WATERBORNE DISEASES

CONTINUING EDUCATION
PROFESSIONAL DEVELOPMENT COURSE

1St Edition

Technical Learning College

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This course booklet does not have the assignment. Please visit our website and download the assignment also.

You can obtain a printed version from TLC for an additional $69.95 plus shipping charges.

All downloads are electronically tracked and monitored for security purposes.

We require the final exam to be proctored.

Do not solely depend on TLC's Approval list for it may be outdated.

A second certificate of completion for a second State Agency $25 processing fee.

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.

Responsibility

This course contains EPA's federal rule requirements. Please be aware that each state implements drinking water/wastewater/safety regulations may be more stringent than EPA's or OSHA's regulations. Check with your state environmental agency for more information. You are solely responsible in ensuring that you abide with your jurisdiction or agency's rules and regulations.

Contributing Editors
Joseph Camerata has a BS in Management with honors (magna cum laude). He retired as a Chemist in 2006 having worked in the field of chemical, environmental, and industrial hygiene sampling and analysis for 40 years. He has been a professional presenter at an EPA analytical conference at the Biosphere in Arizona and a presenter at an AWWA conference in Mesa, Arizona. He also taught safety classes at the Honeywell and City of Phoenix, and is a motivational/inspirational speaker nationally and internationally.

Dr. Eric Pearce S.M.E., chemistry and biological review.

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Jack White, Environmental, Health, Safety expert, Art Credits.

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

Technical Learning College's Scope and Function

Welcome to the Program,

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

TLC's delivery method of continuing education can include traditional types of classroom lectures and distance-based courses or independent study. TLC's distance based or independent study courses are offered in a print- based format and you are welcome to examine this material on your computer with no obligation. 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 do finish the material on your leisure. Students can also receive course materials through the mail. The CEU course or e-manual will contain all your lessons, activities and 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.

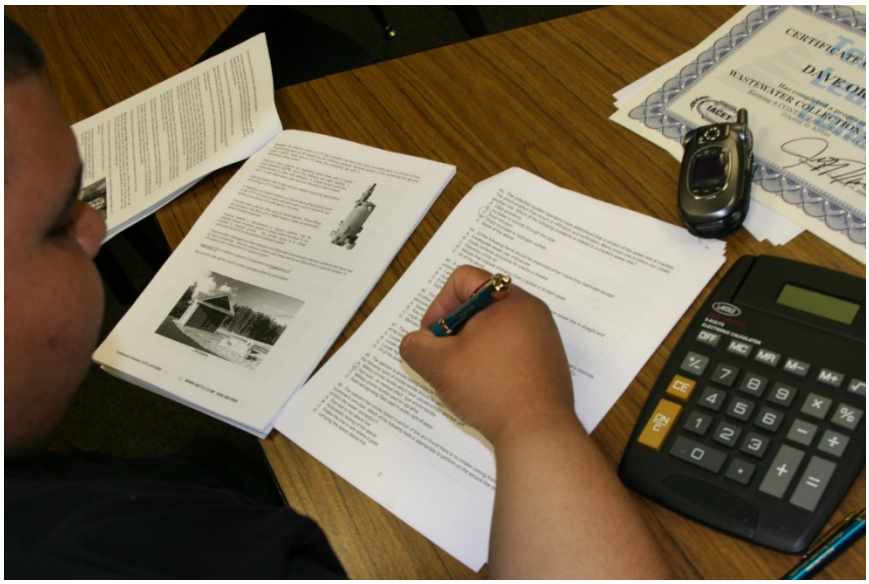
Flexible Learning
At TLC, there are no scheduled online sessions or passwords you need contend with, nor are you required to participate in learning teams or groups designed for the "typical" younger campus based student. You will work at your own pace, completing assignments in time frames that work 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 are assigned at the beginning of each course providing the academic support you need to successfully complete each course.

No Data Mining Policy
Unlike most online training providers, we do not use passwords or will upload intrusive data mining software onto your computer. We do not use any type of artificial intelligence in our program. Nor will we sell you any other product or sell your data to others as with many of our competitors. Unlike our training competitors, we have a telephone and we humanly answer.

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.

We welcome you to complete the assignment in Word.

Once we grade it, we will mail a certificate of completion to you. Call us if you need any help.

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

CEU COURSE INTRODUCTION

WATERBORNE DISEASES CEU TRAINING COURSE

Review of commonly found water and wastewater diseases, symptoms, and identification techniques. This course will cover the federal rules concerning water and wastewater sampling techniques, waterborne disease control, general water quality operations and definitions; disease symptoms; disease diagnosis; history; susceptibility; and disease sources of contamination. This course will apply to all categories of water treatment/distribution and wastewater treatment/collection. 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 designed for Water Laboratory Analysts, but can be utilized by Wastewater Treatment, Collections, Water Distribution, Well Drillers, Pump Installers, and Water Treatment Operators. The target audience for this course is any person that has at least 2 years of college lecture and laboratory course work in microbiology or a closely related field. This person should have at least 6 months of continuous bench experience with environmental protozoa detection techniques and must have successfully analyzed at least 50 water and/or wastewater samples for *Cryptosporidium* and *Giardia*. Six months of additional experience in the above areas may be substituted for two years of college. 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 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, website, 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. It is the prerogative of TLC to decide whether to grant the request. All students will be tracked by their social security number or a unique number will be 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 on line 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 Waterborne Diseases distance learning course uses a multiple-choice style answer key. You can find the answer key in the front of the assignment. 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 assignmentor 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 Waterborne Disease 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 to obtain-up-to date or certain sampling information.

Recordkeeping and Reporting Practices

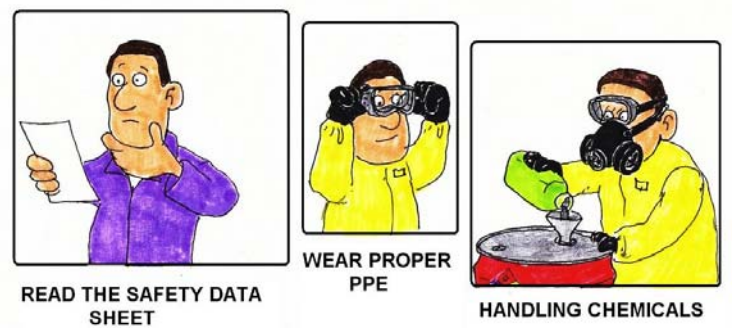
TLC will keep all student records for a minimum of seven years. It is the student's responsibility to give the completion certificate to the appropriate agencies. TLC will not release any records to any party, except to the student. We will send the required information to Texas, Indiana and Pennsylvania 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 possible education service possible. TLC will attempt to make your learning experience an enjoyable opportunity.



Educational Mission

The educational mission of TLC is:

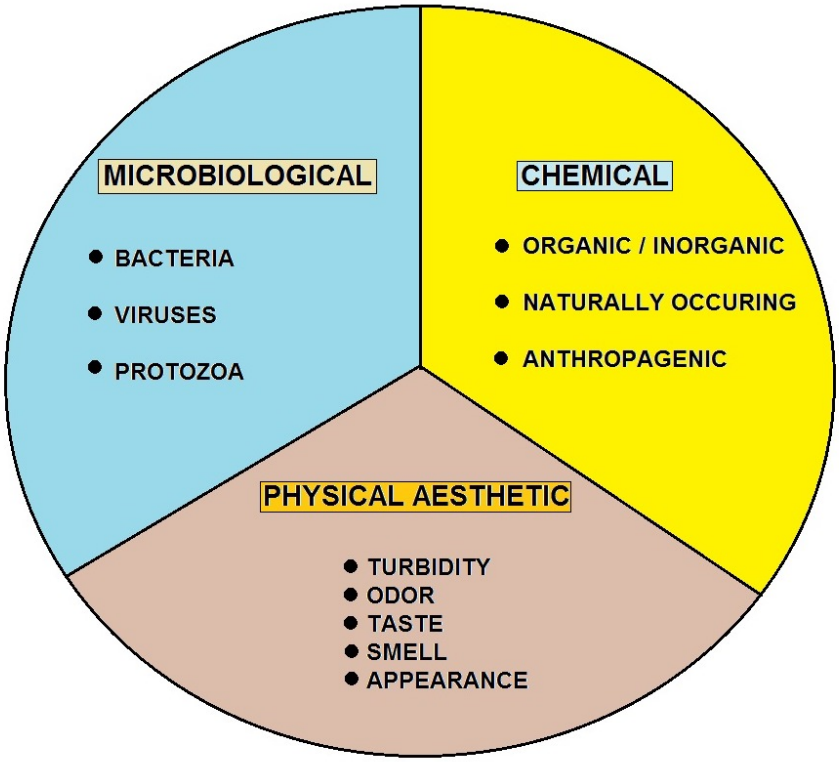
To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

To provide TLC students 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.



WATER QUALITY BROKEN DOWN INTO 3 BROAD CATEGORIES

Table of Contents

Common EPA Definitions **Larger Glossary and detailed Laboratory Terms and Procedures in the rear.**

The following are terms that will be found in this course, especially in the waterborne disease area and laboratory/sampling chapters.

As used in 40 CFR 141, the term:

Best available technology* or *BAT means the best technology, treatment techniques, or other means which the Administrator finds, after examination for efficacy under field conditions and not solely under laboratory conditions, are available (taking cost into consideration). For the purposes of setting MCLs for synthetic organic chemicals, any BAT must be at least as effective as granular activated carbon.

Community water system is a public water system which serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

Compliance cycle is the nine-year calendar year cycle during which public water systems must monitor. Each compliance cycle consists of three three-year compliance periods. The first calendar year cycle begins January 1, 1993 and ends December 31, 2001; the second begins January 1, 2002 and ends December 31, 2010; the third begins January 1, 2011 and ends December 31, 2019.

Compliance period is a three-year calendar year period within a compliance cycle. Each compliance cycle has three three-year compliance periods. Within the first compliance cycle, the first compliance period runs from January 1, 1993 to December 31, 1995; the second from January 1, 1996 to December 31, 1998; the third from January 1, 1999 to December 31, 2001.

Contaminant is any physical, chemical, biological, or radiological substance or matter in water.

Maximum contaminant level is the maximum permissible level of a contaminant in water which is delivered to any user of a public water system.

Maximum contaminant level goal* or *MCLG is the maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur, and which allows an adequate margin of safety. Maximum contaminant level goals are non-enforceable health goals.

Non-transient non-community water system* or *NTNCWS is a public water system that is not a community water system and that regularly serves at least 25 of the same persons over 6 months per year.

Point-of-entry treatment device (POE) is a treatment device applied to the drinking water entering a house or building for the purpose of reducing contaminants in the drinking water distributed throughout the house or building.

Point-of-use treatment device (POU) is a treatment device applied to a single tap used for the purpose of reducing contaminants in drinking water at that one tap.

Public water system is a system for the provision to the public of water for human consumption through pipes or, after August 5, 1998, other constructed conveyances, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. Such term includes: any collection, treatment, storage, and distribution facilities under control of the operator of such system and used primarily in connection with such system; and any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system. Such term does not include any **"*special irrigation district*."**

A public water system is either a ***"community water system"*** or a ***"noncommunity water system."***

State means the agency of the State or Tribal government which has jurisdiction over public water systems. During any period when a State or Tribal government does not have primary enforcement responsibility pursuant to section 1413 of the Act, the term ***"State"*** means the Regional Administrator, of the U.S. Environmental Protection Agency.

Surface water means all water which is open to the atmosphere and subject to surface runoff.

Microbes **Basic definitions, more detailed information in the next section.**

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

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

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

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems. The EPA and 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, cramps).

Waterborne Disease CEU Course Introduction

Bacteria, viruses and protozoans that cause disease are known as pathogens. Most pathogens are generally associated with diseases that cause intestinal illness and affect people in a relatively short amount of time, generally a few days to two weeks. They can cause illness through exposure to small quantities of contaminated water or food or from direct contact with infected people or animals. 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 the feces has been broken up, dispersed and diluted into microscopic particles. These particles, containing pathogens, may remain in the water and be passed to humans or animals unless adequately treated.

Only proper treatment will ensure eliminating the spread of disease. In addition to water, other methods exist for spreading pathogens by the fecal-oral route. The foodborne route is one of the more common methods. A frequent source is a food handler who does not wash his hands after a bowel movement and then handles food with ***"unclean"*** hands. The individual who eats feces-contaminated food may become infected and ill. It is interesting to note the majority of foodborne diseases occur in the home, not restaurants.

Day care centers are another common source for spreading pathogens by the fecal-oral route. Here, infected children in diapers may get feces on their fingers, then put their fingers in a friend's mouth or handle toys that other children put into their mouths. You will usually be asked to sample at these facilities for 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've digested within the past few days.

Chain of Transmission

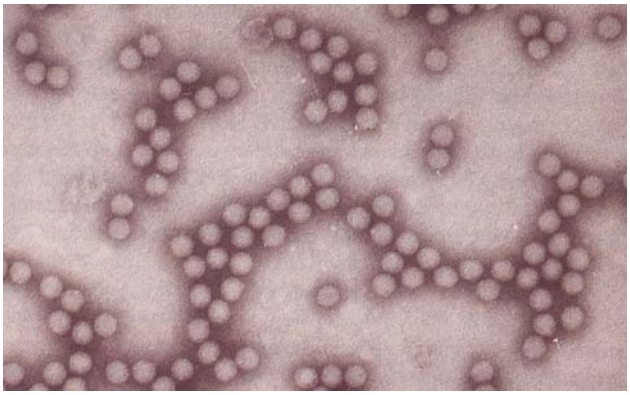
When water is contaminated with feces, this contamination may be of human or animal origin. If the human or animal source is not infected with a pathogen (disease-causing bacteria, viruses or protozoa), no disease will result.

The pathogens must survive in the water. This depends on the temperature of the water and the length of time the pathogens are in the water. Some pathogens will survive for only a short time in water, others, such as Giardia or Cryptosporidium, may survive for months.The pathogens in the water must enter the water system's intake and in numbers sufficient to infect people. The water is either not treated or inadequately treated for the pathogens present. A susceptible person must drink the water that contains the pathogen in order for illness (disease) to occur. This chain lists the events that must occur for the transmission of disease via drinking water. By breaking the chain at any point, the transmission of disease will be prevented.

Bacterial Diseases (More detailed information in the next chapters)
Campylobacteriosis is the most common diarrheal illness caused by bacteria. Symptoms include abdominal pain, malaise, fever, nausea and vomiting, and usually begin three to five days after exposure. The illness is frequently over within two to five days and usually lasts no more than 10 days. Campylobacteriosis outbreaks have most often been associated with food, especially chicken and unpasteurized milk as well as unchlorinated 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.

Viral Diseases or Viruses



Hepatitis A is a common example of a 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.

Terrorism
Recent investigations have shown proof the terrorist organizations have been able to reproduce most of these pathogens and have the technology and funding to attack our public water supply system. You will need to know these diseases and how to deal with these issues. Even diseases that we have not seen in years are easily and readily available for a terrorist to backflow into our distribution system, or pour into a wellhead or clearwell.

The Main Players- History and Biology Chapter 1

Before we define the major waterborne diseases, let's first examine the germs and other creatures that cause the diseases. Most of the following information may be simple or instruction that you already know. But to be safe, let's review the basics.

History of 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 Dimitri 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.

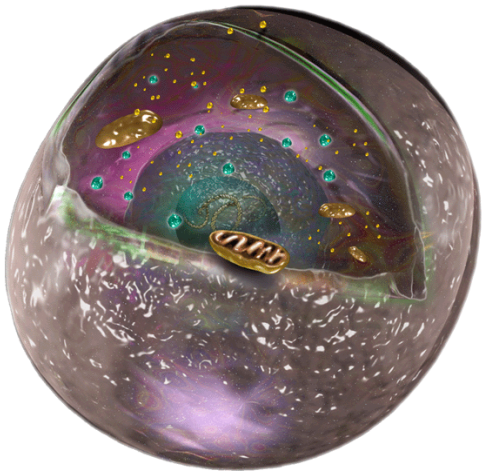
Koch's postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.

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 Thedore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.



Rudolph Virchow completed the cell theory with the idea that all cells must arise from preexisting cells.

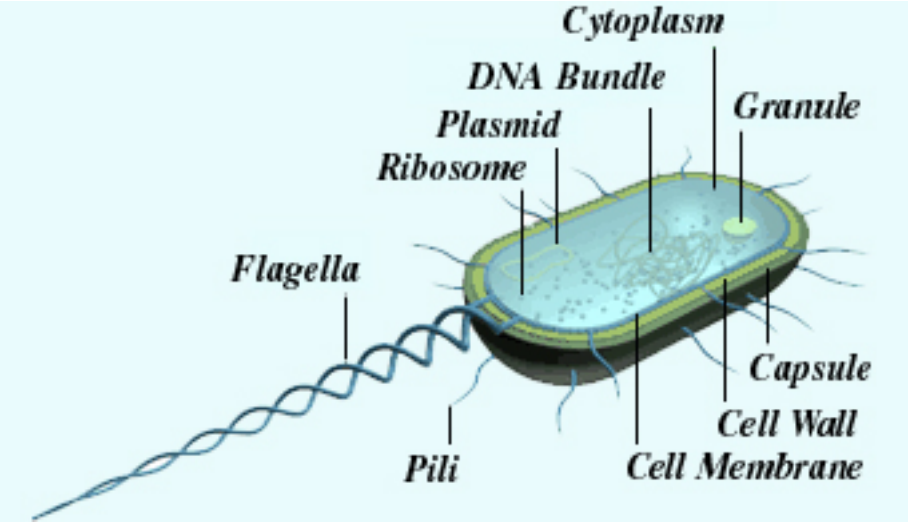
In biology, a cell is defined as the fundamental living unit of any organism and exhibits the basic characteristics of life. A cell obtains food from the environment to produce energy and nutrients for metabolism.

Metabolism
Metabolism is a term that describes all the chemical reactions by which food is transformed for use by the cells.

Through its metabolism, a cell can grow, reproduce and it can respond to changes in its environment. As a result of accidental changes in its environment, a cell can undergo changes in its genetic material. This is called mutation. The genetic changes may adapt the organism for better survival and development into a new species of organism.

Bacteria

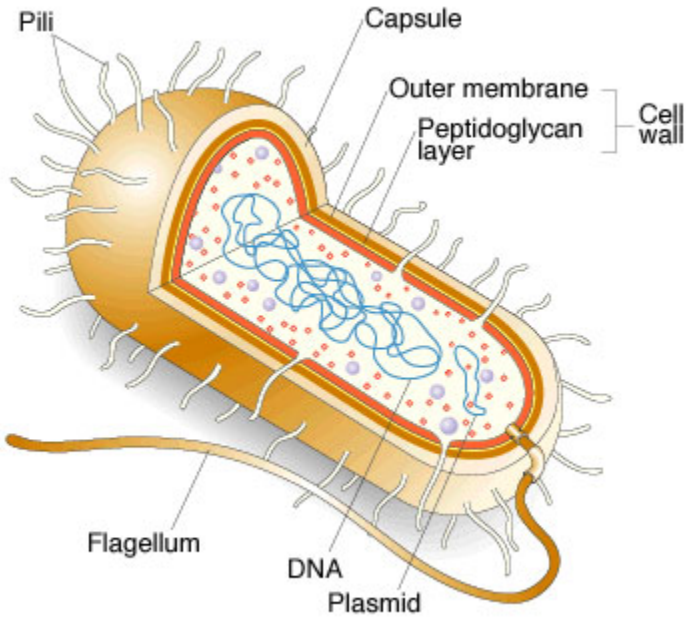
Bacteria consist of only a single cell, but don't let their small size and seeming simplicity fool you. They're an amazingly complex and fascinating group of creatures. Bacteria have been found that can live in temperatures above the boiling point and in cold that would freeze your blood. They "eat" everything from sugar and starch to sunlight, sulfur and iron. There's even a species of bacteria—*Deinococcus radiodurans*—that can withstand blasts of radiation 1,000 times greater than would kill a human being.



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**.

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.



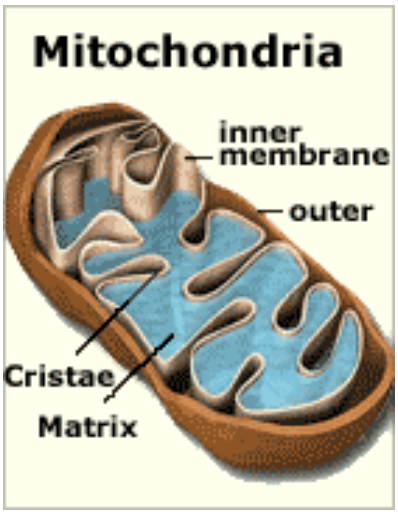
These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they're not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_2, while others are poisoned or killed by it.

Prokaryotes
Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes *(you-carry-oats)*, creatures whose cells have nuclei.

(Note: viruses are not considered true cells, so they don't fit into either of these categories; this will be covered in the next few pages.)

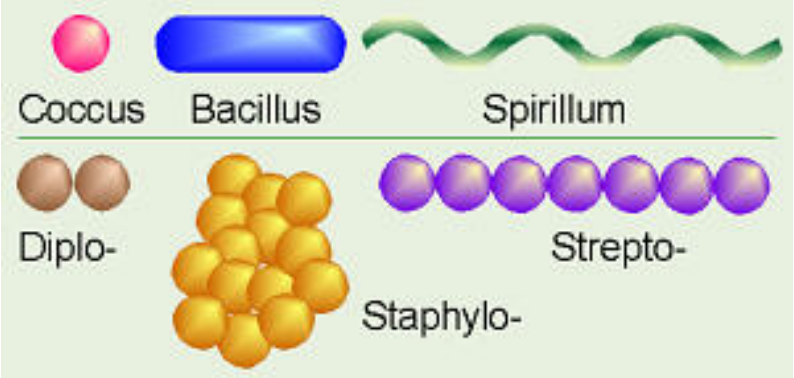
Early Origins
Bacteria are among the earliest forms of life that appeared on Earth billions of years ago. Scientists think that they helped shape and change the young planet's environment, eventually creating atmospheric oxygen that enabled other, more complex life forms to develop. Many believe that more complex cells developed as once free-living bacteria took up residence in other cells, eventually becoming the organelles in modern complex cells.

The mitochondria *(mite-oh-con-dree-uh)* that make energy for your body cells is one example of such an organelle.



There are thousands of species of bacteria, but all of them are basically one of three different shapes. Some are rod - or stick-shaped and called bacilli *(buh-sill-eye)*.

Others are shaped like little balls and called cocci *(cox-eye)*.

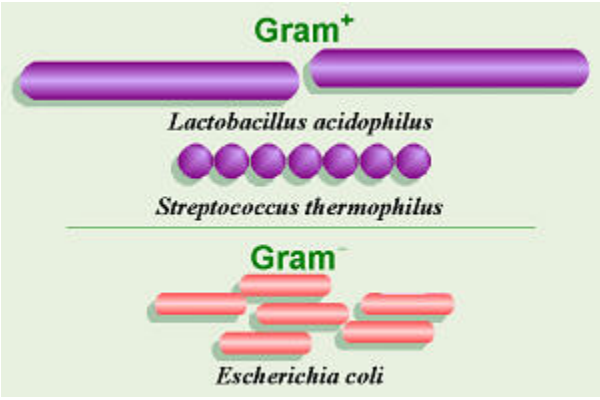


Others still are helical or spiral in shape. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other groupings.

Bacteria live on or in just about every material and environment on Earth from soil to water to air, and from your house to arctic ice to volcanic vents. Each square centimeter of your skin averages about 100,000 bacteria. A single teaspoon of topsoil contains more than a billion (1,000,000,000) bacteria.

Peptidoglycan
Most bacteria secrete a covering for themselves which we call a **cell wall**.

However, bacterial cell walls are a totally different thing than the cell walls we talk about plants having. Bacterial cell walls do **NOT** contain cellulose like plant cell walls do. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.



Some antibiotics, like penicillin, inhibit the formation of the chemical cross linkages needed to make peptidoglycan.

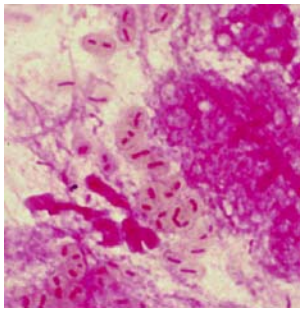
These antibiotics don't kill the bacteria outright, just stop them from being able to make more cell wall so they can grow.

That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.

Gram Stain

However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. Dr. Hans Christian Gram, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Gram stain**. In this process, the amount of peptidoglycan in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained; thus, bacterial cells can be $Gram^{+}$ or Gram $^{-}$. $Gram^{+}$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color. $Gram^{-}$ bacteria have more complex cell walls with less peptidoglycan, thus absorb less of the purple dye used and stain a pinkish color instead. Also, $Gram^{-}$ bacteria often incorporate toxic chemicals into their cell walls, and thus tend to cause worse reactions in our bodies. Because $Gram^{-}$ bacteria have less peptidoglycan, antibiotics like penicillin are less effective against them. As we have discussed before, taking antibiotics that don't work can be bad for you, thus a good doctor should always have a culture done before prescribing antibiotics to make sure the person is getting something that will help.

Pseudomonas aeruginosa is a strictly aerobic, oxidase positive, gram-negative nonfermentative bacterium. The Gram-stain appearance is not particularly characteristic although rods are somewhat thinner than those seen for the enteric-like bacteria. Mucoid strains that produce an extracellular polysaccharide are frequently isolated from patients with cystic fibrosis and this capsular material can be seen in the photo.



Two types of cells- Procaryotes and Eucaryotes
A Procaryotic cell exhibits all the characteristics of life but it lacks the complex system of membranes and organelles. ***Example***: Bacterial cells and cyanobacteria.

A Eucaryotic cell has a complex structure. It contains a true nucleus and many membrane bound organelles. ***Example***: Protozoa, fungi, algae, all plants and animal cells.

Structure of a Eucaryotic 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 eucaryotic cells possess relatively long and thin structures called flagella. These are organs of locomotion. Cilia are also organs of locomotion but are shorter and more numerous

Structure of a Procaryotic Cell
All bacteria are procaryotes and are simple cells. They divide by binary fission.

Chromosome
The chromosome of a procaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell. A 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 eucaryotic 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 noncapsulated

bacteria produce rough and dry colonies. Capsules enable the bacterial species to attach to mucus membranes and protect the bacteria from phagocytosis.

Flagella
Flagella are thread like proteins that enable the bacteria to move. Flagellated bacteria are said to be motile while non flagellated bacteria are generally non-motile. The number and arrangement of flagella are species specific and can be used to classify bacteria.
Peritrichous bacteria- possess flagella over the entire surface.
Lophotrichous bacteria-possess a tuft of flagella at one or both ends.
Amphitrichous bacteria-bacteria with one flagellum at each end.
Monotrichous bacteria-bacteria with a single polar flagellum.

Pili or Fimbriae
Pili or Fimbriae are thin hair like structures observed on gram negative bacteria. They are not associated with motility. They enable the bacteria to attach to other bacteria or to membrane surfaces such as intestinal linings or RBC. They are also used to transfer genetic material from one bacteria cell to another.

Spores
Some bacteria are capable of forming spores (also called endospore) as a means of survival under adverse conditions. During sporulation the genetic material is enclosed in several protein coats that are resistant to heat, drying and most chemicals. Spores have been shown to survive in soil or dust. When the dried spore lands on a nutrient rich surface, it forms a new vegetative cell. Spore formation is related to the survival of bacterial cells, not reproduction.

Bacterial Nutrition
All life has the same basic nutritional requirements which include:
Energy. This may be light (the sun or lamps) or inorganic substances like sulfur, carbon monoxide or ammonia, or preformed organic matter like sugar, protein, fats etc. Without energy life cannot exist and quickly dies or becomes inactive.
Nitrogen. This may be nitrogen gas, ammonia, nitrate/nitrite, or a nitrogenous organic compound like protein or nucleic acid.
Carbon. This can be carbon dioxide, methane, carbon monoxide, or a complex organic material.
Oxygen. All cells use oxygen in a bound form and many require gaseous oxygen (air), but oxygen is lethal to many microbes.
Phosphorous, Sulfur, Magnesium, Potassium and Sodium.
Calcium. Most cells require calcium in significant quantities, but some seem to only need it in trace amounts.
Water. All life requires liquid water in order to grow and reproduce; which is why the Mars Mission is so interested in water on Mars. Some resting stages of cells, like bacterial spores, can exist for long periods without free water, but they do not grow or metabolize.
Iron, Zinc, Cobalt. These are called trace metals that are required by some enzymes to function.

The sources of these various requirements define an organism, so a description of every organism should include this information.

