detect other viruses that cause gastroenteritis are not in routine use.

How is Viral Gastroenteritis Treated?
The most important of treating viral gastroenteritis in children and adults is to prevent severe loss of fluids (dehydration). This treatment should begin at home. Your physician may give you specific instructions about what kinds of fluid to give.

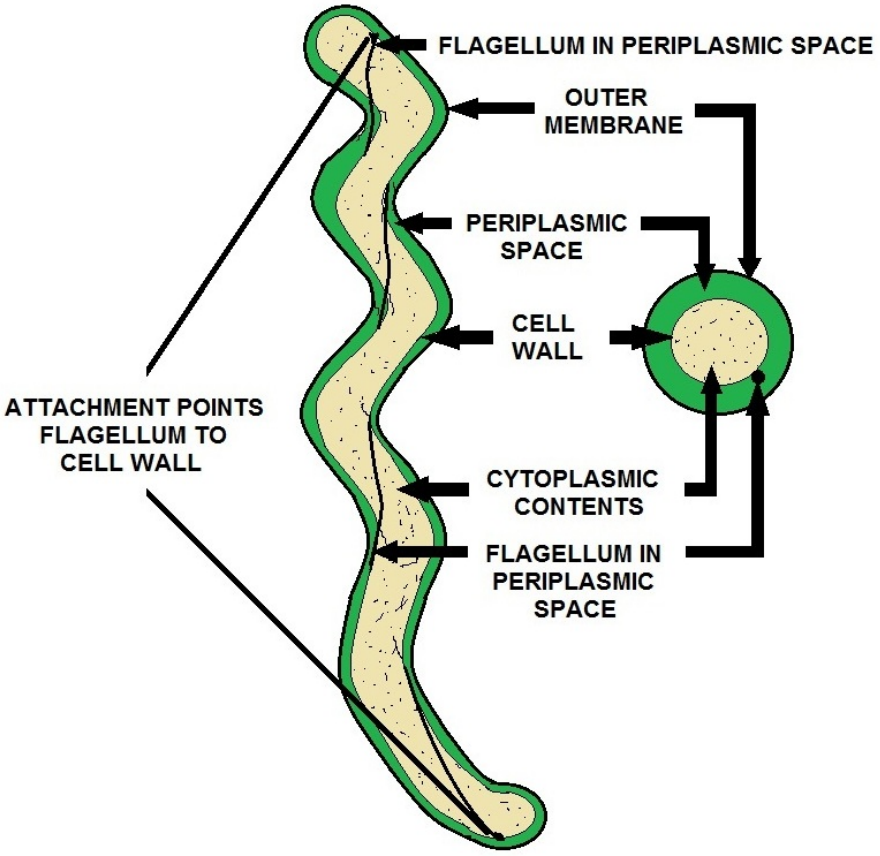
CDC recommends that families with infants and young children keep a supply of oral rehydration solution (ORS) at home at all times and use the solution when diarrhea first occurs in the child. ORS is available at pharmacies without a prescription.

Follow the written directions on the ORS package, and use clean or boiled water. Medications, including antibiotics (which have no effect on viruses) and other treatments, should be avoided unless specifically recommended by a physician.

Can Viral Gastroenteritis be Prevented?
Yes, persons can reduce their chance of getting infected by frequent handwashing, prompt disinfection of contaminated surfaces with household chlorine bleach-based cleaners, and prompt washing of soiled articles of clothing. If food or water is thought to be contaminated, it should be avoided.

Is there a Vaccine for Viral Gastroenteritis?
There is no vaccine or medicine currently available that prevents viral gastroenteritis. A vaccine is being developed, however, that protects against severe diarrhea from rotavirus infection in infants and young children.

Leptospirosis- Leptospira Sub-Section



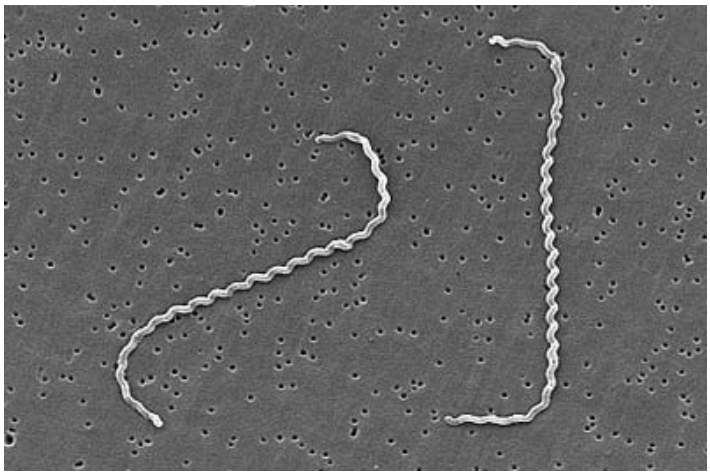
LEPTOSPIRA

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.

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.



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.

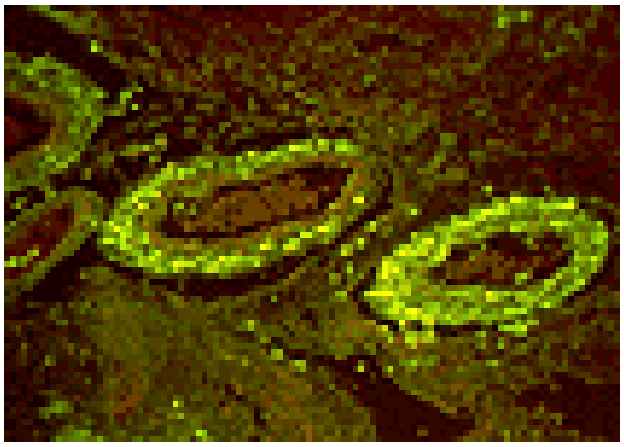
Diagnosis
Clinical diagnosis is usually confirmed by serology. Isolation of spirochetes is possible, but it is time-consuming and requires special media.

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 Sub-Section

Refer to amoebic meningoencephalitis (PAM), Naegleria fowleri and granulomatous 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.

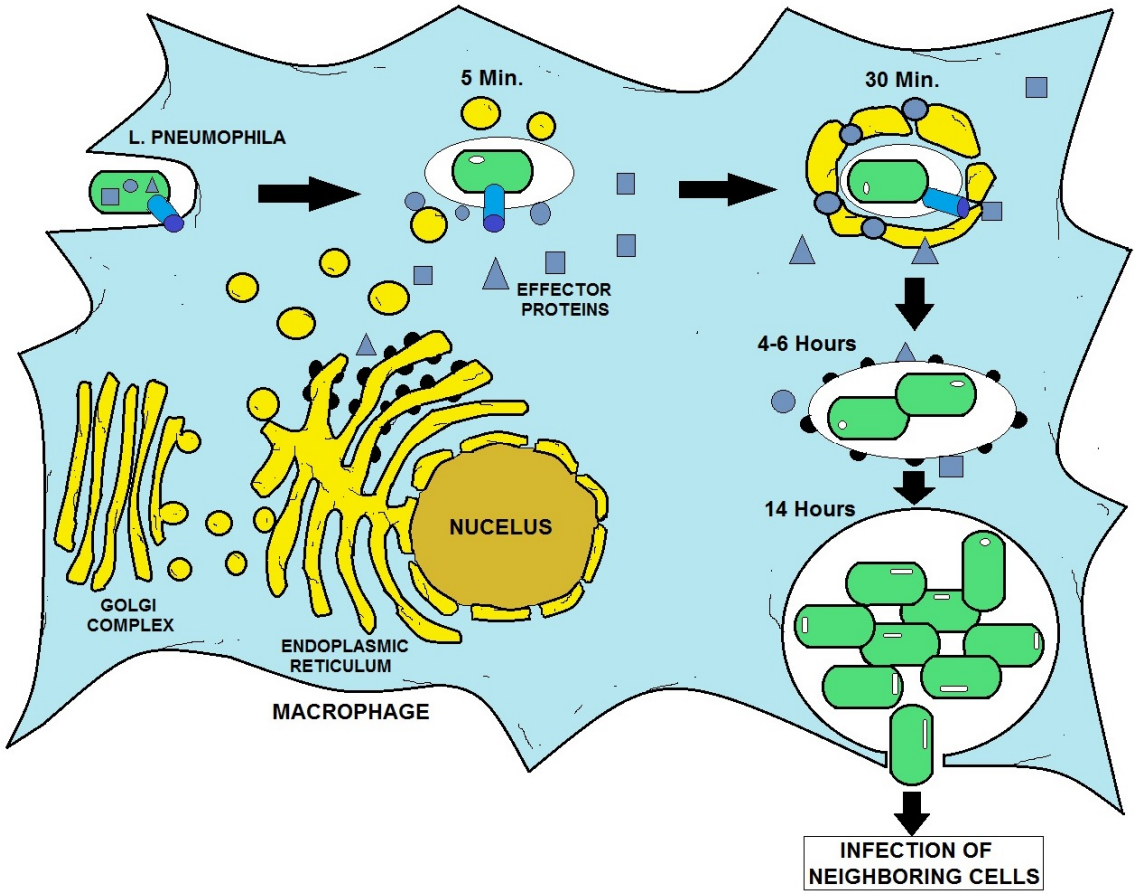
There was also necrotizing amoebic panniculitis in subcutaneous, peripancreatic, mesenteric and peri-aortic tissue. There were occasional amoebae in the liver, but not enough to account for all of the patient's liver disease, so part of his liver disease was most likely due to sepsis due to his disseminated amoebiasis.

Pseudomonas Aeruginosa Sub-Section

Pseudomonas aeruginosa 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.



INFECTION CYCLE OF L. PNEUMOPHILA

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.

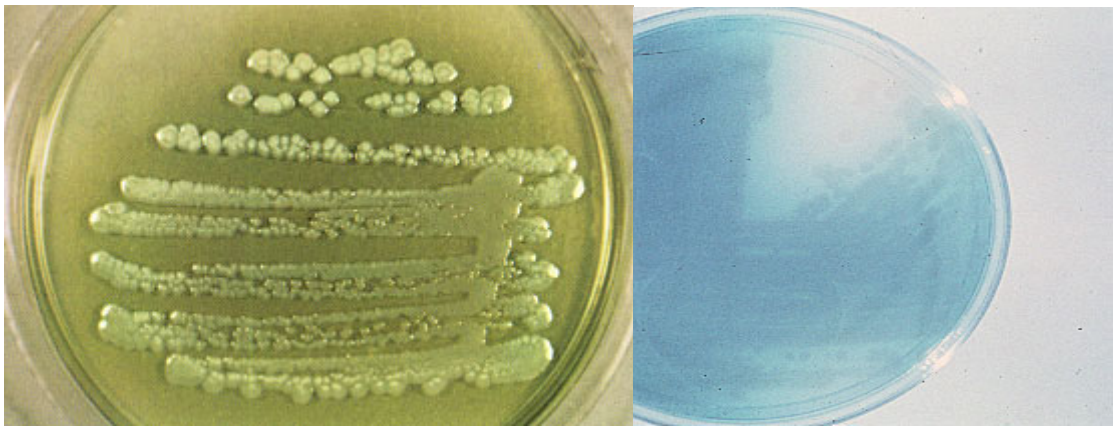
--Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C.

--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. The soluble blue pigment pyocyanin is produced by many, but not all, strains of Pseudomonas aeruginosa.

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.

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.

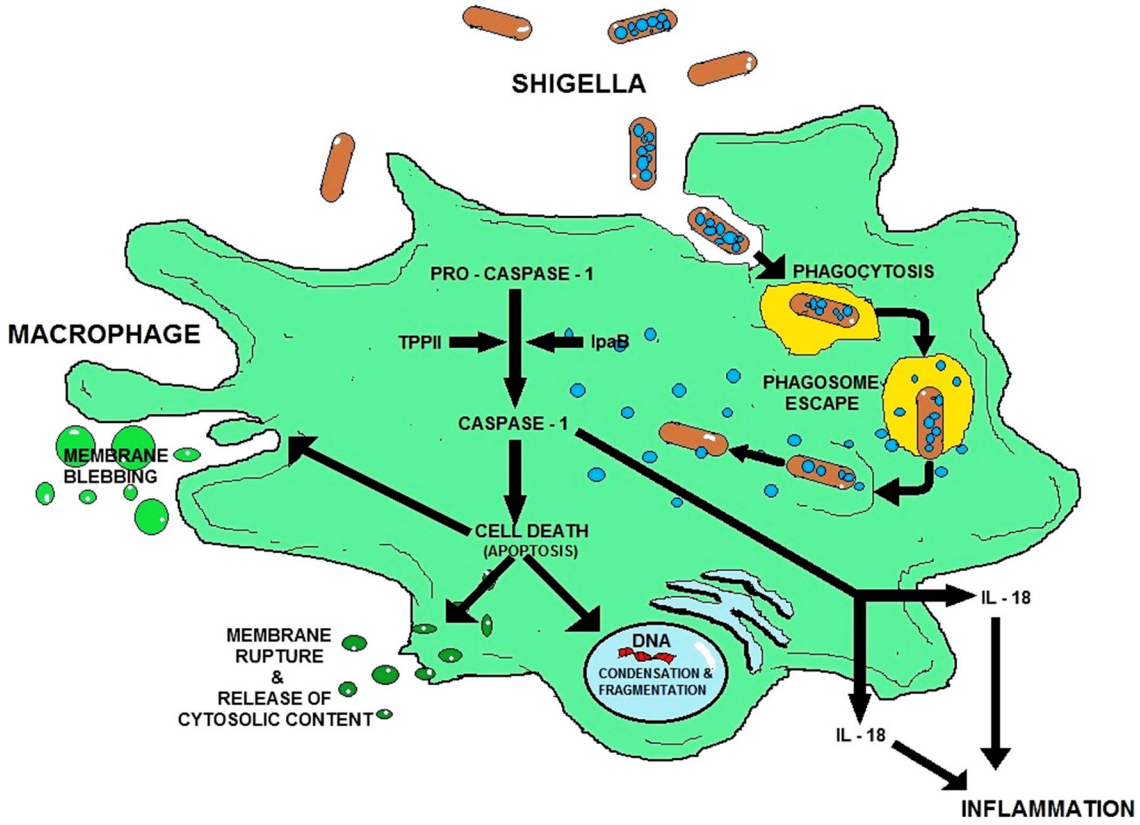
Colonization
Although colonization usually precedes infections by Pseudomonas aeruginosa, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment. It is sometimes present as part of the normal flora of humans, although the prevalence 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.

In addition, 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.

Shigellosis Shigella Sub-Section



SHIGELLOSIS DIAGRAM

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.

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.

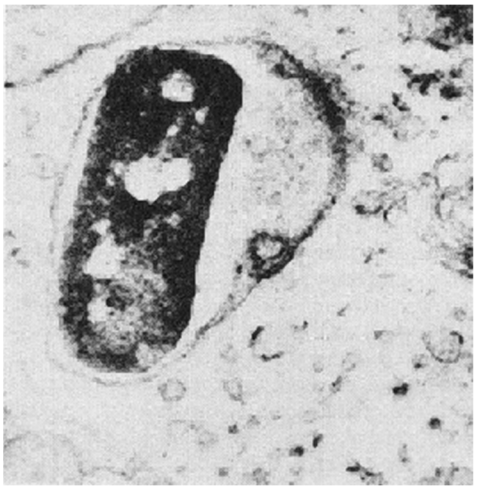
A second type, Shigella flexneri, or "group B" Shigella, accounts for almost all of the rest. Other types of Shigella are rare in this country, though they continue to be important causes of disease in the developing world. One type found in the developing world, Shigella dysenteriae type 1, causes deadly 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.



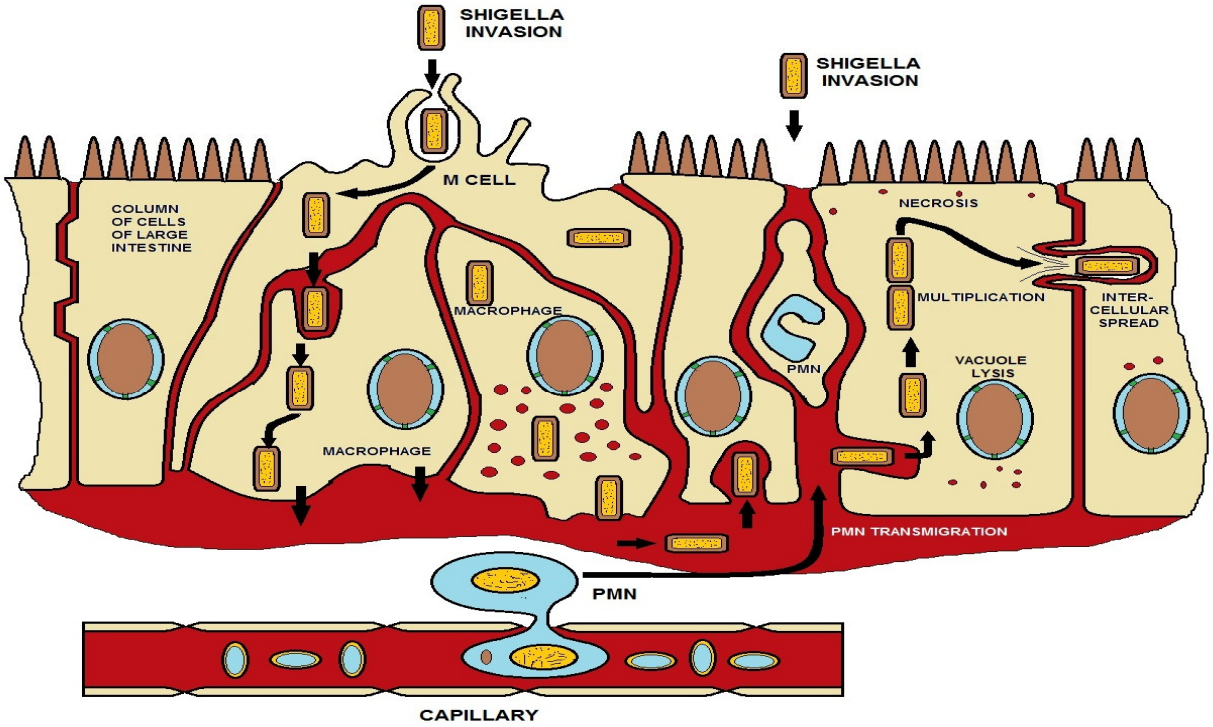
Micrograph of intra-epithelial membrane-enclosed Shigella
(from Microbiology: Fundamentals and Applications by R. M. Atlas, p. 609)

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.



SHIGELLA LIFE CYCLE BEGINS WITH THE PENETRATION OF COLONIC MUCOSA

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.

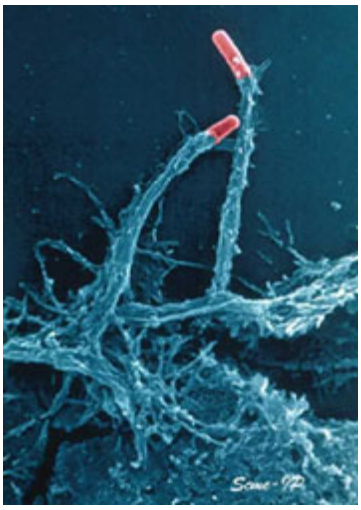
About one million people die of Shigella infections every year, most of them children. The bacterium Shigella flexneri causes sudden and severe diarrhea in humans, known as shigellosis.

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.

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.



Highly infectious microbe Shigella flexneri. Courtesy P. Sansonetti, Institut Pasteur, Paris, France

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.

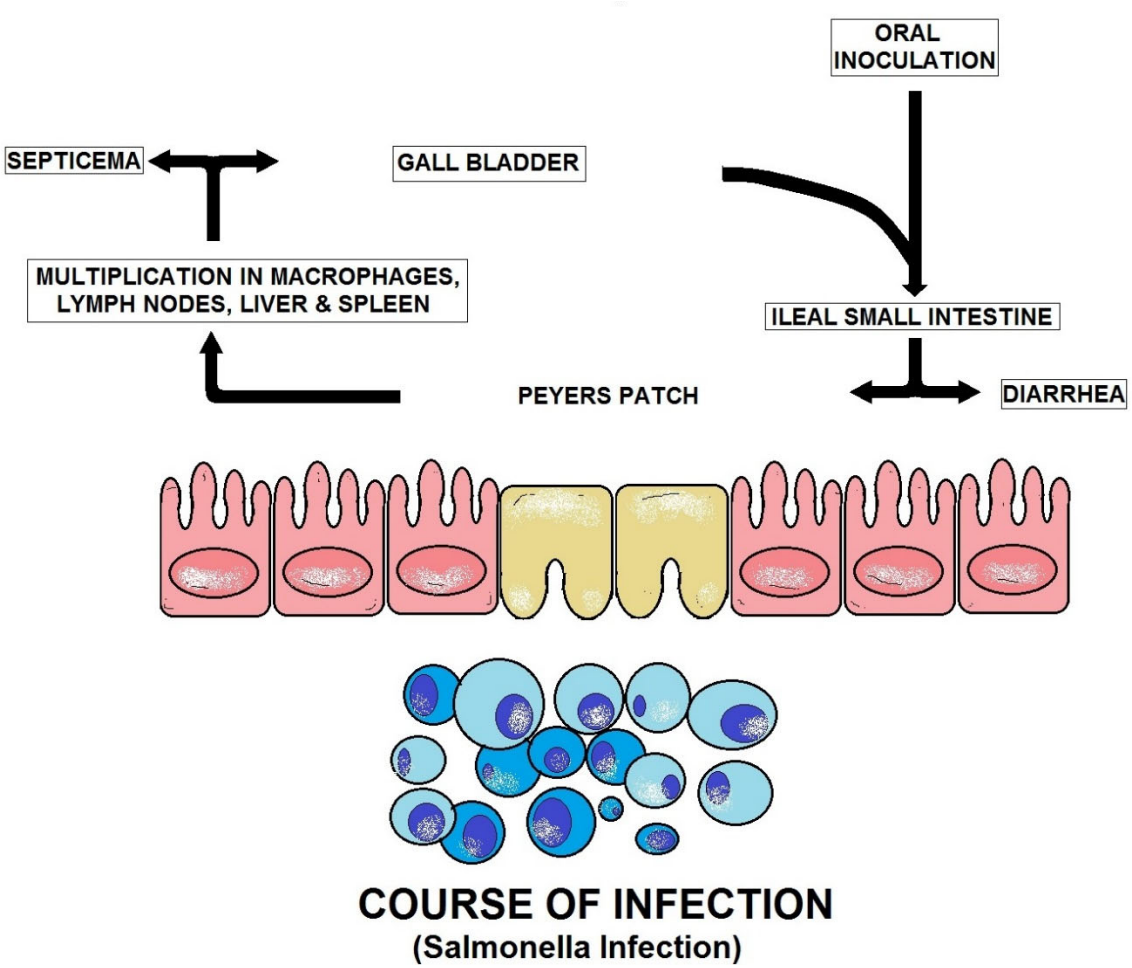
The Food and Drug Administration inspects imported foods, and promotes better food preparation techniques in restaurants and food processing plants. The Environmental Protection Agency regulates and monitors the safety of our drinking water supplies. The government has also maintained active research into the development of a Shigella vaccine.

Salmonellosis

Salmonella enterocolitis is an infection in the lining of the small intestine caused by Salmonella bacteria. Salmonellosis is an infection with Salmonella bacteria. Most people infected with Salmonella develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In most cases, the illness lasts four to seven days, and most people recover without treatment.

However, in some cases the diarrhea may be so severe that the patient becomes dangerously dehydrated and must be taken to a hospital. At the hospital, the patient may receive intravenous fluids to treat the dehydration, and may be given medications to provide symptomatic relief, such as fever reduction.

In severe cases, the Salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness. Some people afflicted with salmonellosis later experience reactive arthritis, which can have long-lasting, disabling effects. There are different kinds of Salmonella, including S. bongori and S. enterica.



COURSE OF INFECTION
(Salmonella Infection)

The type of Salmonella usually associated with infections in humans, non-typhoidal Salmonella, is usually contracted from sources such as:

- Poultry, pork, and beef, if the meat is prepared incorrectly or is infected with the bacteria after preparation.
- Infected eggs, egg products, and milk when not prepared, handled, or refrigerated properly.
- Reptiles, such as turtles, lizards, and snakes, which may carry the bacteria in their intestine.
- Tainted fruits and vegetables.