Fastidious

Many bacteria can synthesize every complex molecule they need from the basic minerals, but others, said to be fastidious, require preformed organic molecules like vitamins, amino acids, nucleic acids, carbohydrates; humans are ***fastidious***. In general bacterial pathogens need more preformed organic molecules than do nonpathogens, 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			

What in the World is an Eukaryote ?

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 superkingdom, empire, or domain. The name comes from the Greek *eus* or true and *karyon* or nut, referring to the nucleus.

What are Protists?

- They are **eukaryotes** because they all have a **nucleus**.
- Most have **mitochondria** although some have later lost theirs. Mitochondria were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
- Many have **chloroplasts** with which they carry on photosynthesis. Chloroplasts were derived from photosynthetic **cyanobacteria** (also prokaryotes) living within their cells.

Eukaryotic Cells

Eukaryotic cells are generally much larger than prokaryotes, typically with a thousand times their volumes. They have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of microtubules and microfilaments, which plays an important role in defining the cell's organization. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among prokaryotes.

Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or vacuoles, can form by budding off of other membranes. Many cells ingest food and other materials through a process of endocytosis, where the outer membrane invaginates and then pinches off to form a vesicle. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles.

The nucleus is surrounded by a double membrane, with pores that allow material to move in and out. Various tube- and sheet-like extensions of the nuclear membrane form what is called the endoplasmic reticulum or ER, which is involved in protein transport. It includes rough sections where ribosomes are attached, and the proteins they synthesize enter the interior space or lumen. Subsequently, they generally enter vesicles, which bud off from the smooth section. In most eukaryotes, the proteins may be further modified in stacks of flattened vesicles, called Golgi bodies or dictyosomes.

Vesicles may be specialized for various purposes. For instance, lysosomes contain enzymes that break down the contents of food vacuoles, and peroxisomes are used to break down peroxide which is toxic otherwise.

Contractile Vacuoles
Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure.

Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short, that are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles
Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division.

Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

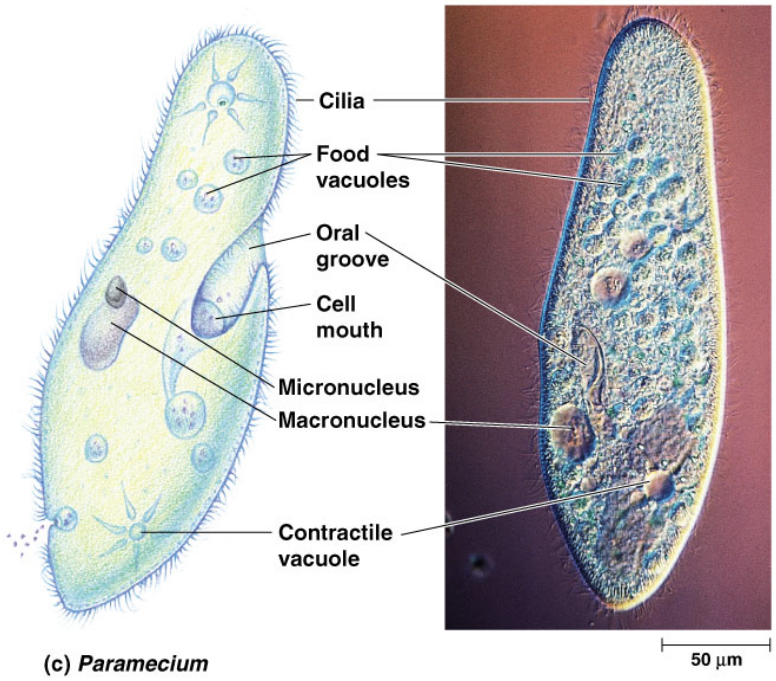
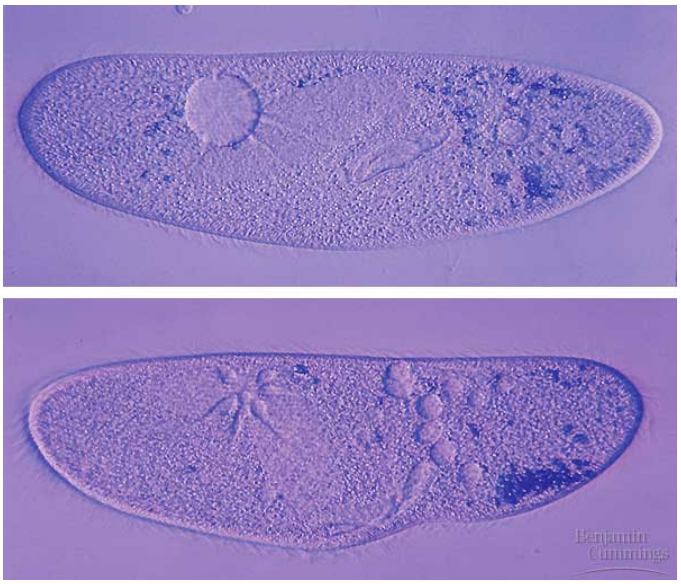


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.



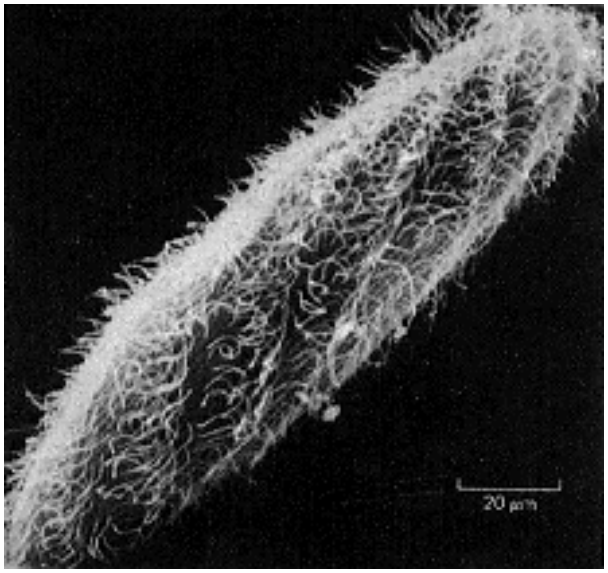
Contractile Vacuoles

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

Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration.

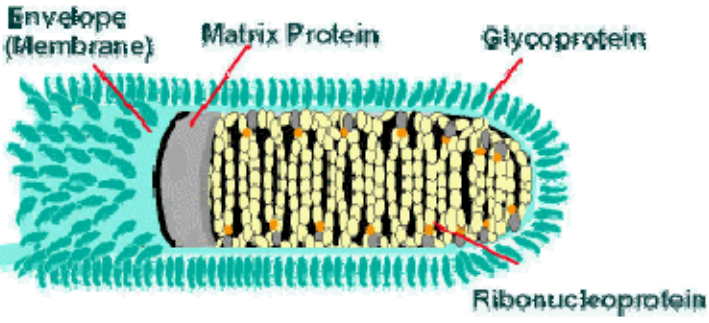
If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).



Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce.

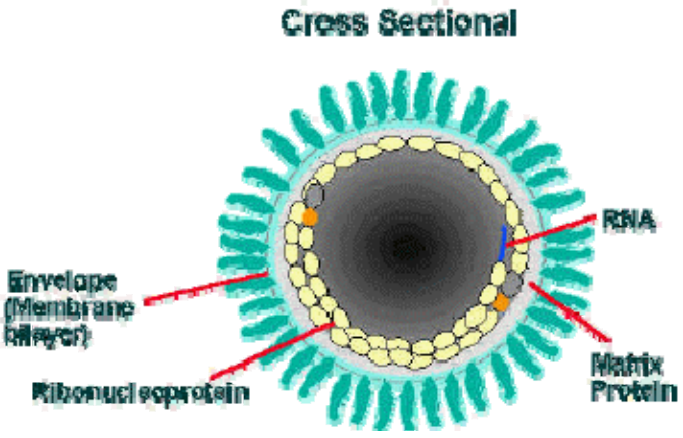
A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



Size

Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication.

Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.



Virions

Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA.

Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or nonsegmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.

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 and to direct capsid production and virus particle assembly inside the cell.

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

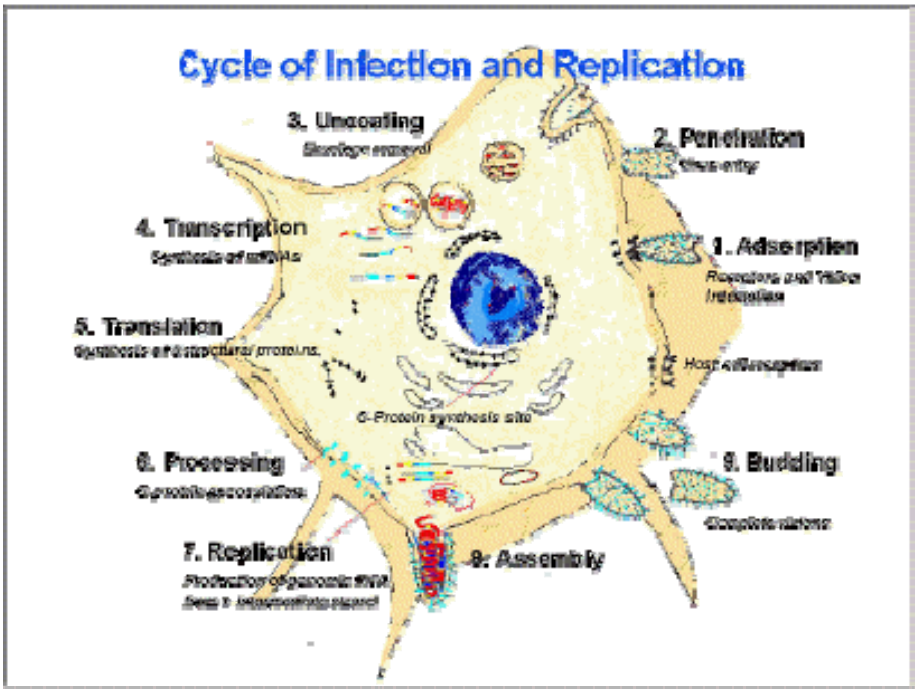
The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

Replication

The first contact between a virus particle and its host cell occurs when an outer viral structure docks with a specific molecule on the cell surface. For example, a glycoprotein called gp120 on the surface of the human immunodeficiency virus (HIV, the cause of acquired immunodeficiency syndrome, or AIDS) virion specifically binds to the CD4 molecule found on certain human T lymphocytes (a type of white blood cell). Most cells that do not have surface CD4 molecules generally cannot be infected by HIV.

After binding to an appropriate cell, a virus must cross the cell membrane. Some viruses accomplish this goal by fusing their lipid envelope to the cell membrane, thus releasing the nucleocapsid into the cytoplasm of the cell.



Other viruses must first be endocytosed (enveloped by a small section of the cell's plasma membrane that pokes into the cell and pinches off to form a bubblelike 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.

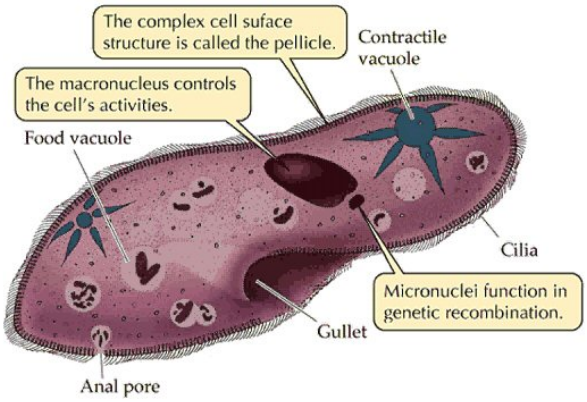
Co-infection of the same cell with different related viruses allows for genetic re-assortment (exchange of genome segments) and intramolecular recombination. Genetic alterations can alter virulence or allow viruses to gain access to new cell types or new animal hosts.

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.

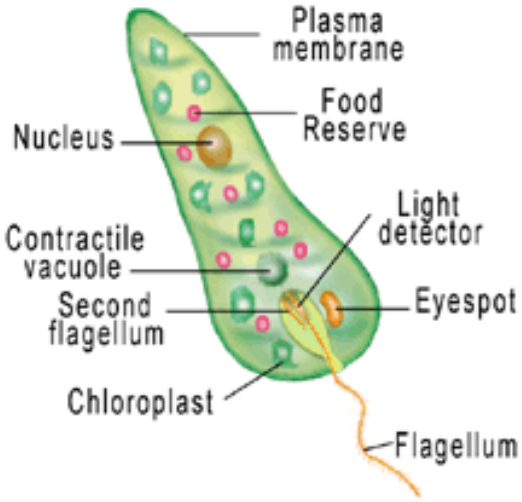
Protozoa Section

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).



Paramecium

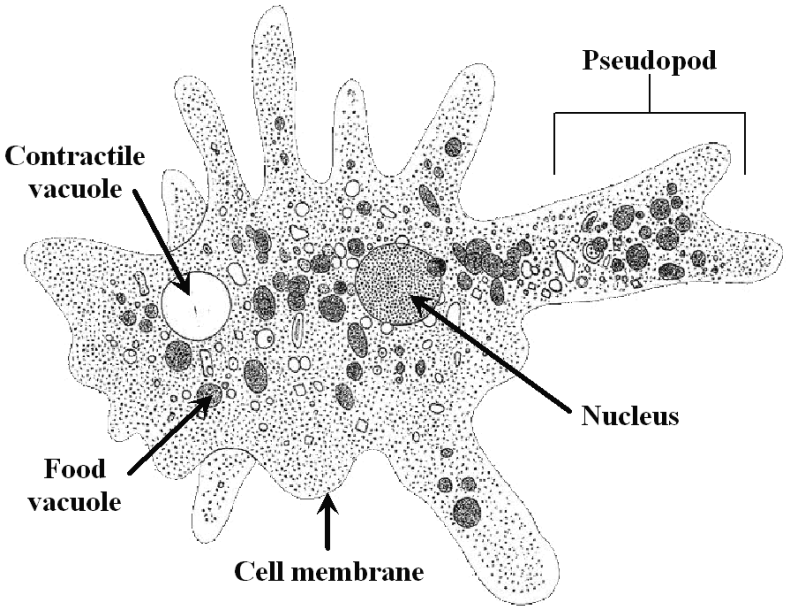


Euglena

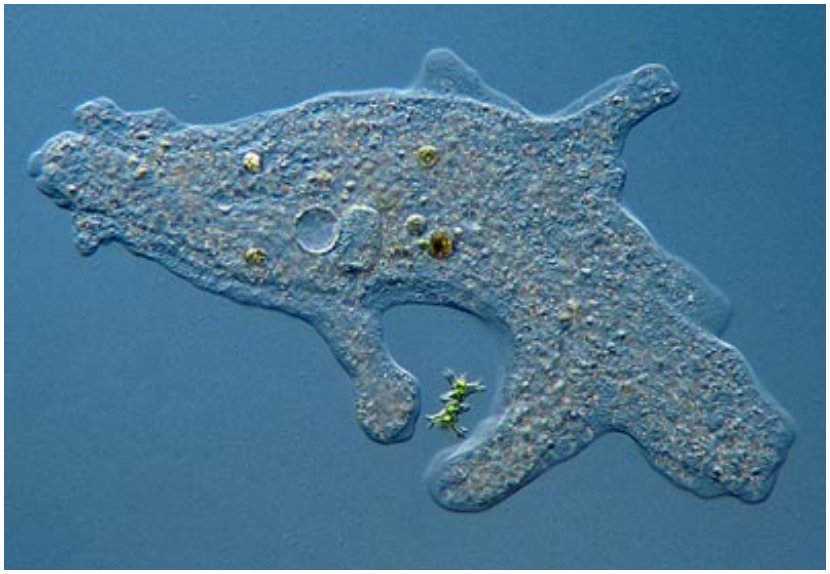
Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.



Amoeba



Amoeba proteus*, pseudopods slowly engulf the small desmid *Staurastrum

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey, they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information
Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa
Parasites
Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease
The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following chapters.

Symbionts
Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa
While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

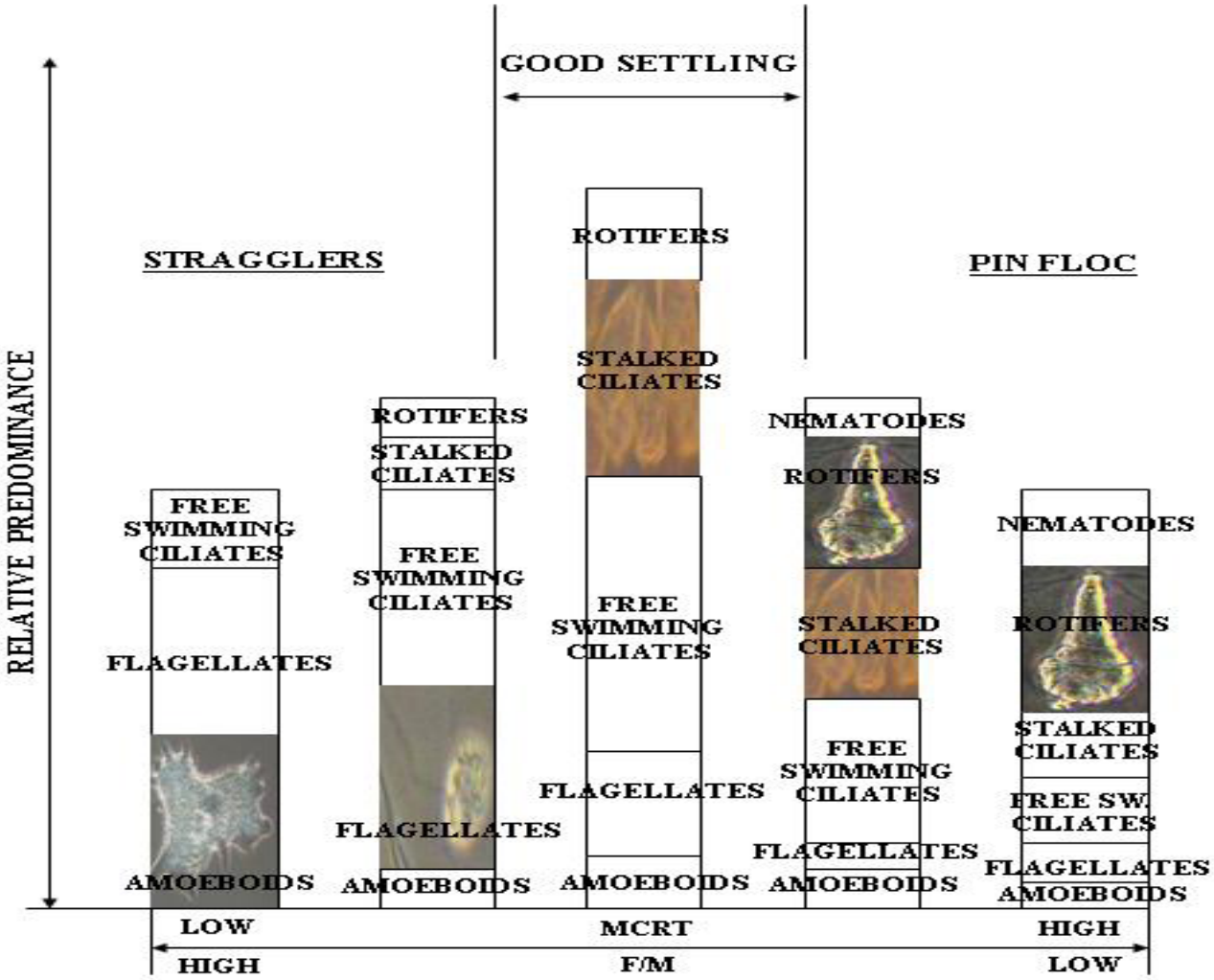
Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

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

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).



Commonly found organisms that are present in wastewater treatment

Wastewater Treatment Biology

Four (4) groups of bugs do most of the ***"eating"*** in the activated sludge process. The first group is the bacteria which eat the dissolved organic compounds. The second and third groups of bugs are microorganisms known as the free-swimming and stalked ciliates. These larger bugs eat the bacteria and are heavy enough to settle by gravity. The fourth group is a microorganism, known as Suctoria, which feed on the larger bugs and assist with settling.

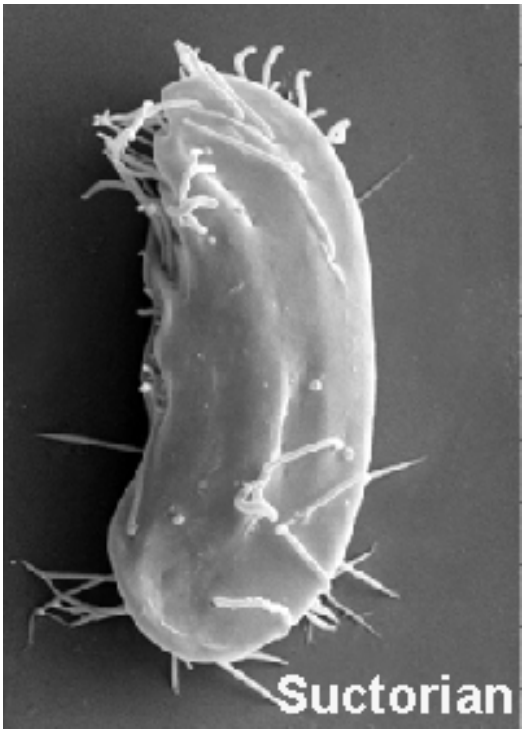
The interesting thing about the bacteria that eat the dissolved organics, is that they have no mouth. The bacteria have an interesting property, their "fat reserve" is stored on the outside of their body. This fat layer is sticky and is what the organics adhere to.

Once the bacteria have "contacted" their food, they start the digestion process. A chemical enzyme is sent out through the cell wall to break up the organic compounds. This enzyme, known as hydrolytic enzyme, breaks the organic molecules into small units which are able to pass through the cell wall of the bacteria.

In wastewater treatment, this process of using bacteria-eating-bugs in the presence of oxygen to reduce the organics in water is called activated sludge. The first step in the process, the contact of the bacteria with the organic compounds, takes about 20 minutes. The second step is the breaking up, ingestion and digestion processes, which takes four (4) to 24 hours.

The fat storage property of the bacteria is also an asset in settling. As the bugs "bump" into each other, the fat on each of them sticks together and causes flocculation of the non-organic solids and biomass.

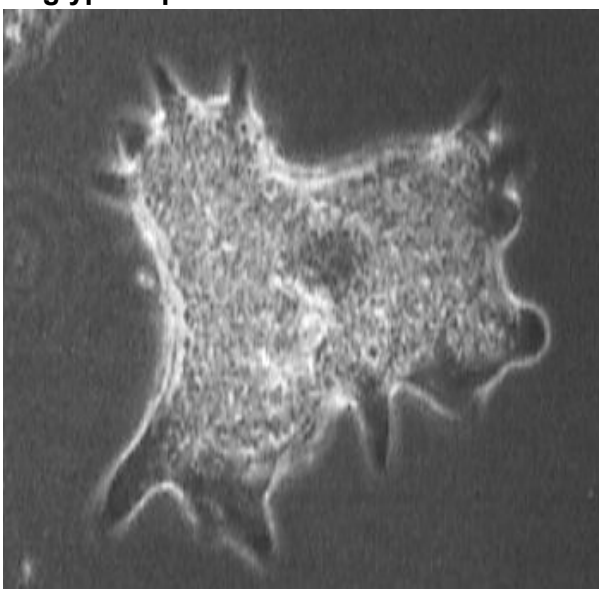
From the aeration tank, the wastewater, now called mixed liquor, flows to a secondary clarification basin to allow the flocculated biomass of solids to settle out of the water. The solids biomass, which is the activated sludge, contains millions of bacteria and other microorganisms, is used again by returning it to the influent of the aeration tank for mixing with the primary effluent and ample amounts of air.



Urostyla or maybe Euplotes

Wastewater Treatment Microlife

Euglypha sp.

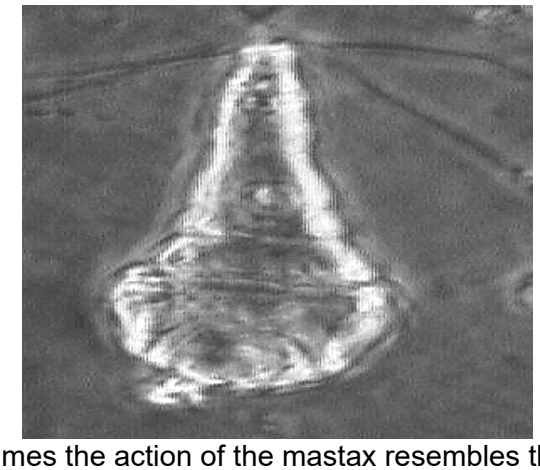


Euglypha (70-100 æm) is a shelled (testate) amoeba. Amoebas have jelly-like bodies. Motion occurs by extending a portion of the body (pseudopodia) outward. Shelled amoebas have a rigid covering which is either secreted or built from sand grains or other extraneous materials. The secreted shell of this Euglypha sp. consists of about 150 oval plates. Its spines project backward from the lower half of the shell. Euglypha spines may be single or in groups of two or three. The shell has an opening surrounded by 8-11 plates that resemble shark teeth under very high magnification.

The shell of Euglypha is often transparent, allowing the hyaline (watery) body to be seen inside the shell. The pseudopodia extend outward in long, thin, rays when feeding or moving. Euglypha primarily eats bacteria. Indicator: Shelled amoebas are common in soil, treatment plants, and stream bottoms where decaying organic matter is present. They adapt to a wide range of conditions and therefore are not good indicator organisms.

Euchlanis sp.

This microscopic animal is a typical rotifer. Euchlanis is a swimmer, using its foot and cilia for locomotion. In common with other rotifers, it has a head rimmed with cilia, a transparent body, and a foot with two strong swimming toes.



The head area, called the "corona," has cilia that beat rhythmically, producing a strong current for feeding or swimming. Euchlanis is an omnivore, meaning that its varied diet includes detritus, bacteria, and small protozoa. Euchlanis has a glassy shell secreted by its outer skin. The transparent body reveals the brain, stomach, intestines, bladder, and reproductive organs.

A characteristic of rotifers is their mastax, which is a jaw-like device that grinds food as it enters the stomach. At times the action of the mastax resembles the pulsing action of a heart. Rotifers, however, have no circulatory system. Indicator: Euchlanis is commonly found in activated sludge when effluent quality is good. It requires a continual supply of dissolved oxygen, evidence that aerobic conditions have been sustained.

Protozoan Diseases

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

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days.

Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.

Summary of Common Waterborne Diseases

Name Causative organism Source of organism Disease

Viral gastroenteritis ***Rotavirus*** mostly in young children; Human feces; Diarrhea or vomiting.

Norwalk-like viruses Human feces; also, shellfish; lives in polluted waters; Diarrhea and vomiting.

Salmonellosis ***Salmonella*** (bacterium) Animal or human feces; Diarrhea or vomiting.

Escherichia coli-- E. coli O157:H7 (bacterium) Other *E. coli* organisms. Human feces; Symptoms vary with type caused; gastroenteritis

Typhoid ***Salmonella typhi*** (bacterium) Human feces, urine Inflamed intestine, enlarged spleen, high temperature— sometimes fatal.

Shigellosis ***Shigella*** (bacterium) Human feces Diarrhea.

Cholera ***Vibrio cholerae*** (bacterium) Human feces; also, shellfish; lives in many coastal waters; Vomiting, severe diarrhea, rapid dehydration, mineral loss —high mortality.

Hepatitis A virus Human feces; shellfish grown in polluted waters; Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain — low mortality, lasts up to four months.

Amebiasis ***Entamoeba histolytica*** Human feces; Mild diarrhea, dysentery, (protozoan) extra intestinal infection.

Giardiasis ***Giardia lamblia*** (protozoan) Animal or human feces; Diarrhea, cramps, nausea, and general weakness — lasts one week to months.

Cryptosporidiosis ***Cryptosporidium parvum*** (protozoan) Animal or human feces Diarrhea, stomach pain — lasts days to weeks.

Source: Adapted from American Water Works Association, *Introduction to Water Treatment: Principles and Practices of Water Supply Operation*s, Denver CO, 1984.

The Best Method to kill most of these Bugs

Disinfection is usually synonymous with chlorination. That is because chlorine addition is by far the most common form of disinfection used today. In this section, the main emphasis will be on chlorination: how it works, safety, types of chlorine, basic chemistry of chlorine and an introduction to **CT** values. Disinfection is the process of killing microorganisms in water that might cause disease (pathogens). Disinfection, however, should not be confused with sterilization, which is the destruction of all microorganisms. Disinfection is concerned only with killing pathogens. Cryptosporidium parvum and Giardia lamblia will require proper water treatment techniques.

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Chapter 1 Review

Example Question, fill-in-the blank with one correct answer.
_____ is usually credited for dispelling the notion of spontaneous generation and proving that organisms reproduce new organisms.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

Review, you can check your answers at the end of this section.
1. The German scientist _____, a student of Jacob Henle, and the British surgeon Joseph Lister developed techniques for growing cultures of single organisms that allowed the assignment of specific bacteria to specific diseases.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

2. The first experimental transmission of a viral infection was accomplished in about 1880 by the German scientist _____, when he demonstrated that extracts from infected tobacco leaves could transfer tobacco mosaic disease to a new plant, causing spots on the leaves.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

3. Because _____ was unable to isolate a bacterium or fungus from the tobacco leaf extracts, he considered the idea that tobacco mosaic disease might be caused by a soluble agent, but he concluded incorrectly that a new type of bacteria was likely to be the cause.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

4. The Russian scientist _____ extended Mayer's observation and reported in 1892 that the tobacco mosaic agent was small enough to pass through a porcelain filter known to block the passage of bacteria.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

5. In 1917 the French-Canadian scientist _____ discovered that viruses of bacteria, which he named bacteriophage, could make holes in a culture of bacteria.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

6. In 1935 the American biochemist _____ crystallized tobacco mosaic virus to demonstrate that viruses had regular shapes, and in 1939 tobacco mosaic virus was first visualized using the electron microscope.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

7. In 1898 the German bacteriologists Friedrich August Johannes Löffler and Paul F. Frosch (both trained by _____) described foot-and-mouth disease virus as the first filterable agent of animals.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

8. In 1900, the American bacteriologist _____ and colleagues recognized yellow fever virus as the first human filterable agent.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

9. For several decades viruses were referred to as filterable agents, and gradually the term virus (Latin for "_____" or "poison") was employed strictly for this new class of infectious agents.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

10. Through the 1940s and 1950s many critical discoveries were made about viruses through the study of _____ because of the ease with which the bacteria they infect could be grown in the laboratory.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

11. Between 1948 and 1955, scientists at the National Institutes of Health (NIH) and at Johns Hopkins Medical Institutions revolutionized the study of animal viruses by developing _____ that permitted the growth and study of many animal viruses in laboratory dishes.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

12. Louis Pasteur along with _____ developed the germ theory of disease which states that "a specific disease is caused by a specific type of microorganism."
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

13. In 1876, _____ established an experimental procedure to prove the germ theory of disease. This scientific procedure is known as Koch's postulates.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

14. _____ postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

15. _____ observed small empty chambers in the structure of cork with the help of his crude microscope. He called them cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

16. Two German biologists _____ and Thedore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

17. _____ completed the cell theory with the idea that all cells must arise from preexisting cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

18. There's even a species of _____—Deinococcus radiodurans—that can withstand blasts of radiation 1,000 times greater than would kill a human being.
A. Metabolism
B. Cell
C. Mutation
D. Bacteria
E. None of the above

19. “Bacteria” is a singular word. The plural for this word is “bacterium” (**bacter** = rod, staff).
A. True
B. False

20. Bacteria are prokaryotes (Kingdom Monera), which means that they have a large nucleus.
A. True
B. False

21. Bacteria do have one chromosome of single-stranded DNA in a ring. They reproduce by binary fission.
A. True
B. False

22. Most bacteria lack or have very few internal membranes, which means that they don’t have some kinds of organelles (like mitochondria or chloroplasts). Most bacteria are **benign** (**benign** = good, friendly, kind) or beneficial, and only a few are “bad guys” or **pathogens**.
A. True
B. False

23. Kingdom Monera is a very diverse group. All bacteria relatives can do photosynthesis—because they have chloroplasts, because chlorophyll and other needed chemicals are built into their cell membranes.
A. True
B. False

24. These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they’re not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_3, while others are poisoned/killed by it.
A. True
B. False

25. Bacteria fall into a category of life called the Prokaryotes (pro-carry-oats). Prokaryotes' genetic material, or DNA, is not enclosed in a cellular compartment called the nucleus.
A. True
B. False

26. Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes (you-carry-oats), creatures whose cells have nuclei.
A. True
B. False

27. The mitochondria (mite-oh-con-dree-uh) that make energy for your body cells is one example of such an organelle.
A. True
B. False

28. There are less than 200 hundred of species of bacteria, but all of them are basically one of five different shapes.
A. True
B. False

29. Some bacteria are rod - or stick-shaped and are called _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

30. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

31. Most bacteria secrete a covering for themselves which we call a **cell wall**.
A. True
B. False

32. Bacterial cell walls do contain cellulose like plant cell walls do.
A. True
B. False

33. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.
A. True
B. False

34. All antibiotics, like penicillin, prohibit the formation of the chemical cross linkages needed to make peptidoglycan.
A. True
B. False

35. That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.
A. True
B. False

36. However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls.
A. True
B. False

37. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. Dr. Hans Christian Gram, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Hetertropic Gram Count**.
A. True
B. False

38. In this process, the amount of cellulose in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained, thus bacterial cells can be $Gram^{+}$ or $Gram^{-}$.
A. True
B. False

39. $Gram^{+}$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color.
A. True
B. False

40. Human cells have 46 (23 pairs) chromosomes. Each _____ consists of many genes.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

41. A gene is a coiled unit made up of _____ and proteins that codes for, or determines, a particular characteristic of an individual organism.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

42. The cellular material outside the _____; composed of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, centrioles, microtubules, lysosomes and vacoules.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

43. Some eukaryotic cells possess relatively long and thin structures called _____. These are organs of locomotion.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

44. _____ are also organs of locomotion but are shorter and more numerous.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

45. All bacteria are _____.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

46. The _____ of a procaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

47. A typical bacterial _____ contains approximately 10,000 genes.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

48. Semi-liquid, surrounds the chromosome and is contained within the plasma membrane. Within the _____ are located several ribosomes-which are the sites of protein synthesis.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

49. Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei.
A. True
B. False

50. The eukaryotes share a common origin, and are often treated formally as a superkingdom, empire, or domain.
A. True
B. False

51. The name “eukaryotes” comes from the Greek ***eus*** or true and ***karyon*** or nut, referring to the nucleus.
A. True
B. False

52. Protists are eukaryotes because they all have a nucleus.
A. True
B. False

53. Protists have mitochondria, although some have later lost theirs. _____ were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

54. Many Protists have chloroplasts with which they carry on photosynthesis. _____ were derived from photosynthetic cyanobacteria (also prokaryotes) living within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

55. Eukaryotic cells are generally much larger than _____, typically with a thousand times their volumes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

56. Eukaryotic cells have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of _____ and microfilaments, which plays an important role in defining the cell's organization.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

57. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among _____.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

58. Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or _____, can form by budding off of other membranes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

1.E, 2.D, 3.D, 4.C, 5.C, 6.D, 7.B, 8.E, 9.A, 10.B, 11.C, 12.A, 13.A, 14.A, 15.E, 16.B, 17.C, 18.D, 19.B, 20.B, 21.B, 22.A, 23.B, 24.B, 25.A, 26.A, 27.A, 28.B, 29.B, 30. E, 31.A, 32.B, 33.A, 34.B, 35.A, 36.A, 37.B, 38.B, 39.A, 40.C, 41.D, 42.B, 43.A, 44.B, 45.C, 46.E, 47.E, 48.D, 49.A, 50.A, 51.A, 52.A, 53.B, 54.E, 55.C, 56.D, 57.C, 58.A

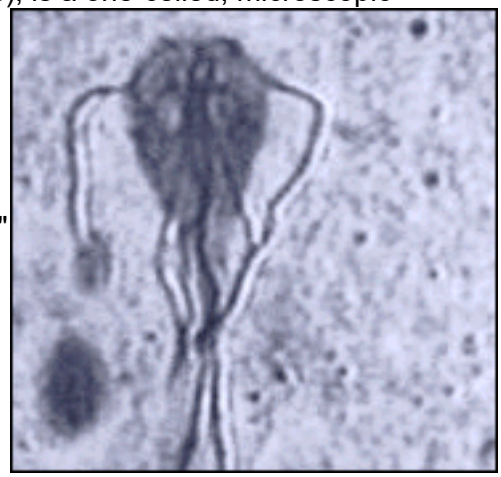
Microorganisms

Contaminant	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Cryptosporidium	zero	TT [3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and fecal animal waste
Giardia lamblia	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Heterotrophic plate count	n/a	TT[3]	HPC has no health effects; it is an analytic method used to measure the variety of bacteria that are common in water. The lower the concentration of bacteria in drinking water, the better maintained the water system is.	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT[3]	Legionnaire's Disease, a type of pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)	zero	5.0%[4]	Not a health threat in itself; it is used to indicate whether other potentially harmful bacteria may be present[5]	Coliforms are naturally present in the environment; as well as feces; fecal coliforms and *E. coli* only come from human and animal fecal waste.
Turbidity	n/a	TT[3]	Turbidity is a measure of the cloudiness of water. It is used to indicate water quality and filtration effectiveness (e.g., whether disease-causing organisms are present). Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.	Soil runoff
Viruses (enteric)	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste

Giardiasis *Giardia lamblia* Chapter 2

Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella. In Europe, it is sometimes referred to as Lamblia intestinalis.

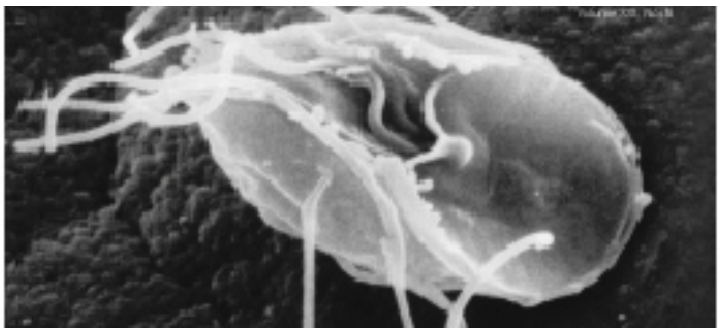
Giardiasis is the most frequent cause of non-bacterial diarrhea in North America. *Giardia duodenalis*, cause of giardiasis (GEE-are-DYE-uh-sis), is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.



Approximately one week after ingestion of the *Giardia* cysts, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.

It is possible to experience some, not all, of the symptoms, yet still shed cysts and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.

The organism exists in two different forms--a hardy, dormant cyst that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the parasite. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.



Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', which suggest that the condition of amitochondrialism is not primitive to eukaryotes but instead is a result of reductive evolution.

The microaerophilic Giardia uses these mitosomes in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.

Nature of Disease

Organisms that appear identical to those that cause human illness have been isolated from domestic animals (dogs and cats) and wild animals (beavers and bears). A related but morphologically distinct organism infects rodents, although rodents may be infected with human isolates in the laboratory. Human giardiasis may involve diarrhea within 1 week of ingestion of the cyst, which is the environmental survival form and infective stage of the organism.

Normally illness lasts for 1 to 2 weeks, but there are cases of chronic infections lasting months to years. Chronic cases, both those with defined immune deficiencies and those without, are difficult to treat.

The disease mechanism is unknown, with some investigators reporting that the organism produces a toxin while others are unable to confirm its existence. The organism has been demonstrated inside host cells in the duodenum, but most investigators think this is such an infrequent occurrence that it is not responsible for disease symptoms. Mechanical obstruction of the absorptive surface of the intestine has been proposed as a possible pathogenic mechanism, as has a synergistic relationship with some of the intestinal flora.

Giardia can be excysted, cultured and encysted in vitro; new isolates have bacterial, fungal, and viral symbionts. Classically, the disease was diagnosed by demonstration of the organism in stained fecal smears.

Several strains of *G. lamblia* have been isolated and described through analysis of their proteins and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.

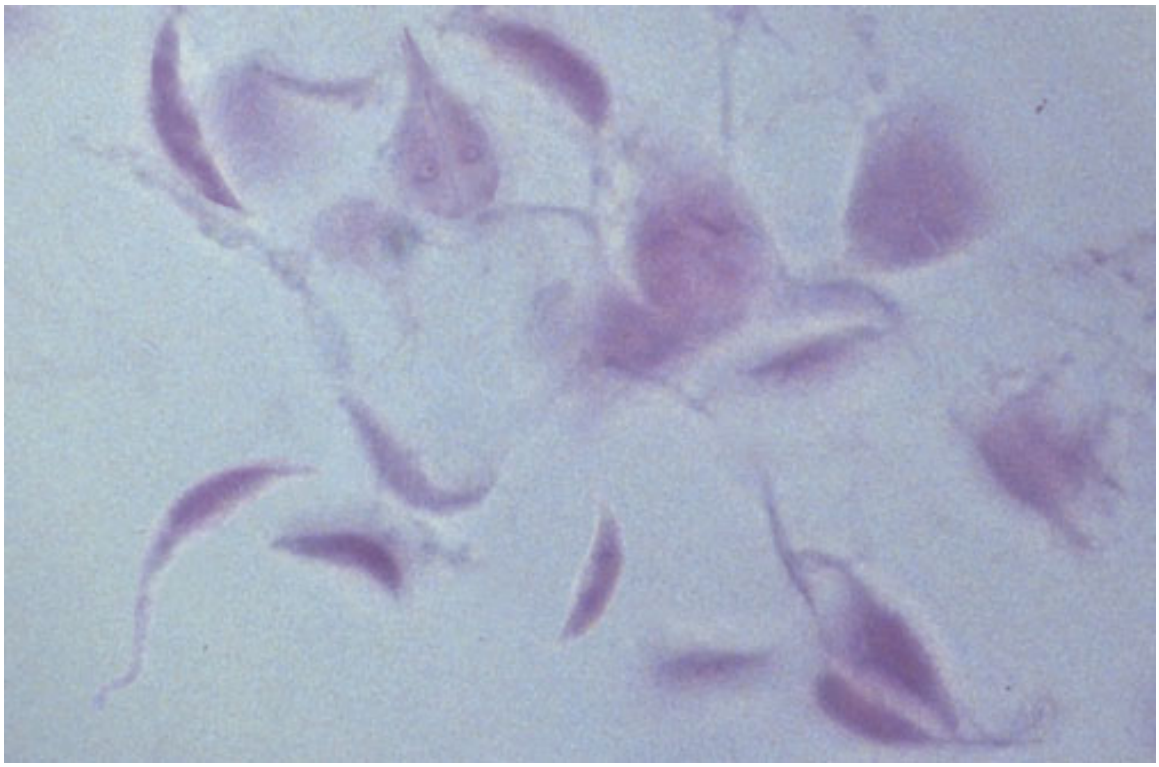
Diagnosis of Human Illness

Giardia lamblia is frequently diagnosed by visualizing the organism, either the trophozoite (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. An enzyme linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.

Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the survival of the organism.

Relative Frequency of Disease
Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically. The overall incidence of infection in the United States is estimated at 2% of the population. This disease afflicts many homosexual men, both HIV-positive and HIV-negative individuals. This is presumed to be due to sexual transmission. The disease is also common in child day care centers, especially those in which diapering is done.

Acute outbreaks appear to be common with infants and is not usually associated with water but is related to child care and diaper changing hygiene procedures. When I worked for a major water provider, I would receive 2-3 calls a week about infants diagnosed with Giardiasis. The problem lies with the water provider in that we are obligated to investigate and analyze all water customer complaints and make sure that our water is safe.



This is an example of infectious diarrhea due to Giardia lamblia infection of the small intestine. The small pear-shaped trophozoites live in the duodenum and become infective cysts that are excreted. They produce a watery diarrhea. A useful test for diagnosis of infectious diarrheas is stool examination for ova and parasites.

Course of Disease and Complications
About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.

Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.

Target Populations
Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.

Major Outbreaks
Major outbreaks are associated with contaminated water systems that do not use sand filtration or have a defect in the filtration system.

In April 1988, the Albuquerque Environmental Health Department and the New Mexico Health and Environment Department investigated reports of giardiasis among members of a church youth group in Albuquerque. The first two members to be affected had onset of diarrhea on March 3 and 4, respectively; stool specimens from both were positive for Giardia lamblia cysts. These two persons had only church youth group activities in common.

On August 8, 1983, the Utah Department of Health was notified by the Tooele County Health Department (TCHD) of an outbreak of diarrheal illness in Tooele, Utah, possibly associated with a contaminated public water supply that resulted from flooding during Utah's spring thaw.

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Giardia Images

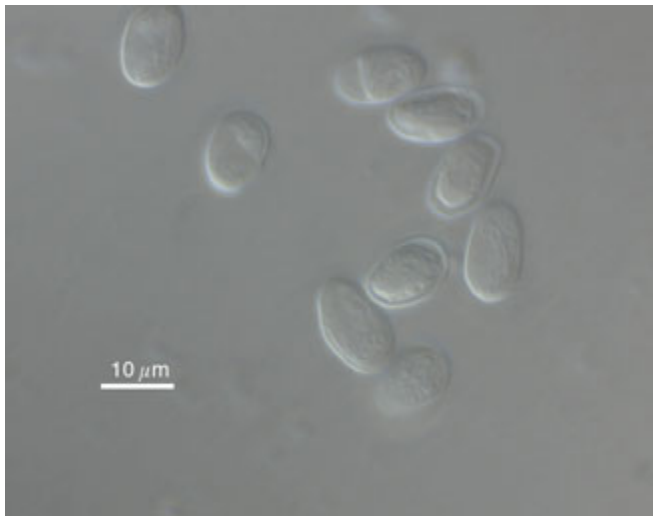


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Differential interference contrast (DIC) image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. Cysts are ovoidal or elipsoidal objects, usually 11-14 microns in length. Cysts may contain as many as 4 nuclei, and residual structures from their trophozoite or vegetative form. These residua include central axonemes, remnants of the striated disk, and remnant median bodies. In some cysts these structures will be indistinct. Scale bar is 10 microns.

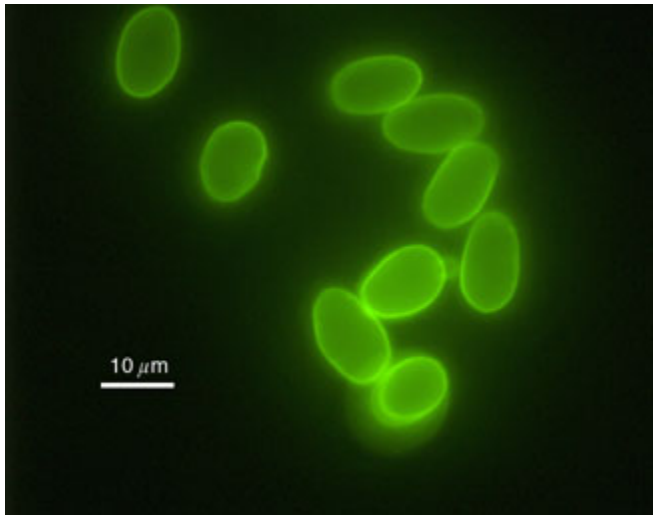


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Immunofluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with commercially available immunofluorescent antibodies. Cysts should have an intense apple green fluorescence on the periphery of their cyst wall, and measure 11-14 microns in length. Scale bar is 10 microns.

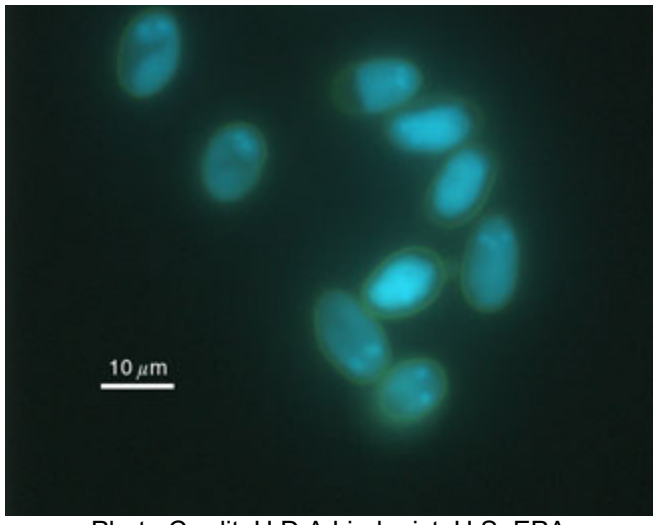


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Fluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with 4,6-diamidino 2-phenyl-indole dihydrochloride (DAPI). DAPI interacts with nucleic acids and stains the nuclei within the cyst. There should be 4 nuclei in each cyst. Cysts that appear to have fewer than 4 stained nuclei may have 4 nuclei with the others not visible in this plane of focus.

Cysts with no nuclei visible, may be dead, may be resistant to DAPI staining, or may be organisms other than *G. lamblia*.

Giardiasis *Giardia lamblia* Chapter 2 Review

1. Giardia duodenalis, cause of giardiasis, is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.
A. True
B. False

2. Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella.
A. True
B. False

3. In Europe, Giardia lamblia is sometimes referred to as Lamblia intestinalis.
A. True
B. False

4. Giardiasis is the least frequent cause of non-bacterial diarrhea in North America.
A. True
B. False

5. Approximately one week after ingestion of the Giardia _____, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

6. It is possible to experience some, not all, of the symptoms, yet still shed _____ and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

7. The organism exists in two different forms--a hardy, dormant _____that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

8. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the _____. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

9. Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', which suggest that the condition of _____ is not primitive to eukaryotes but instead is a result of reductive evolution.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

10. The microaerophilic Giardia uses these _____ in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

11. Several strains of G. lamblia have been isolated and described through analysis of their _____ and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

12. Giardia lamblia is frequently diagnosed by visualizing the organism, either the _____ (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

13. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. An _____linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

14. Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the _____ of the organism.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

15. Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically.
A. True
B. False

16. The disease is also common in child day care centers, especially those in which diapering is done.
A. True
B. False

17. Acute outbreaks appear to be common with infants and are not usually associated with water but are related to child care and diaper changing hygiene procedures.
A. True
B. False

18. About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.
A. True
B. False

19. Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment.
A. True
B. False

20. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.
A. True
B. False

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

1.B, 2.A, 3.A, 4.B,5.A, 6.A, 7.A, 8.C, 9.D, 10.E, 11.D,12.B, 13.E, 14.A, 15.A, 16.A, 17.A, 18.A, 19.A, 20.A, 21.A

Cryptosporidiosis *Cryptosporidium* Chapter 3

Introduction

Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of *Cryptosporidium parvum*, or the disease it causes, cryptosporidiosis. Today, however, public health and water utility officials are increasingly called on to provide information and make decisions about the control of this protozoan found in public water supplies, recreational water and other areas.

Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death. Those with CD4 counts above 200 may recover from the symptoms of cryptosporidiosis yet maintain the infection asymptomatically, with symptoms potentially returning if their CD4 count later drops.

Other diseases besides AIDS can cause immunosuppression severe enough to lead to chronic cryptosporidiosis. Persons with these diseases should also be concerned about becoming infected. These diseases include congenital agammaglobulinemia, congenital IgA deficiency and cancer. Persons taking corticosteroids, for cancer and bone marrow or organ transplants, also need to be concerned about becoming infected. Even though persons who are taking immunosuppressive drugs may develop chronic and/or severe cryptosporidiosis, the infection usually resolves when these drugs are decreased or stopped. Persons taking immunosuppressive drugs need to consult with their healthcare provider if they believe they have cryptosporidiosis.

Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis.

Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.

Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.

AIDS

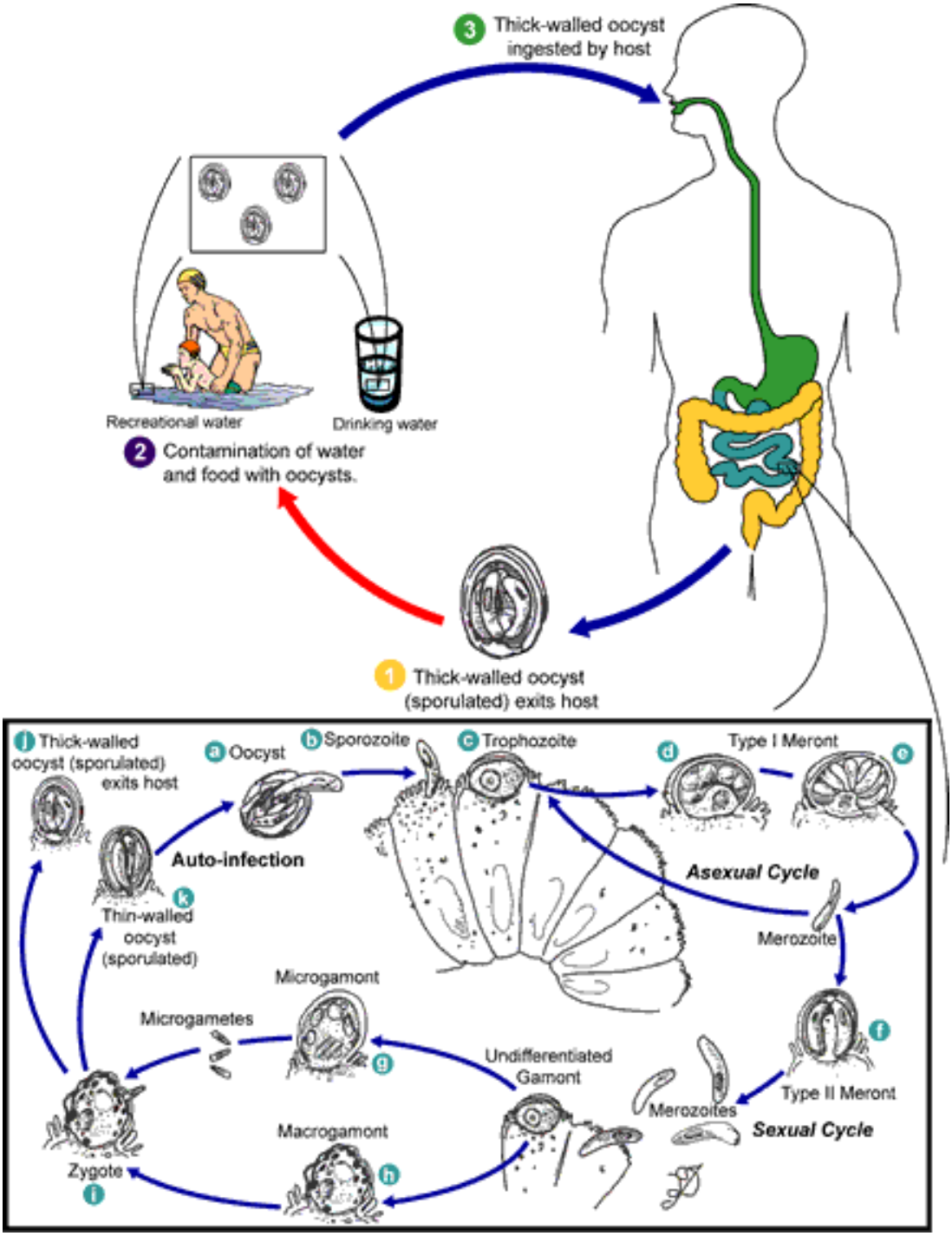
Patients with AIDS can have a large number of stools per day for months or even years. There is currently no cure for cryptosporidiosis, though drug research is continuing. Patients who suspect they may have cryptosporidiosis should drink extra fluids and may wish to drink oral rehydration therapy liquid,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

HIV-positive Individuals
HIV-positive individuals who suspect they have cryptosporidiosis should contact their healthcare provider. Infected individuals should be advised to wash their hands frequently, especially before preparing food and after going to the toilet.

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

Prevention
Washing hands is the most effective means of preventing cryptosporidiosis transmission.

For the immunocompromised, sex, including oral sex, that involves possible contact with stool should be avoided. Immunocompromised individuals should also avoid the stool of all animals and wash their hands thoroughly after any contact with animals or the living areas of animals. Immunocompromised persons may also wish to wash, peel, or cook all vegetables and to take extra measures, such as boiling or filtering their drinking water, to ensure its safety.



Life cycle of *Cryptosporidium parvum* and *C. hominis*.

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 1.

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 2.

Following ingestion (and possibly inhalation) by a suitable host 3, excystation a occurs. The sporozoites are released and parasitize epithelial cells (b, c) of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) (d, e, f) and then sexual multiplication (gametogony) producing microgamonts (male) g and macrogamonts (female) h.