The typhoidal form of Salmonella can lead to typhoid fever. Typhoid fever is a life-threatening illness, and about 400 cases are reported each year in the United States, and 75% of these are acquired while traveling out of the country. It is carried only by humans and is usually contracted through direct contact with the fecal matter of an infected person. Typhoidal Salmonella is more commonly found in poorer countries, where unsanitary conditions are more likely to occur, and can affect as many as 21.5 million persons each year.

Causes, incidence, and risk factors

Salmonella infection is one of the most common types of food poisoning. It occurs when you swallow food or water that contains the salmonella bacteria. The salmonella germs may get into the food you eat (called contamination) in several ways.

You are more likely to get this type of infection if you have:

- Eaten foods such as turkey, turkey dressing, chicken, or eggs that have not been cooked well or stored properly.
- Family members with recent salmonella infection.
- Been or worked in a hospital, nursing home, or other long-term health facility.
- A pet iguana or other lizards, turtles, or snakes (reptiles are carriers of salmonella).
- A weakened immune system.
- Regularly used medicines that block acid production in the stomach.
- Crohn's disease or ulcerative colitis.
- Used antibiotics in the recent past.
- Most people with this condition are younger than 20.

Symptoms

Salmonellosis is an infection with bacteria called Salmonella. Most persons infected with Salmonella develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. The time between infection and symptoms is 8 - 48 hours.

Symptoms include:

- Abdominal pain, cramping, or tenderness
- Chills
- Diarrhea
- Fever
- Muscle pain
- Nausea
- Vomiting

Signs and tests
The health care provider will perform a physical exam. You may have signs of a tender abdomen and tiny pink spots on the skin called rose spots.

Tests that may be done include:
- Blood culture
- Complete blood count with differential
- Febrile/cold agglutinins (test for specific antibodies)
- Stool culture for salmonella

Treatment
The goal is to make you feel better and avoid dehydration. Dehydration means your body does not have as much water and fluids as it should.

These things may help you feel better if you have diarrhea:
- Drink 8 to 10 glasses of clear fluids every day. Water is best.
- Drink at least 1 cup of liquid every time you have a loose bowel movement.
- Eat small meals throughout the day, instead of three big meals.
- Eat some salty foods, such as pretzels, soup, and sports drinks.
- Eat some high-potassium foods, such as bananas, potatoes without the skin, and watered-down fruit juices.

Give your child fluids for the first 4 to 6 hours. At first, try 1 ounce (2 tablespoons) of fluid every 30 to 60 minutes.
- Infants should continue to breastfeed and receive electrolyte replacement solutions as recommended by your health care provider.
- You can use an over-the-counter drink, such as Pedialyte or Infalyte. Do not water down these drinks.
- You can also try Pedialyte popsicles.
- Watered-down fruit juice or broth may also help.

Medicines that slow down diarrhea are usually not given because they may make the infection last longer. If you have severe symptoms, your health care provider may prescribe antibiotics.
If you take water pills or diuretics, you may need to stop taking them when you have diarrhea. Ask your health care provider.

Expectations (prognosis)
In otherwise healthy people, symptoms should go away in 2 - 5 days, but they may last for 1 - 2 weeks. The bacteria can be shed in the feces of some treated patients for months to a year after the infection. Food handlers who carry salmonella in their body can pass the infection to the people who eat their food.

Calling your health care provider
Call your health care provider if:
- There is blood or pus in your stools.
- You have diarrhea and are unable to drink fluids due to nausea or vomiting.
- You have a fever above 101°F or your child has a fever above 100.4°F, along with diarrhea.
- You have signs of dehydration (thirst, dizziness, light-headedness).
- You have recently traveled to a foreign country and developed diarrhea.
- Your diarrhea does not get better in 5 days (2 days for an infant or child), or it gets worse.

- Your child has been vomiting for more than 12 hours (in a newborn under 3 months you should call as soon as vomiting or diarrhea begins.
- Your child has reduced urine output, sunken eyes, sticky or dry mouth, or no tears when crying

Prevention

Learning how to prevent food poisoning can reduce the risk of this infection.

- Proper food handling and storage can help reduce the risk. Good hand washing when handling eggs, poultry, and other foods is important.
- If you own a reptile, wear gloves when handling the animal or its feces because salmonella can easily pass to humans.

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.

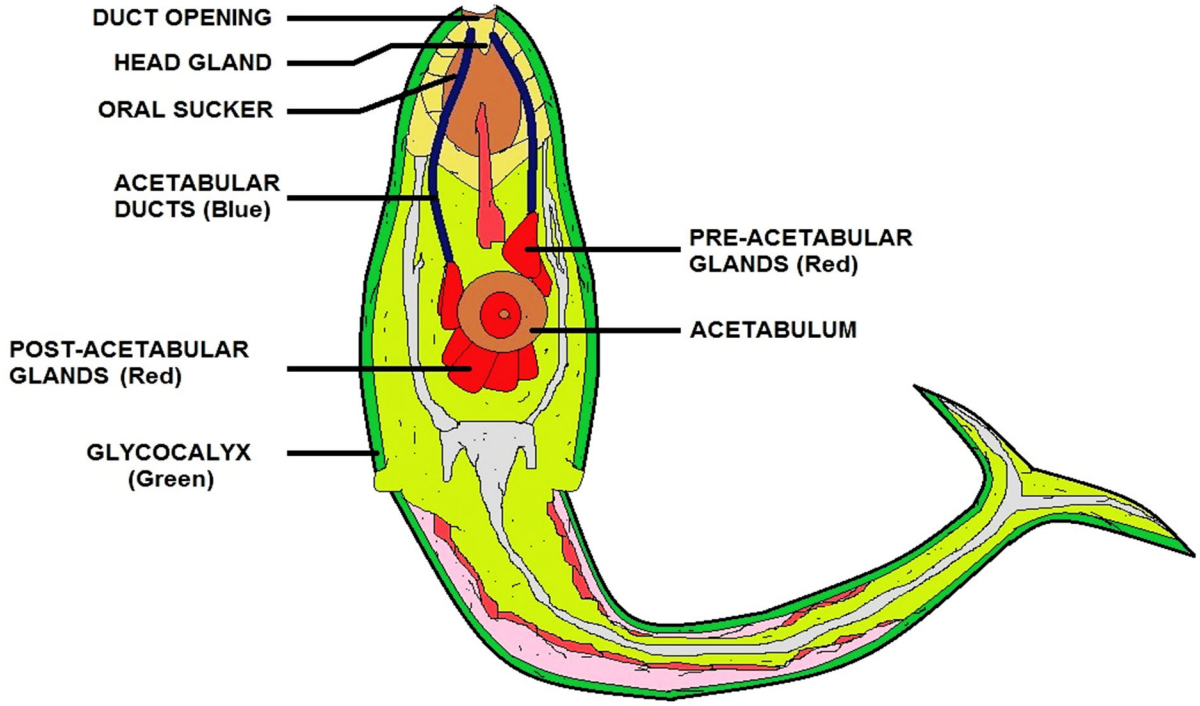


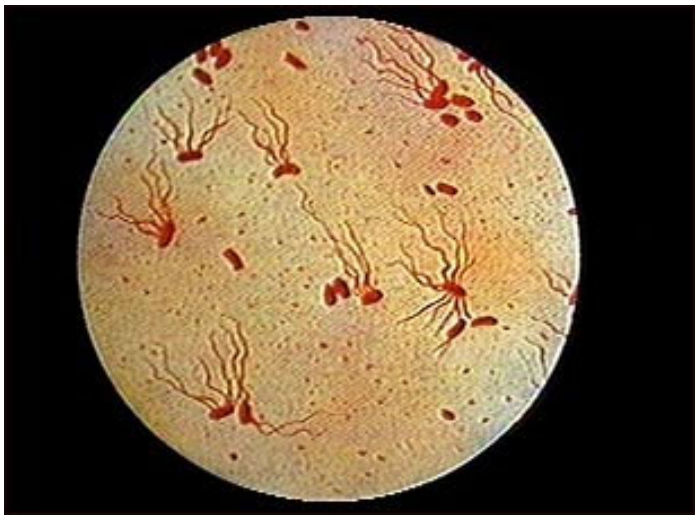
DIAGRAM OF A SCHISTOSOMA

Control
As a control measure, exposure to parasite larvae in water and food should be prevented. Treatment with praziquantel is effective.

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.

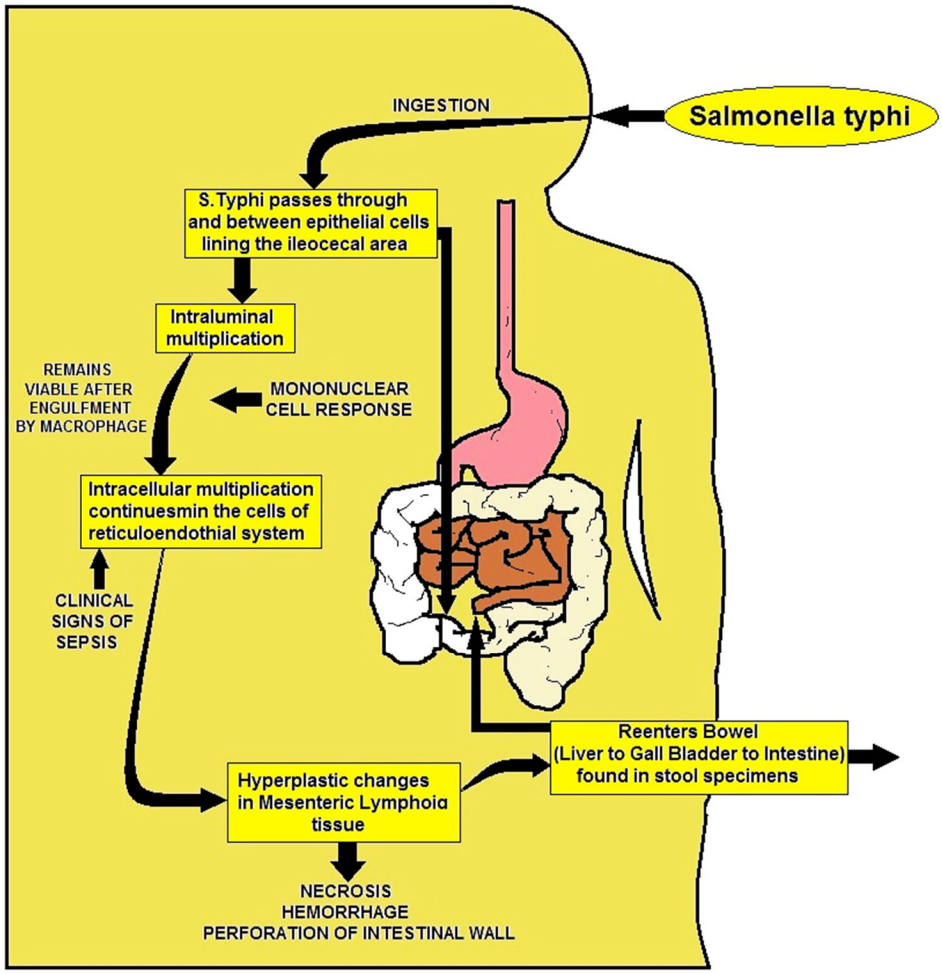


Salmonella typhi

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.



SALMONELLA TYPHI ROUTE OF ENTRY

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

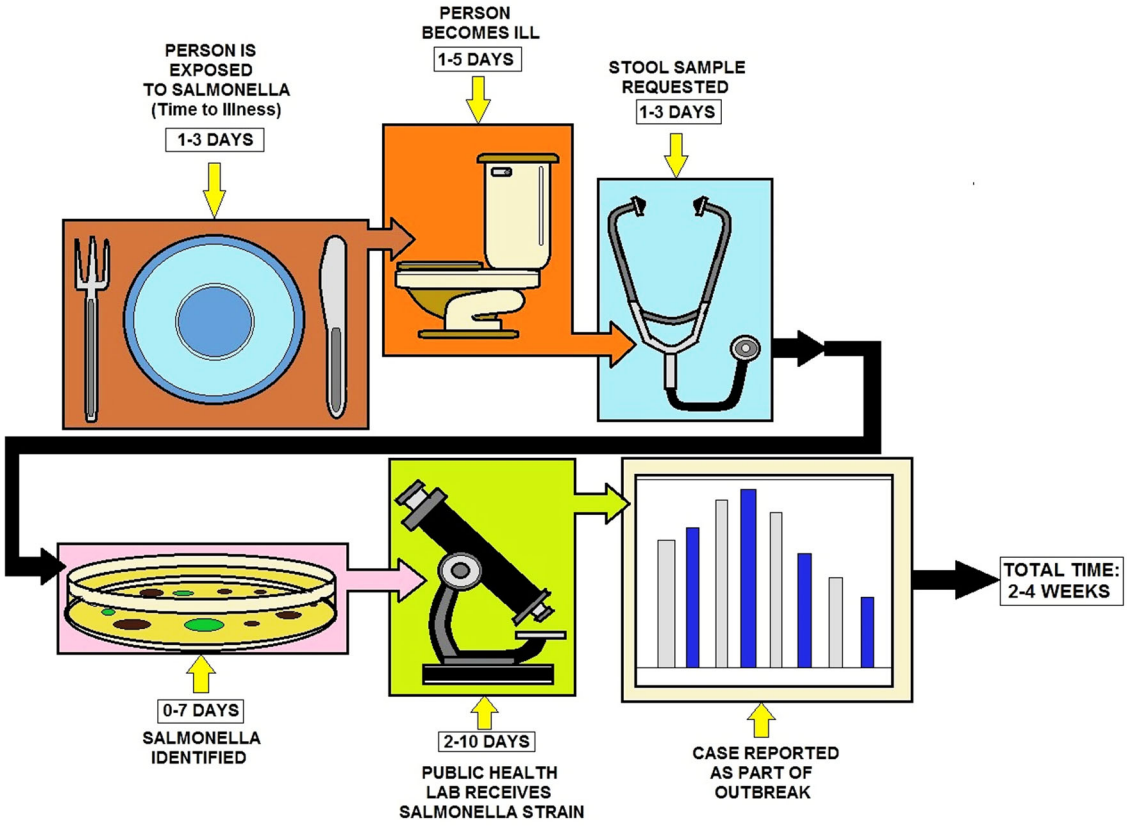
If you are traveling to a country where typhoid is common, you should consider being vaccinated against typhoid. Visit a doctor or travel clinic to discuss your vaccination options.

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.

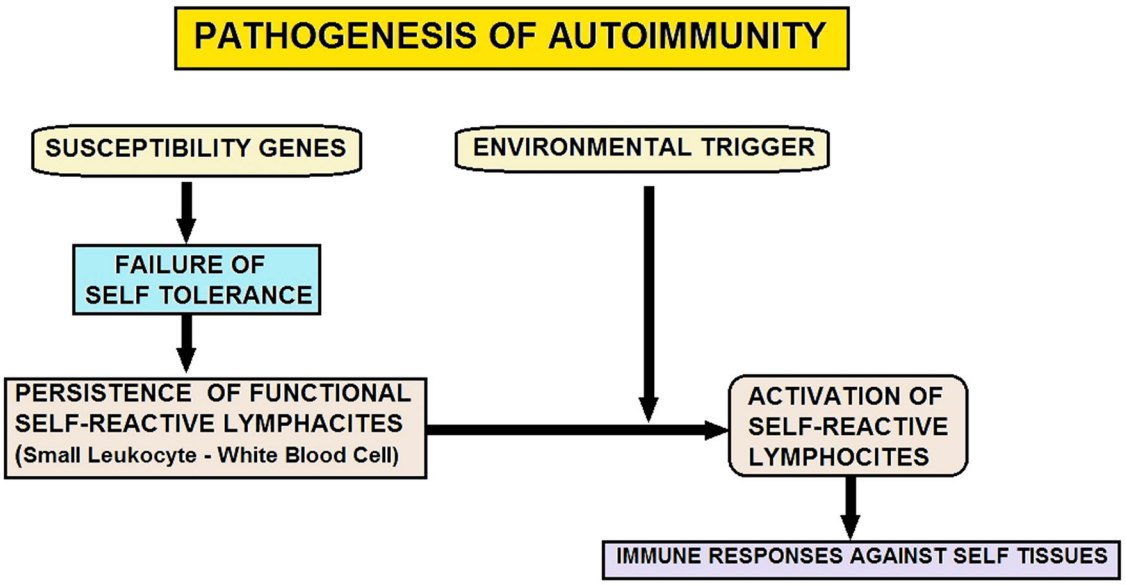
The chart below provides basic information on typhoid vaccines that are available in the United States.

Vaccine Name	How given	Number of doses necessary	Time between doses	Total time needed to set aside for vaccination	Minimum age for vaccination	Booster needed every...
Ty21a (Vivotif Berna, Swiss Serum and Vaccine Institute)	1 capsule by mouth	4	2 days	2 weeks	6 years	5 years
ViCPS (Typhim Vi, Pasteur Merieux)	Injection	1	N/A	1 week	2 years	2 years

The parenteral heat-phenol-inactivated vaccine (manufactured by Wyeth-Ayerst) has been discontinued.



TIMELINE FOR REPORTING A CASE OF SALMONELLA INFECTION



PATHOGENESIS
(The Manner in Which a Disease Develops)

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).

Tularemia is a disease that can infect animals and people. Rabbits, hares, and rodents are especially susceptible and often die in large numbers during outbreaks.

People can become infected in several ways, including:
- Tick and deer fly bites
- Skin contact with infected animals
- Drinking contaminated water
- Inhaling contaminated aerosols or agricultural and landscaping dust
- Laboratory exposure

In addition, people could be exposed as a result of bioterrorism.

What are the Symptoms of Tularemia?
Symptoms vary depending how the person was infected. Tularemia can be life-threatening, but most infections can be treated successfully with antibiotics.

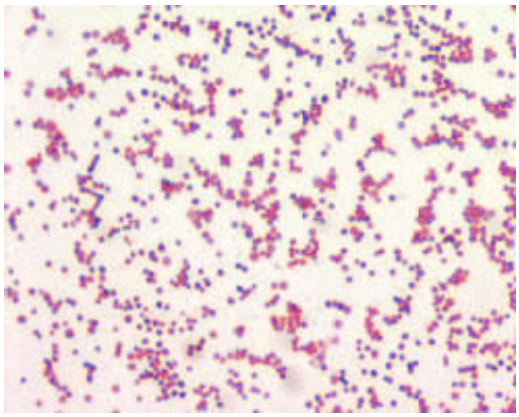
Symptoms of tularemia could include:
- *sudden fever*
- *chills*
- *headaches*
- *diarrhea*
- *muscle aches*
- *joint pain*
- *dry cough*
- *progressive weakness*

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

Steps to prevent tularemia include:

- Using insect repellent
- Wearing gloves when handling sick or dead animals
- Avoiding mowing over dead animals

In the United States, naturally occurring infections have been reported from all states except Hawaii.

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.

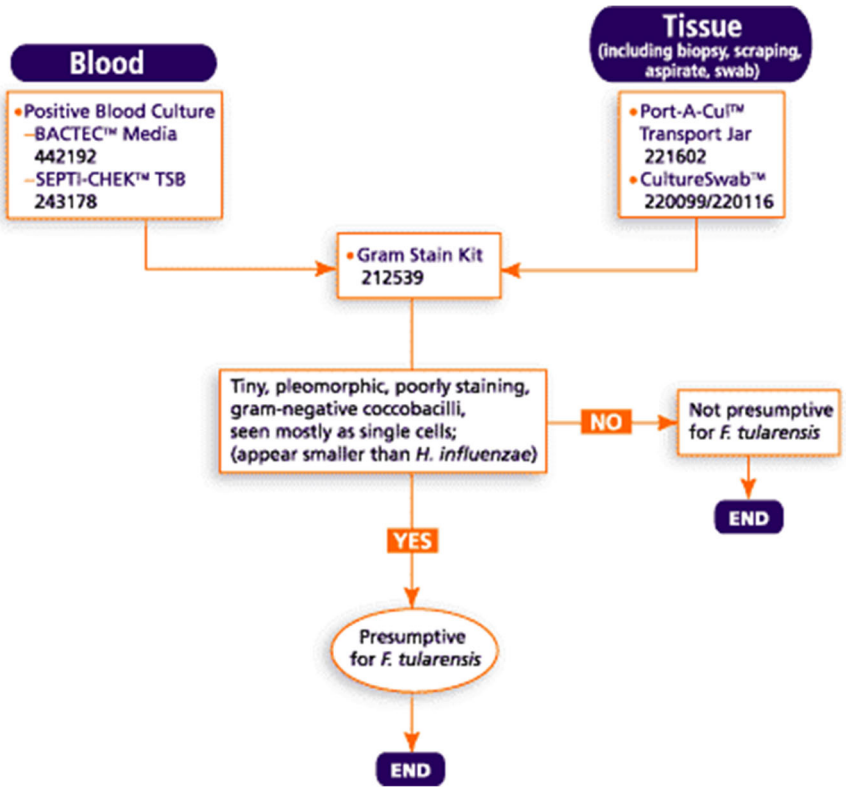
Clinical diagnosis can be difficult since the disease mimics a slew of other illnesses. Pathogenesis varies greatly depending on mode of infection.

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.


Blood
•Positive Blood Culture
–BACTEC™ Media
442192
–SEPTI-CHEK™ TSB
243178
Tissue
(including biopsy, scraping, aspirate, swab)
•Port-A-Cul™
Transport Jar
221602
•CultureSwab™
220099/220116
•Gram Stain Kit
212539
Tiny, pleomorphic, poorly staining, gram-negative coccobacilli, seen mostly as single cells; (appear smaller than H. influenzae)
NO
Not presumptive for F. tularensis
END
YES
Presumptive for F. tularensis
END

Virus Sub-Section

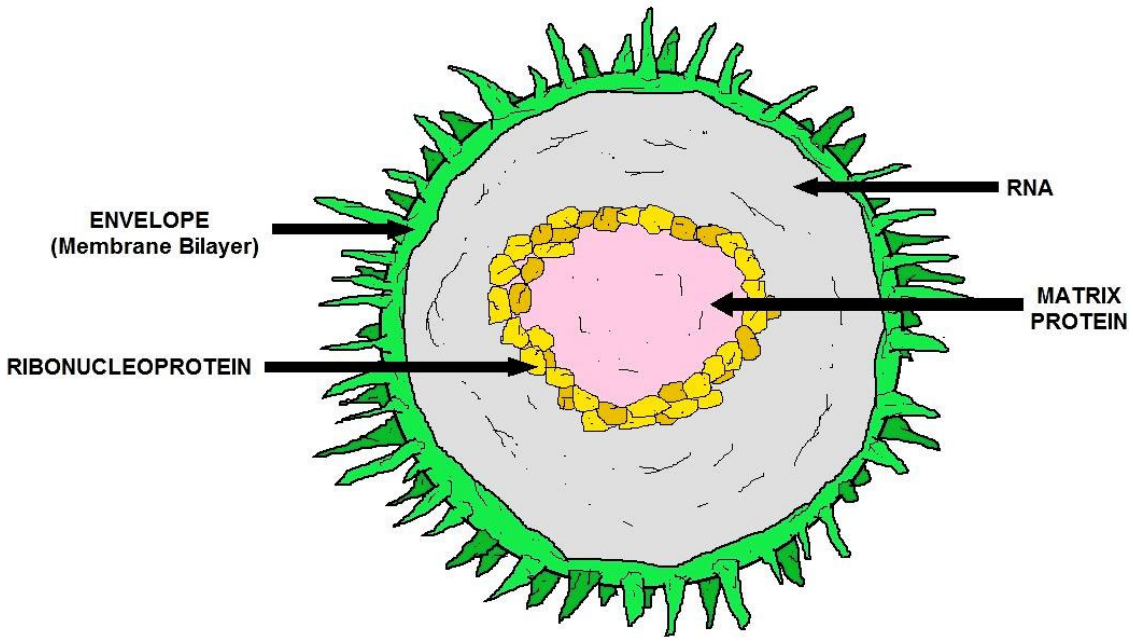
Both bacteria and viruses are microorganisms regulated by EPA's Maximum Contaminant Levels (MCLs) criteria. Viruses are the smallest form of microorganisms capable of causing disease, particularly those of a fecal origin infectious to humans by waterborne transmission; bacteria are typically single-celled microorganisms that can also cause health problems in humans, animals or plants, despite many form's ability to aid in water pollution control.

- Enteroviruses are small viruses, such as polioviruses, echoviruses and coxsackieviruses, living in the intestines of infected humans or animals; in addition to the three different polioviruses are 62-nonpolio enteroviruses that can cause disease in humans ranging from gastroenteritis to meningitis.