Upon fertilization of the macrogamonts by the microgametes (i), oocysts (j, k) develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host j, and the thin-walled oocyst k, 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

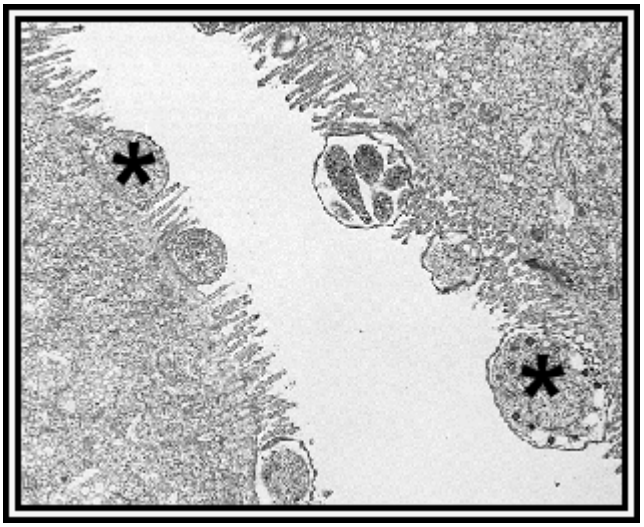
Genus *Cryptosporidium*

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.

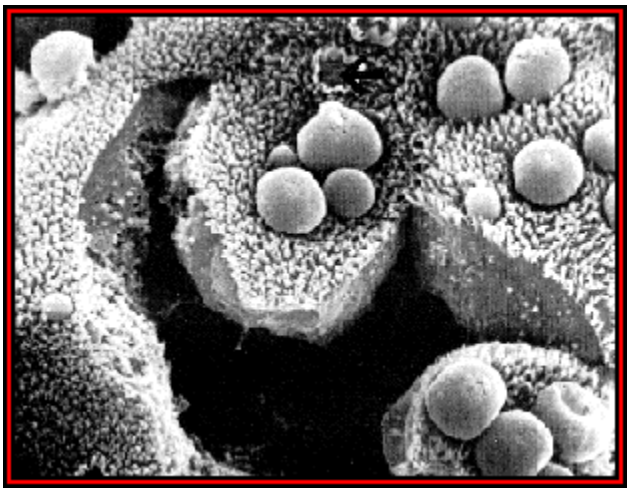
Cryptosporidium is a small parasite, measuring about 3-5 µm. It lives on (or just under) the surface of the cells lining the small intestine, reproduces asexually, and oocysts are passed in the feces. Transmission of the infection occurs via the oocysts. Many human infections have been traced to the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

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."

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.

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.

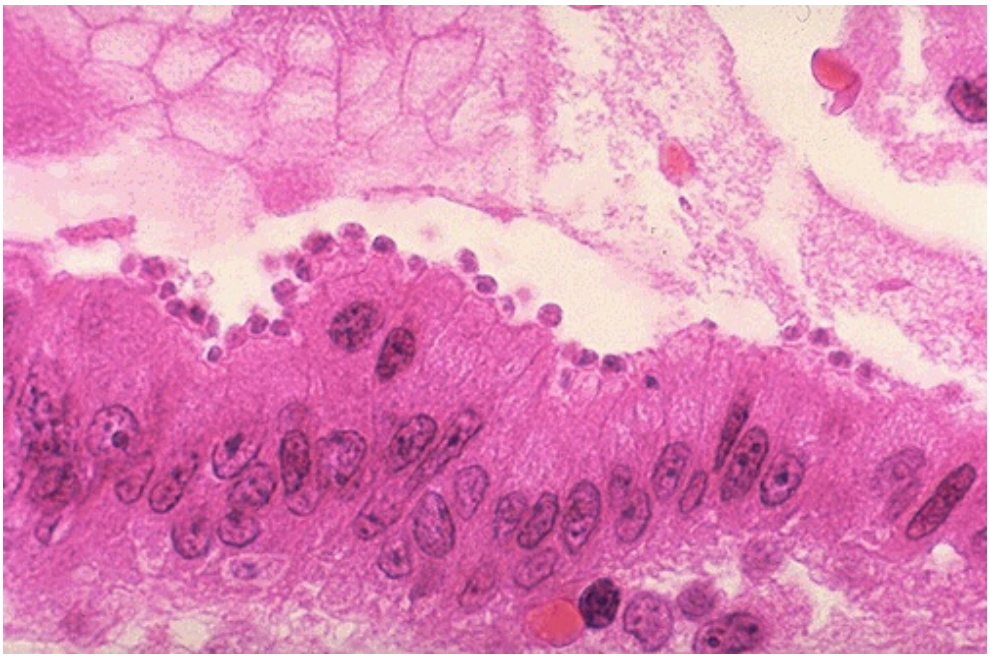
Avoid food that might be contaminated.

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

Take extra care when traveling.

If you travel to developing nations, you may be at a greater risk for *Cryptosporidium* infection because of poorer water treatment and food sanitation. Warnings about food, drinks, and swimming are even more important when visiting developing countries. Avoid foods and drinks, in particular raw fruits and vegetables, tap water, or ice made from tap water, unpasteurized milk or dairy products, and items purchased from street vendors. These items may be contaminated with *Cryptosporidium*. Steaming-hot foods, fruits you peel yourself, bottled and canned processed drinks, and hot coffee or hot tea are probably safe. Talk with your health care provider about other guidelines for travel abroad.

Avoid fecal exposure during sexual activity.



This is another infectious agent that is becoming more frequent in immunocompromised patients, particularly those with AIDS. The small round blue organisms at the lumenal border are cryptosporidia. Cryptosporidiosis produces a copious watery diarrhea.

A Guide to Water Filters and Bottled Water

Filtering tap water
Many, but not all, available home water filters remove Cryptosporidium. Some filter designs are more suitable for removal of *Cryptosporidium* than others. Filters that have the words "reverse osmosis" on the label protect against *Cryptosporidium*. Many other types of filters that work by micro-straining also work. Look for a filter that will remove particles that are less than or equal to 1 micron in diameter.

There are two types of these - "absolute 1 micron" filters and "nominal 1 micron" filters. The absolute 1 micron filter will more consistently remove *Cryptosporidium* than a nominal filter. Some nominal 1 micron filters will allow 20% to 30% of 1 micron particles to pass through.

NSF-International
NSF-International (NSF) does independent testing of filters to determine if they remove *Cryptosporidium*. To find out if a particular filter is certified to remove *Cryptosporidium*, you can look for the NSF trademark plus the words "cyst reduction" or "cyst removal" on the product label information. You can also contact the NSF at 789 N. Dixboro Road, Ann Arbor, MI 48105 USA, toll free1-877-867-3435, fax 313-769-0109, email info@nsf.org, or visit their Web site at www.nsf.org/certified/DWTU/.

At their Web site, you can enter the model number of the unit you intend to buy to see if it is on their certified list, or you can look under the section entitled "Reduction claims for drinking water treatment units - Health Effects" and check the box in front of the words "Cyst Reduction." This will display a list of filters tested for their ability to remove *Cryptosporidium*.

Because NSF testing is expensive and voluntary, some filters that may work against *Cryptosporidium* have not been NSF-tested. If you choose to use a product not NSF-certified, select those technologies more likely to reduce *Cryptosporidium*; this includes filters with reverse osmosis and those that have an absolute pore size of 1 micron or smaller.

Package and Label information for purchasing water filters:

Filters designed to remove crypto ***(any of the four messages below on a package label indicate that the filter should be able to remove crypto***	**Filters labeled only with these words may NOT be designed to remove crypto**
Reverse osmosis (with or without NSF testing)	*Nominal* pore size of 1 micron or smaller
Absolute pore size of 1 micron or smaller (with or without NSF testing)	One micron filter
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst removal	Effective against *Giardia*
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst reduction	Effective against parasites
	Carbon filter
	Water purifier
	EPA approved — Caution: EPA does not approve or test filters
	EPA registered — Caution: EPA does not register filters based on their ability to remove Cryptosporidium
	Activated carbon
	Removes chlorine
	Ultraviolet light
	Pentiodide resins
	Water softener

Note: Filters collect germs from water, so someone who is not HIV infected or immune impaired should change the filter cartridges. Anyone changing the cartridges should wear gloves and wash hands afterwards. Filters may not remove *Cryptosporidium* as

well as boiling does because even good brands of filters may sometimes have manufacturing flaws that allow small numbers of *Cryptosporidium* to get in past the filter. Selection of NSF-Certified filters provides additional assurance against such flaws. Also, poor filter maintenance or failure to replace the filter cartridges as recommended by the manufacturer can cause a filter to fail.

If you drink bottled water, read the label and look for this information:

Water so labeled has been processed by method effective against crypto	Water so labeled may not have been processed by method effective against crypto
Reverse osmosis treated	Filtered
Distilled	Micro-filtered
Filtered through an *absolute* 1 micron or smaller filter	Carbon-filtered
"One micron absolute"	Particle-filtered
	Multimedia-filtered
	Ozonated
	Ozone-treated
	Ultraviolet light-treated
	Activated carbon-treated
	Carbon dioxide-treated
	Ion exchange-treated
	Deionized
	Purified
	Chlorinated

Bottled water labels reading "well water," "artesian well water," "spring water," or "mineral water" do not guarantee that the water does not contain crypto. However, water that comes from protected well or protected spring water sources is less likely to contain crypto than bottled water or tap water from less protected sources, such as rivers and lakes.

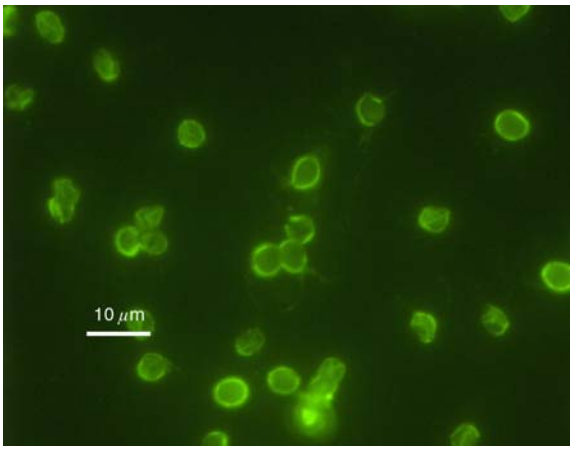
Home distillers: You can remove crypto and other germs from your water with a home distiller. If you use one, you need to carefully store your water as recommended for storing purified water.

Other drinks: Soft drinks and other beverages may or may not contain crypto. You need to know how they were prepared to know if they might contain crypto.

If you consume prepared beverages, look for drinks from which crypto has been removed:

Crypto killed or removed in preparation	Crypto may not be killed or removed in preparation
Canned or bottled soda, seltzer, and fruit drinks	Fountain drinks
Steaming hot (175 degrees F or hotter) tea or coffee	Fruit drinks you mix with tap water from frozen concentrate
Pasteurized drinks	Iced tea or coffee

Juices made from fresh fruit can also be contaminated with crypto. Several people became ill after drinking apple cider made from apples contaminated with crypto. You may wish to avoid unpasteurized juices or fresh juices if you do not know how they were prepared.



Immunofluorescence image of *Cryptosporidium parvum* oocysts, purified from murine fecal material. (Same field of view) Oocysts were stained with commercially available immunofluorescent antibodies. Oocysts should have an intense apple green fluorescence on the periphery of their oocyst wall, and measure 4 to 6 microns in diameter. Scale bar is 10 microns. Photo Credit: H.D.A Lindquist, U.S. EPA.

Cryptosporidiosis Chapter 3 Review

1. Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of Cryptosporidium parvum, or the disease it causes, cryptosporidiosis.
A. True
B. False

2. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.
A. True
B. False

3. Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible.
A. True
B. False

4. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.
A. True
B. False

5. Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death.
A. True
B. False

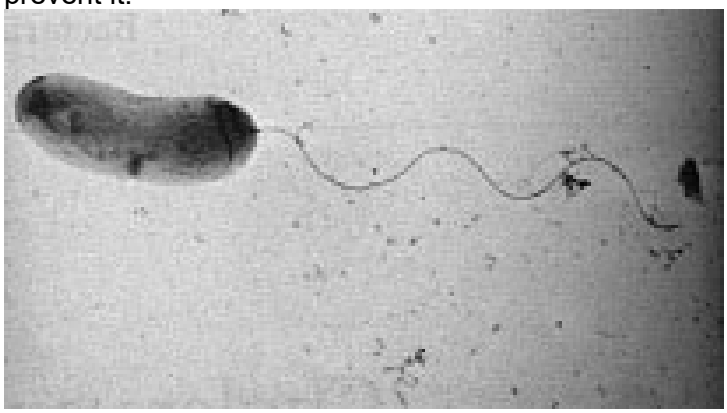
6. Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis.
A. True
B. False

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

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

Cholera *Vibrio cholerae* Chapter 4

Cholera has been very rare in industrialized nations for the last 100 years; however, the disease is still common today in other parts of the world, including the Indian subcontinent and sub-Saharan Africa. Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.



Vibrio cholerae

Cholera, which is derived from a Greek term meaning "flow of bile," is caused by *Vibrio cholerae* and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of hours of infection.

In 1883, Robert Koch discovered *V cholerae* during a cholera outbreak in Egypt. The organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. *V cholerae* O1 or O139 are associated with epidemic cholera. *V cholerae* O1 has 2 major biotypes: classic and El Tor.

Currently, El Tor is the predominant cholera pathogen. Organisms in both biotypes are subdivided into serotypes according to the structure of the O antigen, as follows:

- Serotype Inaba - O antigens A and C
- Serotype Ogawa - O antigens A and B
- Serotype Hikojima - O antigens A, B, and C

How does a person get cholera?

A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.

The cholera bacterium may also live in the environment in brackish rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.

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 is the risk for cholera in the United States?
In the United States, cholera was prevalent in the 1800s but has been virtually eliminated by modern sewage and water treatment systems. However, as a result of improved transportation, more persons from the United States travel to parts of Latin America, Africa, or Asia where epidemic cholera is occurring. U.S. travelers to areas with epidemic cholera may be exposed to the cholera bacterium. In addition, travelers may bring contaminated seafood back to the United States; foodborne outbreaks have been caused by contaminated seafood brought into this country by travelers.

Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat. The last major outbreak of cholera in the United States was in 1911. However, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.

What should travelers do to avoid getting cholera?
The risk for cholera is very low for U.S. travelers visiting areas with epidemic cholera. When simple precautions are observed, contracting the disease is unlikely.

All travelers to areas where cholera has occurred should observe the following recommendations:

- Drink only water that you have boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made with boiled water and carbonated, bottled beverages with no ice.
- Eat only foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish, including ceviche.
- Make sure all vegetables are cooked, avoid salads.
- Avoid foods and beverages from street vendors.
- Do not bring perishable seafood back to the United States.

A simple rule of thumb is "***Boil it, cook it, peel it, or forget it***."

Treatment

The objective of treatment is to replace fluid and electrolytes lost through diarrhea. Depending on the condition of the person, oral or intravenous fluid will be given. Tetracycline and other antibiotics may shorten the duration of the symptoms.
Note: Tetracycline is usually not prescribed for children until after all the permanent teeth have come in, because it can permanently discolor teeth that are still forming.

The World Health Organization (WHO) has developed an oral rehydration solution that is cheaper and easier to use than the typical intravenous fluid. This solution of sugar and electrolytes is now being used internationally.

Expectations (prognosis)

Severe dehydration can cause death. Given adequate fluids, most people will make a full recovery.

Complications

- Severe dehydration.

Calling your health care provider

Call your health care provider if profuse watery diarrhea develops.

Call your health care provider if signs of dehydration occur, including rapid pulse (heart rate), dry skin, dry mouth, thirst, "glassy" eyes, lethargy, sunken eyes, no tears, reduced or no urine, and unusual sleepiness or tiredness.

Susceptibility

Recent genetic research has determined that a person's susceptibility to cholera and other diarrheas) is affected by their blood type. Those with type O blood are the most susceptible. Those with type AB are the most resistant, virtually immune. Between these two extremes are the A and B blood types, with type A being more resistant than type B.

Carriers of the cystic fibrosis gene are protected from the severe effects of cholera because they don't lose water as quickly. This explains the high incidence of cystic fibrosis among populations which were formerly exposed to cholera.

Epidemic control and preventive measures
When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an adequate supply of safe drinking water, and good food hygiene. Effective food hygiene measures include cooking food thoroughly and eating it while still hot; preventing cooked foods from being contaminated by contact with raw foods, including water and ice, contaminated surfaces or flies; and avoiding raw fruits or vegetables unless they are first peeled. Washing hands after defecation, and particularly before contact with food or drinking water, is equally important.

Routine treatment of a community with antibiotics, or "mass chemoprophylaxis", has no effect on the spread of cholera, nor does restricting travel and trade between countries or between different regions of a country. Setting up a *cordon sanitaire* at frontiers uses personnel and resources that should be devoted to effective control measures, and hampers collaboration between institutions and countries that should unite their efforts to combat cholera.

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.

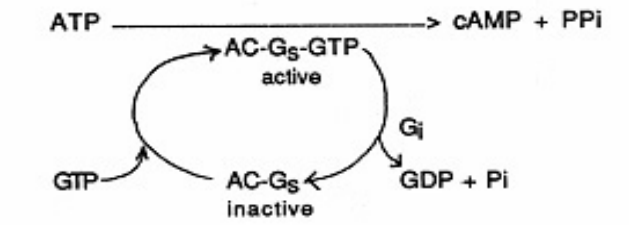
Cholera Toxin

Cholera toxin **activates the adenylate cyclase enzyme in cells of the intestinal mucosa** leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin.

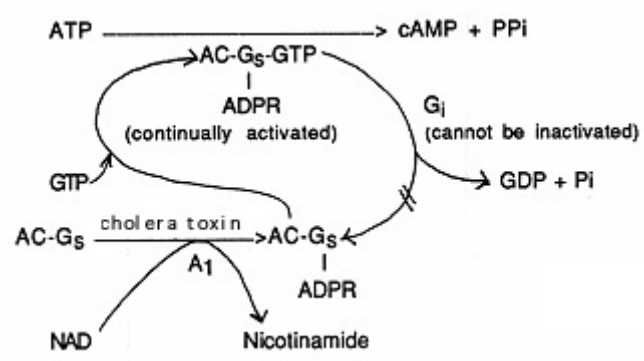
The toxin has been characterized and contains **5 binding (B) subunits** of 11,500 daltons, an active **(A1) subunit** of 23,500 daltons, and a **bridging piece (A2)** of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells.

Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP.

The normal situation is described as follows.



The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated. This situation can be illustrated as follows.



Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-.

The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes, causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.

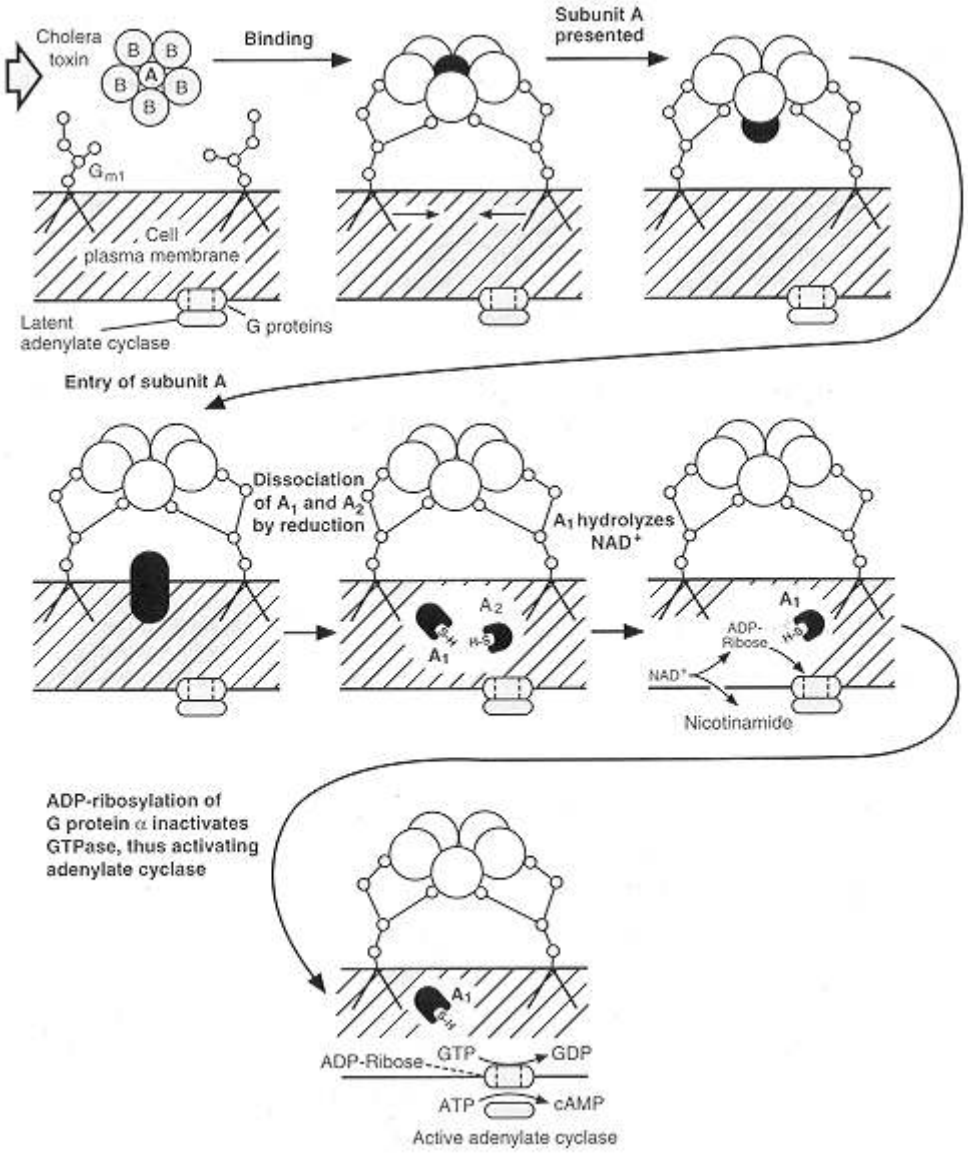
Last Word

E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT toxin is on a plasmid that can be transferred to other *E. coli* strains and probably to other enteric bacteria, as well. Close relationships between the genetic code for LT toxin and the cholera toxin undoubtedly exist but have not been documented as yet.

The genetic information for the toxin in *V. cholerae* is located on the bacterial chromosome. Other bacterial enterotoxins related to cholera toxin have been reported in non-group O *Vibrio* strains and a strain of *Salmonella*.

Enterotoxins, toxins which act in the GI tract, are produced by a wide variety of bacteria.

The family of heat-stable (ST) enterotoxins of *E. coli*, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin. Other enterotoxins, which elicit cytotoxic effects on intestinal epithelial cells, have been described from *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Aeromonas*, *Pseudomonas*, *Shigella*, *V. parahaemolyticus*, *Campylobacter*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium perfringens*, *C. difficile*, and *Staphylococcus aureus*.



Mechanism of action of cholera enterotoxin according to Finkelstein in Baron, Chapter 24. Cholera toxin approaches target cell surface. B subunits bind to oligosaccharide of GM1 ganglioside. Conformational alteration of holotoxin occurs, allowing the presentation of the A subunit to cell surface. The A subunit enters the cell. The disulfide bond of the A subunit is reduced by intracellular glutathione, freeing A1 and A2. NAD is hydrolyzed by A1, yielding ADP-ribose and nicotinamide. One of the G proteins of adenylate cyclase is ADP-ribosylated, inhibiting the action of GTPase and locking adenylate cyclase in the "on" mode.

Cholera Chapter 4 Review

1. Cholera, which is derived from a Greek term meaning “Running to the bathroom,” is caused by Vibrio cholerae and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of minutes of infection.
A. True
B. False

2. In 1883, Louis Pasteur discovered V cholerae during a cholera outbreak in Egypt.
A. True
B. False

3. Cholera has been very common in industrialized nations for the last 100 years.
A. True
B. False

4. Cholera is always life-threatening, it is easily prevented and treated with choromines.
A. True
B. False

5. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.
A. True
B. False

6. The V cholerae organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen.
A. True
B. False

7. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. V cholerae O1 or O139 are associated with epidemic cholera. V cholerae O1 has 2 major biotypes: classic and El Tor.
A. True
B. False

8. Currently, El Leche is the predominant cholera pathogen.
A. True
B. False

9. A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.
A. True
B. False

10. The cholera bacterium may also live in the environment in brackish rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.
A. True
B. False

11. Cholera (also called Asiatic flu) is a disease of the respiratory tract caused by the Vibrio cholerae bacterium. These bacteria are typically ingested by drinking water contaminated by improper sanitation or by eating improperly cooked fish, especially shellfish.
A. True
B. False

12. About one hundred Vibrio cholerae bacteria must be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a strong immune system, individuals with increased gastric acidity, or those who are malnourished.
A. True
B. False

13. Vibrio cholerae causes disease by producing a toxin that disables the _____ of G proteins which are part of G protein-coupled receptors in intestinal cells. This has the effect that the G proteins are locked in the "on position" binding GTP (normally, the G proteins quickly return to "off" by hydrolyzing GTP to GDP).
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

14. The _____ then cause adenylate cyclases to produce large amounts of cyclic AMP (cAMP) which results in the loss of fluid and salts across the lining of the gut.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

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

16. When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an _____ supply of safe drinking water, and good food hygiene.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. None of the above

17. _____ variation plays an important role in the epidemiology and virulence of cholera. The emergence of the Bengal strain is an example.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

18. The _____ of V. cholerae are shared with many water vibrios and therefore are of no use in distinguishing strains causing epidemic cholera.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

19. O antigens, however, do distinguish strains of V. cholerae into 139 known _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

20. Almost all strains of V. cholerae are _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

21. Until the emergence of the Bengal strain (which is "non-O1") a single serotype, designated O1, has been responsible for epidemic cholera. However, there are three distinct O1 biotypes, named Ogawa, Inaba and Hikojima, and each biotype may display the "classical" or El Tor _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

22. E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT _____ is on a plasmid that can be transferred to other E. coli strains and probably to other enteric bacteria, as well.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

23. Close relationships between the genetic code for LT _____ and the cholera toxin un-doubtedly exist but have not been documented as yet.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

24. The genetic information for the toxin in V. cholerae is located on the bacterial chromo-some. Other bacterial _____ related to cholera toxin have been reported in non-group O Vibrio strains and a strain of Salmonella.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

25. _____, toxins which act in the GI tract, are produced by a wide variety of bacteria. The family of heat-stable (ST) enterotoxins of E. coli, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

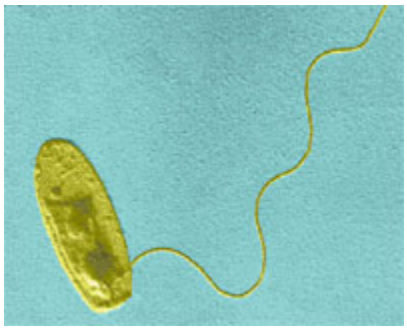
26. Other _____, which elicit cytotoxic effects on intestinal epithelial cells, have been described from Escherichia, Klebsiella, Enterobacter, Citrobacter, Aeromonas, Pseudomonas, Shigella, V. parahaemolyticus, Campylobacter, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, C. difficile, and Staphylococcus aureus.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

1.B, 2.B, 3.B, 4.B, 5.A, 6.A, 7.A, 8.B, 9.A,10.A, 11.B, 12.B, 13.A, 14.B, 15.C, 16.E, 17.D, 18.E, 19.C, 20.B, 21.D,22.A, 23.A, 24.D, 25.D, 26.D

Legionnaires' Disease *Legionella* Chapter 5

Introduction **Genus:** *Legionella* **Species:** *pneumophila*

The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of pneumonia at an American Legion convention led to 29 deaths. The causative agent, what would come to be known as Legionella pneumophila, was isolated and given its own genus. The organisms classified in this genus are Gram-negative bacteria that are considered intracellular parasites. The disease has two distinct forms:

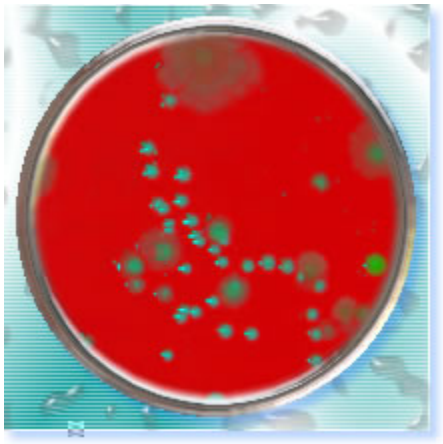


- Legionnaires' disease, the more severe form of infection which includes pneumonia, and
- Pontiac fever, a milder illness.

What have been the water sources for Legionnaires' disease?

The major source is water distribution systems of large buildings, including hotels and hospitals. Cooling towers have long been thought to be a major source for *Legionella*, but new data suggest that this is an overemphasized mode of transmission. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for Legionnaires' disease. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.

Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine aerosol containing Legionella bacteria. Legionella bacteria are naturally found in environmental water sources such as rivers, lakes and ponds and may colonize man-made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.



How do people contract Legionella?

The most popular theory is that the organism is aerosolized in water and people inhale the droplets containing *Legionella*. However, new evidence suggests that another way of contracting *Legionella* is more common. "Aspiration" is the most common way that bacteria enter into the lungs to cause pneumonia. Aspiration means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent aspiration is defective in patients who smoke or have lung disease. Aspiration now appears to be the most common mode of transmission.

Legionella may multiply to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.

Within one month, Legionella can multiply, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.

Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a routine maintenance program because equipment may become corroded.

Property owners have been sued for the spread of Legionella, resulting in expensive settlements. Regular monitoring with a battery of DFA monoclonal antibodies for several serogroups and species of Legionella morphologically intact bacteria provides a means for exercising 'reasonable care' to deter potential litigation.

Currently, there are no United States government regulations concerning permissible numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.

Epifluorescence Microscopy DFA Method
The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by ultraviolet light and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.

Most labs will provide a quantitative epifluorescence microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species (listed below).

Legionella anisa	*Legionella bozemanii* sg 1 & 2
Legionella dumoffi	*Legionella feeleii* sg 1 & 2
Legionella gormanii	*Legionella hackeliae* sg 1 & 2
Legionella jordanis	*Legionella longbeachae* sg 1& 2
Legionella maceachernii	*Legionella micdadei*
Legionella oakridgensis	*Legionella parisiensis*
Legionella pneumophila sg 1-14	*Legionella sainthelensi*
Legionella santicrucis	*Legionella wadsworthii*

Heterotrophic bacterial CFU are often inversely proportional to numbers of Legionella in cooling tower samples, in our experience. Routine biocide treatments will not eradicate Legionella bacteria in the environment, only in laboratory studies.

Culture methods are good during outbreaks for biotyping; but culture methods lack sensitivity for routine, quantitative monitoring. Many factors will inhibit growth or identification of legionella on BCYE with or without antimicrobial agents, heat or acid treatment.

Culture methods will not identify non-culturable legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.

Direct fluorescent antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.

Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly aerobic rods.

The U.S. Environmental Protection Agency and the U.S. Occupational Safety and Health Administration recommend routine maintenance of water-containing equipment. Most State health departments recommend monthly testing for Legionella as part of a routine maintenance program.

As far as we know, there are no federal or state certification programs for laboratories that perform Legionella testing of environmental samples. Therefore, care must be taken when selecting a testing laboratory.

More on Legionnaires' Disease Medical Aspects
Legionnaires' disease is caused by bacteria that belong to the family Legionellaceae. This family now includes 48 species and over 70 serogroups. Approximately half of these species have been implicated in human disease. Legionella pneumophila is responsible for approximately 90% of infections.

Most cases are caused by L. pneumophila, serogroup 1. Legionella species are small (0.3 to 0.9 μm in width and approximately 2 μm in length) faintly staining Gram-negative rods with polar flagella (except L. oakridgensis). They generally appear as small coccobacilli in infected tissue or secretions. They are distinguished from other saccharolytic bacteria by their requirement for L-cysteine and iron salts for primary isolation on solid media and by their unique cellular fatty acids and ubiquinones.

They grow well on buffered charcoal yeast extract agar, but it takes about five days to get sufficient growth. When grown on this medium, Legionella colonies appear off-white in color and circular in shape. Laboratory identification can also include microscopic examination in conjunction with a direct flourescent antibody (DFA) test. Since the initial discovery, many species have been added to the Legionella genus, but only two are within the scope of our discussion.

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

Prevention and Control
In the prevention and control of Legionnaires disease (legionella) causing microbes, chlorine dioxide has taken an eminent roll. The specific characteristics of the disinfectant make sure ClO^2 gets the job done where others fail.

Biofilm in the piping can protect legionella from most of the disinfectants.

Chlorine dioxide however removes the biofilm and kills the bacteria, spores and viruses. Other advantages are:
1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. The required contact time for ClO^2 is lower;
3. Chlorine dioxide has better solubility;
4. Chlorine dioxide does not react with NH3 or NH4+;
5. It destroys THM precursors and increases coagulation;
6. ClO^2 destroys phenols and has no distinct smell.

Hot and cold water systems
The advantages in using chlorine dioxide with hot and cold water systems have already been shown at the descriptions on legionella. There are however more advantages:

1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. Chlorine dioxide is clearly superior to chlorine in the destruction of spores, bacteria's, viruses and other pathogen organisms on an equal residual base (even cryptosporidium and giardia);
3. The required contact time for ClO^2 is lower;
4. Chlorine dioxide has better solubility;
5. No corrosion associated with high chlorine concentrations. Reduces long term maintenance costs;
6. Chlorine dioxide does not react with NH3 or NH4+;
7. It destroys THM precursors and increases coagulation;
8. ClO^2 destroys phenols and has no distinct smell;
9. It is better at removing iron and magnesia compounds than chlorine, especially complex bounds.

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Legionella Chapter 5 Review

1. The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of _____ at an American Legion convention led to 29 deaths.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

2. The causative agent, what would come to be known as _____, was isolated and given its own genus.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

3. The organisms classified in this genus are Gram-negative bacteria that are considered _____ .
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Intracellular parasites
E. None of the above

4. Legionnaires' disease is the more severe form of infection which includes pneumonia; _____is a milder illness.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

5. The major source is water distribution systems of large buildings including hotels and hospitals. Cooling towers have long been thought to be a major source for _____, but new data suggest that this is an overemphasized mode of transmission.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella
E. None of the above

6. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for _____. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

7. _____ is the most common way that bacteria enter into the lungs to cause pneumonia. It means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent it is defective in patients who smoke or have lung disease.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

8. Legionella may _____ to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

9. Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine _____ containing Legionella bacteria.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

10. Legionella bacteria are _____ in environmental water sources such as rivers, lakes and ponds and may colonise man made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

11. Within one month, Legionella can _____, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

12. Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a _____ because equipment may become corroded.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

13. Currently, there are no United States government regulations concerning _____ numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

14. The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by _____ and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

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

16. Culture methods will not identify _____ legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

17. Direct _____ antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

18. Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly _____.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

19. Legionnaires' disease is caused by bacteria that belong to the family _____.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

20. This family now includes 48 species and over 70 _____. Approximately half of these species have been implicated in human disease.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

21. _____ is responsible for approximately 90% of infections.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

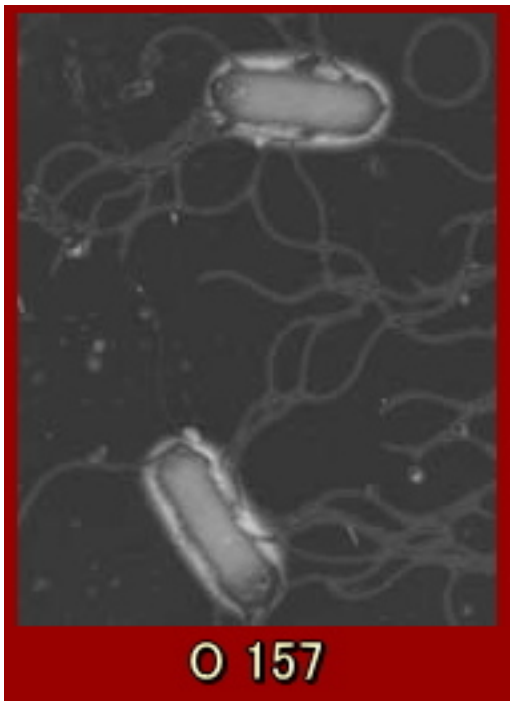
1.B, 2.D, 3.D, 4.C, 5.D, 6.A, 7.B, 8.E, 9.C, 10.D, 11.E, 12.A, 13.B, 14.C, 15.E, 16.B, 17.D, 18.A, 19.C, 20.D, 21.E

Escherichia Coli Chapter 6

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.....1FC
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 not the thing 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.

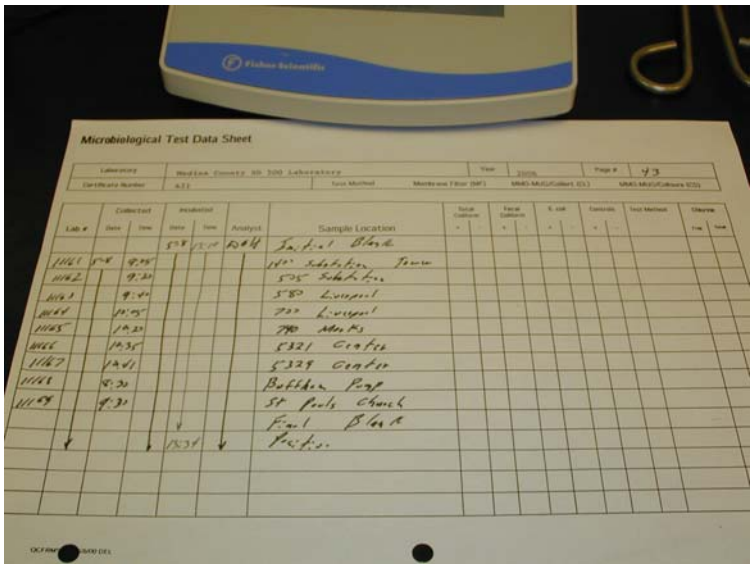
Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment 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 at Medina County for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers by Medina County.

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:
Counters are alcohol wiped.
Bench sheets are filled out
Samples are removed from refrigeration.
Sterile wrapped utensils are placed on counters
Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
Sterile petri dishes are labeled
The samples closures are clipped
The sample is shaken 25 times 1 foot in length within 7 seconds
100 mL is filtered and rinsed with sterile distilled water 3 times
The membrane filter is aseptically removed from filter holder
A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
The sterile labeled lid is placed on the petri dish
2 blanks and a known is run with each series of samples
The samples are placed in the 35°C ± .5°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 – see picture)
After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
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.
Report as Total Coliform per 100 mL
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.

Picture and Credits to Mary McPherson
Aran™ Aqua Analytical Laboratory Director

Escherichia coli

Two types of pathogenic Escherichia coli, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.

E. coli O157:H7
E. coli O157:H7 (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.

Escherichia coli O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illnesses have been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water.

Consumers can prevent *E. coli* O157:H7 infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully. Because the organism lives in the intestines of healthy cattle, preventive measures on cattle farms and during meat processing are being investigated.

What is *Escherichia coli* O157:H7?
E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.

E. coli O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli.*

Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces.

Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains are capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*.

How does *E. coli* or other fecal coliforms get in the water?
E. coli comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water.

How is water treated to protect me from *E. coli*?
The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli.* Systems using surface water sources are required to disinfect to ensure that all bacterial contamination is inactivated, such as *E. coli*. Systems using ground water sources are not required to disinfect, although many of them do.

How does the U.S. Environmental Protection Agency regulate *E. coli*?
According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the Safe Drinking Water Act. If a system is not a public water system as defined by EPA regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities.

Under the Safe Drinking Water Act, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

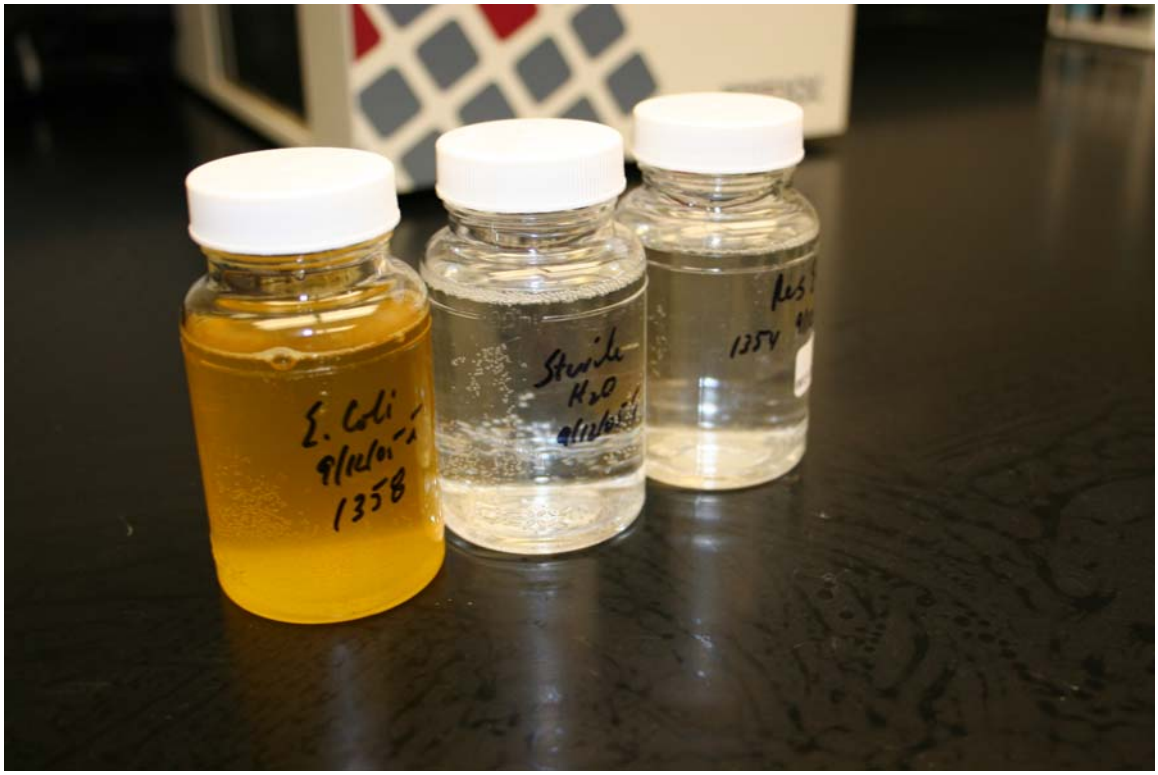
The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect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

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection will kill *E. coli* O157:H7.

What can I do to protect myself from *E. coli* O157:H7 in drinking water?
Approximately 89 percent of Americans are receiving water from community water systems that meet all health-based standards. Your public water system is required to notify you if, for any reason, your drinking water is not safe. If you wish to take extra precautions, you can boil your water for one minute at a rolling boil, longer at higher altitudes.

To find out more information about your water, see the Consumer Confidence Report from your local water supplier or contact your local water supplier directly. You can also obtain information about your local water system on the EPA's website at www.epa.gov/safewater/dwinfo.htm.

Positive Tests
If you draw water from a private well, you can contact your state health department to obtain information on how to have your well tested for total coliforms and *E. coli* contamination. If your well tests positive for *E. coli*, there are several steps that you should take: (1) begin boiling all water intended for consumption, (2) disinfect the well according to procedures recommended by your local health department, and (3) monitor your water quality to make certain that the problem does not recur. If the contamination is a recurring problem, you should investigate the feasibility of drilling a new well or install a point-of-entry disinfection unit, which can use chlorine, ultraviolet light, or ozone.



How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment may get into raw milk. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7 can cause infection. Contaminated meat looks and smells normal. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

Among other known sources of infection are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water. Bacteria in diarrheal stools of infected persons can be passed from one person to another if hygiene or handwashing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected. Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms.

What illness does *E. coli* O157:H7 cause?
E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Hemorrhagic colitis is the name of the acute disease caused by *E. coli* O157:H7.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.

How is *E. coli* O157:H7 infection diagnosed?
Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not test for *E. coli* O157:H7, so it is important to request that the stool specimen be tested on sorbitol-MacConkey (SMAC) agar for this organism. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli* O157:H7.

How is the illness treated?
Most persons recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (Imodium), should also be avoided. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood

transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5%.

What are the long-term consequences of infection?
Persons who only have diarrhea usually recover completely. About one-third of persons with hemolytic uremic syndrome have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of persons with hemolytic uremic syndrome have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed.

What can be done to prevent the infection?
E. coli O157:H7 will continue to be an important public health concern as long as it contaminates meat. Preventive measures may reduce the number of cattle that carry it and the contamination of meat during slaughter and grinding. Research into such prevention measures is just beginning.

What can you do to prevent *E. coli* O157:H7 infection?
Cook all ground beef and hamburger thoroughly. Because ground beef can turn brown before disease-causing bacteria are killed, use a digital instant-read meat thermometer to ensure thorough cooking. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 160º F. Persons who cook ground beef without using a thermometer can decrease their risk of illness by not eating ground beef patties that are still pink in the middle.

If you are served an undercooked hamburger or other ground beef product in a restaurant, send it back for further cooking. You may want to ask for a new bun and a clean plate, too. Avoid spreading harmful bacteria in your kitchen. Keep raw meat separate from ready-to-eat foods. Wash hands, counters, and utensils with hot soapy water after they touch raw meat. Never place cooked hamburgers or ground beef on the unwashed plate that held raw patties. Wash meat thermometers in between tests of patties that require further cooking. Drink only pasteurized milk, juice, or cider.

Commercial juice with an extended shelf-life that is sold at room temperature (e.g. juice in cardboard boxes, vacuum sealed juice in glass containers) has been pasteurized, although this is generally not indicated on the label. Juice concentrates are also heated sufficiently to kill pathogens.

Wash fruits and vegetables thoroughly, especially those that will not be cooked. Children under 5 years of age, immunocompromised persons, and the elderly should avoid eating alfalfa sprouts until their safety can be assured. Methods to decontaminate alfalfa seeds and sprouts are being investigated.

Drink municipal water that has been treated with chlorine or other effective disinfectants. Avoid swallowing lake or pool water while swimming. Make sure that persons with diarrhea, especially children, wash their hands carefully with soap after bowel movements to reduce the risk of spreading infection, and that persons wash hands after changing soiled diapers. Anyone with a diarrheal illness should avoid swimming in public pools or lakes, sharing baths with others, and preparing food for others.

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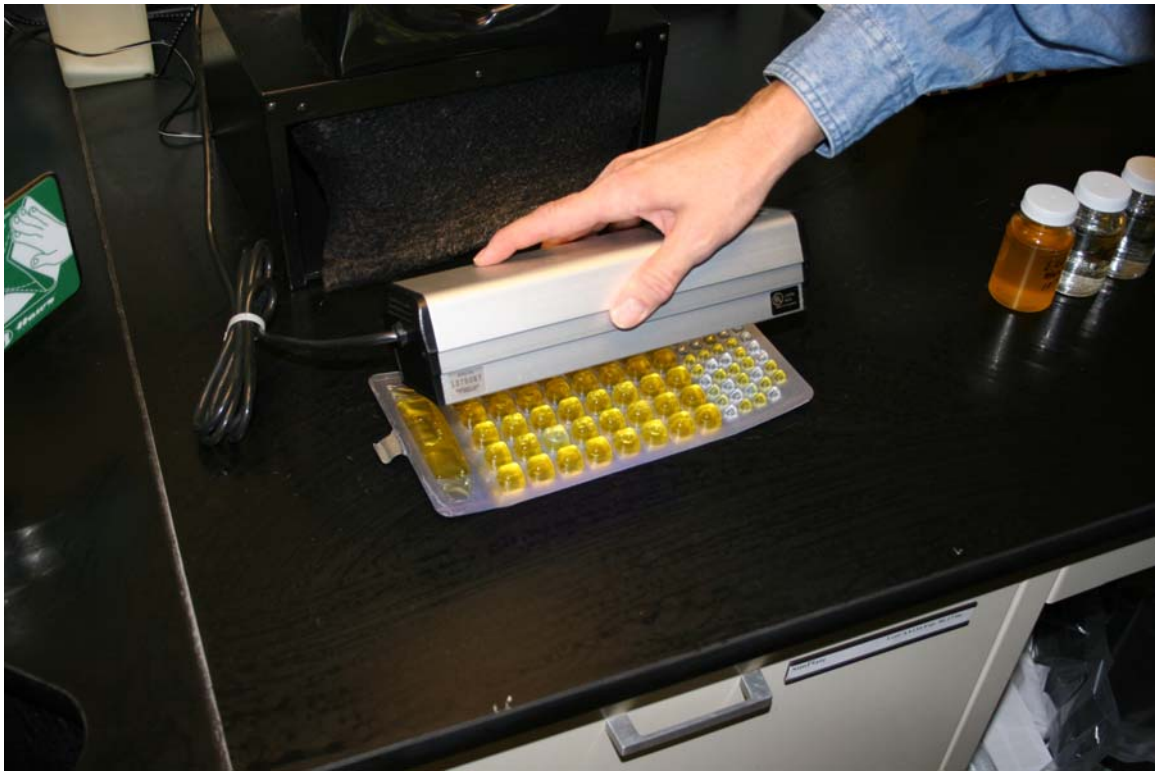

Pathogenic *Escherichia coli* MLST Database – Clonal Group Definition

Clonal Group	Class	Reference Strain	Comments
0	N/A	TW08017	Undefined Clonal Group
1	atypical B13	TW08889	cluster within E. coli with Boydii 13 (B13) antigen
2	STEC 13	TW08045	includes STEC R:H18 strain
3	EPEC 3	TW06584	O86:H34 strains from infant diarrhea
4	H51	TW08260	cluster with H51 antigen
5	NT-1	TW08997	no common traits
6	EPEC 1	TW06375	Classical EPEC with H6
7	EPEC 4	TW03173	O119:H6 strains, basal to EPEC 1
8	NT-2	TW08983	no common traits
9	NT-3	TW08990	no common traits
10	Shigella 3	TW08837	Reeves Shigella Group 3 including serotypes F1a, F2a, F3a, F3b, F4, F5a FY
11	EHEC 1	TW08264	O157:H7 and relatives including atypical EPEC O55:H7
12	STEC 12	TW00964	STEC O145 strains
13	ETEC P	TW00601	ETEC from pigs including O157:H43 clone
14	EHEC 2	TW00970	O26:H11 and O111:H8 and relatives including RDEC
15	NT-4	TW09177	no common traits
16	EIEC 2	TW01095	Invasive strains with O type 29, 124, 152, 164
17	EPEC 2	TW01120	Classical EPEC with H2 antigen
18	STEC 8	TW04909	Serotype O104:H21, includes Montana outbreak strain G5506
19	STEC 9	TW08580	Serotype O174:H8
20	STEC 10	TW07618	Various serotypes
21	STEC 11	TW07613	Serotype O111:H28
22	EIEC 1	TW01116	Invasive strains with O types 29, 124, 152, 164
23	ECOR A	TW00073	Includes atypical EPEC O111:H12
24	STEC 3	TW08023	Serotype O121:H19
25	Shigella 1	TW07572	Reeves Shigella Group 1 including serotypes F6, D3, D6, D7, D9, D11, D12, B1, B2, B3, B4, B8, B10, B14, B15, B18
26	Shigella 2a	TW02615	Reeves Shigella Group 2 including serotypes D2, D4, B9, B15
27	Shigella 2b	TW01151	Reeves Shigella Group 2 including serotypes B5, B11, B17
28	EPEC 5	TW04892	Atypical EPEC with serotype O111:H9, includes Finland outbreak strain 921
29	Sonnei	TW01150	Shigella sonnei
30	STEC 2	TW01391	Includes serotype O113:H21 and ECOR 30
31	NT-5	TW00676	no common traits
32	NT-6	TW07795	no common traits

33	NT-13	TW09011	no common traits
34	STEC 1	TW01393	STEC with H21 antigen including B2F1
35	SMEC 1	TW02268	Extraintestinal strains including RS218
36	NT-7	TW07608	no common traits
37	Sand 2	TW09237	Environmental E. coli clone
38	UTI 1	TW08018	Uropathogens including CFT073
39	STEC 4	TW07995	Mix of serotypes O121, O116, also includes an EIEC 1758-70
40	NT-8	TW09214	no common traits
41	STEC 14	TW08574	Serotype O174:H2
42	NT-7	TW07612	no common traits
43	STEC	TW01670	serotype O8:H19
44	NT-10	TW10091	no comment traits
45	NT-11	TW08942	no comment traits
46	NT-12	TW09069	no comment traits

Quebec Colony Counter

Escherichia Coli Chapter 6 Review

1. _____ is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

2. _____ was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

3. Two types of pathogenic _____, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

4. _____ (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

5. _____O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

6. The combination of letters and numbers in the name of the _____ refers to the specific markers found on its surface and distinguishes it from other types of E. coli.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

7. Currently, there are four recognized classes of enterovirulent E. coli (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the _____ (EHEC) strain designated E. coli O157:H7.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

8. E. coli is a normal inhabitant of the intestines of all animals, including humans. When _____ are used, E. coli is the dominant species found in feces.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

9. _____ are bacteria that are associated with human or animal wastes. They usually live in human or animal intestinal tracts, and their presence in drinking water is a strong indication of recent sewage or animal waste contamination.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

10. The water can be treated using _____, ultra-violet light, or ozone, all of which act to kill or inactivate E. coli. Systems using surface water sources are required to disinfect to ensure that all bacterial contamination is inactivated, such as E. coli. Systems using ground water sources are not required to disinfect, although many of them do.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

11. According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the _____.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

12. If a system is not a public water system as defined by EPA regulations, it is not regulated under the _____, although it may be regulated by state or local authorities.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

13. Under the _____, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or E. coli. Both are indicators of contamination with animal waste or human sewage.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

14. The largest public water systems (serving millions of people) must take at least 50 samples per month. Smaller systems must take at least 20 samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last year.
A. True
B. False

15. Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect.
A. True
B. False

16. Disinfection will kill E. coli O157:H7.
A. True
B. False

1.C, 2.C, 3.B, 4.C, 5.B, 6.D, 7.A, 8.E, 9.D, 10.E, 11.A, 12.A, 13.A, 14.B, 15.A, 16.A

Related Diseases and Associated Illnesses Chapter 7

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
A nonspecific prodrome (i.e., fever, dyspnea, cough, and chest discomfort) follows inhalation of infectious spores. Approximately 2--4 days after initial symptoms, sometimes after a brief period of improvement, respiratory failure and hemodynamic collapse ensue. Inhalational anthrax also might include thoracic edema and a widened mediastinum on chest radiograph. Gram-positive bacilli can grow on blood culture, usually 2--3 days after onset of illness. Cutaneous anthrax follows deposition of the organism onto the skin, occurring particularly on exposed areas of the hands, arms, or face. An area of local edema becomes a pruritic macule or papule, which enlarges and ulcerates after 1--2 days. Small, 1--3 mm vesicles may surround the ulcer. A painless, depressed, black eschar, usually with surrounding local edema, subsequently develops. The syndrome also may include lymphangitis and painful lymphadenopathy.

Plague
Clinical features of pneumonic plague include fever, cough with muco-purulent sputum (gram-negative rods may be seen on gram stain), hemoptysis, and chest pain. A chest radiograph will show evidence of bronchopneumonia.

Botulism
Clinical features include symmetric cranial neuropathies (i.e., drooping eyelids, weakened jaw clench, and difficulty swallowing or speaking), blurred vision or diplopia, symmetric descending weakness in a proximal to distal pattern, and respiratory dysfunction from respiratory muscle paralysis or upper airway obstruction without sensory deficits. Inhalational botulism would have a similar clinical presentation as foodborne botulism; however, the gastrointestinal symptoms that accompany foodborne botulism may be absent.

Smallpox (variola)
The acute clinical symptoms of smallpox resemble other acute viral illnesses, such as influenza, beginning with a 2--4 day nonspecific prodrome of fever and myalgias before rash onset. Several clinical features can help clinicians differentiate varicella (chickenpox) from smallpox. The rash of varicella is most prominent on the trunk and develops in successive groups of lesions over several days, resulting in lesions in various stages of development and resolution. In comparison, the vesicular/pustular rash of smallpox is typically most prominent on the face and extremities, and lesions develop at the same time.

Inhalational tularemia
Inhalation of *F. tularensis* causes an abrupt onset of an acute, nonspecific febrile illness beginning 3--5 days after exposure, with pleuropneumonitis developing in a substantial proportion of cases during subsequent days.

Hemorrhagic fever (such as would be caused by Ebola or Marburg viruses).
After an incubation period of usually 5--10 days (range: 2--19 days), illness is characterized by abrupt onset of fever, myalgia, and headache. Other signs and symptoms include nausea and vomiting, abdominal pain, diarrhea, chest pain, cough, and pharyngitis. A maculopapular rash, prominent on the trunk, develops in most patients approximately 5 days after onset of illness. Bleeding manifestations, such as petechiae, ecchymoses, and hemorrhages, occur as the disease progresses (*8*).

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>.