Bacteria and viruses can also be listed as "indicators," which at a level outside of identified limits, may reflect "a problem in the treatment process or in the integrity of distribution system," according to the EPA

Description

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 OF A VIRUS

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 non-segmented (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.

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.

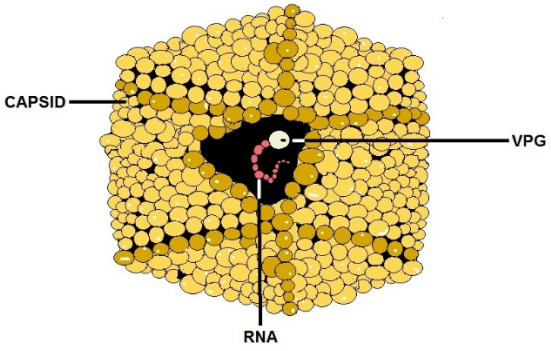
Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule. The single-stranded RNA circle collapses on itself to form a rod-like structure. The only 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.

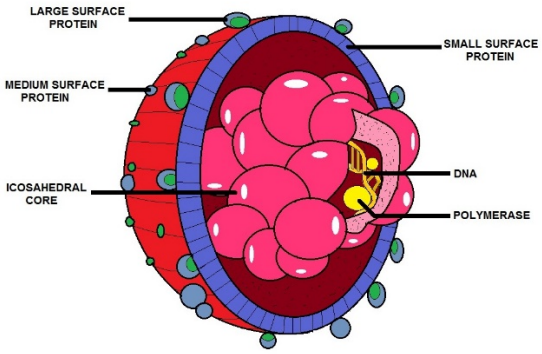
Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. 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.

	CHLORINE AS A DISINFECTANT	ULTRAVIOLET GERMICIDAL IRRADIATION (UV) AS A DISINFECTANT
DISINFECTION BYPRODUCTS (DBPs)	X	No
CHEMICAL RESIDUAL	YES	No
CORROSIVE	X	No
COMMUNITY SAFETY RISKS	X	No
EFFECTIVE AGAINST CRYPTOSPORIDIUM AND GIARDIA	DEPENDS ON TREATMENT PROCESS	Yes
WELL-SUITED FOR CHANGING REGULATIONS	DEPENDS ON TREATMENT PROCESS	Yes

CHLORINE vs. UV FOR DISINFECTION



HEPATITUS A VIRUS



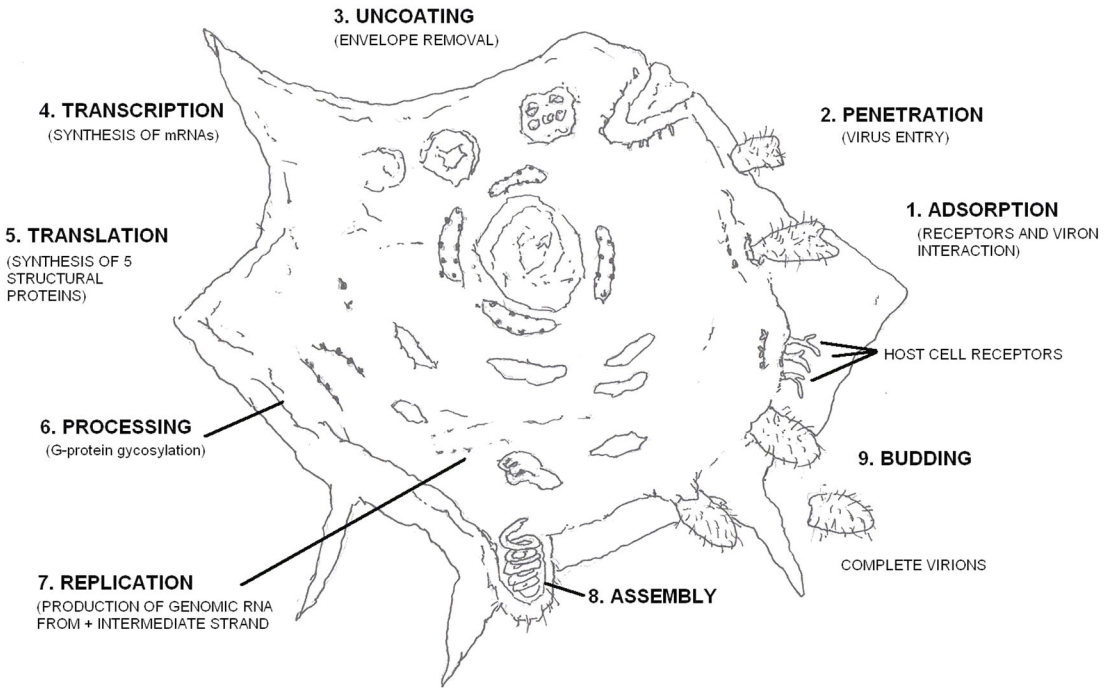
HEPATITUS B VIRUS

HEPATITUS C VIRUS DIAGRAM

Virus 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.



CYCLE OF VIRUS INFECTION AND REPLICATION DIAGRAM

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.

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

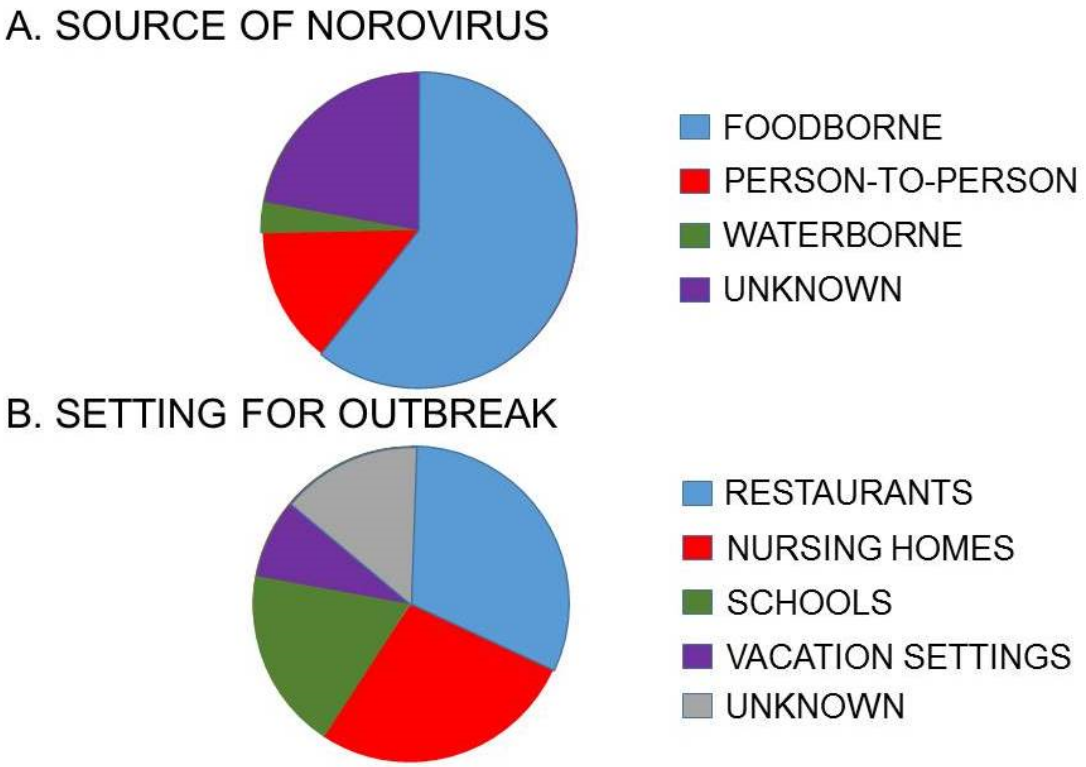
Viruses undergo very high rates of mutation (genetic alteration) largely because they lack the repair systems that cells have to safeguard against mutations. A high mutation rate enables the virus to continually adapt to new intracellular environments and to escape from the host immune response.

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.

Norovirus Sub-Section

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).



Noroviruses are found in every part of the United States and throughout the world. Noroviruses may be found in water sources, such as private wells, that have been contaminated with the feces of infected humans. Waste can enter the water through various ways, including sewage overflows, sewage systems that are not working properly, and polluted storm water runoff. Wells may be more vulnerable to such contamination after flooding, particularly if the wells are shallow, have been dug or bored, or have been submerged by floodwater for long periods of time.

To kill or inactivate noroviruses, bring your water to a rolling boil for one minute (at elevations above 6,500 feet, boil for three minutes) Water should then be allowed to cool, stored in a clean sanitized container with a tight cover, and refrigerated. Because of the small size of the virus, using a point-of-use filter will not remove it from your water.

Note that noroviruses are moderately tolerant to chlorination, so you should contact your local health department for recommended procedures.

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.

Can norovirus infections be prevented?
Yes. You can decrease your chance of coming in contact with noroviruses by following these preventive steps:

- Frequently wash your hands, especially after toilet visits and changing diapers and before eating or preparing food.
- Carefully wash fruits and vegetables, and steam oysters before eating them.
- Thoroughly clean and disinfect contaminated surfaces immediately after an episode of illness by using a bleach-based household cleaner.
- Immediately remove and wash clothing or linens that may be contaminated with virus after an episode of illness (use hot water and soap).
- Flush or discard any vomitus and/or stool in the toilet and make sure that the surrounding area is kept clean.

Persons who are infected with norovirus should not prepare food while they have symptoms and for 3 days after they recover from their illness. Food that may have been contaminated by an ill person should be disposed of properly.

Rotavirus Sub-Section

Rotavirus
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.

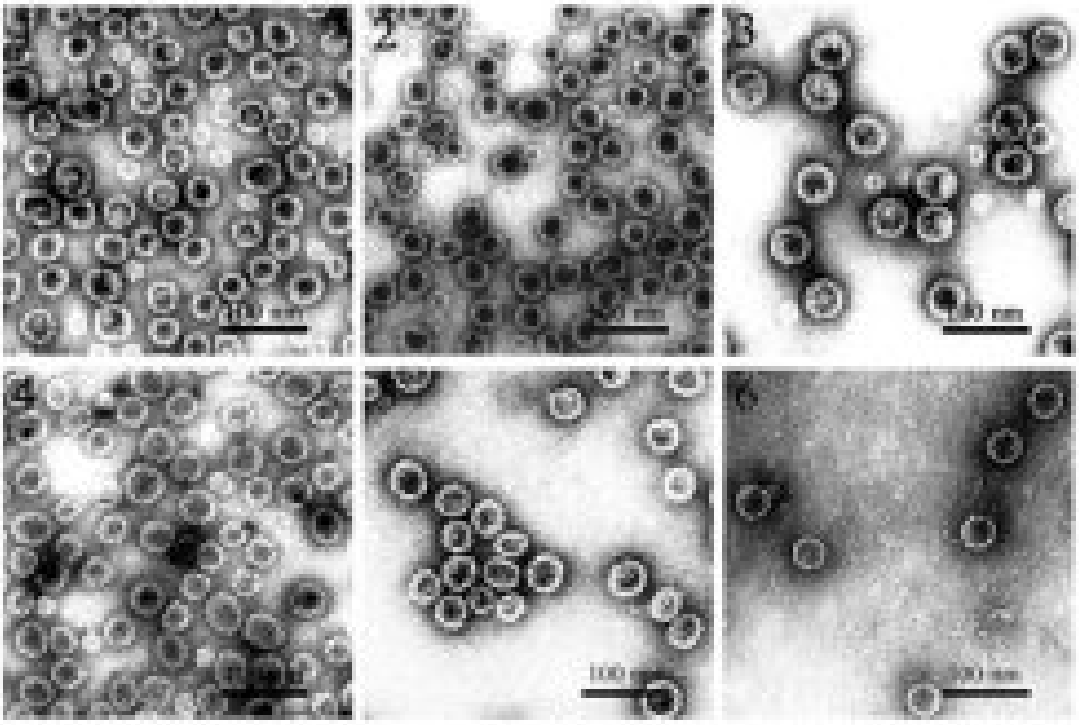
Mode of Transmission and Communicability
Fecal to oral route is the most likely mode of transmission. This virus is easily transmitted. Patients are communicable during the acute phase of the illness and up to 48 hours after the symptoms resolve.

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.

Hepatitis Sub-Section

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.

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.

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Cahill, Mathew. *Handbook of Diagnostic Tests.* Springhouse, PA: Springhouse Corporation, 1995.

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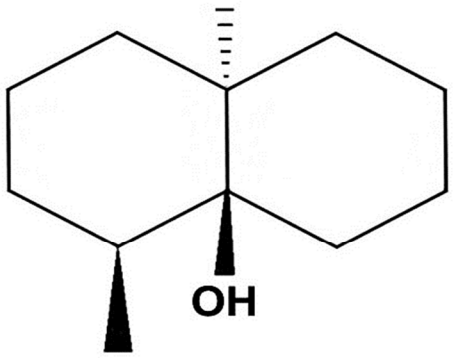
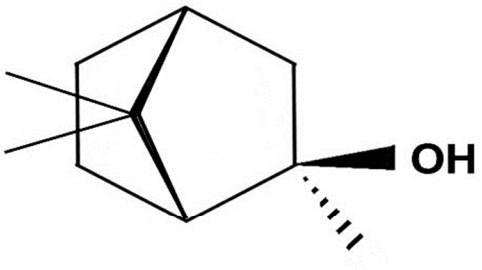
Pagana, Kathleen Deska. *Mosby's Manual of Diagnostic and Laboratory Tests.* St. Louis: Mosby, Inc., 1998.

MIB and Geosmin Sub-Section





(-)- GEOSMIN **(-)-2- METHYLISOBORNEOL**

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 as Spring began. 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.

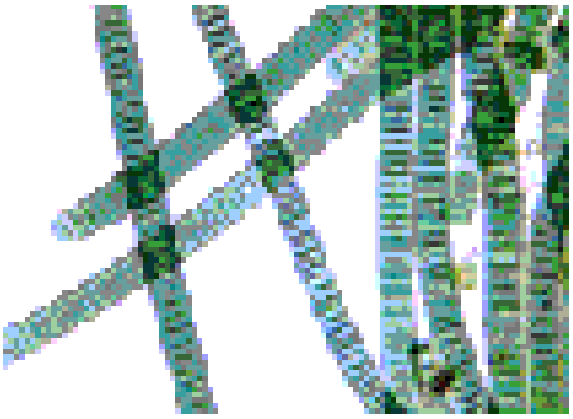
These stable complex compounds present in parts per trillion are difficult to remove with current technology. The detection of these compounds is dependent upon an individual's olfactory sensitivity.

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.

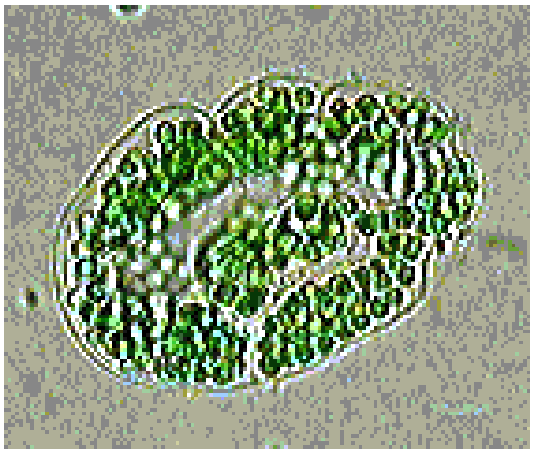
Growing algae produce numerous volatile and nonvolatile organic substances, including aliphatic alcohols, aldehydes, ketones, esters, thioesters, and sulfides.

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.

Chemical Related Water Related Diseases

Arsenic

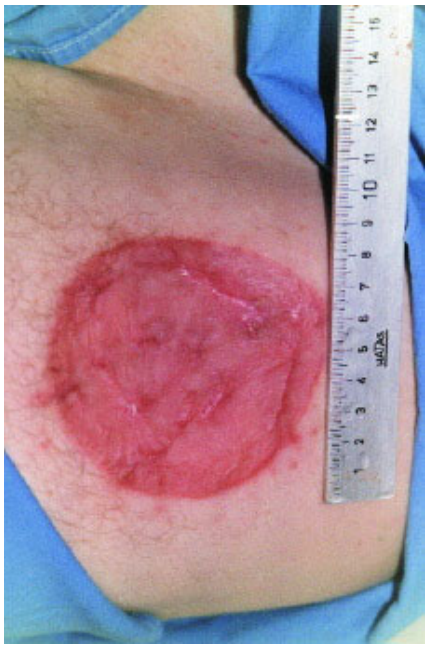
Arsenic is a naturally occurring element used since ancient times and has long been known to be toxic to humans. Arsenic in ground water is largely the result of minerals dissolving from weathered rocks and soils. Several types of cancer have been linked to arsenic in water. In 2001 the US Environmental 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.

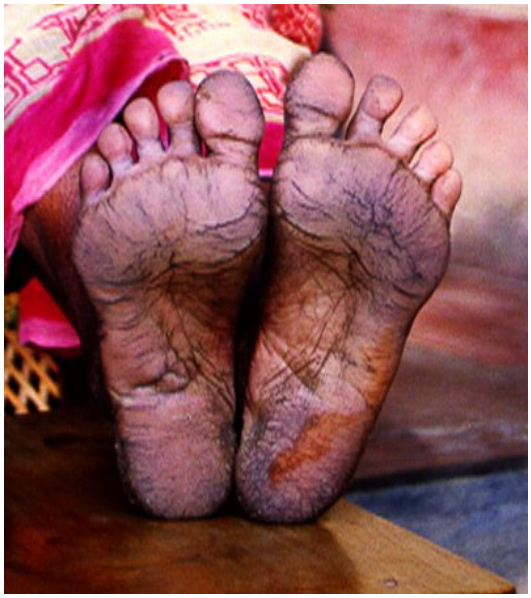
Some studies also found that arsenic harms the central and peripheral nervous systems as well as heart and blood vessels. Arsenic has been associated with birth defects and reproductive problems.



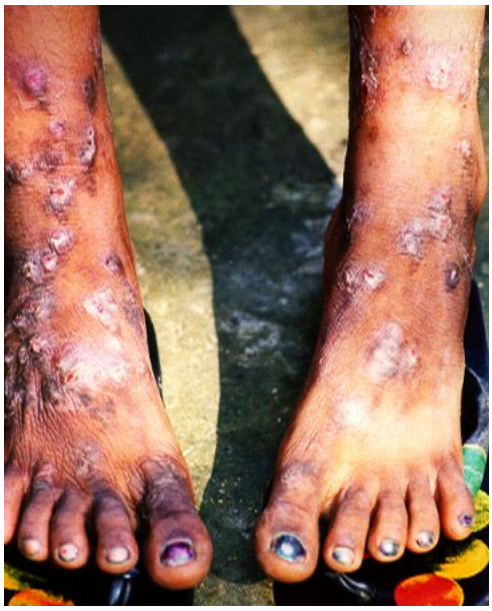
Conservative estimates based on all these data suggest that more than 34 million Americans drink tap water supplied by systems containing average levels of arsenic that pose unacceptable cancer risks. In October 2001, the Environmental Protection Agency implemented new standards for arsenic in drinking 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.

METHEMOGLOBIN CONCENTRATION	% TOTAL HEMOGLOBIN	SYMPTOMS
< 1.5 g/dL	10%	NONE
1.5 - 3.0 g/dL	10 - 20%	CYANOTIC SKIN DISCOLORATION
3.0 - 4.5 g/dL	20 - 30%	ANXIETY, LIGHTHEADEDNESS, HEADACHE, TACHYCARDIA
4.5 - 7.5 g/dL	30 - 50%	FATIGUE, CONFUSION, DIZZINESS, TACHYPNEA, TACHYCARDIA
7.5 - 10.5 g/dL	50 - 70%	COMA, SEIZURES, ANHYTHMIAS, ACIDOSIS
>10.5 g/dL	> 70%	DEATH
KEY: g/dL = grams per deciLiter		
METHEMOGLOBIN - A FORM OF HEMOGLOBIN THAT IS INCAPABLE OF CARRYING OXYGEN, SOMETIMES FOUND IN THE BLOOD AFTER CERTAIN POISONINGS SUCH AS ANILINE, NITRATE & OTHER COMPOUNDS. CAUSES BLUISH COLORING OF THE SKIN		

METHEMOGLOBINEMIA DIAGRAM

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.

Other Diseases that have Returned in the US

Illness Patterns- Diagnostic Clues to Disease

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.

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

Anthrax is a serious infectious disease caused by gram-positive, rod-shaped bacteria known as *Bacillus anthracis*. Although it is rare, people can get sick with anthrax if they come in contact with infected animals or contaminated animal products. The type of illness a person develops depends on how anthrax enters the body. Typically, anthrax gets into the body through the skin, lungs, or gastrointestinal system. All types of anthrax can eventually spread throughout the body and cause death if they are not treated with antibiotics.

Cutaneous

Most common form of anthrax infection, and is considered to be the least dangerous. Infection usually develops from 1 to 7 days after exposure.

Inhalation

Inhalation anthrax is considered to be the most deadly form of anthrax. Infection usually develops within a week after exposure, but it can take up to 2 months

Gastointestinal
Gastrointestinal anthrax has rarely been reported in the United States. Infection usually develops from 1 to 7 days after exposure.

Injection
This type of infection has never been reported in the United States.

Inhalation of Infectious Spores
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
Plague is a disease that affects humans and other mammals. It is caused by the bacterium, *Yersinia pestis*. Humans usually get plague after being bitten by a rodent flea that is carrying the plague bacterium or by handling an animal infected with plague. Plague is infamous for killing millions of people in Europe during the Middle Ages. Today, modern antibiotics are effective in treating plague. Without prompt treatment, the disease can cause serious illness or death. Presently, human plague infections continue to occur in rural areas in the western United States, but significantly more cases occur in parts of Africa and Asia.

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
Botulism (“BOT-choo-liz-um”) is a rare but serious illness caused by a toxin that attacks the body’s nerves and causes difficulty breathing, muscle paralysis, and even death. This toxin is made by Clostridium botulinum and sometimes Clostridium butyricum and clostridium baratii bacteria. These bacteria can be spread by food and sometimes by other means.

The bacteria that make botulinum toxin are found naturally in many places, but it’s rare for them to make people sick. These bacteria make spores, which act like protective coatings. Spores help the bacteria survive in the environment, even in extreme conditions. The spores usually do not cause people to become sick, even when they’re eaten. But under certain conditions, these spores can grow and make one of the most lethal toxins known.

The conditions in which the spores can grow and make toxin are:
- Low-oxygen or no oxygen (anaerobic) environment
- Low acid
- Low sugar
- Low salt
- A certain temperature range

- A certain amount of water

For example, improperly home-canned, preserved, or fermented foods can provide the right conditions for spores to grow and make botulinum toxin. When people eat these foods, they can become seriously ill, or even die, if they don't get proper medical treatment quickly.

Smallpox (Variola)
Before smallpox was eradicated, it was a serious infectious disease caused by the **variola virus**. It was contagious—meaning, it spread from one person to another. People who had smallpox had a fever and a distinctive, progressive skin rash. Most people with smallpox recovered, but about 3 out of every 10 people with the disease died. Many smallpox survivors have permanent scars over large areas of their body, especially their faces. Some are left blind.

Thanks to the success of vaccination, smallpox was eradicated, and no cases of naturally occurring smallpox have happened since 1977. The last natural outbreak of smallpox in the United States occurred in 1949

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).
Viral hemorrhagic fevers (VHFs) refer to a group of illnesses that are caused by several distinct families of viruses. In general, the term "viral hemorrhagic fever" is used to describe a severe multisystem syndrome (multisystem in that multiple organ systems in the body are affected). Characteristically, the overall vascular system is damaged, and the body's ability to regulate itself is impaired. These symptoms are often accompanied by hemorrhage (bleeding); however, the bleeding is itself rarely life-threatening. While some types of hemorrhagic fever viruses can cause relatively mild illnesses, many of these viruses cause severe, life-threatening disease.

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).

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>.

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.

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.

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.

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. 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.

Outbreaks Associated with Drinking Water
Results *All related tables and figures are in the rear of this section*

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) (Figure 3). 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 (*27*), 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 (*27*). 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 (*22*).

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 (*28*).

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 (*30*).

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).

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 (*32*). 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 (*2*). 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. 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.

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 (*40*,*41*). 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 aide 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.

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.

Table 1

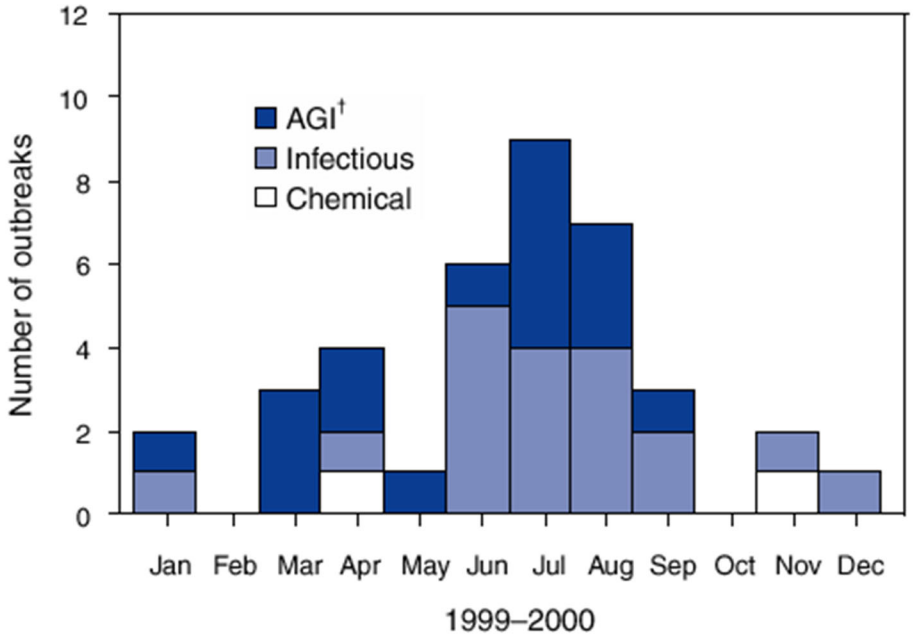
TABLE 1. Classification of investigations of waterborne-disease outbreaks — United States*

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.

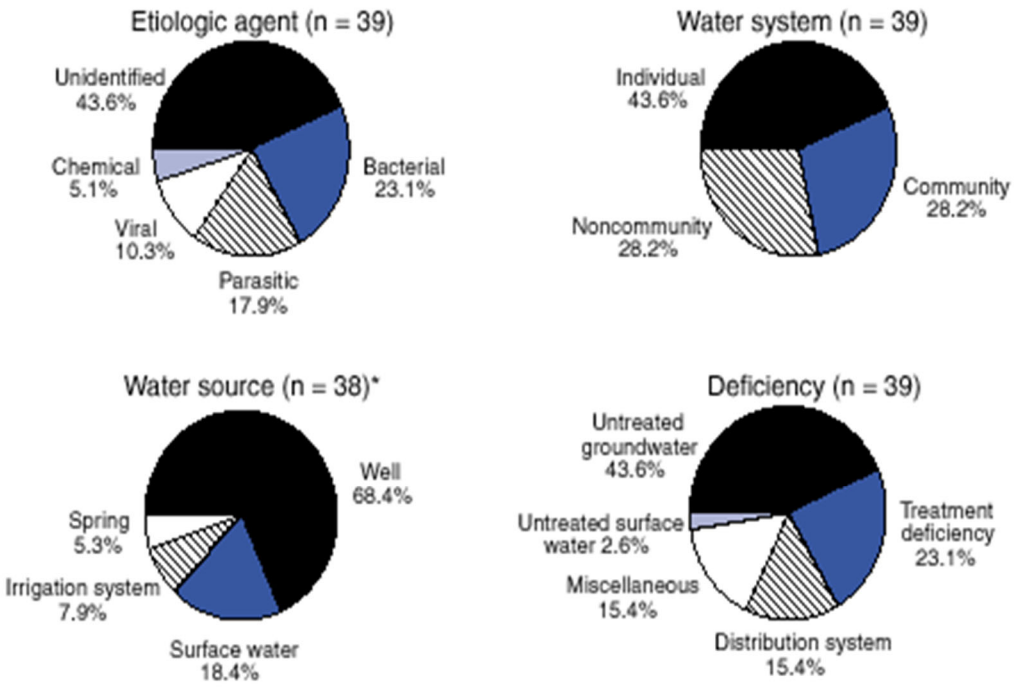
§§ Three persons had stool specimens that tested positive for small round-structured virus, and one person's stool specimen tested positive for *Ca. jejuni*.

¶¶ 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*.

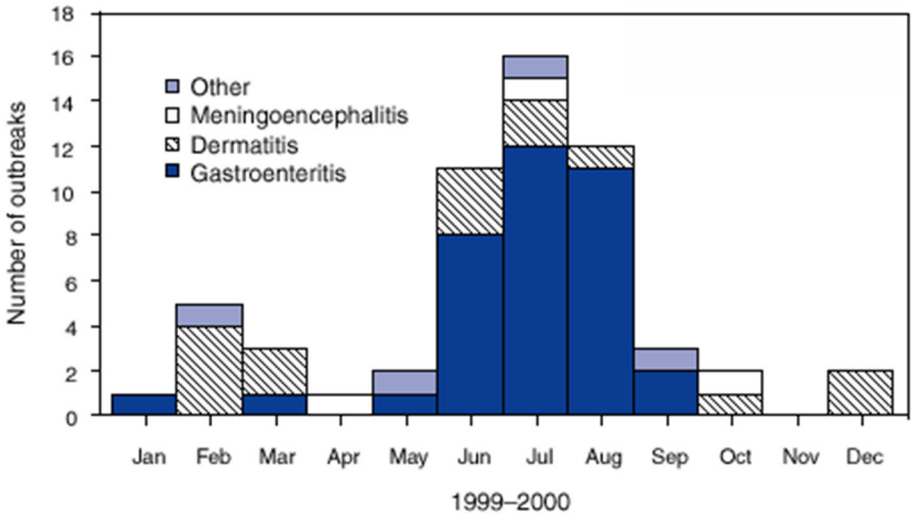
*** Type of water was not specified on report form.

††† 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

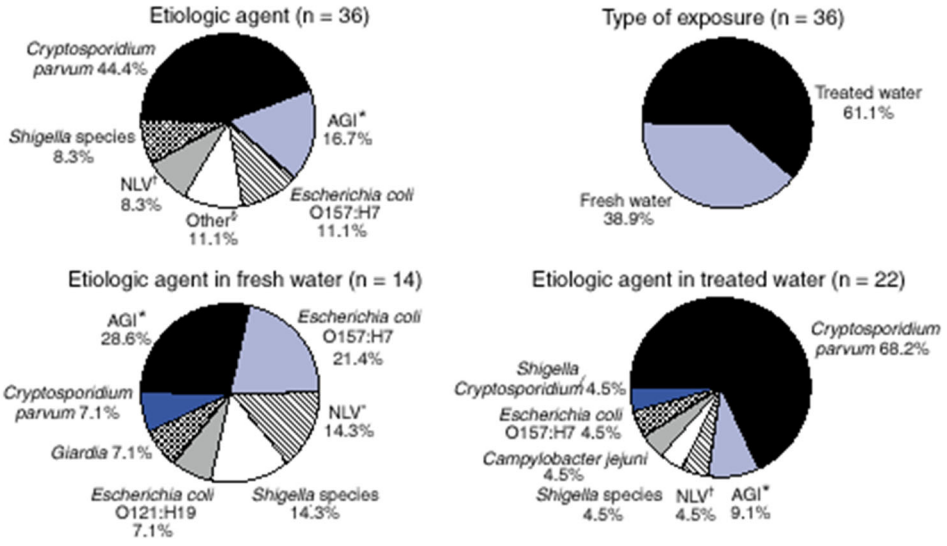
TABLE 4. Waterborne-disease outbreaks associated with drinking water, by etiologic agent and type of water system — United States, 1999–2000 (n = 39)

	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)

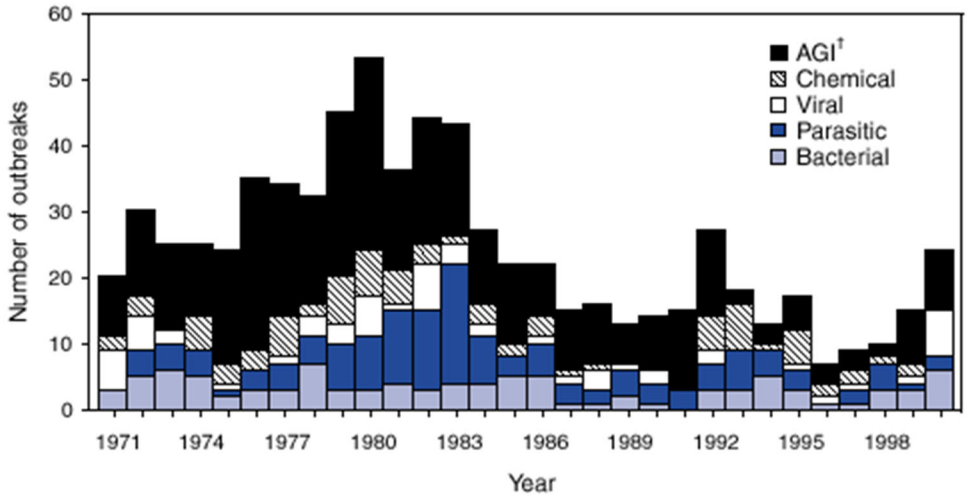
	Type of water system*							
	Community		Noncommunity		Individual		Total	
Type of deficiency†	Outbreaks	%	Outbreaks	%	Outbreaks	%	Outbreaks	%
Untreated surface water	0	0	0	0	1	5.9	**1**	**2.6**
Untreated groundwater	1	9.0	8	72.7	8	47.0	**17**	**43.6**
Inadequate treatment	5	45.5	3	27.3	1	5.9	**9**	**23.1**
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

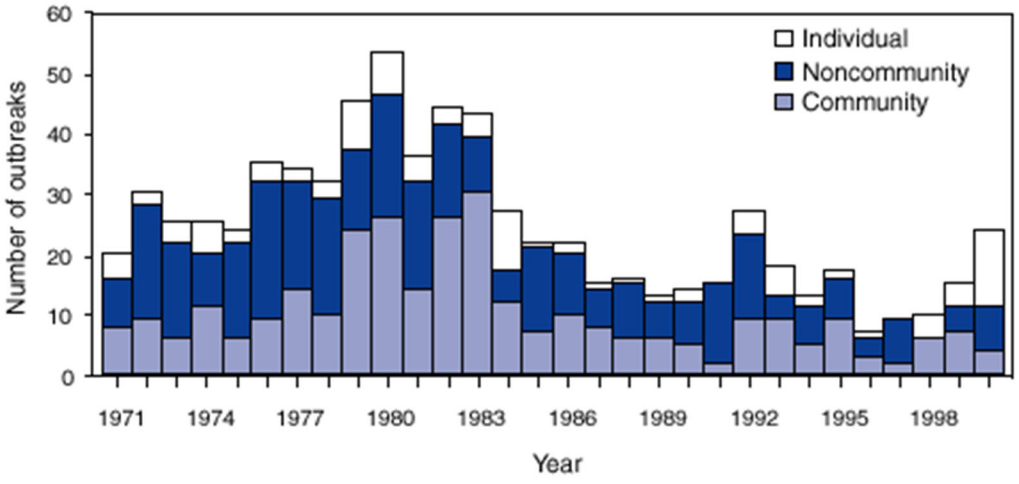
TABLE 6. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 1999 (n = 15)

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.

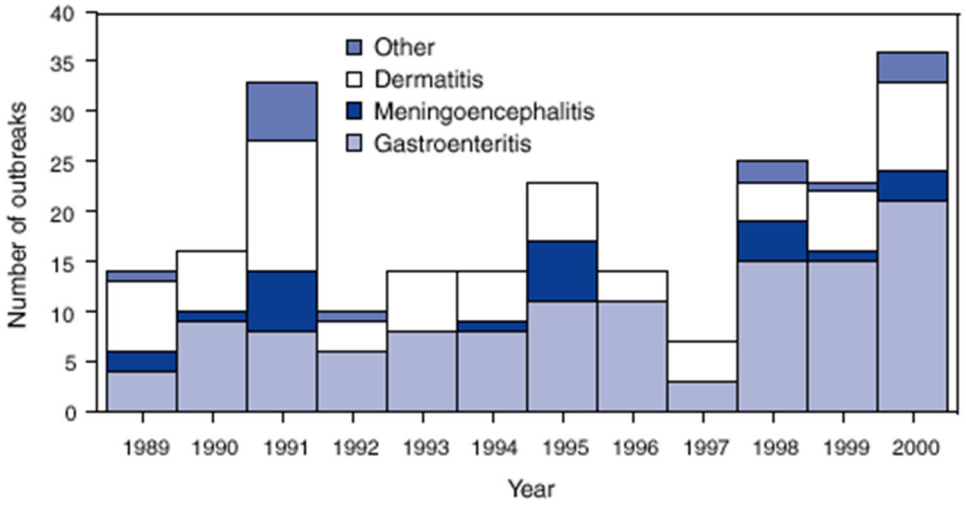
§ Persons swam in a community pool and an inflatable pool.

¶ 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)

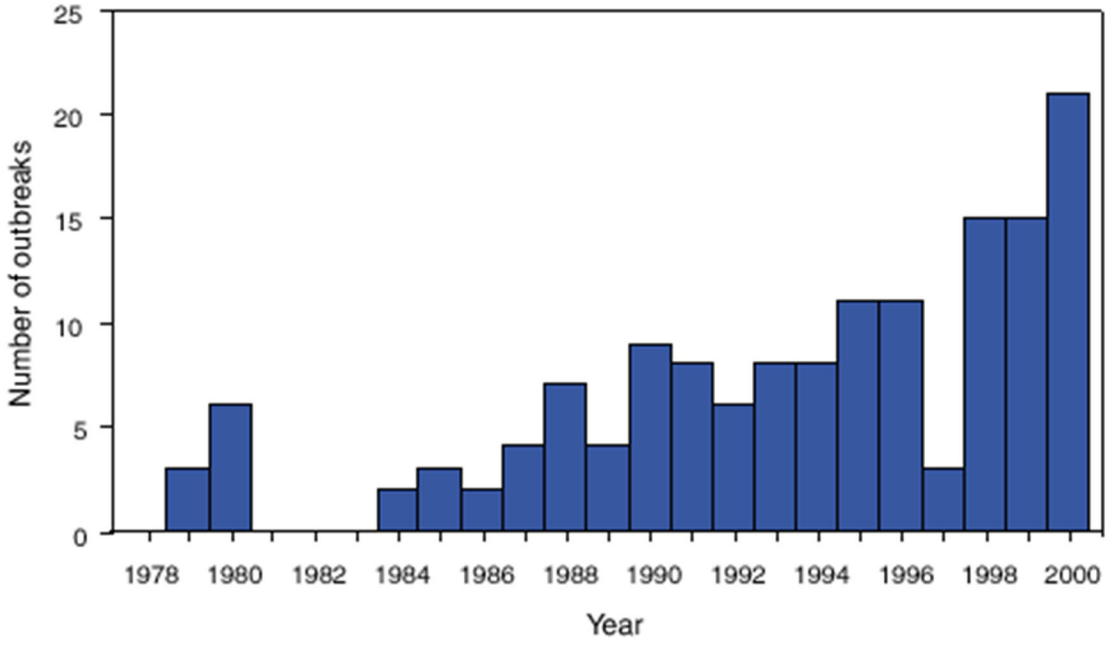


Table 9

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)

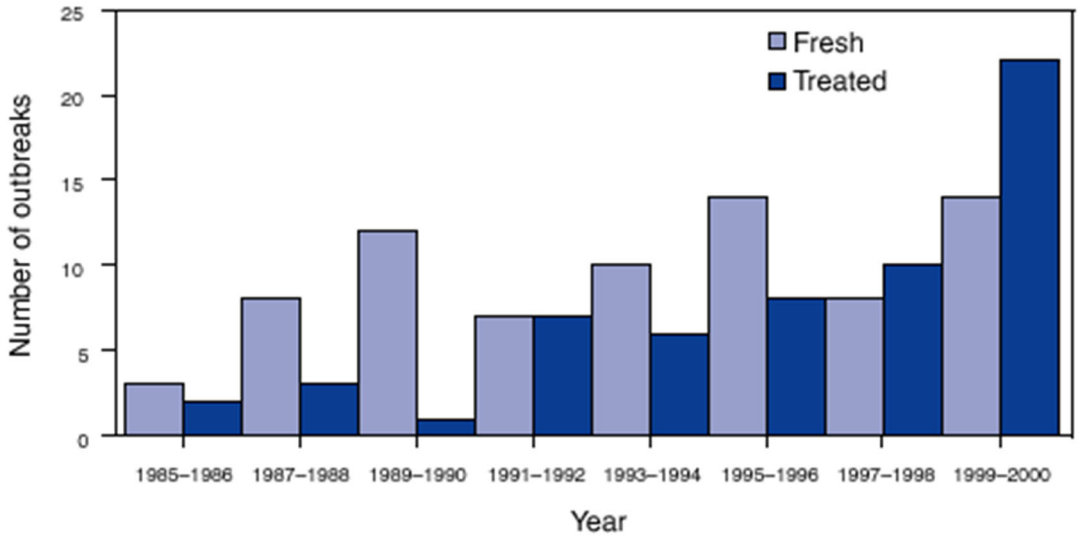


Table 10

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 References

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Related Diseases and Associated Illnesses -Post Quiz

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 _____.
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. Calciviruses
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. Calciviruses
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. Calciviruses
E. None of the above

31. The main symptoms of viral _____ are watery diarrhea and vomiting.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Calciviruses
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. Calciviruses
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. Calciviruses
E. None of the above

Rotavirus 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.
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 ____, 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.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

57. 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 _____.
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

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.
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.
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. None of above

92. 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

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.
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

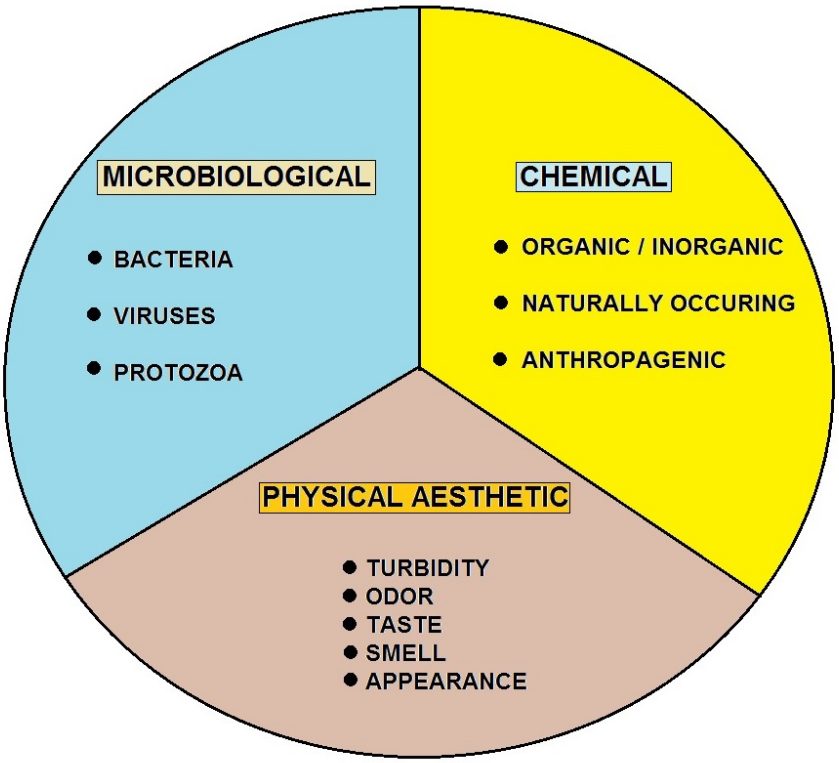
Answers

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

Laboratory Analysis - Chapter 8

Section Focus: You will learn the basics of water laboratory analysis with an emphasis on Chlorine and microorganisms. At the end of this section, you will be able to describe disinfection related testing and microbial examination techniques. 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: Laboratory analysis of water quality refers primarily to the chemical, physical, biological, and radiological characteristics of water. It is a measure of the condition of water relative to compliance or process control requirements. Laboratory analysis is frequently used by reference to a set of standards against which compliance, generally achieved through treatment of the water, can be assessed



WATER QUALITY BROKEN DOWN INTO 3 BROAD CATEGORIES

Quality of Water – Primary Factors

If you classified water by its characteristics and could see how water changes as it passes on the surface and below the ground it would be in these four categories:

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

Chemical characteristics are the elements found that are considered alkali, metals, and non-metals such as fluoride, sulfides or acids. The consumer relates it to scaling of faucets or staining.

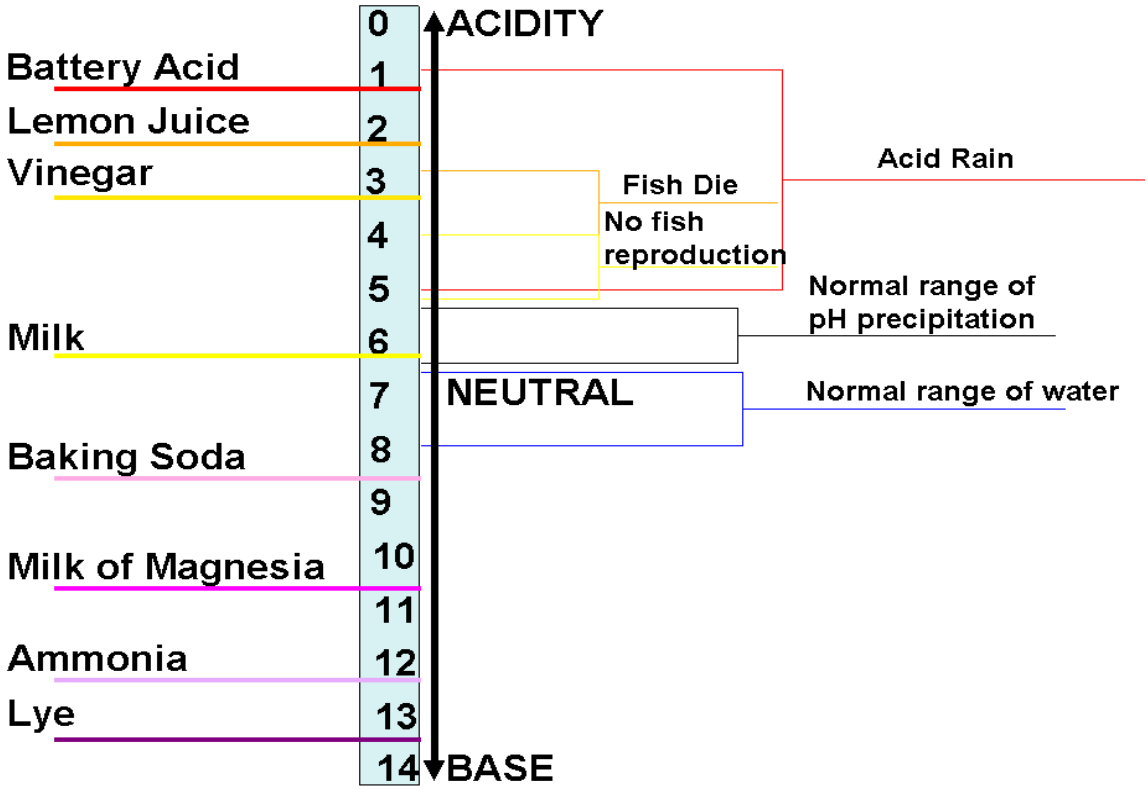
Biological characteristics are the presence of living or dead organisms. This will also interact with the chemical composition of the water. The consumer will become sick or complain about hydrogen sulfide odors--the rotten egg smell.

Radiological characteristics are the result of water coming in contact with radioactive materials. This could be associated with atomic energy. We will not cover this concern in this course.