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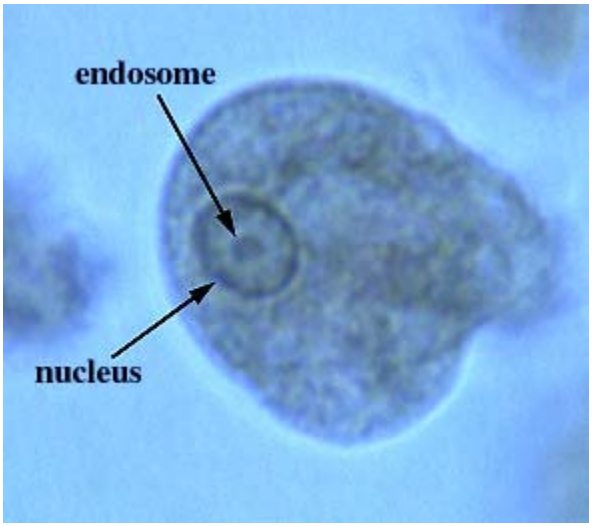
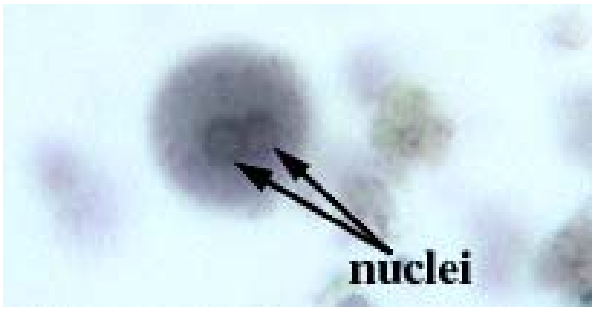
Amebiasis *Entamoeba histolytica* Section

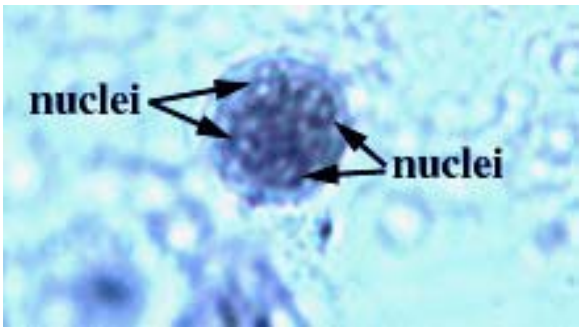
The life cycle of *Entamoeba histolytica* involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and cysts that are passed in the host's feces. Humans are infected by ingesting cysts, most often via food or water contaminated with human fecal material (view diagram of the life cycle). The trophozoites can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, *E. histolytica* is potentially the most pathogenic.



Entamoeba histolytica *trophozoite*



Entamoeba histolytica *immature cyst*



Entamoeba histolytica *mature cyst*

Entamoeba histolytica is an amoeboid protozoan parasite of the intestinal tract, and in some cases other visceral organs especially the liver. There are several species in this genus, distinguished by their number of nuclei in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen. *Entamoeba histolytica* has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal crypts, feeding on intestinal contents and host tissue, and multiplying by fission.

Trophozoites
The trophozoites can be carried out in the feces. As the feces pass through the colon they dehydrate. The dehydration of the feces causes the trophozoites to begin the process of encystment. Undigested food is discharged, and the trophozoite condenses and forms a spherical shape to form what is called the pre-cyst, and the cyst wall is secreted. Within the cyst there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst. The cyst can resist desiccation for 1-2 weeks. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 trophozoites. In some cases the trophozoites secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.

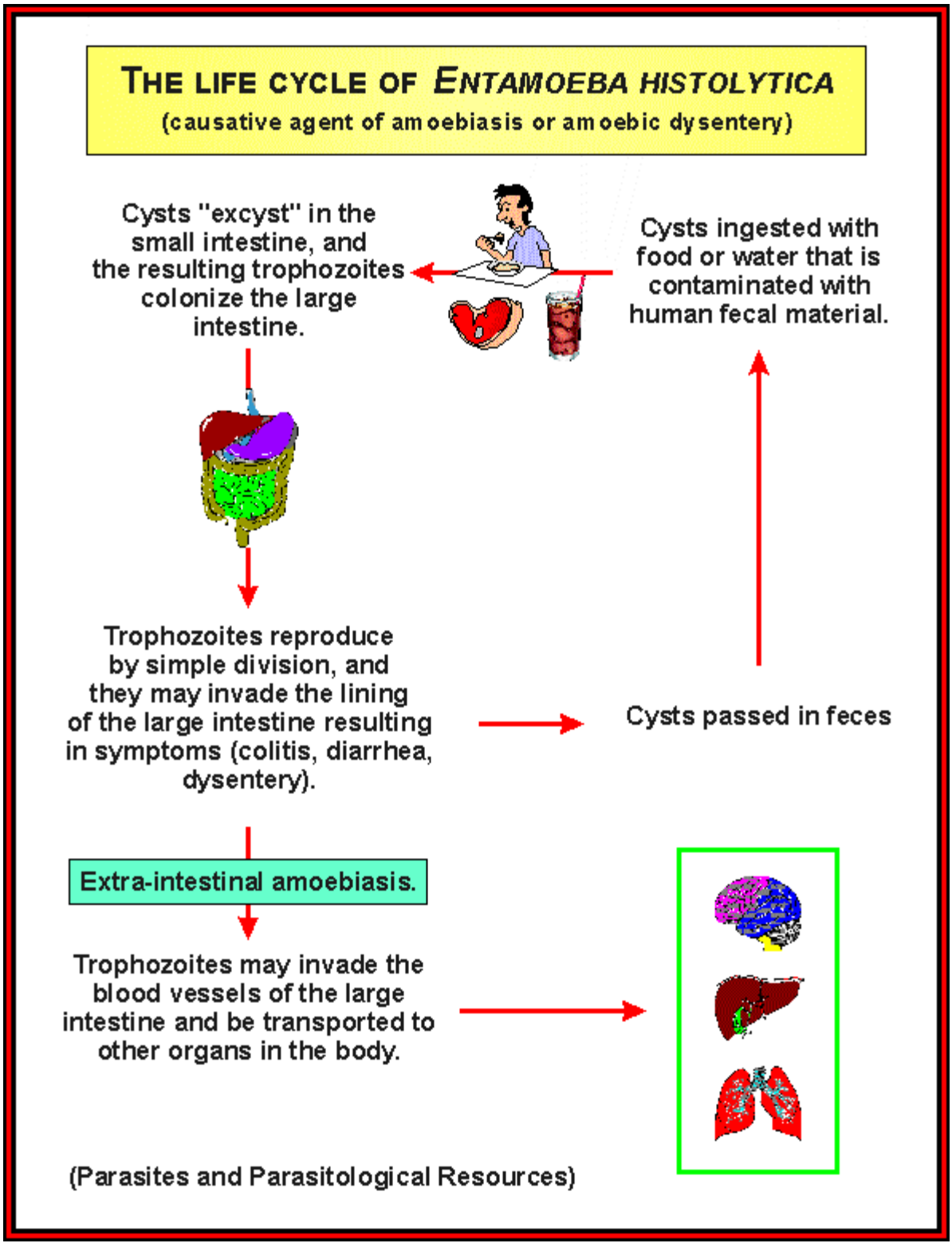
Extensive Tissue Destruction
These can form large abscesses that may allow the parasite to enter the blood stream and be carried to the liver and other organs. In these extra-intestinal sites the trophozoites also can cause extensive tissue destruction. If the intestinal tissue has been invaded the feces can be bloody and diarrheic.

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

Accurate diagnosis of this parasite is important to prevent unnecessary treatment of a non-pathogenic strain, and to ensure treating a pathogenic strain. Definitive diagnosis is based on morphological characteristics of the trophozoites and cysts, the presence of erythrocytes in the trophozoites, and clinical symptoms.

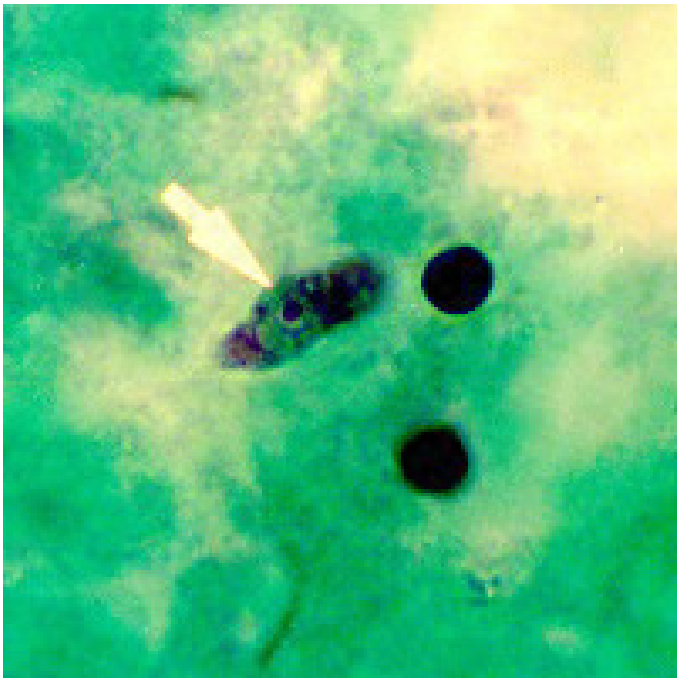
Symptoms of Amoebiasis
In most infected humans the symptoms of "amoebiasis" (or "amebiasis") are intermittent and mild (various gastrointestinal upsets, including colitis and diarrhea). In more severe cases the gastrointestinal tract hemorrhages, resulting in dysentery. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal.

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


THE LIFE CYCLE OF ENTAMOEBA HISTOLYTICA
(causative agent of amoebiasis or amoebic dysentery)
Cysts "excyst" in the small intestine, and the resulting trophozoites colonize the large intestine.
Cysts ingested with food or water that is contaminated with human fecal material.
Trophozoites reproduce by simple division, and they may invade the lining of the large intestine resulting in symptoms (colitis, diarrhea, dysentery).
Cysts passed in feces
Extra-intestinal amoebiasis.
Trophozoites may invade the blood vessels of the large intestine and be transported to other organs in the body.
(Parasites and Parasitological Resources)

Amebic Meningoencephalitis PAM *Naegleria fowleri*

What is primary amebic meningoencephalitis (PAM)?
Primary Amebic Meningoencephalitis (PAM) is a rare and usually deadly disease caused by infection with the ameba (a single-celled organism that constantly changes shape) *Naegleria fowleri*. *[Naegleria fowleri] [Acanthamoeba spp.] [Balamuthia mandrillaris]*



Naegleria fowleri trophozoite in spinal fluid. Trichrome stain. Note the typically large karyosome and the monopodial locomotion. Image contributed by Texas SHD.

What are the symptoms of PAM? What does PAM cause?
Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.

How common is PAM?
The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. *Naegleria* thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.

Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.

Who should be especially careful about PAM?
Cases are usually reported in children and young adults who have had recent exposure to freshwater lakes or streams.

How is PAM spread? How do people get *Naegleria* infection?
The ameba is believed to enter the body through the nose and travel to the brain via the olfactory (smell) nerve. The disease is not spread from person to person.

How do I protect myself from PAM?
To protect yourself against *Naegleria* or any harmful organism that is present in the water:

- Never swim in stagnant or polluted water.
- Do not swim in areas posted as "No Swimming."
- Hold your nose or use nose plugs when jumping or diving into water.
- Avoid swallowing water from rivers, lakes, streams, or stock ponds.
- Use earplugs, swim goggles, or masks if you tend to get ear or eye infections.
- Swim only in properly maintained pools.
- Keep wading pools clean and change the water daily.
- Wash open skin cuts and scrapes with clean water and soap.

What do I do if I think I have PAM?
Seek immediate medical attention and mention any recent fresh water exposure.

How is PAM diagnosed?
The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the patient's spinal chord 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 hyperchlorinate swimming pools.

Supply to the affected area was switched to a chlorinated surface water source, and a flushing program with hyperchlorinated water was carried out to remove possible contamination from the water distribution system.

One of the victims lived in Peoria and the other in the neighboring town of Glendale, some four miles away. They attended separate schools, however the Glendale boy frequently visited his grandparents' home a few blocks from the other boy's residence in Peoria. Both boys became ill on 9 October and died a few days later on 12 and 13 October respectively. Health authorities then began investigating possible common sources of Naegleria exposure including drinking water, pools, bathtubs, spas and fountains.

About 100,000 of Peoria's 120,000 residents receive chlorinated drinking water from the municipal supply. This supply is predominantly drawn from surface water sources but is supplemented by groundwater in times of high demand. As Arizona state law prevents counties from supplying water to areas outside the incorporated municipal zones, the remaining 20,000 residents in the rapidly growing town are served by private water companies which mainly rely on groundwater sources. Some of these companies chlorinate their groundwater supplies and some do not.

The suspect water supply is drawn from a deep aquifer and is not routinely chlorinated, although periodic chlorination has been used after new connections, line breaks or incidents that might allow ingress of microbial contamination.

Tests by the Centers for Disease Control and Prevention have detected N. fowleri in three samples:

· one pre-chlorination water sample from a municipal well that was routinely chlorinated.
· one tank water sample from the suspect unchlorinated groundwater system.
· the refrigerator filter from the home of the grandparents of one of the boys.

The chlorinated well is believed unlikely to be the source of infection as chlorination is effective in killing *N. fowleri*.

Naegleria fowleri is a free living amoeba which is common in the environment and grows optimally at temperatures of 35 to 45 degrees C. Exposure to the organism is believed to be relatively common but infections resulting in illness are rare. The disease was first described in 1965 by Dr Malcolm Fowler, an Australian pathologist, who identified the amoeba in a patient who had died from meningitis.

Most reported cases of N. fowleri meningitis are associated with swimming in natural surface freshwater bodies, and infection occurs through introduction of the organism into the nasal cavities. Cases are often reported to be associated with jumping or falling into the water, providing conditions where water is forced into the nose at pressure. The amoeba may then penetrate the cribiform plate, a semiporous barrier, and spread to the meninges (the membrane surrounding the brain) and often to the brain tissue itself. The cribiform plate is more permeable in children, making them more susceptible to infection than adults. People with immune deficiencies may also be more prone to infection.

The incubation period is usually 2 to 5 days, and the infection cannot be transmitted from person to person. In early studies, transmission by contaminated dust was suspected as an infection route but this has since been discounted as the organism does not survive desiccation.

N. fowleri meningitis causes non-specific symptoms such as fever, drowsiness, confusion, vomiting, irritability, high pitched crying and convulsions. Similar symptoms also occur in viral and bacterial forms of meningitis which are much more common than the amoebic form. Most cases of *N. fowleri* meningitis are fatal, with only four survivors known among about 100 cases in the US since 1965.

Cases of disease have also been associated with swimming pools where disinfection levels were inadequate, and inhalation of tap water from surface water supplies that have been subject to high temperatures.

The involvement of tap water supplies was first documented in South Australia, where a number of cases occurred in the 1960s and 70s in several towns served by unchlorinated surface water delivered through long above-ground pipelines. About half of the cases in the state did not have a recent history of freshwater swimming, but had intra-nasal exposure to tap water through inhaling or squirting water into the nose.

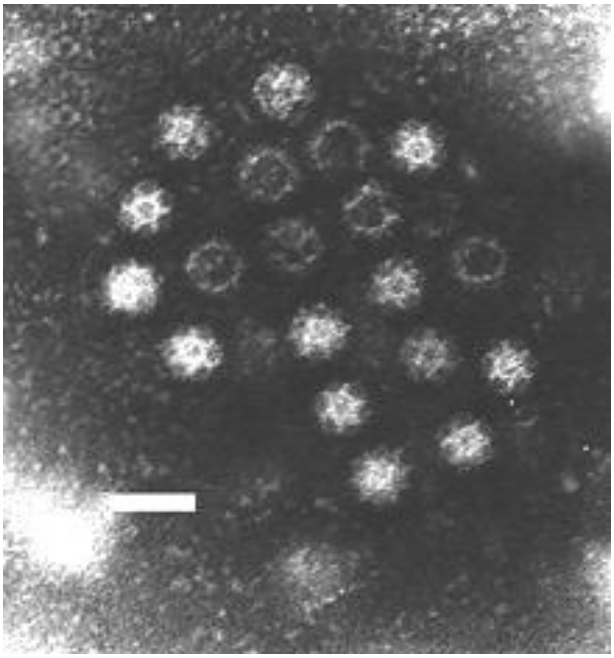
Investigators found *N. fowleri* in the water supply pipelines, and concluded that the high water temperatures reached in summer provided a suitable environment for growth of the organism. Tap water may also have been the primary source of infections attributed to swimming pools in these towns. The incidence of disease was greatly reduced by introduction of reliable chlorination facilities along the above-ground pipelines and introduction of chloramination in the 1980s led to virtual elimination of *N. fowleri* from the water supplies. Cases of disease have also been recorded in Western Australia, Queensland and New South Wales, and *N. fowleri* has been detected in water supplies in each of these states as well as the Northern Territory.

Prior to the incidents in Peoria, *N. fowleri* infections had not been reported to be associated with groundwater supplies. However as the organism may be found in moist soil, it is feasible that the amoeba may penetrate poorly constructed bores or be introduced by occasional contamination events.

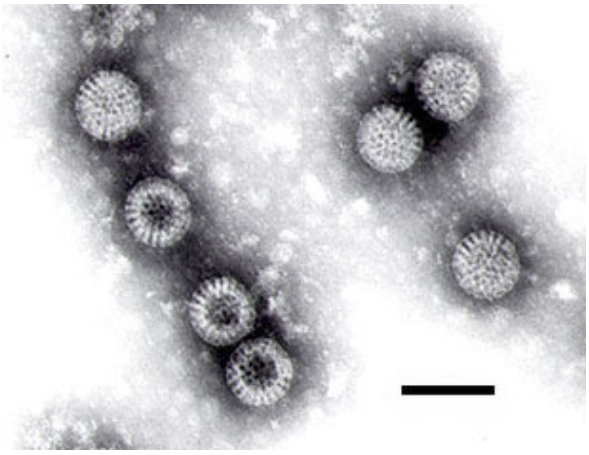
Warm water conditions and the absence of free chlorine may then allow it to proliferate in the system. Local health authorities in Arizona are continuing their investigation into the two deaths with assistance from CDC personnel. Plans are also underway to install a continuous chlorination plant on the groundwater supply, and some residents have called for the municipality to purchase the private water company and take over its operations.

Calicivirus

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



Note the 'Star of David' image exhibited by individual virus particles. This is distinct from the star-like images exhibited by astrovirus particles. Bar = 50 nanometers.
Source: Stool sample from an individual with gastroenteritis.
Method: Negative-stain Transmission Electron Microscopy



Rotovirus
Note the wheel-like appearance of some of the rotavirus particles. The observance of such particles gave the virus its name ('rota' being the Latin word meaning wheel). Bar = 100 nanometers.
Source: Cell culture.
Method: Negative-stain Transmission Electron Microscopy

Photographs and information courtesy from the U.S. EPA and F.P. Williams, U.S. EPA

Schistosomes and Other Trematodes

Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except malaria. Almost unknown in industrialized countries, schistosomiasis infects 200 million people in 76 countries of the tropical developing world.

A Flatworm that spends part of its life in a freshwater snail host causes schistosomiasis. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.

Multiplication and Life Cycle
Free-swimming larvae (cercariae) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes). After entering a human the larvae develop into adult males and females (schistosomes) or hermaphrodites (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.

Cercariae

Pathogenesis
In schistosomiasis, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.

Host Defenses
Host defenses against schistosomiasis include antibody or complement-dependent cellular cytotoxicity and modulation of granulomatous hypersensitivity. The defenses against hermaphroditic flukes are unknown.

Epidemiology
Most infected individuals show no overt disease. In a relatively small proportion of individuals, heavy infections due to repeated exposure to parasitic larvae will lead to the development of clinical manifestations. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other trematodes).

Diagnosis
Diagnosis is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.

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

Clinical Manifestations
Signs and symptoms are related largely to the location of the adult worms. Infections with *Schistosoma mansoni* and *S japonicum* (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis. *Schistosoma haematobium* (vesical venules) causes dysuria, hema turia, and uremia. *Fasciola hepatica, Clonorchis sinensis,* and *Opisthorchis viverrini* (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice. Infections with *Paragonimus westermani* (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. *Fasciolopsis buski* (intestines) causes abdominal pain, diarrhea, and edema.

Structure
Trematodes are multicellular eukaryotic helminths.

Gastroenteritis

What is viral gastroenteritis?
Gastroenteritis means inflammation of the stomach and small and large intestines. Viral gastroenteritis is an infection caused by a variety of viruses that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.

What causes viral gastroenteritis?
Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, caliciviruses, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as *Salmonella* or *Escherichia coli*) or parasites (such as *Giardia*), or by medications or other medical conditions, although the symptoms may be similar. Your doctor can determine if the diarrhea is caused by a virus or by something else.

What are the symptoms of viral gastroenteritis?
The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which virus causes the illness.

Is viral gastroenteritis a serious illness?
For most people, it is not. People who get viral gastroenteritis almost always recover completely without any long-term problems. Gastroenteritis is a serious illness, however, for persons who are unable to drink enough fluids to replace what they lose through vomiting or diarrhea. Infants, young children, and persons who are unable to care for themselves, such as the disabled or elderly, are at risk for dehydration from loss of fluids. Immune compromised persons are at risk for dehydration because they may get a more serious illness, with greater vomiting or diarrhea. They may need to be hospitalized for treatment to correct or prevent dehydration.

Is the illness contagious? How are these viruses spread?
Yes, viral gastroenteritis is contagious. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.

How does food get contaminated by gastroenteritis viruses?
Food may be contaminated by food preparers or handlers who have viral gastroenteritis, especially if they do not wash their hands regularly after using the bathroom. Shellfish may be contaminated by sewage, and persons who eat raw or undercooked shellfish harvested from contaminated waters may get diarrhea. Drinking water can also be contaminated by sewage and be a source of spread of these viruses.

Where and when does viral gastroenteritis occur?
Viral gastroenteritis affects people in all parts of the world. Each virus has its own seasonal activity. For example, in the United States, rotavirus and astrovirus infections occur during the cooler months of the year (October to April), whereas adenovirus

infections occur throughout the year. Viral gastroenteritis outbreaks can occur in institutional settings, such as schools, child care facilities, and nursing homes, and can occur in other group settings, such as banquet halls, cruise ships, dormitories, and campgrounds.

Who gets viral gastroenteritis?
Anyone can get it. Viral gastroenteritis occurs in people of all ages and backgrounds. However, some viruses tend to cause diarrheal disease primarily among people in specific age groups. Rotavirus infection is the most common cause of diarrhea in infants and young children under 5 years old. Adenoviruses and astroviruses cause diarrhea mostly in young children, but older children and adults can also be affected. Norwalk and Noroviruses are more likely to cause diarrhea in older children and adults.

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.

Rotovirus Information

Clinical Features
Rotavirus is the most common cause of severe diarrhea among children, resulting in the hospitalization of approximately 55,000 children each year in the United States and the death of over 600,000 children annually worldwide. The incubation period for rotavirus disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.

The Virus
A rotavirus has a characteristic wheel-like appearance when viewed by electron microscopy (the name rotavirus is derived from the Latin rota, meaning "wheel"). Rotaviruses are nonenveloped, double-shelled viruses. The genome is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.

Epidemiologic Features
The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces. In the United States and other countries with a temperate climate, the disease has a winter seasonal pattern, with annual epidemics occurring from November to April. The highest rates of illness occur among infants and young children, and most children in the United States are infected by 2 years of age. Adults can also be infected, though disease tends to be mild.

Diagnosis
Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by enzyme immunoassay or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.

Treatment
For persons with healthy immune systems, rotavirus gastroenteritis is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children with rotavirus gastroenteritis will require hospitalization for intravenous fluids.

Prevention
In 1998, the U.S. Food and Drug Administration approved a live virus vaccine (Rotashield) for use in children. However, the Advisory Committee on Immunization Practices (ACIP) recommended that Rotashield no longer be recommended for infants in the United States because of data that indicated a strong association between Rotashield and intussusception (bowel obstruction) among some infants during the first 1-2 weeks following vaccination.

More information about rotavirus vaccine is available from the National Immunization Program.

Recent Newspaper Article

Norwalk Virus
The Gila County Department of Health is currently investigating an outbreak of viral gastroenteritis in the Globe / Miami area. The outbreak has been laboratory confirmed by the Arizona State Laboratory as Norwalk virus. Please be aware of the following symptoms and recommendations.

Norwalk Symptoms
Usually a mild to moderate infection that often occurs in outbreaks with clinical symptoms of nausea, vomiting, diarrhea, abdominal pain, low grade fever, or any combination of these symptoms. Gastrointestinal symptoms will characteristically last 24 to 48 hours, resolving on their own.

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

If you are experiencing symptoms consistent with this disease, please exclude yourself from school, work, or any group activity. Hand washing and disinfection are essential to stop the spread of this virus. Anyone experiencing severe complications from this ailment should seek medical attention.

Please report all suspected group outbreaks to the Gila County Department of Health by phone immediately. (928) 425-3189

Noroviruses

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).

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:

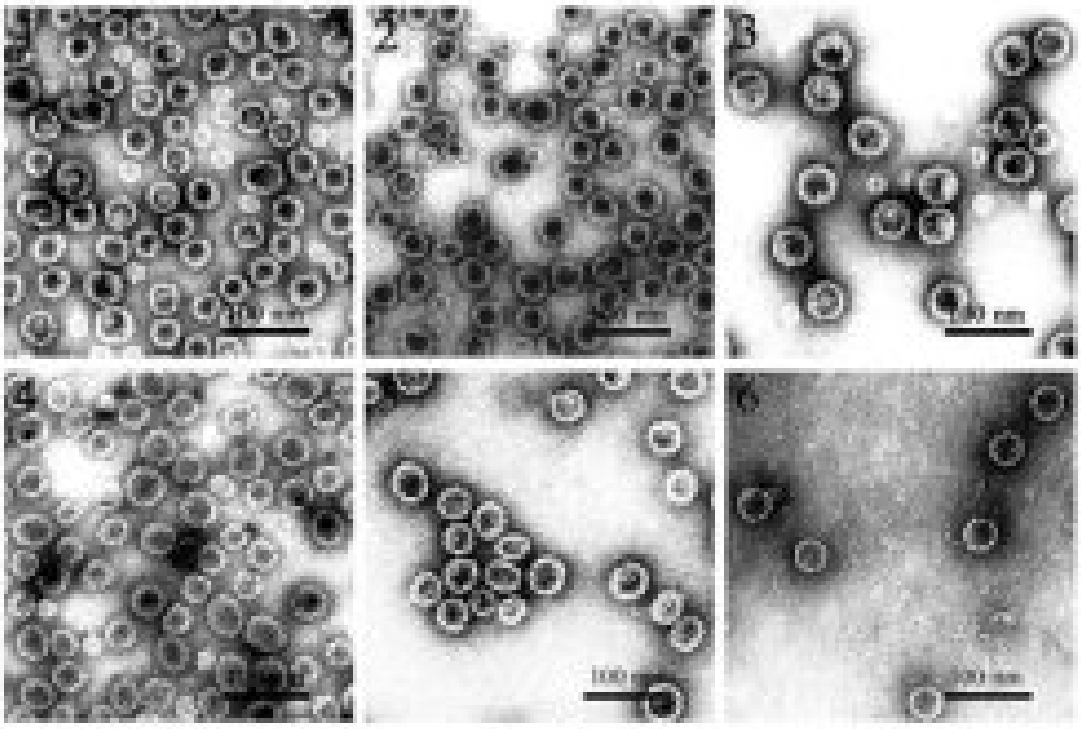
- Frequently wash your hands, especially after toilet visits and changing diapers and before eating or preparing food.
- Carefully wash fruits and vegetables, and steam oysters before eating them.
- Thoroughly clean and disinfect contaminated surfaces immediately after an episode of illness by using 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.

Hepatitis 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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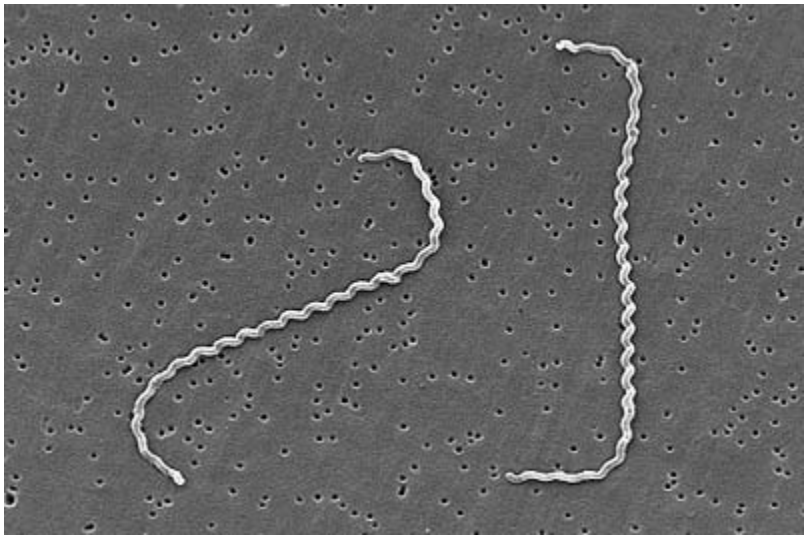
Leptospirosis *Leptospira* Section

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. *Leptospira interrogans* causes leptospirosis, a usually mild febrile illness that may result in liver or kidney failure.