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

WATER QUALITY FACTORS

pH Section



Basics

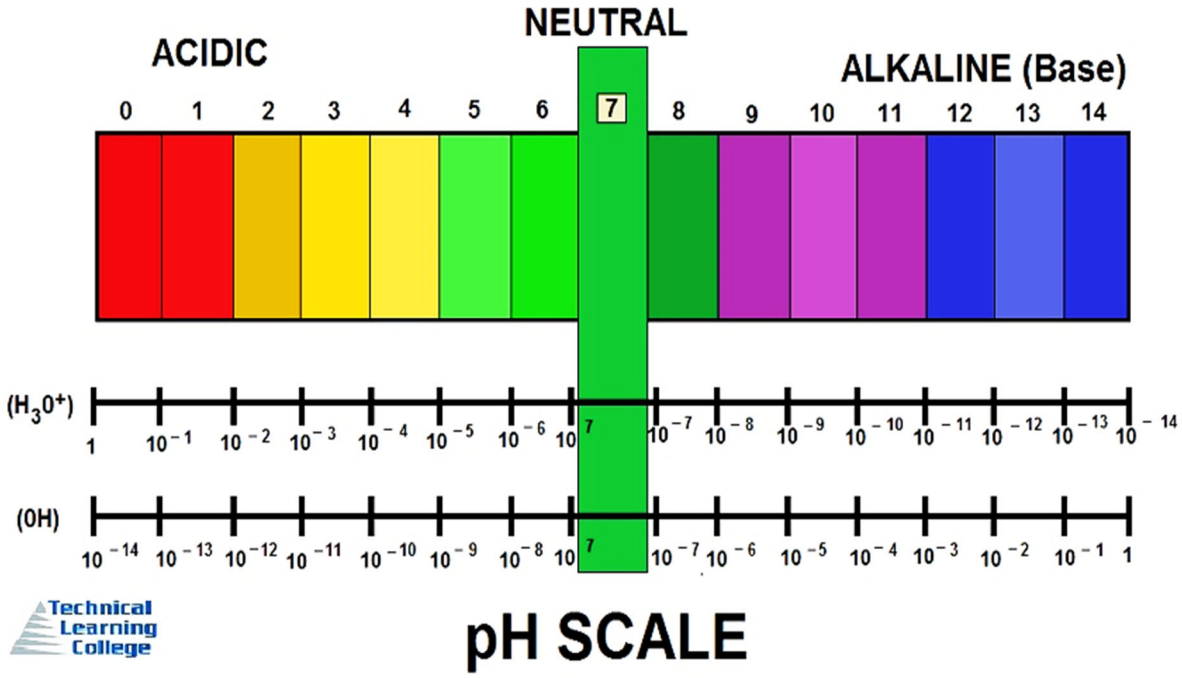
pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral.
A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity.

A pH of 7, there are an equal amount or number of hydroxyl (OH-) and Hydrogen (H+) ions in the solution.

A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. 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.

pH = (**Power of Hydroxyl Ion Activity**).
The acidity of a water sample is measured on a pH scale. This scale ranges from **0** (maximum acidity) to **14** (maximum alkalinity). The middle of the scale, **7**, represents the neutral point. The acidity increases from neutral toward **0**.

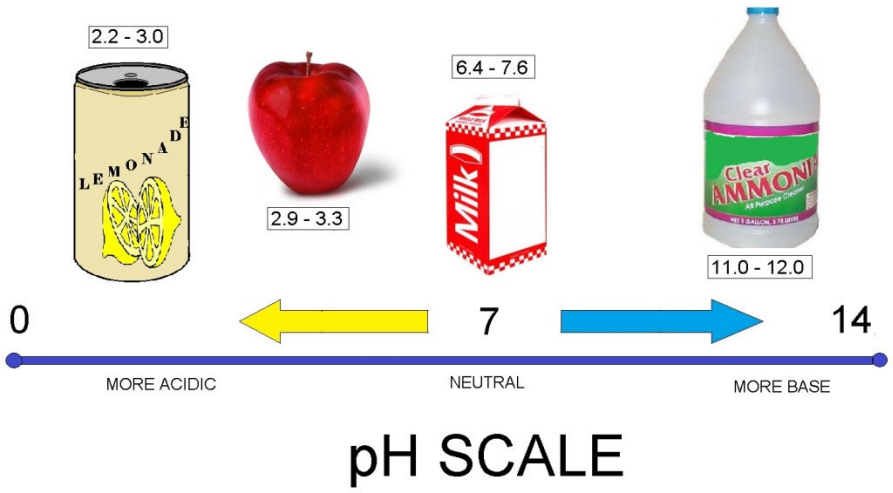
Because the scale is logarithmic, a difference of one pH unit represents a tenfold change. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on.



Hydrogen Ion pH Comparison Chart

pH	Hydrogen Ion Concentration, mmol/L
14	0.00000000000001
13	0.0000000000001
12	0.000000000001
11	0.00000000001
10	0.0000000001
9	0.000000001
8	0.00000001
7	0.0000001
6	0.000001
5	0.00001
4	0.0001
3	0.001
2	0.01
1	0.1

pH Testing Section



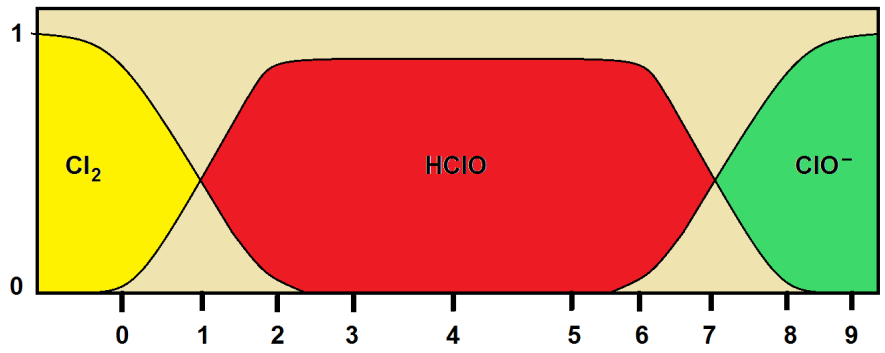
pH SCALE

As a water treatment operator, you will need to master pH sampling and testing. 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, and many other applications.

In water and wastewater processes, **pH** is a measure of the acidity or basicity of an aqueous solution.

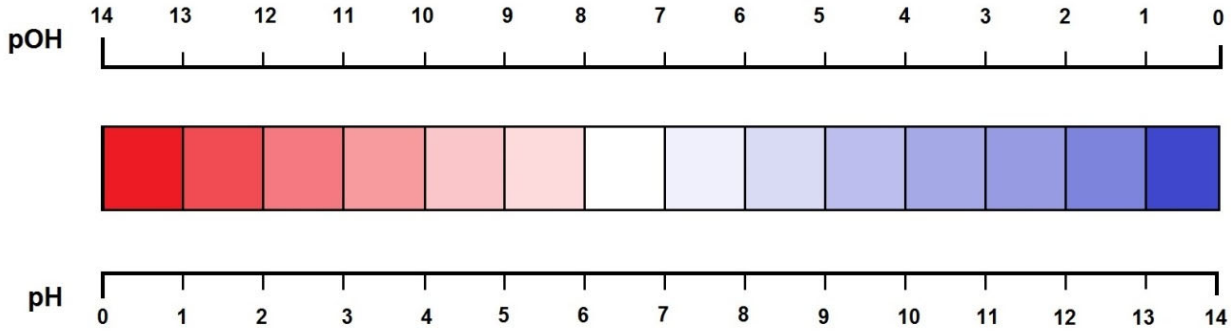
The pH scale is traceable to a set of standard solutions whose pH is established by international agreement.

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 a silver chloride electrode. 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 VALUES CHANGE WITH THE ADDITION OF DIFFERENT TYPES OF CHLORINE

Mathematically, pH is the measurement of hydroxyl ion (H+) 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.



RELATIONSHIP BETWEEN p(OH^-) & p (H^+)
red = ACIDIC / blue = BASIC)

History
The scientific discovery of the p[H] concept 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.

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

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

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.

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 human caused carbon dioxide emissions. pH measurement can be complicated by the chemical properties of seawater, and several distinct pH scales exist in chemical oceanography.

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 $\mathbf{pH_T}$. The bottom line: do not use a fresh water pH meter to measure the pH of seawater.

Calculation 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

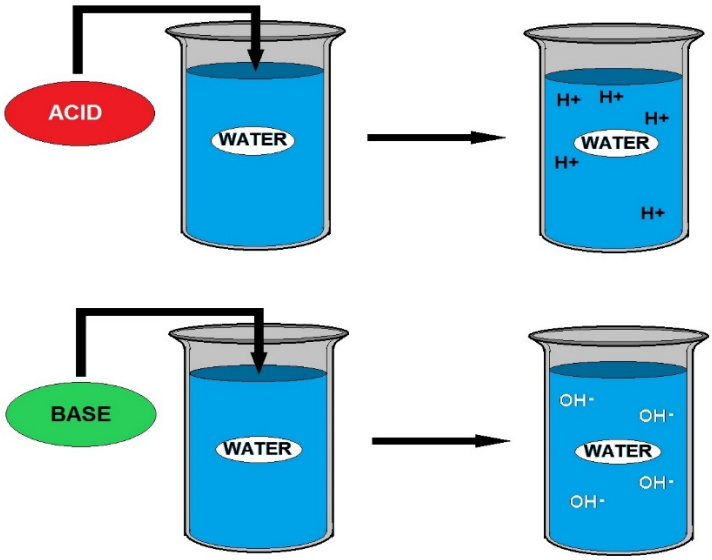
$$K_w = [H^+][OH^-]$$

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.

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.

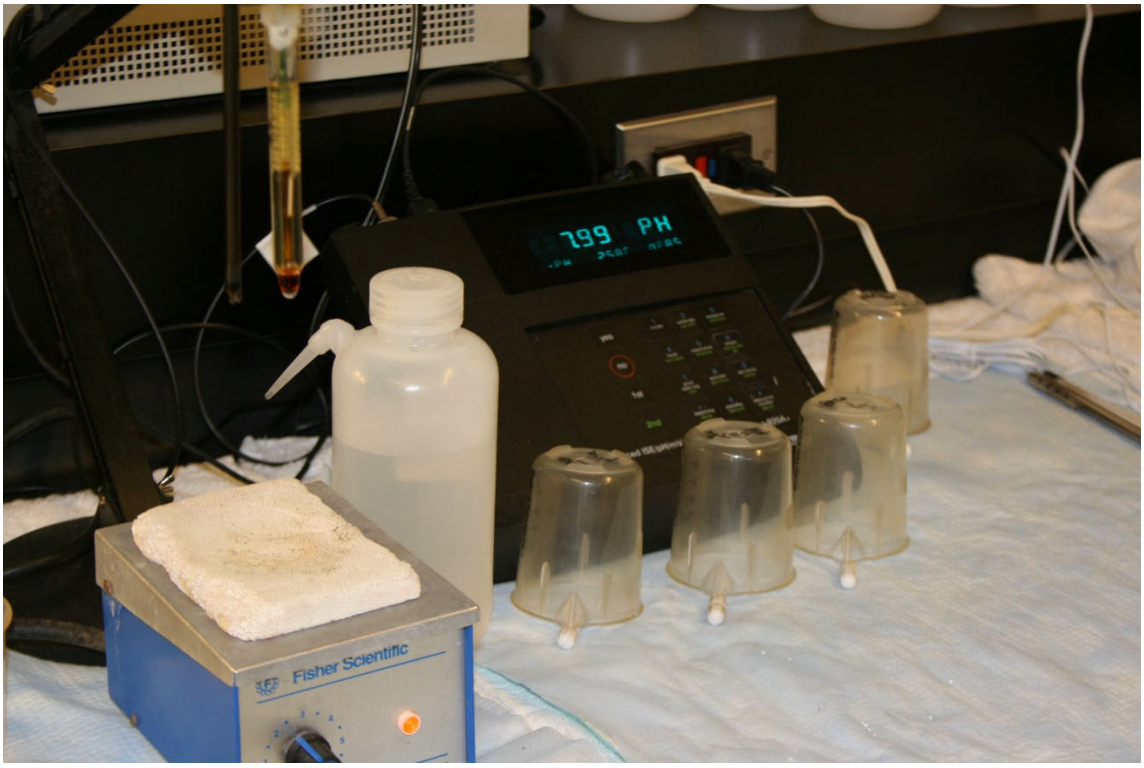
One way to provide them is to apply the law of mass conservation in terms of the two "reagents" H and A.

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

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.

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



Digital pH Meter

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 and pH Adjustment

Adjusting pH and alkalinity is the most common corrosion control method because it is simple and inexpensive. pH is a measure of the concentration of hydrogen ions present in water; alkalinity is a measure of water's ability to neutralize acids.

Generally, water pH less than 6.5 is associated with uniform corrosion, while pH between 6.5 and 8.0 can be associated with pitting corrosion. Some studies have suggested that systems using only pH to control corrosion should maintain a pH of at least 9.0 to reduce the availability of hydrogen ions as electron receptors. However, pH is not the only factor in the corrosion equation; carbonate and alkalinity levels affect corrosion as well.

Generally, an increase in pH and alkalinity can decrease corrosion rates and help form a protective layer of scale on corrodible pipe material.

Chemicals commonly used for pH and alkalinity adjustment are hydrated lime ($CaOH_2$ or calcium hydroxide), caustic soda (NaOH or sodium hydroxide), soda ash (Na_2CO_3 or sodium carbonate), and sodium bicarbonate ($NaHCO_3$, essentially baking soda).

Care must be taken, however, to maintain pH at a level that will control corrosion but not conflict with optimum pH levels for disinfection and control of disinfection by-products.

Corrosion Inhibitors

Inhibitors reduce corrosion by forming protective coatings on pipes. The most common corrosion inhibitors are inorganic phosphates, sodium silicates and mixtures of phosphates and silicates. These chemicals have proven successful in reducing corrosion in many water systems.

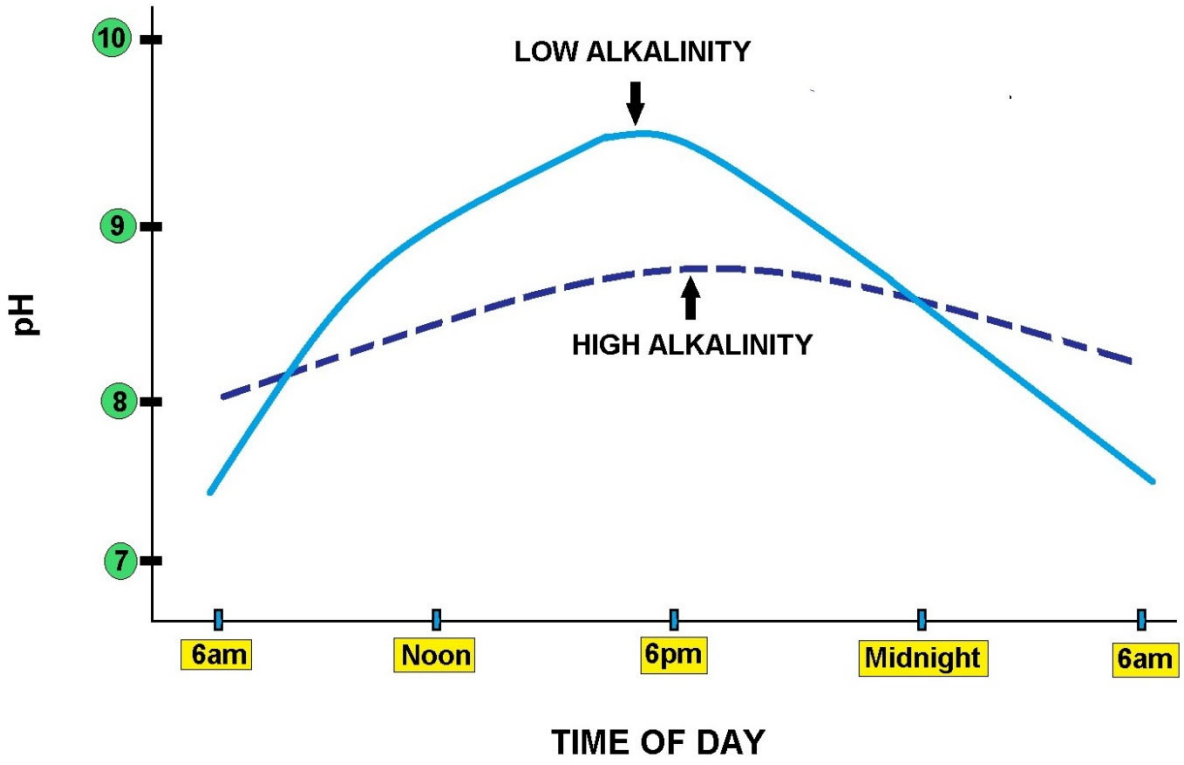
The phosphates used as corrosion inhibitors include polyphosphates, orthophosphates, glassy phosphates and bimetallic phosphates. In some cases, zinc is added in conjunction with orthophosphates or polyphosphates.

Glassy phosphates, such as sodium hexametaphosphate, effectively reduce iron corrosion at dosages of 20 to 40 mg/l.

Glassy phosphate has an appearance of broken glass and can cut the operator. Sodium silicates have been used for over 50 years to inhibit corrosion. The effectiveness depends on the water pH and carbonate concentration.

Sodium silicates are particularly effective for systems with high water velocities, low hardness, low alkalinity and a pH of less than 8.4.

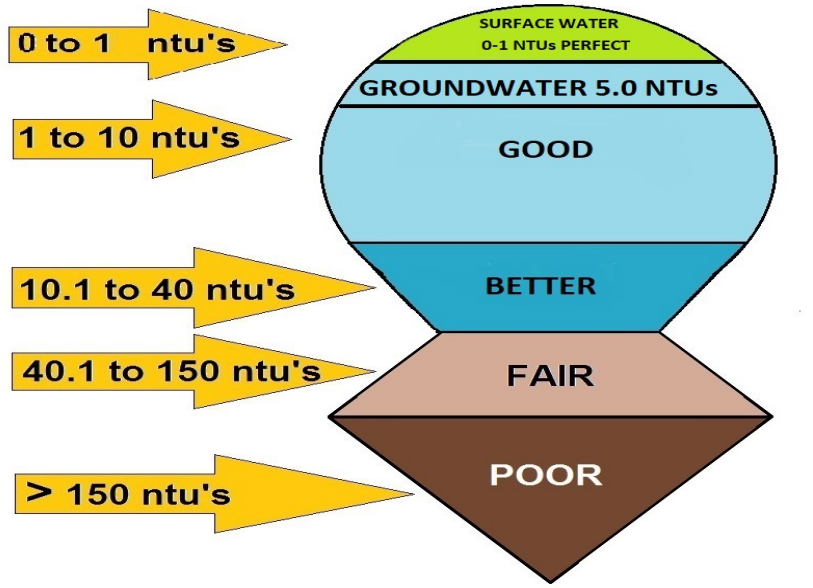
Typical coating maintenance doses range from 2 to 12 mg/1. They offer advantages in hot water systems because of their chemical stability. For this reason, they are often used in the boilers of steam heating systems.



ALKALINITY CAN CHANGE THROUGHOUT THE DAY DIAGRAM

Turbidity Testing Sub-Section

Suspension of particles in water interfering with passage of light is called turbidity. Turbidity is caused by wide variety of suspended matter that range in size from colloidal to coarse dispersions, depending upon the degree of turbulence, and ranges from pure inorganic substances to those that are highly organic in nature. Turbid waters are undesirable from an aesthetic point of view in drinking water supplies. Turbidity is measured to evaluate the performance of water treatment plants.



TURBIDITY PARAMETERS (NTU) FOR WATER QUALITY

Surface Water (SW) System Compliance

- 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)

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.

Turbidity Key

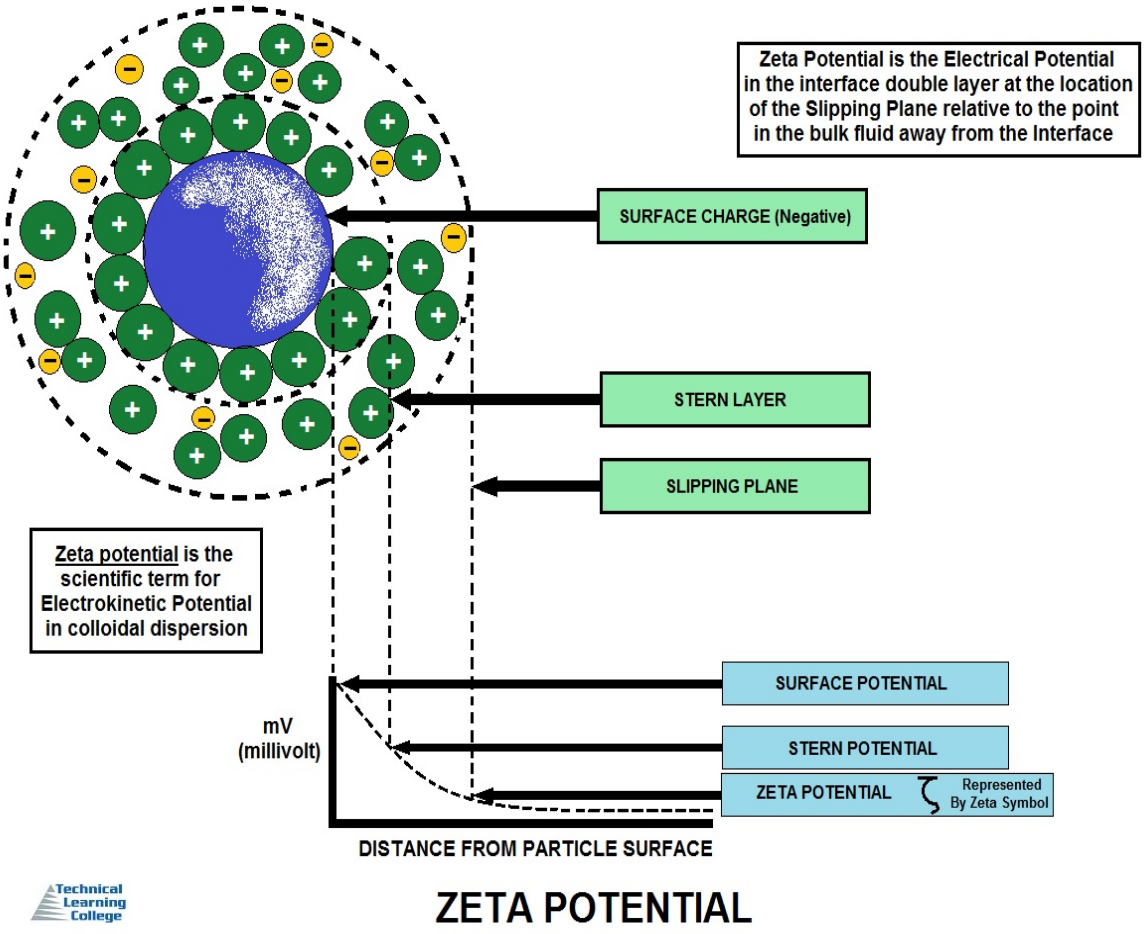
- Turbidity can also be measured in ppm (parts per million) and its size is measured in microns. Turbidity can be particles in the water consisting of finely divided solids, larger than molecules, but not visible by the naked eye; ranging in size from .001 to .150mm (1 to 150 microns).

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

Cloudy Water

Particles less than or about 1 to 10 μm in diameter (primarily colloidal particles) will not settle out by gravitational forces, therefore making them very difficult to remove. These particles are the primary contributors to the turbidity of the raw water causing it to be *"cloudy".* The most important factor(s) contributing to the stability of colloidal particles is not their mass, but their surface properties.

This idea can be better understood by relating the colloidal particles' large surface area to their small volume (S/V) ratio resulting from their very small size. In order to remove these small particles, we must either filter the water or somehow incorporate gravitational forces such that these particles will *settle* out. In order to have gravity affect these particles, we must somehow make them larger, somehow have them come together (agglomerate); in other words, somehow make them *"stick"* together, thereby increasing their size and mass.



The two primary forces that control whether or not colloidal particles will agglomerate are:

Repulsive Force

An electrostatic force called the **"Zeta Potential**" - $$\zeta = \frac{4 \pi q d}{D}$$

Where:

ζ = Zeta Potential
q = charge per unit area of the particle
d = thickness of the layer surrounding the shear surface through which the charge is effective
D = dielectric constant of the liquid

Attractive force

Force due to van der Waals forces

Van der Waals forces are weak forces based on a polar characteristic induced by neighboring molecules. When two or more nonpolar molecules, such as He, Ar, H_2, are in close proximity, the nucleus of each atom will weakly attract electrons in the counter atom resulting, at least momentarily, in an asymmetrical arrangement of the nucleus.

This force, van der Waals force, is inversely proportional to the sixth power of the distance ($1/d^6$) between the particles. As can clearly be seen from this relationship, decay of this force occurs exponentially with distance.

Ways to Measure Turbidity

1.) Jackson Candle Test
2.) Secchi Disk - a black and white disk divided like a pie in 4 quadrants about 6" in diameter. This device is lowered by a rope into the water until it cannot be seen and then the rope is measured.
3.) Turbidimeter - Light is passed through a sample. A sensitive photomultiplier tube at a 90^o angle from the incident light beam detects the light scattered by the particles in the sample. The photomultiplier tube converts the light energy into an electrical signal, which is amplified and displayed on the instrument. The reading is expressed in
Nephelometric Turbidity Unit (NTU) or Formazin Turbidity Unit (FTU).