Structure, Classification, and Antigenic Types
Leptospira is a flexible, spiral-shaped, Gram-negative spirochete with internal flagella. *Leptospira interrogans* has many serovars based on cell surface antigens.

How do people get Leptospirosis?
Outbreaks of leptospirosis are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with broken skin.

The disease is not known to be spread from person to person.

Pathogenesis
Leptospira enters the host through mucosa and broken skin, resulting in bacteremia. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.

Host Defenses
Serum antibodies are responsible for host resistance.

Epidemiology
Leptospirosis is a worldwide zoonosis affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.

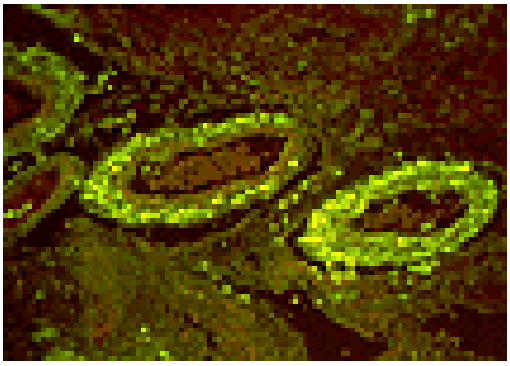
Diagnosis
Clinical diagnosis is usually confirmed by serology. Isolation of spirochetes is possible, but it is time-consuming and requires special media.

Control
Animal vaccination and eradication of rodents are important. Treatment with tetracycline and penicillin G is effective. No human vaccine is available.

Can Leptospirosis be prevented?
The risk of acquiring leptospirosis can be greatly reduced by not swimming or wading in water that might be contaminated with animal urine. Protective clothing or footwear should be worn by those exposed to contaminated water or soil because of their job or recreational activities.

Meningoencephalitis

Refer to amoebic meningoencephalitis (PAM), *Naegleria fowleri* and granulomatious amoebic encephalitis (GAE), acanthamoebic keratitis or acanthamoebic uveitis. These organisms are ubiquitous in the environment, in soil, water, and air. Infections in humans are rare and are acquired through water entering the nasal passages (usually during swimming) and by inhalation.



Granulomatous Amoebic Encephalitis due to *Acanthamoeba castellanii*

With immunofluorescent antibody techniques, the amoebae showed distinct fluorescence with anti-*A. castellanii* at 1:20 and 1:50 dilutions, but they were negative or weakly positive with anti-*A. culbertsoni*, *A. polyphaga*, *A. rhysodes*, and *A. astronyxis* sera. Protozoa were also seen in autopsy lung tissue, and identified as *A. castellanii*. There was also necrotizing amoebic panniculitis in subcutaneous, peripancreatic, mesenteric and peri-aortic tissue. There were occasional amoebae in the liver, but not enough to account for all of the patient's liver disease, so part of his liver disease was most likely due to sepsis due to his disseminated amoebiasis.

Pseudomonas aeruginosa 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.

Pseudomonas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics. The most serious complication of cystic fibrosis is respiratory tract infection by the ubiquitous bacterium *Pseudomonas aeruginosa*. Cancer and burn patients also commonly suffer serious infections by this organism, as do certain other individuals with immune system deficiencies.

Unlike many environmental bacteria, *P. aeruginosa* has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited. *P. aeruginosa* can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs. This bacterium is also noted for its resistance to many antibiotics.

P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its metabolic capability and environmental versatility. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, and systems involved in sensing and responding to environmental changes.

The typical *Pseudomonas* bacterium in nature might be found in a biofilm, attached to some surface or substrate, or in a planktonic form, as a unicellular organism, actively swimming by means of its flagellum. *Pseudomonas* is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples.

In its natural habitat *Pseudomonas aeruginosa* is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis.

--*Pseudomonas aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *Pseudomonas aeruginosa* consists of acetate for carbon and ammonium sulfate for nitrogen.

--*P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth.

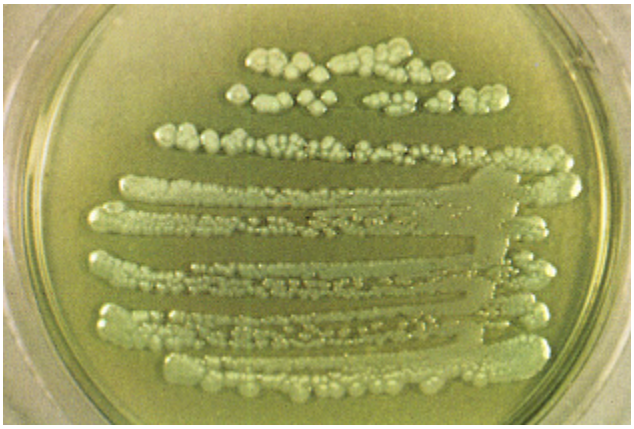
--Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C.

--It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

--*Pseudomonas aeruginosa* has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

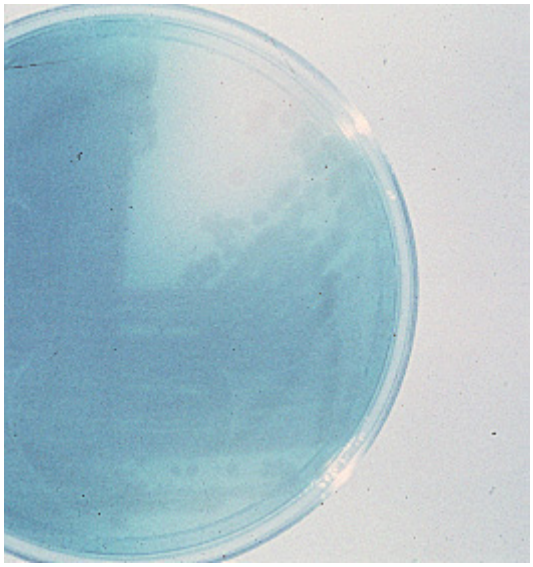
These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominance as a nosocomial pathogen.

P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large and smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.



***Pseudomonas aeruginosa* colonies on agar**

Pyoverdin and the blue pigment Pyocyanin
P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment **pyoverdin** and the blue pigment **pyocyanin**. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus" which is a characteristic of suppurative infections caused by *Pseudomonas aeruginosa.*



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

Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally-occurring antibiotics. Moreover, *Pseudomonas* maintains antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation.

Only a few antibiotics are effective against Pseudomonas, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The futility of treating Pseudomonas infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated.

Diagnosis
Diagnosis of P. aeruginosa infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42° C. Fluorescence under ultraviolet light is helpful in early identification of P. aeruginosa colonies. Fluorescence is also used to suggest the presence of P. aeruginosa in wounds.

Pathogenesis
For an opportunistic pathogen such as Pseudomonas aeruginosa, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of Pseudomonas infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis.

Most Pseudomonas infections are both invasive and toxinogenic. The ultimate Pseudomonas infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization; (2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease.

Colonization
Although colonization usually precedes infections by Pseudomonas aeruginosa, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment. It is sometimes present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is relatively low (estimates range from 0 to 24 percent depending on the anatomical locale).

The fimbriae of Pseudomonas will adhere to the epithelial cells of the upper respiratory tract and, by inference, to other epithelial cells as well. These adhesions appear to bind to specific galactose or mannose or sialic acid receptors on epithelial cells. Colonization of the respiratory tract by Pseudomonas requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play a role in colonization of the respiratory tract since P. aeruginosa will adhere to tracheal epithelial cells of mice infected with Influenza virus but not to normal tracheal epithelium.

This has been called opportunistic adherence, and it may be an important step in Pseudomonas keratitis and urinary tract infections, as well as infections of the respiratory tract. The receptor on tracheal epithelial cells for Pseudomonas pili is probably sialic acid (N-acetylneuraminic acid). Mucoid strains, which produce an exopolysaccharide (alginate) have an additional or alternative adhesion which attaches to the tracheobronchial mucin (N-acetylglucosamine). Besides pili and the mucoid polysaccharide, there are possibly two other cell surface adhesions utilized by Pseudomonas to colonize the respiratory epithelium or mucin. Also, it is likely that surface-bound exoenzyme S could serve as an adhesion for glycolipids on respiratory cells. The mucoid exopolysaccharide produced by P. aeruginosa is a repeating polymer of mannuronic and glucuronic acid referred to as alginate. Alginate slime forms the matrix of the Pseudomonas biofilm which anchors the cells to their environment and, in medical situations, it protects the bacteria from the host defenses such as lymphocytes, phagocytes, the ciliary action of the respiratory tract, antibodies and complement. Biofilm mucoid strains of P. aeruginosa are also less susceptible to antibiotics than their planktonic counterparts. Mucoid strains of P. aeruginosa are most often isolated from patients with cystic fibrosis and they are usually found in post mortem lung tissues from such individuals.

Shigellosis *Shigella* Section

Shigella dysenteriae type 1(or bacillary dysentery) is the only cause of epidemic dysentery. This organism is generally found in the stool of infected individuals, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days. Shigella can even survive in dust particles for six weeks at room temperature.

Infected humans act as host for this particular organism, as well as primates. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. Transmission occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.

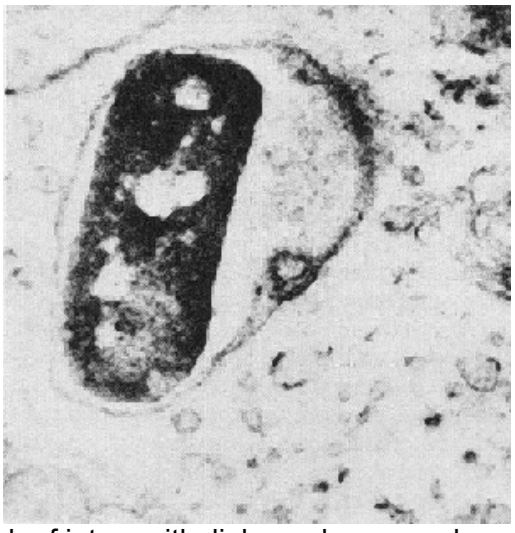
What sort of germ is *Shigella*?
The *Shigella* germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person. *Shigella* were discovered over 100 years ago by a Japanese scientist named Shiga, for whom they are named. There are several different kinds of *Shigella* bacteria: *Shigella sonnei*, also known as "Group D" *Shigella*, accounts for over two-thirds of the shigellosis in the United States. A second type, *Shigella flexneri*, or "group B" *Shigella*, accounts for almost all of the rest. Other types of *Shigella* are rare in this country, though they continue to be important causes of disease in the developing world. One type found in the developing world, *Shigella dysenteriae* type 1, causes deadly epidemics there.

Microbial Characteristics
Shigella dysenteriae is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and lactose and lysine (-). This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.

Shigella dysenteriae is the organism responsible for bacillary dysentery. This disease is most often associated with areas of overcrowding and poor sanitation (developing countries). Illness does, however, tend to be seasonal, happening when it is hot and wet. Symptoms of dysentery due to this organism include mild to severe diarrhea, which is sometimes bloody or watery.

There is also fever and nausea that accompany the diarrhea. Some people, however, also suffer from vomiting and cramping, and some show no symptoms at all. The symptoms of the disease will generally show between 12-96 hours (1-3 days) after becoming infected.

During this incubation period, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.



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

How can *Shigella* infections be diagnosed?
Many different kinds of diseases can cause diarrhea and bloody diarrhea, and the treatment depends on which germ is causing the diarrhea. Determining that *Shigella* is the cause of the illness depends on laboratory tests that identify *Shigella* in the stools of an infected person. These tests are sometimes not performed unless the laboratory is instructed specifically to look for the organism. The laboratory can also do special tests to tell which type of *Shigella* the person has and which antibiotics, if any, would be best to treat it.

How can *Shigella* infections be treated?
Shigellosis can usually be treated with antibiotics. The antibiotics commonly used for treatment are ampicillin, trimethoprim/sulfamethoxazole (also known as Bactrim* or Septra*), nalidixic acid, or ciprofloxacin. Appropriate treatment kills the *Shigella* bacteria that might be present in the patient's stools, and shortens the illness. Unfortunately, some *Shigella* bacteria have become resistant to antibiotics and using antibiotics to treat shigellosis can actually make the germs more resistant in the future.

Persons with mild infections will usually recover quickly without antibiotic treatment. Therefore, when many persons in a community are affected by shigellosis, antibiotics are sometimes used selectively to treat only the more severe cases. Antidiarrheal agents such as loperamide (Imodium*) or diphenoxylate with atropine (Lomotil*) are likely to make the illness worse and should be avoided.

Are there long term consequences to a *Shigella* infection?
Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. About 3% of persons who are infected with one type of Shigella, *Shigella flexneri*, will later develop pains in their joints, irritation of the eyes, and painful urination. This is called Reiter's syndrome. It can last for months or years, and can lead to chronic arthritis which is difficult to treat. Reiter's syndrome is caused by a reaction to *Shigella* infection that happens only in people who are genetically predisposed to it.

Once someone has had shigellosis, they are not likely to get infected with that specific type again for at least several years. However, they can still get infected with other types of *Shigella*.

How do people catch *Shigella*?
The *Shigella* bacteria pass from one infected person to the next. *Shigella* are present in the diarrheal stools of infected persons while they are sick and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person.

This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.

Shigella infections may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. *Shigella* infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with shigellosis swims in it.

What can a person do to prevent this illness?
There is no vaccine to prevent shigellosis. However, the spread of *Shigella* from an infected person to other persons can be stopped by frequent and careful handwashing with soap. Frequent and careful handwashing is important among all age groups. Frequent, supervised handwashing of all children should be followed in day care centers and in homes with children who are not completely toilet-trained (including children in diapers). When possible, young children with a *Shigella* infection who are still in diapers should not be in contact with uninfected children.

People who have shigellosis should not prepare food or pour water for others until they have been shown to no longer be carrying the *Shigella* bacterium.

If a child in diapers has shigellosis, everyone who changes the child's diapers should be sure the diapers are disposed of properly in a closed-lid garbage can, and should wash his or her hands carefully with soap and warm water immediately after changing the diapers. After use, the diaper changing area should be wiped down with a disinfectant such as household bleach, Lysol* or bactericidal wipes.

Basic food safety precautions and regular drinking water treatment prevents shigellosis. At swimming beaches, having enough bathrooms near the swimming area helps keep the water from becoming contaminated.

Simple precautions taken while traveling to the developing world can prevent getting shigellosis. Drink only treated or boiled water, and eat only cooked hot foods or fruits you peel yourself. The same precautions prevent traveler's diarrhea in general.

How common is shigellosis?
Every year, about 18,000 cases of shigellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be twenty times greater. Shigellosis is particularly common and causes recurrent problems in settings where hygiene is poor and can sometimes sweep through entire communities. Shigellosis is more common in summer than winter. Children, especially toddlers aged 2 to 4, are the most likely to get shigellosis. Many cases are related to the spread of illness in child-care settings, and many more are the result of the spread of the illness in families with small children.

In the developing world, shigellosis is far more common and is present in most communities most of the time. Chinese scientists have sequenced the genome of a bacterium that is a leading cause of infant mortality in developing countries. About one million people die of *Shigella* infections every year, most of them children. The bacterium *Shigella flexneri* causes sudden and severe diarrhea in humans, known as shigellosis.

New treatments are needed for this highly infectious microbe because antibiotics are often inadequate and drug-resistant strains are on the rise. Currently, no vaccines exist and the World Health Organization considers the development of a vaccine a priority.

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

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.

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.

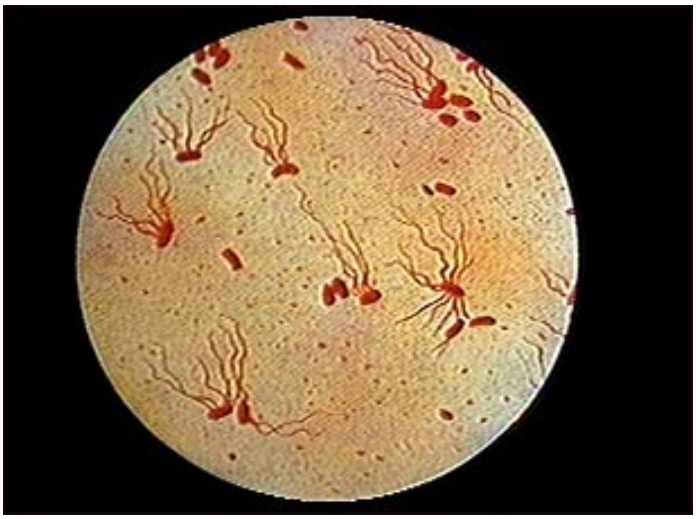
What is the government doing about shigellosis?
The Centers for Disease Control and Prevention (CDC) monitors the frequency of *Shigella* infections in the country, and assists local and State health departments to investigate outbreaks, determine means of transmission and devise control measures. CDC 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.

Typhoid Fever *Salmonella* typhi Section

Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella* Typhi. In the United States about 400 cases occur each year, and 70% of these are acquired while traveling internationally. Typhoid fever is still common in the developing world, where it affects about 12.5 million persons each year.

Typhoid fever can be prevented and can usually be treated with antibiotics. If you are planning to travel outside the United States, you should know about typhoid fever and what steps you can take to protect yourself.



***Salmonella* typhi**

Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed *S.* Typhi in their feces (stool).

You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding *S.* Typhi or if sewage contaminated with *S.* Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage. Once *S.* Typhi bacteria are eaten or drunk, they multiply and spread into the blood-stream. The body reacts with fever and other signs and symptoms.

In 1885, pioneering American veterinary scientist, Daniel E. Salmon, discovered the first strain of Salmonella from the intestine of a pig. This strain was called Salmonella choleraesuis, the designation that is still used to describe the genus and species of this common human pathogen. Salmonella is a type of bacteria that causes typhoid fever and many other infections of intestinal origin. Typhoid fever, rare in the U.S., is caused by a particular strain designated Salmonella typhi. But illness due to other Salmonella strains, just called "salmonellosis," is common in the U.S. Today, the number of known strains (technically termed "serotypes" or "serovars") of this bacteria total over 2300.

Serotypes
Salmonella serotypes typhimurium and enteritidis are the most common serotypes in the United States. In recent years, concerns have been raised because many strains of Salmonella have become resistant to several of the antibiotics traditionally used to treat it, in both animals and humans.

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

Remember that you will need to complete your vaccination at least 1 week before you travel so that the vaccine has time to take effect. Typhoid vaccines lose effectiveness after several years; if you were vaccinated in the past, check with your doctor to see if it is time for a booster vaccination. Taking antibiotics will not prevent typhoid fever; they only help treat it.

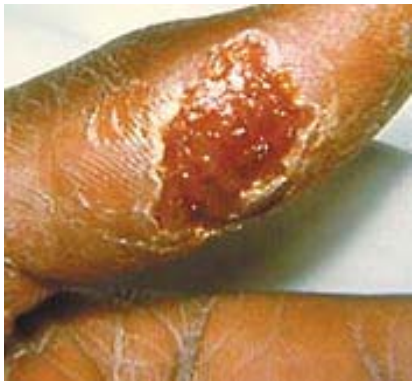
The chart below provides basic information on typhoid vaccines that are available in the United States.

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.

Tularemia *Francisella tularensis* Section

What is Tularemia?
Tularemia is a potentially serious illness that occurs naturally in the United States. It is caused by the bacterium *Francisella tularensis* found in animals (especially rodents, rabbits, and hares).



What are the Symptoms of Tularemia?
Symptoms of tularemia could include:

- sudden fever
- chills
- headaches
- diarrhea
- muscle aches
- joint pain
- dry cough
- progressive weakness

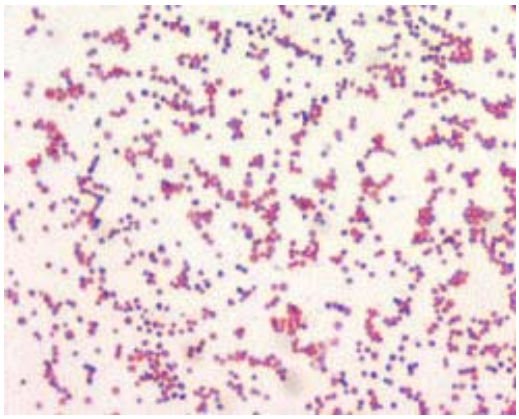
People can also catch pneumonia and develop chest pain, bloody sputum and can have trouble breathing and even sometimes stop breathing.

Other symptoms of tularemia depend on how a person was exposed to the tularemia bacteria. These symptoms can include ulcers on the skin or mouth, swollen and painful lymph glands, swollen and painful eyes, and a sore throat.

How Does Tularemia Spread?
People can get tularemia many different ways:

- being bitten by an infected tick, deerfly or other insect
- handling infected animal carcasses
- eating or drinking contaminated food or water
- breathing in the bacteria, *F. tularensis*

Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated. People who have been exposed to the tularemia bacteria should be treated as soon as possible. The disease can be fatal if it is not treated with the right antibiotics.



F. tularensis
Gram stain

How Soon Do Infected People Get Sick?
Symptoms usually appear 3 to 5 days after exposure to the bacteria, but can take as long as 14 days.

What Should I Do if I Think I Have Tularemia?
Consult your doctor at the first sign of illness. Be sure to let the doctor know if you are pregnant or have a weakened immune system.

How Is Tularemia Treated?
Your doctor will most likely prescribe antibiotics, which must be taken according to the directions supplied with your prescription to ensure the best possible result. Let your doctor know if you have any allergy to antibiotics. A vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States.

What Can I Do To Prevent Becoming Infected with Tularemia?
Tularemia occurs naturally in many parts of the United States. Use insect repellent containing DEET on your skin, or treat clothing with repellent containing permethrin, to prevent insect bites. Wash your hands often, using soap and warm water, especially after handling animal carcasses. Be sure to cook your food thoroughly and that your water is from a safe source.

Note any change in the behavior of your pets (especially rodents, rabbits, and hares) or livestock, and consult a veterinarian if they develop unusual symptoms.

Can Tularemia Be Used As a Weapon?
Francisella tularensis is very infectious. A small number (10-50 or so organisms) can cause disease. If *F. tularensis* were used as a weapon, the bacteria would likely be made airborne for exposure by inhalation. People who inhale an infectious aerosol would generally experience severe respiratory illness, including life-threatening pneumonia and systemic infection, if they are not treated. The bacteria that cause tularemia occur widely in nature and could be isolated and grown in quantity in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication.

F. tularensis is a small Gram-negative aerobic bacillus with two main serotypes: Jellison Type A and Type B. Type A is the more virulent form. The causative agent of the disease was named after Dr. Edward Francis and the location where the organism was discovered, Tulare County, California. Tularemia is frequently spread by direct contact with rabbits, leading to the term "rabbit fever." However, the disease can also be spread by other animals, typically rodents, and by arthropods. It is a primarily rural disease that is found in all 50 states, except Hawaii.

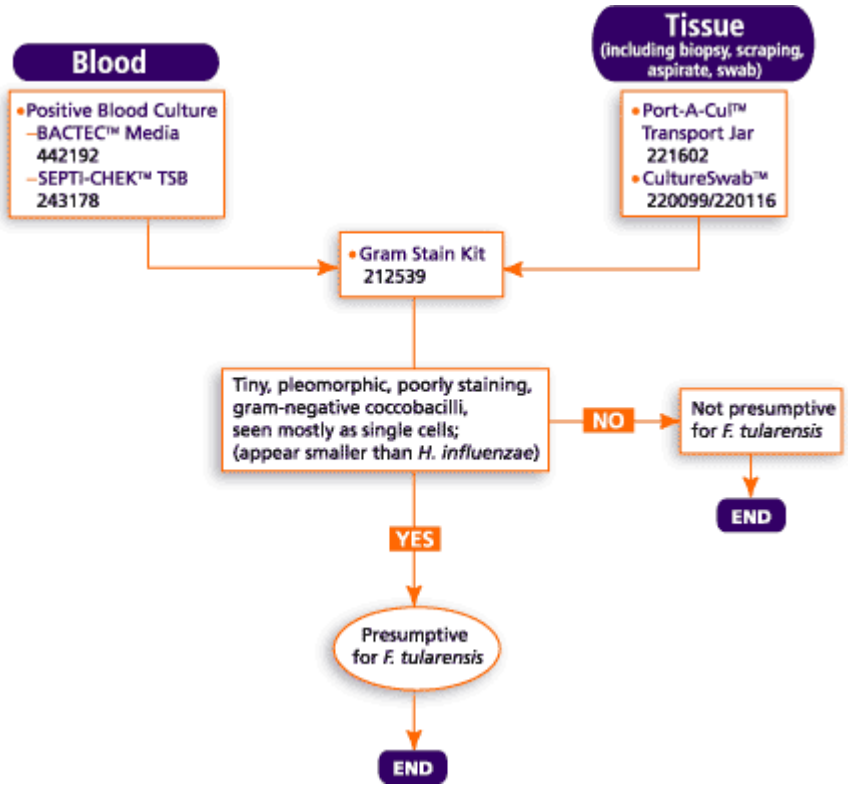
Pathogenesis
Historical commentaries reference the virulence of the disease, indicating that people have been aware of pathogenicity of Francisella for thousands of years. However, there is still much to be learned about this extremely virulent organism. The disease can be contracted by ingestion, inhalation, or by direct skin contact. Tularemia occurs in six different forms: typhoidal, pneumonic, oculoglandular, oropharyngeal, ulceroglandular, and glandular. Clinical diagnosis can be difficult since the disease mimics a slough of other illnesses. Pathogenesis varies greatly depending on mode of infection.

Manifestations
The incubation period is about 3-5 days but it can take as long as two weeks for symptoms to appear. Symptoms vary based on mode of infection, but generally include fever, chills, joint and muscle pain, headache, weakness, and sometimes pneumonia. People who develop pneumonic tularemia experience chest pain, bloody sputum, and difficultly 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.



MIB and Geosmin Section

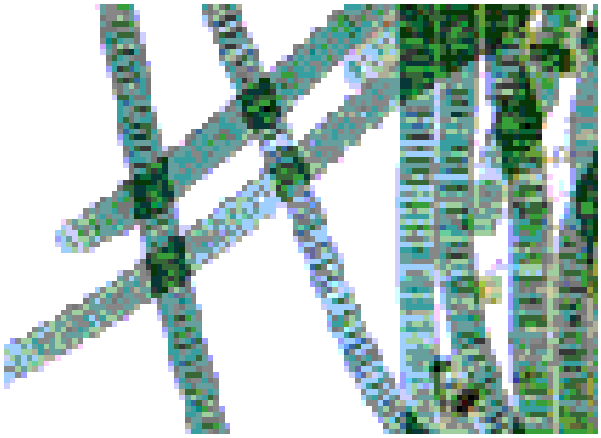
Aesthetics only and not a disease concern
While I was working in the water quality laboratory, we would be overwhelmed by customers calling in and worrying about tastes and odors. While this small section is not really about a waterborne disease, water customers will react to this as if was a disease.

Be prepared
Seasonal occurrences of musty/moldy or earthy tastes and odors may be detected in the system water. Research, by laboratories dedicated to this subject, has determined the culprits are naturally occurring algal and fungal (microbiological) by-products. As algae in the canals die, compounds known as Methyl-Isoborneol (MIB) and Geosmin are released into the water.

These stable complex compounds present in parts per trillion are difficult to remove with current technology. The detection of these compounds is dependent upon an individual's olfactory sensitivity. Many people may never detect them, while others who are sensitive may detect the musty/moldy taste and smell at levels below instrument detection levels. Most water providers use activated carbon to adsorb the MIB and Geosmin, thus alleviating the taste and odor.

Earthy-musty tastes and odors are produced by certain cyanobacteria (blue-green algae), actinomycetes, and a few fungi. The substances are produced by actinomycetes and cyanobacteria that cause tastes and odors in drinking-water include geosmin, methyl-isoborneol (MIB), and cardin-4-ene-1-ol. Growing algae produce numerous volatile and nonvolatile organic substances, including aliphatic alcohols, aldehydes, ketones, esters, thioesters, and sulfides.