How to Treat Turbidity

By supercharging the water supply momentarily with a positive charge, we can upset the charge effect of the particle enough to reduce the Zeta potential (repulsive force), thereby allowing van der Waals forces (attractive forces) to take over.

By introducing aluminum (Al_3^+) into the water in the form of Alum ($Al_2(SO_4)_3 \bullet nH_20$) we can accomplish the supercharging of the water. This is the *coagulation* part of the coagulation/flocculation process; flocculation follows coagulation.

During the *flocculation* process the particles join together to form flocs; the larger the flocs, the faster they will settle within a clarifier.

Other chemical coagulants used are Ferric Chloride and Ferrous Sulfate.

Alum works best in the pH range of natural waters, 5.0 - 7.5. Ferric Chloride works best at lower pH values, down to pH 4.5.

Ferrous Sulfate works well through a range of pH values, 4.5 to 9.5.

During the coagulation process, charged hydroxy-metallic complexes are formed momentarily (i.e. $Al(OH)_2^+$, $Al(OH)_2^{1+}$ etc.). These complexes are charged highly positive, and therefore upset the stable negative charge of the target particles, thereby momentarily displacing the water layer surrounding the charged particle. This upset decreases the distance "d," in turn decreasing the Zeta potential.

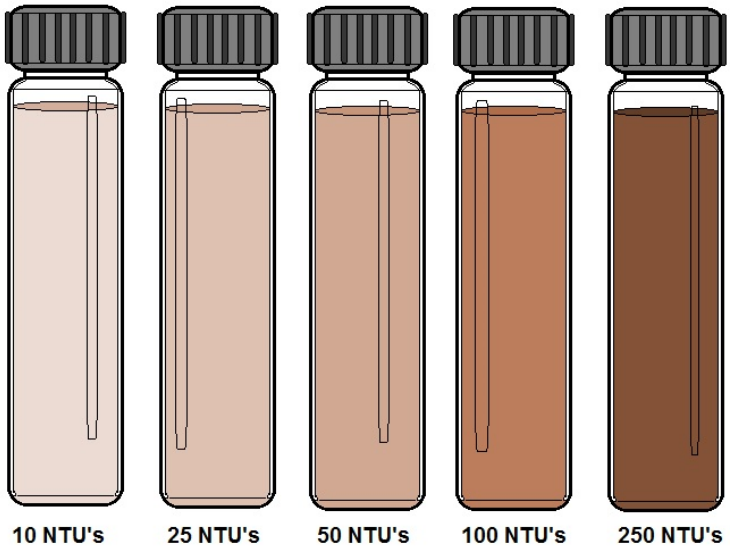
The particles are then able to get close enough together for van der Waals forces to take over and the particles begin to flocculate. The chemical reaction continues until the aluminum ions (Al^+_3) reach their final form, $Al(OH)_3$ (s), and settle out (note – the flocculated particles settle out separately from the precipitated $Al(OH)_3$ (s)).

If too much alum is added, then the opposite effect occurs--the particles form sub complexes with the Al^+_3 and gain a positive charge about them, and the particles re-stabilize.

The final key to obtaining good flocs is the added energy put into the system by way of rotating paddles in the flocculator tanks. By *"pushing"* (adding energy) the particles together we can aid in the flocculation process, forming larger flocs.

It is important to understand that too much energy, i.e. rotating the paddles too fast, would cause the particles to shear (breakup), thereby reducing the size of the particles and increasing the settling time in the clarifier.

Turbidity Analysis



TURBIDITY SAMPLES IN NTU's (Neophelometric Turbidity Unit)

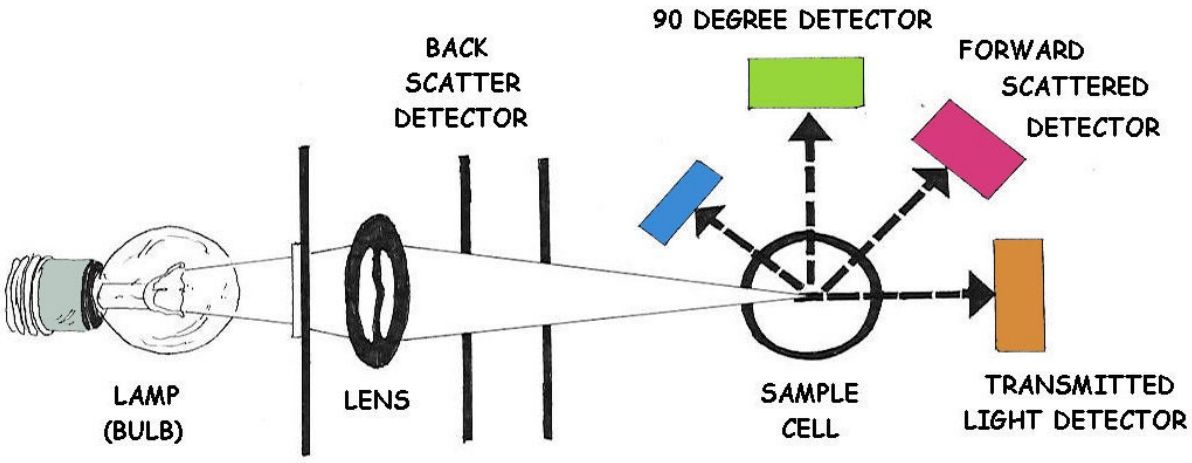
Principle
Turbidity can be measured either by its effect on the transmission of light, which is termed as Turbidimetry, or by its effect on the scattering of light, which is termed as Nephelometry. A Turbidimeter can be used for samples with moderate turbidity and a Nephelometer for samples with low turbidity. The higher the intensity of scattered light, the higher the turbidity.

Interference
Color is the main source of interference in the measurement of turbidity.

Apparatus Necessary: Turbidimeter or Nephelometer.

Reagents
1. Solution I: Dissolve 1.0 gm Hydrazine Sulfate and dilute to 100 mL.
2. Solution II: Dissolve 10.0 gm Hexamethylene tetramine and dilute to 100 mL.
3. Mix 5 mL of I with 5 mL of II. Allow to stand for 24 hrs. at 25 $\pm$ 3°C and dilute to 100 mL. This solution (III) will have turbidity of 400 units (N.T.U.)
4. Standard turbidity suspension: Dilute 10 mL of solution III as prepared above to 100 mL to have solution of the turbidity of 40 units. (N.T.U.)

Procedure
1. Prepare calibration curve in the range of 0-400 units by carrying out appropriate dilutions of solutions III and IV above taking readings on turbidimeter.
2. Take sample or a suitably diluted aliquot and determine its turbidity either by visual comparison with the diluted standards or by reading on turbidimeter.
3. Read turbidity from the standard curves and apply correction due to dilution, if necessary.
4. Report the readings in turbidity units.



HOW AN TURBIDIMETER WORKS

Residual Chlorine Sub-Section
Test Methods Available for Residual Chlorine

Residual Chlorine can be measured using different methods. Iodometric and DPD colorimetric methods are the most common methods. Each method has its own set of reagents and concentration range.

Iodometric Method
Residual Chlorine by Iodometric has a minimum detectable concentration of 40ppb if 0.01N sodium thiosulfate is used. Prepare the sample for titration by adding 5mL of acetic acid and 1g of potassium iodide to the sample. Titrate the sample with 0.01N sodium thiosulfate. Concentrations below 1 mg/L should be measured by using either 0.00564N sodium thiosulfate or 0.00564N phenylarsine oxide.

DPD Colorimetric Method
Residual Chlorine can also be measured by the DPD Colorimetric method. This method has a minimum detectable concentration of 10ppb. In this method, the calibration is either made up from a chlorine solution or a potassium permanganate solution. The typical calibration range for this method is 0.05 to 4mg/L.

The reagents used in this method are a phosphate buffer and N,N-diethyl-p-phenylenediamine indicator solution. The samples are mixed with the reagents and then read on a spectrophotometer at a wavelength of 515nm.

Chlorine in water solutions is not stable. As a result, its concentration in samples decreases rapidly. Exposure to sunlight or other strong light, air, or agitation will further reduce the quantity of chlorine present in solutions.

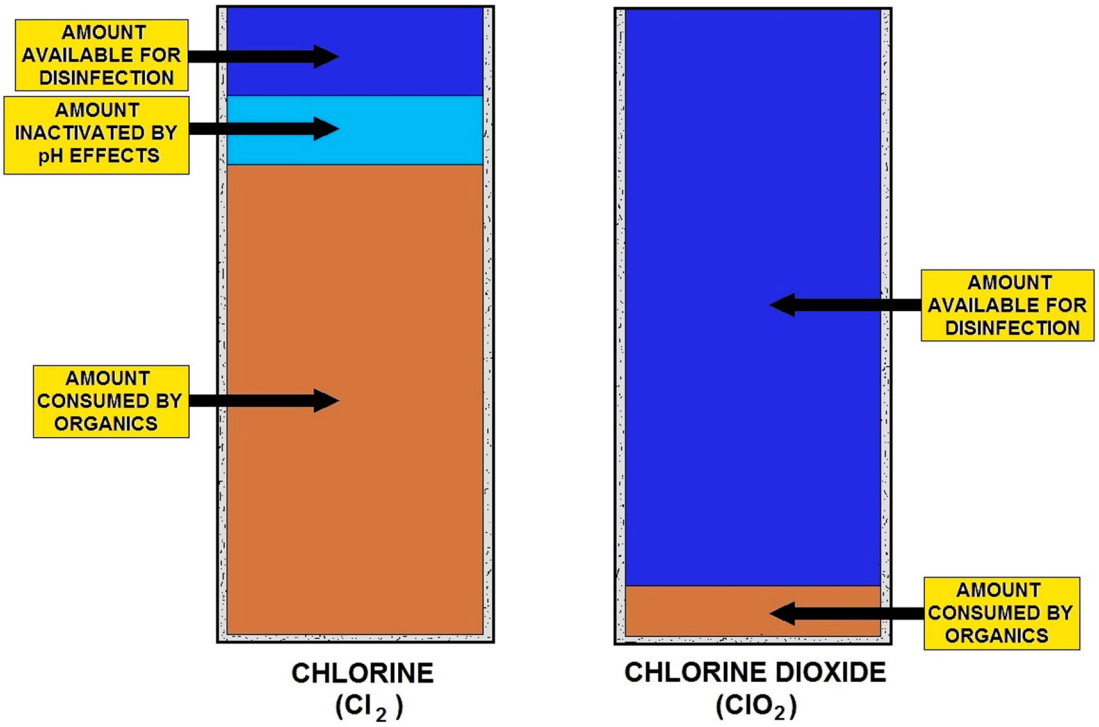
Samples to be analyzed for chlorine cannot be stored or preserved.

Tests must be started immediately after sampling. Therefore, samples taken for the chlorine residual test must be grab samples only and excessive agitation must be avoided.

It is not necessary to use special sampling devices or containers for the chlorine residual test. However, the sampling container should be capable of collecting samples from a representative sampling point following chlorine contact, and should be made of resistant materials that will not rust or corrode, and which can be easily cleaned.

NOTE: A long handled aluminum dipper attached to a wooden handle, or an equivalent device, is acceptable for collecting samples. Do not use coffee cans, bleach bottles, etc.

Preparation of Chemicals
At a minimum, hand and eye protection should be used when handling any of the chemicals mentioned in this section. Before working with any chemical, consult the appropriate Safety Data Sheet (formerly MSDS) (SDS) to determine if other safety precautions are necessary.



THE DIFFERENCE IN USING CHLORINE AND CHLORINE DIOXIDE AS A DISINFECTANT

Chlorine Residual Reagents

Iodometric and Amperometric Methods:

I. Standard Phenylarsine Oxide (PAO) Solution, 0.00564 N

A. Prepare 0.3 N sodium hydroxide solution (NaOH) by dissolving 12.0 g NaOH in 800 mL distilled water and diluting to 1 liter.

B. Prepare a 6.0 N hydrochloric acid solution (HCl) by adding 108 mL concentrated HCl to 800 mL distilled water and diluting to 1 liter. (Caution: Concentrated HCl fumes can burn eyes and lungs—do not breathe fumes!)

C. Prepare an approximately 0.00564 N solution of PAO using the following procedures:

1. Dissolve approximately 0.8 g PAO powder in 150 mL of 0.3 N NaOH solution, and allow to settle.

2. Decant 110 mL into 800 mL distilled water and mix thoroughly.

3. Bring to pH 6 to 7 with 6N HCl and dilute to 950 mL with distilled water. (Caution: PAO is poisonous. Wash thoroughly after use and do not ingest.)

D. Standardization

1. Accurately measure 5 to 10 mL freshly standardized 0.0282 N iodine solution into a flask and add 1 mL potassium iodide solution (50g KI dissolved and diluted to 1 L with freshly boiled and cooled distilled water.

2. Titrate with PAO solution, using starch solution as an indicator, until blue disappears.

3. Normality (N) of PAO = (mL iodine solution x 0.0282)/mL PAO titrated.

4. Adjust PAO to 0.00564 N and recheck.

II. Standard Sodium Thiosulfate Solution, 0.00564 N

A. Prepare a 0.1 N sodium thiosulfate solution by dissolving 25 g $Na_2S_2O_3$ $5H_2O$ in 1000 mL of freshly boiled distilled water. Store reagent for at least 2 weeks to allow oxidation of any bisulfite ion present. Add a few mL of chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize by one of the following methods:

1. Iodate Method

a. Dissolve 3.249 g anhydrous primary standard quality potassium bi-iodate ($KH(IO_3)2$) or 3.567 g potassium iodate (KIO_3) dried at 103 +/-2°C for 1 hour in distilled water and dilute to 1000 mL to yield a 0.1000 N iodate solution. Store in a glass stoppered bottle.

b. Add, with constant stirring, 1 mL concentrated sulfuric acid (H_2SO_4), 10 mL 0.1000 N iodate solution, and 1 g potassium iodide (KI) to 80 mL distilled water. Titrate immediately with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color of the liberated iodine is almost discharged. Add 1 mL starch indicator solution and continue titration until the blue color disappears.

c. The normality (N) of the sodium thiosulfate is calculated as follows: N of $Na_2S_2O_3$ = 1/mL $Na_2S_2O_3$ for titration

2. Dichromate Method

A. Dissolve 4.904 g anhydrous primary standard grade potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1000 mL to yield a 0.1000 N dichromate solution. Store in a glass stoppered bottle.

B. For maximum stability of the standard 0.00564 N sodium thiosulfate solution, prepare by diluting an aged 0.1N $Na_2S_2O_3$ standard solution with freshly boiled distilled water. Add 10 mg Mercuric iodide and 4 g of sodium borate per liter of solution. Standardize daily using 0.00564 N potassium dichromate or iodate solution.

III. Standard Iodine Solution (I_2), 0.1 N

A. Dissolve 40 g potassium iodide (KI) in 25 mL chlorine-demand-free water.

B. Add 13 g resublimed iodine (I_2) and stir until dissolved.

C. Transfer to a 1-liter volumetric flask and dilute to the mark.

D. Standardization

1. Volumetrically measure 40 to 50 mL 0.1 N arsenite solution into a flask.

2. Titrate with 0.1 N iodine solution using starch solution as an indicator.

3. Just before end-point is reached, add a few drops of hydrochloric acid solution to liberate sufficient carbon dioxide (CO_2) to saturate the solution.

4. Titrate until blue color first appears and remains.

5. Normality (N) of iodine = (mL of arsenite solution used x 0.1)/mL of iodine titrated

IV. Standard Iodine Titrant (I_2), 0.0282 N

A. Dissolve 25 g KI in a bottle of distilled water in a 1L volumetric flask.

B. Add the correct amount of the exactly standardized 0.1 N iodine solution to yield a 0.0282 N solution.

C. Dilute to one liter with chlorine-demand-free water.

D. Store iodine solutions in amber bottles or in the dark, and protect from exposure to direct sunlight. Do not use rubber stoppers; keep iodine from all contact with rubber.

E. Check titrant normality daily against 0.00564 N PAO or sodium thiosulfate solution. A procedure for calculating a correction factor for this titrant is given in Appendix C.

V. Standard Potassium Iodate Titrant (KIO3), 0.00564 N

A. Dissolve 201.2 mg primary standard grade potassium iodate (KIO_3), dried for 1 hour at 103°C, or 183.3 mg primary standard grade anhydrous potassium bi-iodate ($KH(IO_3)2V$) in distilled water.

B. Dilute to 1 liter volumetrically.

C. Store in glass bottles in the dark and protect from exposure to direct sunlight.

VI. Potassium Iodide Solution (KI), 5% W/V

A. Dissolve 50 g KI in freshly boiled and cooled distilled water and dilute to 1 liter.

B. Store in a brown glass-stoppered bottle in the dark, preferably at 4°C.

C. Discard when solution becomes yellow.

VII. Acetate Buffer Solution, pH 4.0

A. Dissolve 146 g anhydrous sodium acetate ($NaC_2H_3O_2$ $3H_2O$) in 400 mL distilled water.

B. CAREFULLY add 458 mL concentrated (glacial) acetic acid.

C. Dilute to 1 liter with chlorine-demand-free water.

VIII. Standard Arsenite Solution (As_2O_3), 0.1N

A. Accurately weigh a dried, cooled stoppered weighing bottle.

NOTE: Use forceps or tongs—do not handle weighing bottle with fingers.

B. In weighing bottle, weigh out approximately 4.95 g arsenic trioxide (As_2O_3).

C. Transfer without loss to a 1-liter volumetric flask

NOTE: Do not attempt to brush out remaining arsenic trioxide).

D. Reweigh bottle and record weight of arsenic trioxide transferred.

E. Add enough distilled water to moisten the arsenic trioxide.

F. Add 15 g sodium hydroxide (NaOH) and 100 mL distilled water.

G. Swirl flask gently until As_2O_3 is dissolved.

H. Dilute to 250 mL and saturate the solution with carbon dioxide (CO_2) by bubbling CO_2 gas through the solution for a few minutes.

NOTE: This converts the sodium hydroxide (NaOH) to sodium bicarbonate ($NaHCO_3$).

I. Dilute to the 1-liter mark, stopper, and mix thoroughly.

J. This solution has an almost indefinite shelf life.

CAUTION: This solution is highly poisonous and is a suspected cancer causing agent: handle carefully!

IX. Starch Indicator

A. Weigh out 5 g soluble or potato starch.

B. Add enough distilled water to make a thin paste.

C. Pour into 1 liter boiling distilled water, stir and let settle overnight.

D. Transfer clear supernatant into a storage container and preserve by adding 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide per liter of starch solution.

E. Some commercial starch substitutes or powder indicators are acceptable.

X. Phosphoric Acid solution (H_3PO_4), 1 + 9

A. Carefully add 100 mL of phosphoric acid (H_3PO_4), 85%, to 900 mL of freshly boiled distilled water.

B. Caution should be used when handling this solution, as it can be corrosive.

XI. Phosphoric Acid—Sulfamic Acid Solution

A. Dissolve 20 g sulfamic acid (NH_2SO_3H) in 1 liter of 1 + 9 phosphoric acid (H_3PO_4).

DPD Titrimetric Method

I. Phosphate Buffer Solution

A. Dissolve 24 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) in 400 to 500 mL distilled water.

B. Add 46 g anhydrous potassium dihydrogen phosphate (KH_2PO_4).

C. Dissolve 800 mg disodium ethylenediaminetetraacetate dihydrate (EDTA) in a separate container.

NOTE: This chemical is also known as (ethylenediamine) tetraacetic acid sodium salt.

D. Combine the 2 solutions and dilute to 1 liter.

E. Add 20 mg mercuric chloride to prevent mold growth.

F. Caution: Mercuric chloride is toxic. Take care to avoid ingestion.

II. DPD Indicator Solution

A. Add 8 mL of a 1 + 3 sulfuric acid solution (H_2SO_4) into 500 mL distilled water. Prepare by mixing one part concentrated H_2SO_4 to 3 parts distilled water. (For example, 5 mL H_2SO_4 to 15 mL distilled water.)

B. Add 200 mg EDTA (disodium ethylenediaminetetraacetate dihydrate).

C. Add 1 g DPD Oxalate (N, N-Diethyl-p-phenylenediamine oxalate).

D. Dilute to 1 liter and store in a brown glass-stoppered bottle and discard when discolored.

CAUTION: The DPD oxalate is poisonous, handle carefully!

III. Standard Ferrous Ammonium Sulfate (FAS) Titrant, 0.00282 N

A. Add 1 mL of 1 + 3 sulfuric acid solution (H2SO4) to 500 mL of freshly boiled and cooled distilled water. Prepare by adding one part concentrated H2SO4 to 3 parts distilled water.

B. Dissolve 1.106 g ferrous ammonium sulfate ($Fe(NH_4)2(SO_4)2\ 6H_2O$)

C. Dilute to 1 liter.

D. This standard can be used for 1 month before replacement.

E. Standardize weekly using the following procedure:

1. Measure 100 mL of FAS standard solution into an Erlenmeyer flask.

2. Add 10 mL of 1 + 5 sulfuric acid. Prepare by adding one part concentrated H_2SO_4 to 5 parts distilled water.

3. Add 5 mL concentrated phosphoric acid.

4. Add 2 mL 0.1% barium diphenylamine sulfonate indicator. Prepare by dissolving 0.1 g ($C_6H_5NHC_6H_4$-4-SO_3) Ba in 100 mL distilled water.

5. Titrate with 0.100N potassium dichromate (see Iodometric and amperometric section for preparation directions) to a violet end-point that persists for 30 seconds.

DPD Colorimetric Method

I. Phosphate Buffer Solution

(see DPD Titrimetric Method chemicals)

II. DPD Indicator Solution

(see DPD Titrimetric Method chemicals)

III. Potassium Permanganate Stack Solution

A. Dissolve 891 mg potassium permanganate ($KMnO_4$) in distilled water and dilute to 1000 mL.

IV. Potassium Permanganate Standard Solution

A. Dilute 10 mL of stock solution to 100 mL in a volumetric flask.

B. 1 mL of the standard solution diluted to 100 mL with distilled water will be equivalent to 1.0 mg/L chlorine residual in a DPD reaction.

C. Prepare standard solutions by diluting appropriate volumes to 100 mL with distilled water.

If a direct concentration readout colorimeter is used, the DPD and buffer reagents should be prepared or ordered in accordance with the instrument manufacturer's instructions. If the Hach DR100 colorimeter is used, the prepared DPD powder pillows used with the Hach direct reading colorimeters may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

Orion Model 97-70 Electrode Method

With the exception of the 1 ppm potassium iodate standard and the chlorine water (100 ppm), all of the reagents required for this method can be purchased from Orion Research at the following address:

Orion Research Incorporated
840 Memorial Drive
Cambridge, Massachusetts 02139

I. Prepare a 1 mg/L iodate standard by volumetrically diluting 1 mL of the 100 ppm iodate standard to 100 mL with distilled water.

II. Prepare the chlorine water (approximately 100 ppm) by diluting 1 mL hypochlorite solution (household chlorine bleach) to 500 mL with distilled water.

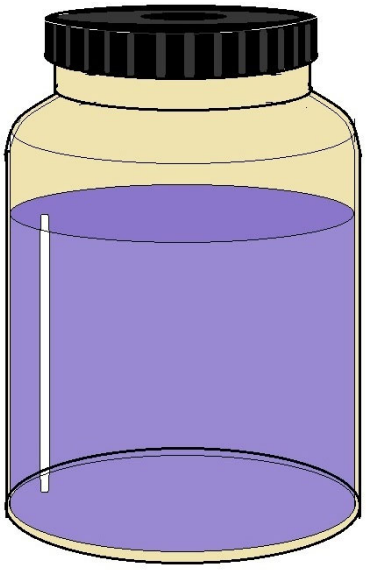
Hach Model CN-66 Test Kit Method

The DPD indicator powder pillows used in the Hach Model CN-66 Test Kit may be purchased from the Hach Company at the following address:

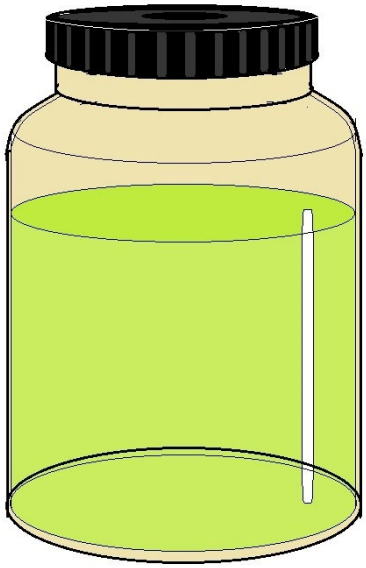
Hach Company
P.O. Box 389
Loveland, Colorado 80539

Bacteriological Sample Processing – Procedures Sub-Section

Examples



COLIFORM POSITIVE SAMPLE



COLIFORM NEGATIVE SAMPLE

COLIFORM BACTERIA COLOR TESTING

Samples need to be kept on ice and shipped to a central laboratory for analysis of coliphage, *C. perfringens*, *Cryptosporidium, Giardia*, and enteric viruses by the current analytical methods. The single-agar layer (SAL), direct plating method with induction of β-galactosidase (Ijzerman and Hagedorn, 1992) is recommended for detection of somatic and F-specific coliphage in streamwater samples. In this method, 100-mL sample volumes are mixed with an agar medium, *E. coli* host culture, chemicals that induce the β-galactosidase enzyme, and appropriate antibiotics. The mixtures are poured into four 150- x 15-mm plates and incubated at 35°C.

Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is dark blue is visible within each plaque. Viral plaques are easily identified and enumerated by the distinct blue circle. Because of contamination by naturally occurring bacteria in streamwater samples, antibiotic- resistant host-culture strains, *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for somatic and F-specific coliphage, respectively. Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water. Because the SAL method is impractical for sample volumes above 100 mL, an alternative method should be used for ground-water sample analysis.

One example, currently being tested by USEPA, is a two-step enrichment presence-absence method (U.S. Environmental Protection Agency, 1999e). Samples for enumeration of *C. perfringens* are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c). Standard MF techniques are used, and the plates are incubated anaerobically for 24 hours at 44.5°C. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as *C. perfringens*. In the laboratory, *C. perfringens* analyses are done on 100-, 30-, and 10-mL volumes of streamwater. In the case of a high-flow or high-turbidity streamwater sample, lower sample volumes may be plated.

Method 1623 (U.S. Environmental Protection Agency, 1999c) is recommended for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. The oocysts are concentrated on a capsule filter from a 10-L water sample, eluted from the capsule filter with buffer, and concentrated by centrifugation. Immunomagnetic separation (IMS) is used to separate the oocysts from other particulates in the sample. In IMS, the oocysts are magnetized by attachment of magnetic beads conjugated to an antibody and then are separated from sediment and debris by means of a magnet.

Fluorescently labeled antibodies and vital dye are used to make the final microscopic identification of oocysts and cysts. The reverse-transcriptase, polymerase chain reaction (RT-PCR) and cell-culture methods are recommended for detection of enteric viruses in water samples (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). To prepare samples for RT-PCR and cell culture, attached viruses are eluted from a 1MDS filter with beef extract (pH 9.5), concentrated using celite (pH 4.0), and eluted with sodium phosphate (pH 9.5).

For RT-PCR analysis, viruses are isolated from the eluate by ultracentrifugation through a sucrose gradient, and trace contaminants are removed by extraction with a solvent mixture. During these steps, the 10-L streamwater sample (or 2,000-L ground-water sample) is concentrated down to 40 µL. An aliquot of the concentrate is used for RT-PCR, wherein any target viral RNA is converted to DNA and amplified by use of an enzymatic process. The RT-PCR products are analyzed by agarose gel electrophoresis and confirmed by hybridization. The enteric viruses detected by use of this method include enterovirus, hepatitis-A, rotavirus, reovirus, and calicivirus.

For cell-culture analysis, the sample eluate is added to a monlayer of a continuous cell line derived from African green monkey kidney cells (U.S. Environmental Protection Agency, 1996c). Each cell culture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water.

QA/QC Activities and Measures

QA/QC activities and measures to take to reduce contamination.

• Use a sterilization indicator, such as autoclave tape, in preparing sample bottles and other equipment for collection of microbiological samples to determine whether adequate temperatures and pressures have been attained during autoclaving.
• Prepare a separate set of sterile equipment for microbiological sampling at each site.
• Before processing samples in the field vehicle, wipe down the area with a disinfectant (such as isopropyl alcohol) to ensure a sterile working surface.

• Monitor the incubators daily to ensure temperatures are appropriate for the methods used.

For bacteria samples, membrane-filtration (MF) equipment and MF procedure blanks are used to estimate analytical bias.

Field personnel should do the following:
• Prepare an MF equipment blank, a 50- to 100-mL aliquot of sterile buffered water plated before the sample—for every sample by field personnel for total coliform, *E. coli*, and enterococci analyses to determine the sterility of equipment and supplies.
• Prepare a MF procedure blank, a 50- to 100-mL aliquot of sterile buffered water plated after the sample— for every fourth sample to measure the effectiveness of the analyst's rinsing technique or presence of incidental contamination of the buffered water.

If contamination from a MF equipment or procedure blank is found, results are suspect and are qualified or not reported. Proper and consistent procedures for counting and identifying target colonies will be followed, as described in Myers and Sylvester (1997).
• After counting, turn the plate 180° and ensure the second count is within 5 percent of the first count. Have a second analyst check calculations of bacterial concentrations in water for errors.

For coliphage, *Cryptosporidium, Giardia*, and enteric virus samples, equipment and field blanks are used to determine sampling and analytical bias. Equipment blanks for these analyses are different from the MF equipment blanks for bacterial analysis. An equipment blank is a blank solution (sterile buffered water) subjected to the same aspects of sample collection, processing, storage, transportation, and laboratory handling as an environmental sample, but it is processed in an office or laboratory. Field blanks are the same as equipment blanks except that they are generated under actual field conditions.

• For enteric virus analysis, collect one equipment blank after collection of the first sample to ensure that equipment cleaning and sterilization techniques are adequate.
• For coliphage, *Cryptosporidium, Giardia*, and enteric virus analyses, collect field blanks periodically.
At a minimum, the number of field blanks should equal 5 percent of the total number of samples collected. Five percent of samples collected for bacterial and viral indicators (total coliforms, *E. coli*, enterococci, *C. perfringens*, and coliphage) should be nested replicate samples to estimate sampling and analytical variability. For streamwater samples, concurrent replicates to estimate sampling variability are collected by alternating subsamples in each vertical between two collection bottles. For ground-water samples, sequential replicates are collected one after another into separate sterile bottles. Concurrent and sequential replicates are then analyzed in duplicate (split replicates) to estimate analytical variability.
• Because of the expense associated with collection and analysis of samples for pathogens (*Cryptosporidium* and enteric viruses), collect only one replicate sample per year at a site wherein detection of pathogens was found in an earlier sample.
To assess analytical bias of the sampling and analytical method, 2 to 5 percent of the samples collected for enteric virus should be field matrix spikes.

• Run all but 10 L of ground water through the 1 MDS filter and collect the remaining 10 L in a carboy. In the laboratory, the poliovirus vaccine will be added to the 10 L and then passed through the same 1MDS filter. Analysis will be done by use of the cell-culture and RT-PCR methods. • All cell-culture positive samples are serotyped to identify or discount laboratory contamination. Because of the variability in the performance of Method 1623 for recovery of *Cryptosporidium* and *Giardia*, each sample will be collected in duplicate—one will be a regular sample and the other a matrix spike. The laboratory will add a known quantity of cysts and oocysts to the matrix spike to determine recovery efficiency, as described in USEPA (1999c).

Quality Assurance and Quality Control in the Laboratory

The following criteria may be used to evaluate each production analytical laboratory: (1) appropriate, approved, and published methods, (2) documented standard operating procedures, (3) approved quality-assurance plan, (4) types and amount of quality-control data fully documented and technical defensible, (5) participation in the standard reference sample project (6) scientific capability of personnel, and (7) appropriate laboratory equipment.

The microbiology laboratories must follow good laboratory practices—cleanliness, safety practices, procedures for media preparation, specifications for reagent water quality—as set forth by American Public Health Association (1998) and Britton and Greeson (1989). Some specific guidelines are listed in the following paragraphs.

Reference cultures are used by the central laboratory to evaluate the performance of the test procedures, including media and reagents. Pure cultures of *E. coli, Enterobacter aerogenes,* and *Streptococcus faecalis* (American Type Culture Collection, Rockville, Md.) are used to ensure that MF culture media and buffered water are performing adequately.

A pure culture of *C. perfringens*, isolated from a sewage sample and verified by standard procedures, is used to evaluate the test procedure and each lot of media and reagents.

Because contamination of samples from coliphage during the analytical procedure is highly probable (Francy and others, 2000), a negative control of host and sterile buffered water is run concurrently with each batch of samples.

In addition, to ensure that the method is being executed properly, a positive-control sewage sample is run with each batch of samples. A laminar flow safety hood is recommended for processing the samples for coliphage analysis.

Alternatively, a separate coliphage room may be established to discourage laboratory contamination during the analytical process. An ultraviolet light is installed and operated for 8 hours every night in the safety hood or coliphage room to reduce contamination.

The laboratory should follow the QA/QC guidelines in Method 1623 (U.S. Environmental Protection Agency, 1999c) for *Cryptosporidium* and *Giardia* and in the cell-culture and RT-PCR analysis for enteric viruses (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c).

Protozoan Pathogens

The principal protozoan pathogens that affect the public health acceptability of waters in the United States are Giardia lamblia (Giardia) and Cryptosporidium parvum (Cryptosporidium). These organisms are widely distributed in the aquatic environment and have been implicated in several recent outbreaks of waterborne disease, including a well-publicized outbreak of cryptosporidiosis in Milwaukee, Wisconsin (Rose and others, 1997). Both Giardia and Cryptosporidium produce environmentally resistant forms (called cysts and oocysts), which allow for the extended survival of the parasites in water and treated water.

Because cysts and oocysts are more resistant to disinfection and survive longer in the environment than bacterial indicators, fecal-indicator bacteria are not adequate indicators for Giardia and Cryptosporidium in source waters. The presence of protozoan pathogens in water, therefore, must be verified by identification of the pathogens themselves.

The USEPA-required method for detection of Giardia and Cryptosporidium in source and drinking water under the ICR involves nominal porosity filtration and indirect fluorescent antibody procedures (U.S. Environmental Protection Agency, 1996c).

The ICR method has been criticized for being difficult to implement, being characterized by poor recovery of target organisms, and yielding highly variable results (U.S. Environmental Protection Agency, 1996b). As a result, the USEPA supported the development of Method 1622 for Cryptosporidium (U.S. Environmental Protection Agency, 1998b), and Method 1623 for Giardia and Cryptosporidium (U.S. Environmental Protection Agency, 1999c). Method 1622 was validated through an Interlaboratory study and revised as a final, valid method in January 1999.

Understanding Routine Coliform Sampling

Streamwater Sample Collection

When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles.

The standard samplers can be used to collect streamwater samples for bacterial and viral indicators, *Cryptosporidium*, and *Giardia* providing that the equipment coming in contact with the water is properly cleaned and sterilized.

For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

- Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.
- Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment
(EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.
- Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI

methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).

• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for *E. coli* and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for *C. perfringens* and coliphage analysis.

Method 1623

For *Cryptosporidium* and *Giardia* analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 20 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.

• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for *Cryptosporidium* analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.). The sampling equipment is obtained from the analyzing laboratory; for example, the USGS Ohio District Laboratory has modified the sampling apparatus (G. Shay Fout, U.S. Environmental Protection Agency, 1997) into a self-contained box with easy-to-use control valves. The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Groundwater Sample Collection
Collecting ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps. Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible. Because this is operationally prohibitive for private domestic wells, a tap that yields water directly from the well and before entering the holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.
• Document the stage of the distribution system from which water was collected and details about the distribution system, including the type of tank and condition of the tank and pipes.

In addition, if the well can easily be opened for inspection, document the condition of the well, including the sanitary seal (if any) and the amount of debris in the well. Any information on the location of the well, including proximity to septic systems or feedlots, should also be documented in the field at the time of sampling.

For wells without in-place pumps, samples should be obtained by use of the following methods
(in descending order from most to least desirable):
(1) a peristaltic or vacuum pump with autoclavable silicon tubing, (2) a sterile bailer, (3) a chlorine-disinfected pump and tubing, or (4) a detergent-cleaned pump and tubing. Pre-sampling activities, such as purging, must be carried out in such a way as to avoid contaminating the well. All equipment must be properly cleaned and sterilized between sites, using a Liquinox wash and a thorough tap water or deionized-water rinse. If using this last method, collect additional field blanks to evaluate the effectiveness of the cleaning procedure. Refer to Myers and Sylvester (1997) for a detailed discussion of ground-water sampling for microbiological analysis.

Because ground water is less prone to microbiological contamination than surface water, larger volumes of ground water are needed than of surface water.
• For regular sampling, collect 3 L of ground water for bacterial and viral indicators.
• Process the sample for total coliforms, *E. coli*, and enterococci using 200-mL sample volumes for each analysis; send the remainder (at least 2.5 L) to the laboratory for coliphage analysis. In the laboratory, coliphage analysis is done using 1 L for somatic and 1 L for F-specific coliphage.
• For enteric virus analysis by RT-PCR and cell culture, use the same sampler for ground-water samples as for streamwater samples; pump 2,000 L of ground water through the sampling apparatus and 1MDS filter.

Sample Preservation and Storage
Holding times for samples before processing are 6 hours for total coliforms, *E. coli*, and enterococci and 24 hours for *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and the 1MDS filters for enteric viruses by RTPCR and cell culture.
• After collection, immediately store the sample on ice.
• Be sure to keep the sample out of direct sunlight, because ultraviolet rays kill microorganisms.
• Add sodium thiosulfate to sample bottles for bacterial and viral indicators if the water collected contains residual chlorine. (Samples may have residual chlorine if the sampling site is downstream from a wastewater-treatment plant that chlorinates its effluents). Add ethylene diaminetetracetic acid to sample bottles when water is suspected to contain trace elements such as copper, nickel, and zinc at concentrations greater than 1 mg/L (Britton and Greeson, 1989, p. 5-6; U.S. Environmental Protection Agency, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). (Sodium thiosulfate or ethylene diaminetetracetic acid are not added to containers for *Cryptosporidium* and *Giardia)*.

Analytical Methods

Field Analysis

Analysis of water samples for total coliforms, *E. coli,* and enterococci, are done by use of membrane filtration (MF) or most-probable number (MPN) methods. Because membrane filtration is easier to use and provides a more precise quantification of bacteria than MPN, MF is recommended for most analyses. Refer to Myers and Sylvester (1997) for complete MF procedures.

Different MF methods are used for quantification of bacteria in ground-water and streamwater samples.

• For examining streamwater samples for *E. coli*, use the USEPA-recommended mTEC agar method (Environmental Protection Agency, 1986b).
• For examining ground-water samples for total coliforms and *E. coli*, use the MI method (Brenner and others, 1993).
• For enterococci, use the mEI method (U.S. Environmental Protection Agency, 1997).
• For streamwater, plate sufficient sample volumes in order to obtain at least one plate in the ideal count range. For ground water, a 200-mL sample volume is usually sufficient.

Testing of new microbiological monitoring methods and comparing the recoveries of new methods to the USEPA-approved method can be done by use of the NAWQA network.

For groundwater samples, for example, one may include a commercially available MPN kit, Colilert (Idexx Laboratories, Westbrook, Maine), for simultaneous detection of total coliforms and *Escherichia coli*.

For streamwater sampling, one may include a single-step modified Mtec medium with 5-bromo-6-chloro-3-indolyl'β-d-glucuronide (Bennett Smith, USEPA, Cincinnati, Ohio, oral commun., 1997); this method was developed to replace the mTEC method. Other new methods can be added to the monitoring program for field testing as they are developed.

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 Sheet (formerly MSDS)s 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-cytometer sorted 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/SDS.

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 aperture. The numerical aperture 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 µL volume 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 ^{o}C to 8 ^{o}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 °C to 25 °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 $^{\circ}$C to 42 $^{\circ}$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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20.6 *ICR Microbial Laboratory Manual*, EPA/600/R-95/178, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 Martin Luther King Drive, Cincinnati, OH 45268 (1996).
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20.9 "Envirochek™ Sampling Capsule," PN 32915, Gelman Sciences, 600 South Wagner Road, Ann Arbor, MI 48103-9019 (1996).
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20.12 USEPA. Implementation and Results of the Information Collection Rule Supplemental Surveys. EPA-815-R-01-003. Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division, Washington, DC (2001).
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21.0 Tables and Figures
Table 1. Method Holding Times (See Section 8.2 for details)

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) Ongoing precision and recovery (percent)	9.7	24 - 100 55 11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery1, 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*

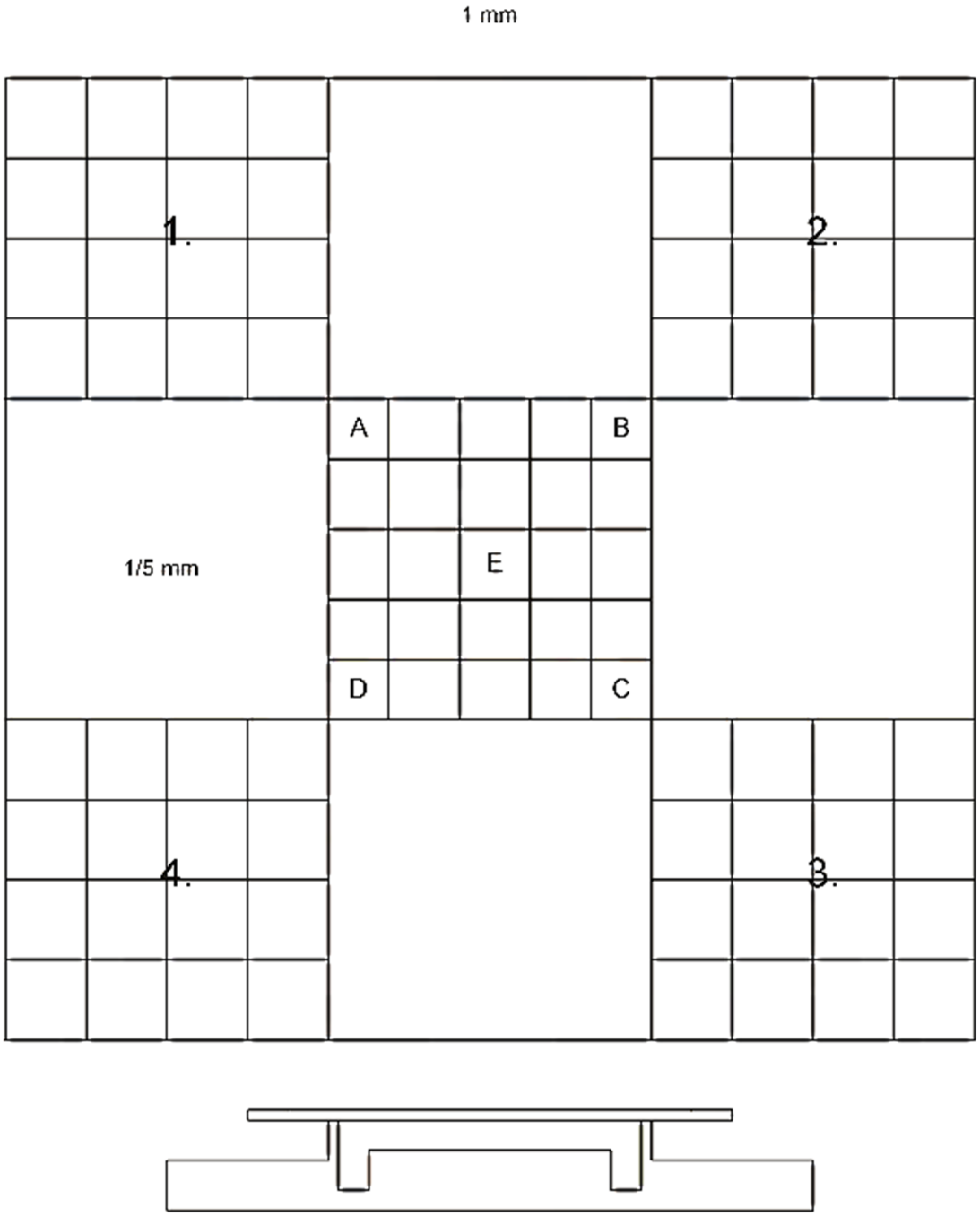
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation) Ongoing precision and recovery (percent)	9.7	24 - 100 49 14 - 100
Matrix spike/matrix 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)

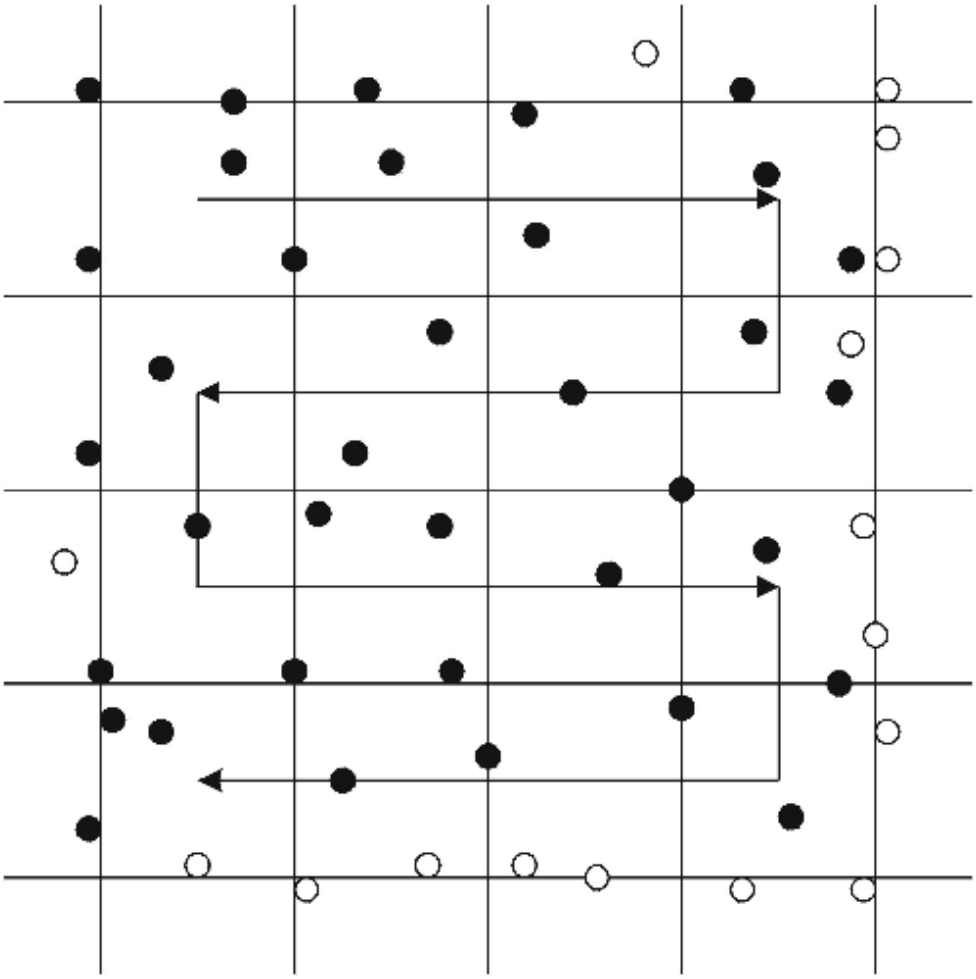
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium* MS Samples in Recovery Range	Percent of 270 *Giardia* MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 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

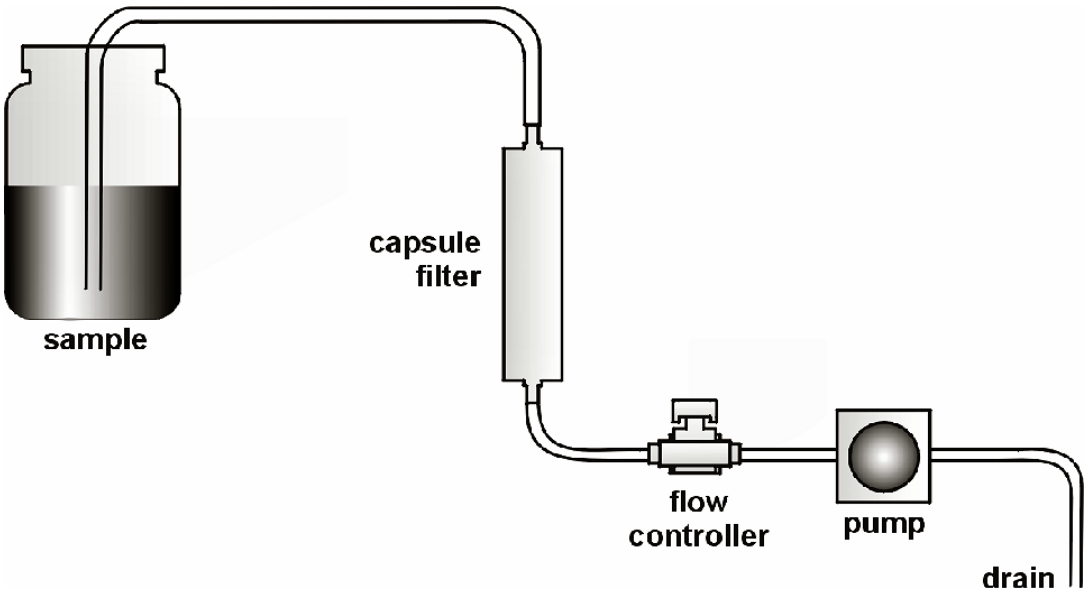
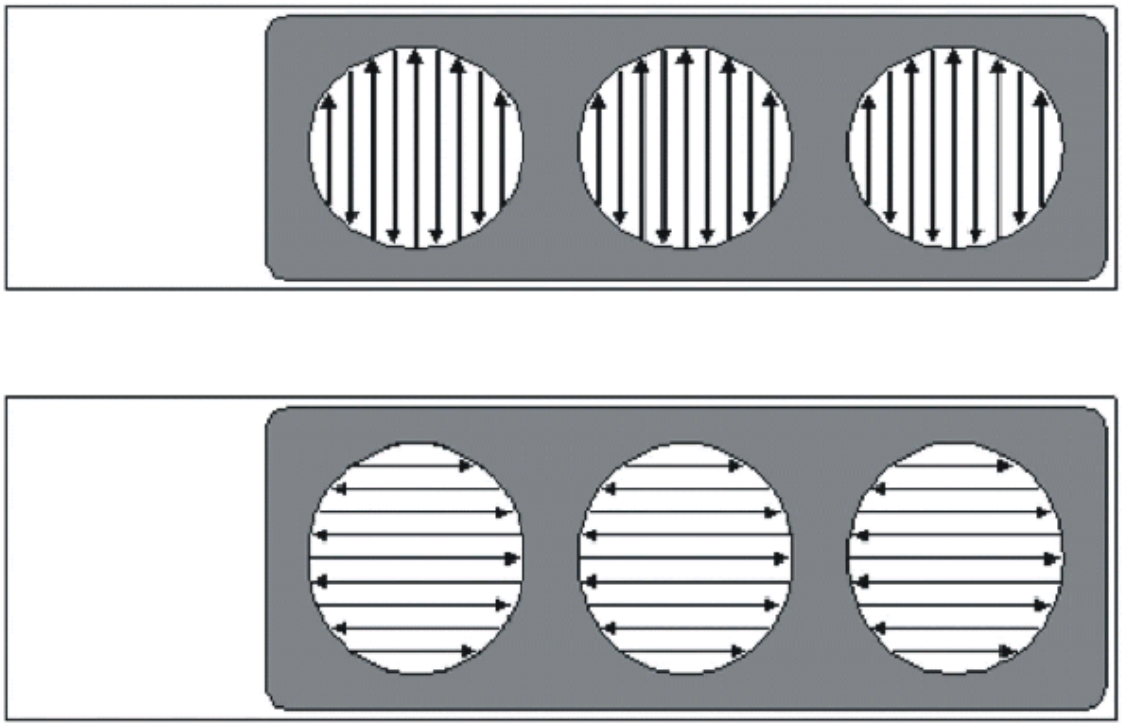


Figure 4. Methods for Scanning a Well Slide



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

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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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16.11 Buntel, C.J. 1995. E. coli $-Glucuronidase (GUS) as a Marker for Recombinant Vaccinia Viruses. BioTechniques 19 (3); 352-353.
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16.13 Federal Register. 1994. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants; Final Rule. *Federal Register* 59: 62456-62471.
16.14 Federal Register. 1999. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule. *Federal Register* 64: 67450-67467.
16.15 U.S. Environmental Protection Agency. 1992. Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, Third Edition. EPA-814B-92-002, Office of Ground Water and Drinking Water, Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

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] $\bar{\chi}$, 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.

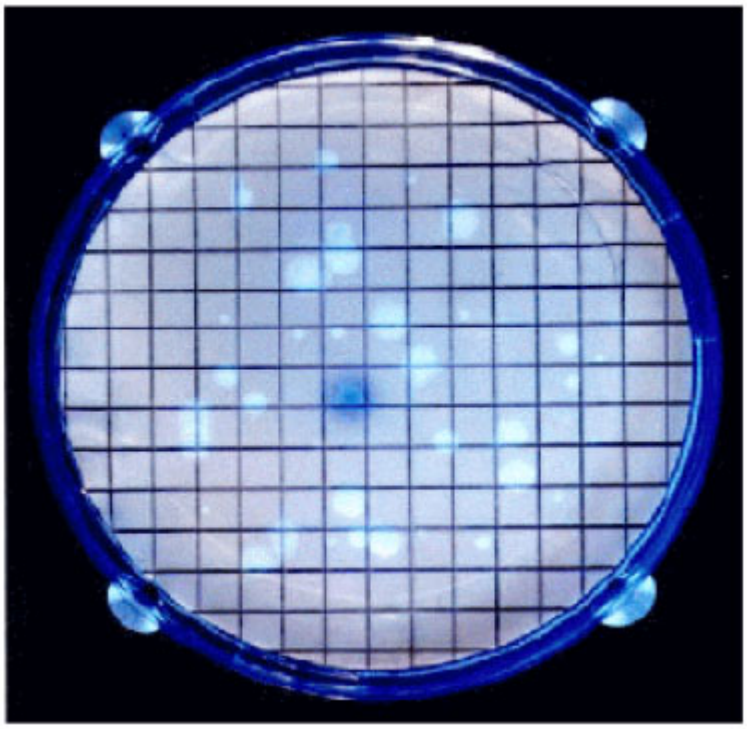

Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

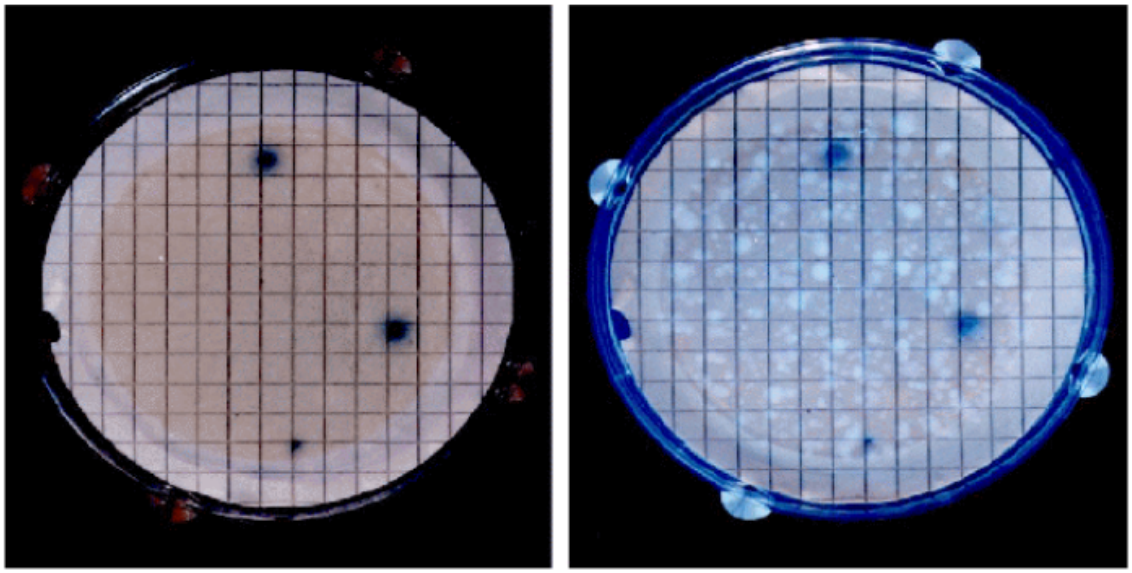

Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other 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).

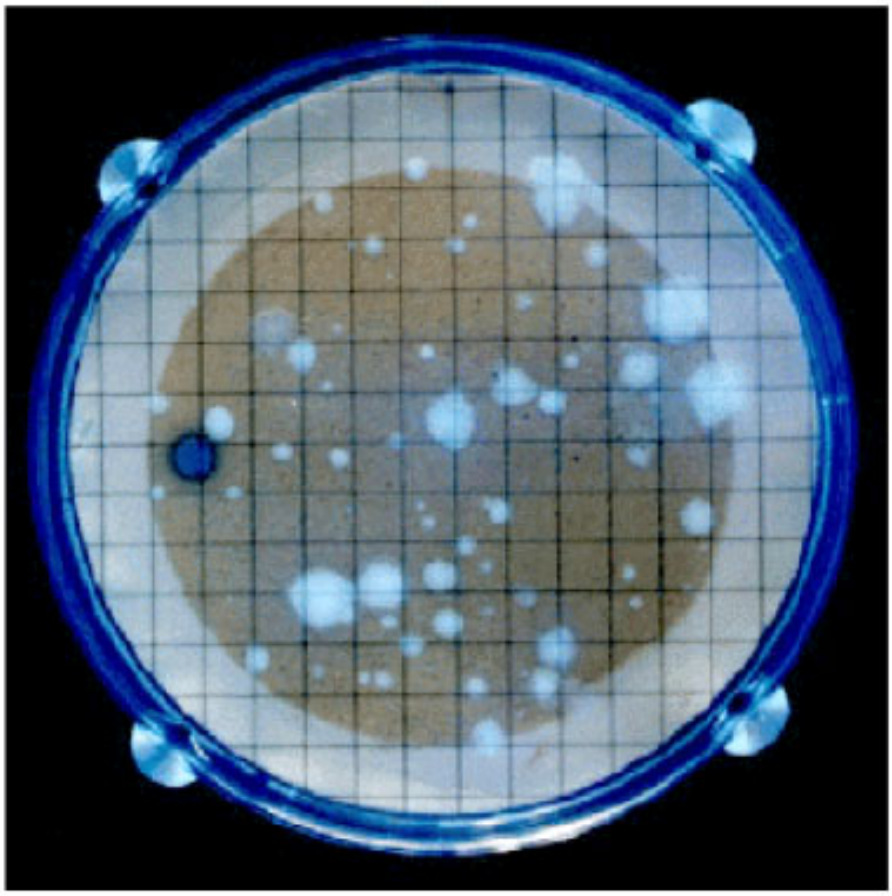

Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.

Summary

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature.

The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Detection and investigation of waterborne disease outbreaks is the primary responsibility of local, state and territorial public health departments, with voluntary reporting to the CDC. The CDC and the U.S. Environmental Protection Agency (EPA) collaborate to track waterborne disease outbreaks of both microbial and chemical origins. Data on drinking water and recreational water outbreaks and contamination events have been collected and summarized since 1971.

While useful, statistics derived from surveillance systems do not reflect the true incidence of waterborne disease outbreaks because many people who fall ill from such diseases do not consult medical professionals.

For those who do seek medical attention, attending physicians and laboratory and hospital personnel are required to report diagnosed cases of waterborne illness to state health departments. Further reporting of these illness cases by state health departments to the CDC is voluntary, and statistically more likely to occur for large outbreaks than small ones.

Despite these limitations, surveillance data may be used to evaluate the relative degrees of risk associated with different types of source water and systems, problems in current technologies and operating conditions, and the adequacy of current regulations. (Craun, Nwachuku, Calderon, and Craun, 2002).

Understanding Cryptosporidiosis

Cryptosporidium is an emerging parasitic protozoan pathogen because its transmission has increased dramatically over the past two decades. Evidence suggests it is newly spread in increasingly popular day-care centers and possibly in widely distributed water supplies, public pools and institutions such as hospitals and extended-care facilities for the elderly. Recognized in humans largely since 1982 and the start of the AIDS epidemic, Cryptosporidium is able to cause potentially life-threatening disease in the growing number of immunocompromised patients.

Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April, 1993. More than 100 deaths are attributed to this outbreak. Cryptosporidium remains a major threat to the U.S. water supply (Ibid.).

The EPA is developing new drinking water regulations to reduce Cryptosporidium and other resistant parasitic pathogens. Key provisions of the Long Term 2 Enhanced Surface Water Treatment Rule include source water monitoring for Cryptosporidium; inactivation by all unfiltered systems; and additional treatment for filtered systems based on source water

Cryptosporidium concentrations. EPA will provide a range of treatment options to achieve the inactivation requirements. Systems with high concentrations of Cryptosporidium in their source water may adopt alternative disinfection methods (e.g., ozone, UV, or chlorine dioxide).

However, most water systems are expected to meet EPA requirements while continuing to use chlorination. Regardless of the primary disinfection method used, water systems must continue to maintain residual levels of chlorine-based disinfectants in their distribution systems.

Understanding Giardia lamblia

Giardia lamblia, discovered approximately 20 years ago, is another emerging waterborne pathogen. This parasitic microorganism can be transmitted to humans through drinking water that might otherwise be considered pristine. In the past, remote water sources that were not affected by human activity were thought to be pure, warranting minimal treatment. However, it is known now that all warm-blooded animals may carry Giardia and that beaver are prime vectors for its transmission to water supplies.

There is a distinct pattern to the emergence of new pathogens. First, there is a general recognition of the effects of the pathogen in highly susceptible populations such as children, cancer patients and the immunocompromised.

Next, practitioners begin to recognize the disease and its causative agent in their own patients, with varied accuracy. At this point, some may doubt the proposed agent is the causative agent, or insist that the disease is restricted to certain types of patients.

Finally, a single or series of large outbreaks result in improved attention to preventive efforts. From the 1960’s to the 1980’s this sequence of events culminated in the recognition of Giardia lamblia as a cause of gastroenteritis (Lindquist, 1999).

Laboratory Analysis Post Quiz

Sample Procedures

1. Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is yellow in color is visible within each plaque.
A. True B. False

2. Viral plaques are easily identified and enumerated by the distinct red circle.
A. True B. False

3. Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water.
A. True B. False

4. Samples for enumeration of _____ are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c).

5. Standard MF techniques are used, and _____ are incubated anaerobically for 24 hours at 44.5°C.

6. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as _____.

7. Method 1623 (U.S. Environmental Protection Agency, 1999c) is recommended for detection of *coliform bacteria* in water. The oocysts are concentrated on a capsule filter from a 10-L water sample, eluted from the capsule filter with buffer, and concentrated by centrifugation.
A. True B. False

8. In IMS, the _____ are magnetized by attachment of magnetic beads conjugated to an antibody and then are separated from sediment and debris by means of a magnet.

9. _____ means that fluorescently labeled antibodies and vital dye were used to make the final microscopic identification of_____.

10. QA/QC activities and measures to take to reduce contamination.
A. True B. False

11. Prepare a separate set of E. coli host cultures for microbiological sampling at each site.
A. True B. False

12. Membrane-filtration (MF) equipment and MF procedure blanks are used to estimate _____.

Field personnel should do the following:

13. Prepare _____, a 50- to 100-mL aliquot of sterile buffered water plated before the sample—for every sample by field personnel for total coliform, *E. coli*, and enterococci analyses to determine the sterility of equipment and supplies.

14. Prepare a _____, a 50- to 100-mL aliquot of sterile buffered water plated after the sample— for every fourth sample to measure the effectiveness of the analyst's rinsing technique or presence of incidental contamination of the buffered water.

15. _____ are the same as equipment blanks except that they are generated under actual field conditions.

Quality Assurance and Quality Control in the Laboratory

16. Production analytical laboratory criteria may be used to evaluate each of the following: (1) appropriate, approved, and published methods, (2) documented standard operating procedures, (3) approved quality-assurance plan, (4) types and amount of quality-control data fully documented and technical defensible, (5) participation in the standard reference sample project (6) scientific capability of personnel, and (7_____.

17. According to the text, microbiology laboratories must follow good laboratory practices—cleanliness, safety practices, procedures for_____, specifications for reagent water quality—as set forth by American Public Health Association.

Answers

1. False, 2. False, 3. True, 4. C. perfringens, 5. The plates, 6. C. perfringens, 7. False, 8. Oocyst(s), 9. Oocysts and cysts, 10. True, 11. False, 12. Analytical bias, 13. An MF equipment blank, 14. MF procedure blank(s), 15. Field blanks, 16. Appropriate laboratory equipment, 17. Media preparation

Math Formulas and Conversions

$$\text{Acid Feed Rate} = \frac{(\text{Waste Flow})\ (\text{Waste Normality})}{\text{Acid Normality}}$$

$$\text{Alkalinity} = \frac{(\text{mL of Titrant})\ (\text{Acid Normality})\ (50{,}000)}{\text{mL of Sample}}$$

Amperage = Voltage ÷ Ohms

Area of Circle = $(0.785)(\text{Diameter}^2)$ OR $(\pi)(\text{Radius}^2)$

Area of Rectangle = (Length)(Width)

$$\text{Area of Triangle} = \frac{(\text{Base})\ (\text{Height})}{2}$$

C Factor Slope = Energy loss, ft. ÷ Distance, ft.

C Factor Calculation = Flow, GPM ÷ [193.75 (Diameter, ft.)2.63(Slope)0.54]

$$\text{Chemical Feed Pump Setting, \% Stroke} = \frac{(\text{Desired Flow})\ (100\%)}{\text{Maximum Flow}}$$

$$\text{Chemical Feed Pump Setting, mL/min} = \frac{(\text{Flow, MGD})\ \text{Dose, mg/L})\ (3.785\text{L/gal})\ (1{,}000{,}000\ \text{gal/MG})}{(\text{Liquid, mg/mL})\ (24\ \text{hr. / day})\ (60\ \text{min/hr.})}$$

Chlorine Demand (mg/L) = Chlorine dose (mg/L) – Chlorine residual (mg/L)

Circumference of Circle = (3.141) (Diameter)

$$\text{Composite Sample Single Portion} = \frac{(\text{Instantaneous Flow})\ (\text{Total Sample Volume})}{(\text{Number of Portions})\ (\text{Average Flow})}$$

$$\text{Detention Time} = \frac{\text{Volume}}{\text{Flow}}$$

$$\text{Digested Sludge Remaining, \%} = \frac{(\text{Raw Dry Solids})\ (\text{Ash Solids})\ (100\%)}{(\text{Digested Dry Solids})\ (\text{Digested Ash Solids})}$$

$$\text{Discharge} = \frac{\text{Volume}}{\text{Time}}$$

Dosage, lbs/day = (mg/L)(8.34)(MGD)

Dry Polymer (lbs.) = (gal. of solution) (8.34 lbs/gal)(% polymer solution)

$$\text{Efficiency, \%} = \frac{\text{(In – Out) (100\%)}}{\text{In}}$$

$$\text{Feed rate, lbs/day} = \frac{\text{(Dosage, mg/L) (Capacity, MGD) (8.34 lbs/gals)}}{\text{(Available fluoride ion) (Purity)}}$$

$$\text{Feed rate, gal/min (Saturator)} = \frac{\text{(Plant capacity, gal/min.) (Dosage, mg /L)}}{\text{18,000 mg/L}}$$

$$\text{Filter Backwash Rate} = \frac{\text{Flow}}{\text{Filter Area}}$$

$$\text{Filter Yield, lbs/hr./sq. ft} = \frac{\text{(Solids Loading, lbs/day) (Recovery, \% / 100\%)}}{\text{(Filter operation, hr./day) (Area, ft}^2\text{)}}$$

Flow, cu. ft./sec. = (Area, Sq. Ft.)(Velocity, ft./sec.)

$$\text{Gallons/Capita/Day} = \frac{\text{Gallons / day}}{\text{Population}}$$

$$\text{Hardness} = \frac{\text{(mL of Titrant) (1,000)}}{\text{mL of Sample}}$$

$$\text{Horsepower (brake)} = \frac{\text{(Flow, gpm) (Head, ft)}}{\text{(3,960) (Efficiency)}}$$

$$\text{Horsepower (motor)} = \frac{\text{(Flow, gpm) (Head, ft)}}{\text{(3960) (Pump, Eff) (Motor, Eff)}}$$

$$\text{Horsepower (water)} = \frac{\text{(Flow, gpm) (Head, ft)}}{\text{(3960)}}$$

$$\text{Hydraulic Loading Rate} = \frac{\text{Flow}}{\text{Area}}$$

Leakage (actual) = Leak rate (GPD) ÷ [Length (mi.) x Diameter (in.)]

Mean = Sum of values ÷ total number of values

$$\text{Mean Cell Residence Time (MCRT)} = \frac{\text{Suspended Solids in Aeration System, lbs}}{\text{SS Wasted, lbs / day + SS lost, lbs / day}}$$

Organic Loading Rate = $\frac{\text{Organic Load, lbs BOD / day}}{\text{Volume}}$

Oxygen Uptake = $\frac{\text{Oxygen Usage}}{\text{Time}}$

Pounds per day = (Flow, MGD) (Dose, mg/L) (8.34)

Population Equivalent = $\frac{\text{(Flow MGD) (BOD, mg/L) (8.34 lbs / gal)}}{\text{Lbs BOD / day / person}}$

RAS Suspended Solids, mg/l = $\frac{1{,}000{,}000}{\text{SVI}}$

RAS Flow, MGD = $\frac{\text{(Infl. Flow, MGD) (MLSS, mg/l)}}{\text{RAS Susp. Sol., mg/l – MLSS, mg/l}}$

RAS Flow % = $\frac{\text{(RAS Flow, MGD) (100 \%)}}{\text{Infl. Flow, MGD}}$

Reduction in Flow, % = $\frac{\text{(Original Flow – Reduced Flow) (100\%)}}{\text{Original Flow}}$

Slope = $\frac{\text{Drop or Rise}}{\text{Run or Distance}}$

Sludge Age = $\frac{\text{Mixed Liquor Solids, lbs}}{\text{Primary Effluent Solids, lbs / day}}$

Sludge Index = $\frac{\text{\% Settleable Solids}}{\text{\% Suspended Solids}}$

Sludge Volume Index = $\frac{\text{(Settleable Solids, \%) (10,000)}}{\text{MLSS, mg/L}}$

Solids, mg/L = $\frac{\text{(Dry Solids, grams) (1,000,000)}}{\text{mL of Sample}}$

Solids Applied, lbs/day = (Flow, MGD)(Concentration, mg/L)(8.34 lbs/gal)

Solids Concentration = $\frac{\text{Weight}}{\text{Volume}}$

Solids Loading, lbs/day/sq. ft = $\frac{\text{Solids Applied, lbs / day}}{\text{Surface Area, sq. ft}}$

Surface Loading Rate = $\frac{\text{Flow}}{\text{Rate}}$

Total suspended solids (TSS), mg/L =
(Dry weight, mg)(1,000 mL/L) ÷ (Sample vol., mL)

Velocity = $\frac{\text{Flow}}{\text{Area}}$ O R $\frac{\text{Distance}}{\text{Time}}$

Volatile Solids, % = $\frac{\text{(Dry Solids - Ash Solids) (100\%)}}{\text{Dry Solids}}$

Volume of Cone = (1/3)(0.785)(Diameter^2)(Height)

Volume of Cylinder = (0.785)(Diameter^2)(Height) OR $(\pi)(r^2)(h)$

Volume of Rectangle = (Length)(Width)(Height)

Volume of Sphere = $[(\pi)(\text{diameter}^3)] \div 6$

Waste Milliequivalent = (mL) (Normality)

Waste Normality = $\frac{\text{(Titrant Volume) (Titrant Normality)}}{\text{Sample Volume}}$

Weir Overflow Rate = $\frac{\text{Flow}}{\text{Weir Length}}$

Conversion Factors

1 acre = 43,560 square feet
1 cubic foot = 7.48 gallons
1 foot = 0.305 meters
1 gallon = 3.785 liters
1 gallon = 8.34 pounds
1 grain per gallon = 17.1 mg/L
1 horsepower = 0.746 kilowatts
1 million gallons per day = 694.45 gallons per minute
1 pound = 0.454 kilograms
1 pound per square inch = 2.31 feet of water
1% = 10,000 mg/L
Degrees Celsius = (Degrees Fahrenheit - 32) (5/9)
Degrees Fahrenheit = (Degrees Celsius * 9/5) + 32
64.7 grains = 1 cubic foot
1,000 meters = 1 kilometer
1,000 grams = 1 kilogram

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