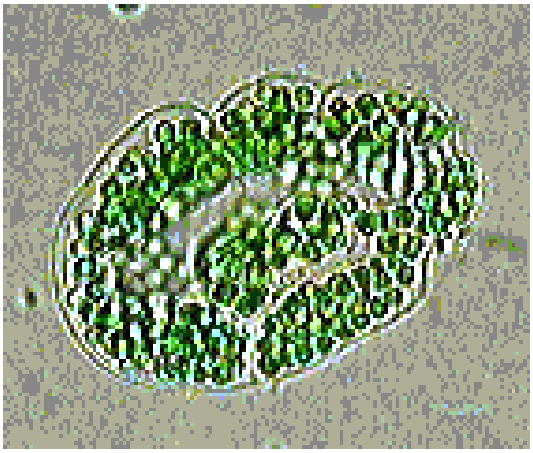
Occasionally, taste and odor problems in water are caused by other bacteria, fungi, zooplankton, and nemathelminthes. Ferrobacteria in water-distribution systems may produce tastes and odors, and some species of Pseudomonas can cause a swampy odor, whereas others can convert sulfur-containing amino acids into hydrogen sulfide, methylthiol, and dimethylpolysulfide.



Oscillatoria chalybea

Blooms

Blooms will produce the noxious substances 2-methyl isoborneol (MIB) and geosmin, compounds that are responsible for causing an off-flavor in catfish. MIB and geosmin are often described as smelling like "***sweaty socks***."



Microcystis aeruginosa

Commonly found in lakes and ponds. In the spring, large numbers floating on the water surface produce a blue-tinge. Blooms of this cyanobacterium are also notorious for producing a liver toxin that in large amount can kill fish and livestock.

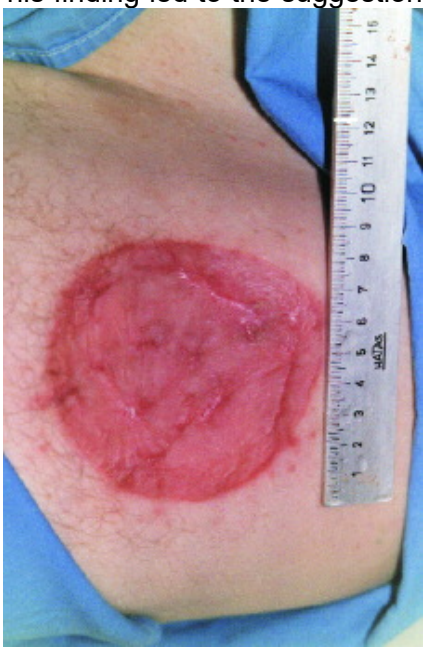
Chemical Related Diseases

Arsenic

Arsenic is a naturally occurring element used since ancient times and has long been known to be toxic to humans. Arsenic in ground water is largely the result of minerals dissolving from weathered rocks and soils. Several types of cancer have been linked to arsenic in water. In 2001 the US Environmental Protection Agency lowered the maximum level of arsenic permitted in drinking water from 50 micrograms per liter (ug/L) to 10 ug/L.

Gastrointestinal and nervous system effects are common and the ingestion of relatively small amounts can result in death. A recent study indicates that arsenic disrupts the activity of glucocorticoids, compounds that have a variety of functions including the regulation of blood sugar.

Interestingly, this same study suggested that arsenic at high levels inhibits those mechanisms that normally suppress tumor production. This finding led to the suggestion that instead of causing cancer arsenic promotes the growth of tumors triggered by other carcinogens. And by the way, arsenic-induced effects appeared at concentrations as low as 2 micrograms per liter. Keep in mind, the new EPA regulation calls for 10 micrograms per liter and the average person ingests 10-15 micrograms per day. These numbers suggest we're getting our minimal daily requirement.



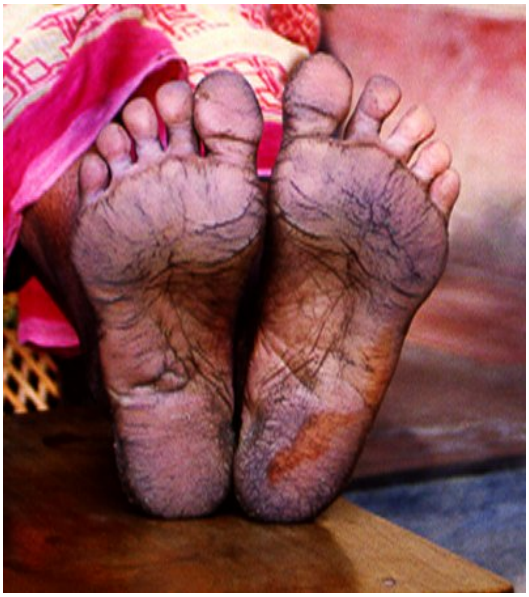
Other epidemiological studies suggest an association between drinking arsenic-tainted water and skin, lung, liver and bladder cancers. A 1999 report by the National Academy of Sciences estimated that daily ingestion of water containing 50 micrograms of arsenic per liter would add about 1 percent to a person's lifetime risk of dying from cancer.

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

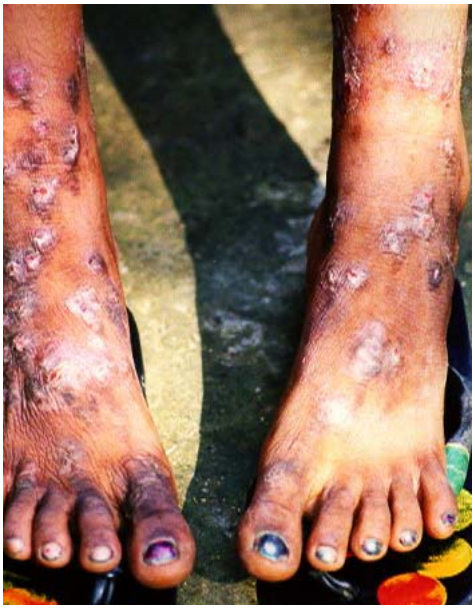
Conservative estimates based on all these data suggest that more than 34 million Americans drink tap water, supplied by systems containing average levels of arsenic that pose unacceptable cancer risks. In October 2001, the Environmental Protection Agency implemented new standards for arsenic in drinking water, lowering the maximum acceptable level in parts per billion from 50ppb to 10ppb.

Exposure to higher than average levels of arsenic occurs mostly in the workplace, near hazardous waste sites, or in areas with high natural levels. At high levels, inorganic arsenic can cause death. Exposure to lower levels for a long time can cause a discoloration of the skin and the appearance of small corns or warts.

Arsenic has been found at 1,014 of the 1,598 National Priority List sites identified by the Environmental Protection Agency (EPA).



Keratosis of the feet



Blackfoot disease

What happens to arsenic when it enters the environment?

- Arsenic cannot be destroyed in the environment. It can only change its form.
- Arsenic in air will settle to the ground or is washed out of the air by rain.
- Many arsenic compounds can dissolve in water.
- Fish and shellfish can accumulate arsenic, but the arsenic in fish is mostly in a form that is not harmful.

Is there a medical test to show whether I've been exposed to arsenic?

There are tests to measure the level of arsenic in blood, urine, hair, or fingernails. The urine test is the most reliable test for arsenic exposure within the last few days. Tests on hair and fingernails can measure exposure to high levels of arsenic over the past 6-12 months.

These tests can determine if you have been exposed to above-average levels of arsenic. They cannot predict how the arsenic levels in your body will affect your health.

Blue Baby Syndrome (Methemoglobinemia) 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.

Related Diseases and Associated Illnesses Chapter 7 Review

Amebiasis Section Entamoeba histolytica

1. The life cycle of Entamoeba histolytica involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and _____ that are passed in the host's feces.
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

2. Humans are infected by ingesting _____, most often via food or water contaminated with human fecal material (view diagram of the life cycle).
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

3. Entamoeba histolytica has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal _____, feeding on intestinal contents and host tissue, and multiplying by fission.
A. Crypts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

4. The _____ can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, E. histolytica is potentially the most pathogenic.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

5. Entamoeba histolytica is an amoeboid _____ of the intestinal tract and in some cases other visceral organs, especially the liver.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

6. There are several species in this genus, distinguished by their number of _____ in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

7. Within the _____ there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst. The cyst can resist desiccation for 1-2 weeks.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

8. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 _____.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

9. In some cases the _____ secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

10. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal. As with most of the amoebae, infections of E. histolytica are often diagnosed by demonstrating cysts or trophozoites in a stool sample.
A. True
B. False

Amebic Meningoencephalitis PAM Section Naegleria fowleri
11. Primary Amebic Meningoencephalitis (PAM) is a common and usually deadly disease caused by infection with the ameba (a multi-celled organism that maintains the original shape).
A. True
B. False

12. Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.
A. True
B. False

13. The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. Naegleria thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.
A. True
B. False

14. Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.
A. True
B. False

15. The ameba is believed to enter the body through the mouth and travel to the stomach. The disease is easily spread from person to person.
A. True
B. False

16. The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the digestive tract or frequently before death through the examination of digestive lining.
A. True
B. False

17. PAM is a mild illness that responds to routine treatments. Aggressive use of some antifungal medications have always been successful. Intensive supportive care is rarely necessary along with the medication.
A. True
B. False

Schistosomes and Other Trematodes Section

18. Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except _____.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

19. Almost unknown in industrialized countries, _____ infects 200 million people in 76 countries of the tropical developing world.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

20. A Flatworm that spends part of its life in a freshwater snail host causes _____. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

21. Free-swimming larvae (_____) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

22. After entering a human the larvae develop into adult males and females (schistosomes) or _____ (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

23. In _____, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

24. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other _____).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

25. _____ is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. None of the above

26. Signs and symptoms are related largely to the location of the adult worms. Infections with Schistosoma mansoni and S japonicum (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis. Schistosoma haematobium (vesical venules) causes dysuria, hema turia, and uremia. Fasciola hepatica, Clonorchis sinensis, and Opisthorchis viverrini (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice. Infections with Paragonimus westermani (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. Fasciolopsis buski (intestines) causes abdominal pain, diarrhea, and edema.
A. True
B. False

27. Trematodes are single cellular eukaryotic helminths.
A. True
B. False

Gastroenteritis Section
28. _____ means inflammation of the stomach and small and large intestines.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

29. Viral gastroenteritis is an infection caused by a variety of _____ that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

30. Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, _____, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as Salmonella or Escherichia coli) or parasites (such as Giardia), or by medications or other medical conditions, although the symptoms may be similar.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

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

32. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which _____ causes the illness.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

33. Viral gastroenteritis is _____. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

Rotovirus Information Section
34. The incubation period for _____ disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

35. A _____ has a characteristic wheel-like appearance when viewed by electron microscopy (the name is derived from the Latin rota, meaning "wheel").
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

36. Rotaviruses are nonenveloped, double-shelled viruses. The _____ is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

37. The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the _____, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

38. Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by _____ or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

39. For persons with healthy immune systems, rotavirus _____ is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children will require hospitalization for intravenous fluids.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

Noroviruses Section

40. Noroviruses (genus Norovirus, family Caliciviridae) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official genus name for the group of viruses provisionally described as “Norwalk-like viruses” (NLV).
A. True
B. False

41. The symptoms of norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. The illness is usually brief, with symptoms lasting only about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.
A. True
B. False

42. Persons who are infected with norovirus should not prepare food while they have symptoms and for 3 weeks after they recover from their illness. Food that may have been contaminated by an ill person can be eaten.
A. True
B. False

43. Illness caused by norovirus infection has several names, including stomach flu – this “stomach flu” is **not** related to the flu (or influenza), which is a respiratory illness caused by influenza virus.
A. True
B. False

44. Noroviruses are found in the stool or vomit of infected people. People can become infected with the virus in several ways, including eating food or drinking liquids that are contaminated with norovirus; touching surfaces or objects contaminated with norovirus, and then placing their hand in their mouth; having direct contact with another person who is infected and showing symptoms (for example, when caring for someone with illness, or sharing foods or eating utensils with someone who is ill).
A. True
B. False

45. Persons working in day-care centers or nursing homes should pay special attention to children or residents who have norovirus illness. This virus is very contagious and can spread rapidly throughout such environments.
A. True
B. False

Hepatitis Section

46. Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). Hepatitis A can affect anyone. In the United States, hepatitis A can occur in situations ranging from isolated cases of disease to widespread _____.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

47. Each year, an estimated 100 persons die as a result of _____ in the United States due to Hepatitis A. Approximately 30 - 50,000 cases occur yearly in the United States and the direct and indirect costs of these cases exceed $300 million.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

48. The unfortunate aspect of these statistics is that with 21st century medicine, Hepatitis A is totally _____, and isolated cases, especially outbreaks relegated to food consumption, need not occur.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

49. Viral Hepatitis is a major public health concern in the United States, and a source of significant morbidity and mortality. The Hepatitis A virus or "HAV" is heat stable and will survive for up to a month at _____ in the environment.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

50. Hepatitis A is a _____ (or contagious) disease that spreads from person to person. (It is not acquired from animals, insects, or other means.) It is transmitted by the "fecal – oral route." This does not mean, or course, that Hepatitis A transmission requires that fecal material from an infectious individual must come in contact directly with the mouth of a susceptible individual. It is almost always true that the virus infects a susceptible individual when he or she ingests it, but it gets to the mouth by an indirect route.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

51. Hepatitis A is found in every part of the United States and throughout the world. When water sources such as private wells are contaminated with feces from infected humans, the water will spread the hepatitis A virus. The _____ can enter the water through various ways, including sewage overflows or broken sewage systems.
A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

52. Hepatitis ____ is a liver disease caused by the hepatitis____ virus, a defective virus that needs the hepatitis B virus to exist. Hepatitis ____ virus is found in the blood of persons infected with the virus.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

53. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus transmitted in much the same way as hepatitis A virus. Hepatitis ____, however, does not occur often in the United States.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

54. Hepatitis ____is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis ____ virus, can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

55. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus, which is found in the blood of persons who have the disease. ____ is spread by contact with the blood of an infected person.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

Leptospirosis Section Leptospira

56. _____ is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus Leptospira. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all.

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

57. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. Leptospira interrogans causes leptospirosis, a usually mild _____ that may result in liver or kidney failure.

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

58. Leptospira is a flexible, spiral-shaped, _____ with internal flagella. Leptospira interrogans has many serovars based on cell surface antigens.

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

59. Outbreaks of _____ are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms.

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

60. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with _____. The disease is not known to be spread from person to person.

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

61. Leptospira enters the host through mucosa and broken skin, resulting in _____. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

62. _____ are responsible for host resistance.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

63. Leptospirosis is a worldwide _____ affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

64. Clinical diagnosis is usually confirmed by _____. Isolation of spirochetes is possible, but it is time-consuming and requires special media.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

65. Animal vaccination and eradication of rodents are important. Treatment with _____ and penicillin G is effective. No human vaccine is available.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

Pseudomonas aeruginosa Section

66. Pseudomonas aeruginosa is the _____ of an opportunistic pathogen of humans.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

67. The bacterium almost never infects _____ tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

68. Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, _____ and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

69. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, _____, and burns. The case fatality rate in these patients is 50 percent.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

70. Pseudomonas aeruginosa is primarily a _____. According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Nosocomial pathogen
D. Uncompromised
E. Gastrointestinal infections

71. Unlike many environmental bacteria, P. aeruginosa has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many _____, from water and soil to plant and animal tissues.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Ecological niches
E. None of the above

72. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to _____ where nutrients are limited.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

73. P. aeruginosa can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that _____ the cell membranes and connective tissues in various organs.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

74. This bacterium is also noted for _____to many antibiotics.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. Its resistance

75. P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its _____ and environmental versatility.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

76. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, _____and systems involved in sensing and responding to environmental changes.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

Shigellosis Section Shigella

77. _____ type 1(or bacillary dysentery) is the only cause of epidemic dysentery.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

78. This organism is generally found in the stool of _____, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

79. _____ can even survive in dust particles for six weeks at room temperature.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

80. Infected humans act as host for this particular organism, as well as _____.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

81. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. _____ occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

82. The _____ germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri

83. A second type, _____, or "group B" Shigella, accounts for almost all of the rest.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

84. Other types of _____ are rare in this country, though they continue to be important causes of disease in the developing world.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

85. One type found in the developing world, _____ type 1, causes deadly epidemics there.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

86. _____ is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

87. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and _____. This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.
Incubation period
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

88. Shigella dysenteriae is the organism responsible for _____.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

89. During this _____, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

90. Most Shigella infections are the result of the _____ from stools or soiled fingers of one person to the mouth of another person. This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

91. _____ may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

92. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. Shigella infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with _____ in it.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

Salmonella typhi Section
93. _____ is a life-threatening illness caused by the bacterium Salmonella Typhi.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

94. _____is still common in the developing world, where it affects about 12.5 million persons each year.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

95. Typhoid fever can be prevented and can usually be treated with _____.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

96. Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called _____, recover from typhoid fever but continue to carry the bacteria. Both ill persons and _____ shed S. Typhi in their feces (stool).
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

97. You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding _____ or if sewage contaminated with S. Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

98. Once S. Typhi bacteria are eaten or drunk, they _____ into the bloodstream. The body reacts with fever and other signs and symptoms.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

1.A, 2.A, 3.A, 4.B, 5.C, 6.D, 7.A, 8.B, 9.B, 10.A, 11.B, 12. A, 13.A, 14.A, 15.B, 16.B, 17.B, 18.C, 19.D, 20.D, 21.A, 22.B, 23.D, 24.E, 25.E, 26.A, 27.B, 28.B, 29.C, 30.D, 31.B, 32. C, 33.A, 34.A, 35.A, 36.C, 37.E, 38.E, 39.D, 40.A, 41.A, 42.B, 43.A, 44.A, 45.A, 46.A, 47.C, 48.B, 49.E, 50.D, 51.E, 52.D, 53.E, 54.B, 55.C, 56.C, 57.E, 58.E, 59.C, 60.E, 61.E, 62.A, 63.B, 64.E, 65.D, 66.C, 67.D, 68.E, 69.B, 70.C, 71.D, 72.D, 73.C, 74.E, 75.B, 76.B, 77.D, 78.E, 79.B, 80.E, 81.E, 82.B, 83.D, 84.B, 85.D, 86.D, 87.A, 88.B, 89.E, 90.C, 91.E, 92.D, 93.B, 94.B, 95.E, 96.D, 97.A, 98.E

EPA Regulations Chapter 8

Proposed Stage 2 DBP Rule – Compliance

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The proposed Stage 2 Disinfection Byproduct Rule (DBPR) is expected to minimize exposure to disinfection byproducts (DBPs) at individual locations in the distribution system and to shave the peak values of DBPs in the system. The Initial Distribution System Evaluation was discussed in the last issued of the Newsletter. In the IDSE process, utilities are required to complete a study that locates new permanent DBP monitoring sites. When the IDSE is complete, an IDSE Report is submitted to the State which includes the proposed new permanent DBP monitoring sites for the utility. Each State may handle the approval of the monitoring sites in a slightly different manner, but the basic process is that that the State can approve the monitoring sites as recommended in the IDSE report, the State can suggest alternative sites, or the State may request additional information.

Stage 2A

Compliance under the Stage 2 DBPR is divided into two phases, Stage 2A and Stage 2B. During the time when utilities are monitoring for the IDSE, compliance continues to be based on the current Stage 1 DBPR requirements. Recall that that Stage 1 DBPR requires utilities to meet MCLs of 80 µg/L and 60 µg/L for TTHMs and HAA5s, respectively, based on a running annual average calculation across the whole system. During that same period, utilities should be preparing themselves to meet the locational running annual average (LRAA) requirements of the Stage 2A portion of the rule. The relative timing of the parts of the rule are shown in Figure 1.

Stage 2A requires all utilities to calculate the locational running annual average (LRAA) of quarterly sample results for TTHMs and HAA5s at each Stage 1 DBPR sampling site. The LRAA is the running annual average at a specific location in the distribution system. All utilities must meet the MCL of 120 µg/L for TTHMs and 100 µg/L for HAA5s on this LRAA basis. Preparation for meeting these LRAA levels should include evaluating the LRAA at each Stage 1 compliance monitoring site. For any sites that cannot meet the MCLs, development and implementation of a plan to bring them into compliance would be wise. The Stage 2A compliance date is three years from the date when the rule is finalized. At that time, all Stage 1 compliance sites must meet the Stage 2A MCLs based on the LRAA calculation method, in addition to maintaining compliance with the running annual average requirements across the system as required by the Stage 1 rule.

Stage 2B

As soon as the IDSE is complete, utilities can begin to prepare for meeting Stage 2B by monitoring at the newly selected compliance sites identified in the IDSE. Obtaining this data prior to the Stage 2B compliance date will provide information to the utility regarding areas in the system that need changes made that will reduce DBPs. Compliance with Stage 2B is based on meeting the MCLs of 80 µg/L and 60 µg/L for TTHMs and HAA5s, respectively, using the locational running annual average of quarterly sample results at each new monitoring site. Once the utility has a some data from the new sites, plans can be made for alterations in treatment or operations to meet MCLs, if needed.

Sufficient time is allowed in the rule for systems to make changes in their system to meet the compliance date for Stage 2B. The Stage 2B compliance date is six years after the rule is finalized for systems serving 10,000 people or more. For small systems the compliance date for Stage 2B is 7.5 or 8.5 years after the rule is finalized, depending on whether the system is required to sample for *Cryptosporidium* under the LT2ESWTR monitoring. Compliance dates for all system sizes for both stages of the rule are shown in Table 1.

TABLE 1
PROPOSED COMPLIANCE SCHEDULE FOR STAGE 2A AND STAGE 2B DBPR

System Size	IDSE Report Due	Comply with Stage 2A	Comply with Stage 2B
≥ 10,000	2 years after publication	3 years after publication	6 years after publication
< 10,000 in combined distribution system	2 years after publication	3 years after publication	6 years after publication
< 10,000	4 years after publication	3 years after publication	7.5/8.5 years after publication

Summary

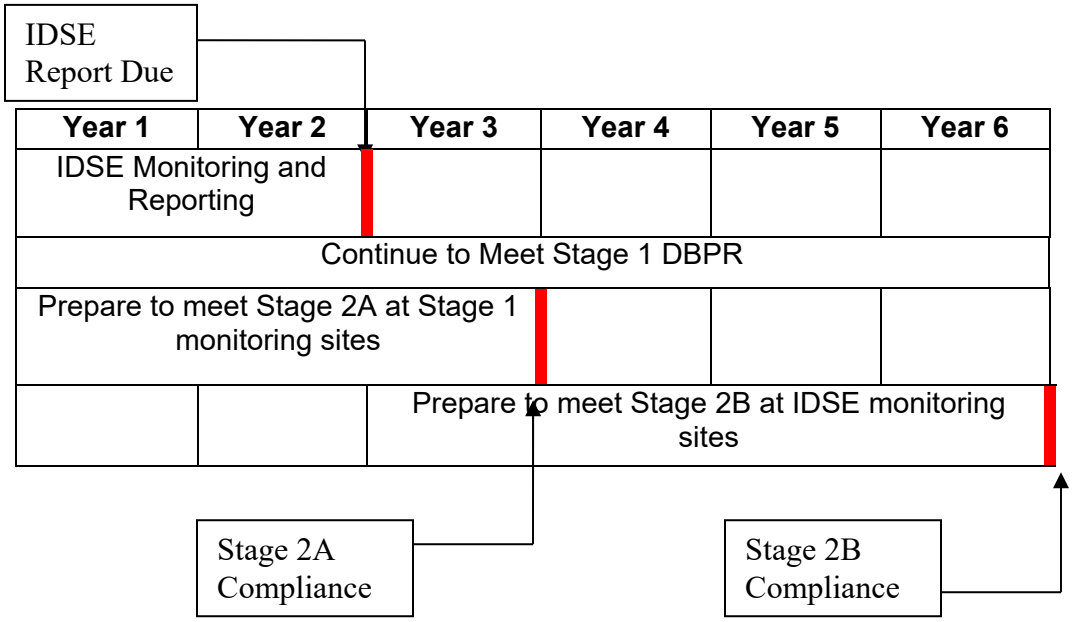
Utilities should keep in mind that the requirements of the Stage 1 DBPR continue to remain in force. This means that the percent removal requirements for total organic carbon (TOC) continue, as do the alternative compliance criteria for TOC removal. A summary of the Stage 2 DBPR phased compliance requirements is shown in Table 2.

Ground water serving10,000 people or more and surface water systems serving 500 or more people must monitor quarterly, taking at least one sample during the peak historical month for DBP concentration. Very small surface water plants and small ground water plants are on a yearly sampling schedule.

TABLE 2
PROPOSED STAGE 2 DBPR PHASED COMPLIANCE WITH MCLs

	Compliance Monitoring Sites	Compliance Calculation Basis	TTHM MCL □g/L	HAA5 MCL □g/L
Stage 2A	Stage 1	LRAA	120	100
	Stage 1	RAA	80	60
Stage 2B	Stage 2	LRAA	80	60

Figure 1
Schedule For DBP Compliance Monitoring



Notes:

- Applies to Large and Medium Systems and Small Systems in Larger Combined Distribution Systems
- Based on years after Stage 2 DBPR is promulgated.

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Drinking Water Rules and Disease Relationship

Public water systems are regulated under the Safe Drinking Water Act (SDWA) of 1974 and its subsequent 1986 and 1996 amendments (*7--9*). Under SDWA, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants. The 1996 SDWA amendments require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated. The first list was called the drinking water Contaminant Candidate List (CCL). CCL contained 60 contaminants/ contaminant groups, included 10 pathogens, and was published in the *Federal Register* on March 2, 1998 (*10*). A decision concerning whether to regulate ≥5 contaminants from the CCL was required by August 2001.

Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the Surface Water Treatment Rule (SWTR) of 1989 (*11--13*). SWTR covers all water systems that use surface water or groundwater under the direct influence of surface water (Glossary). SWTR is intended to protect against exposure to *Giardia intestinalis*, viruses, and *Legionella*, as well as selected other pathogens. In 1998, the EPA promulgated the Interim Enhanced Surface Water Treatment Rule (IESWTR) (*14*), which provides additional protection against *Cryptosporidium* and other waterborne pathogens for systems that serve ≥10,000 persons.

In 2002, the EPA finalized the Long Term 1 Enhanced SWTR (LT1ESWTR) for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons (*15*). LT1ESWTR was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2001 (*16,17*).

The 1996 Amendments require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; the EPA has proposed the Ground Water Rule (GWR) to meet this mandate (*18*). GWR specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).

This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. GWR does not apply to privately owned wells. Additional protection of groundwater from both chemical and microbial contamination from shallow wells (including cesspools) is expected to be provided as a result of recent revisions to the Underground Injection Control Regulations, published December 7, 1999 (*19*).

To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the Information Collection Rule in 1996 (*20*), which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.

Surface water systems are also required to monitor for the presence of *Cryptosporidium*, *Giardia*, total culturable viruses, and total* and fecal coliforms or *Escherichia coli* ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.

The EPA also made minor changes in 2000 to the Lead and Copper Rule to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same (*21*).

Recreational Water
Regulation of recreational water is determined by state and local governments.

Standards for operating, disinfecting, and filtering public swimming and wading pools are regulated by state and local health departments and, as a result, are varied. In 1986, the EPA established a guideline for microbiological water quality for recreational freshwater (e.g., lakes and ponds) and marine water (*22*). The guideline recommends that the monthly geometric mean concentration of organisms in freshwater should be ≤33/100 mL for enterococci or ≤126/100 mL for *Es. coli*.

States have latitude regarding their guidelines or regulations and can post warning signs to alert potential bathers until water quality improves. Unlike treated venues where disinfection can be used to address problems with microbiological quality of the water, contaminated freshwater can require weeks or months to improve or return to normal. Prompt identification of potential sources of contamination and remedial action is necessary to return bathing water to an appropriate quality for recreational use (*23*).

The EPA's Action Plan for Beaches and Recreational Waters (Beach Watch) was developed as part of the Clean Water Action Plan.† The intent of Beach Watch is to assist state, tribal, and local authorities in strengthening and extending programs that specifically protect users of recreational waters. As part of the Beaches Act of 2000, the U.S. Congress directed the EPA to also develop a new set of guidelines for recreational water based on new water-quality indicators.

Data collected as part of the national WBDO surveillance system are used to describe the epidemiology of waterborne diseases in the United States. Data regarding water systems and deficiencies implicated in these outbreaks are used to assess whether regulations for water treatment and monitoring of water quality are adequate to protect the public against disease. Surveillance also enables identifying etiologic agents and environmental or behavioral risk factors that are responsible for these outbreaks.

This information is used to inform public health and regulatory agencies, water utilities, pool operators, and other stakeholders of new or reemerging trends that might necessitate different interventions and changes in policies and resource allotment.

Data Sources
State, territorial, and local public health agencies have primary responsibility for detecting and investigating WBDOs, and they voluntarily report them to CDC on a standard form (CDC form 52.12, which is available at http:// www.cdc.gov/.

The form solicits data related to 1) characteristics of the outbreak, including person, place, time, and location of the outbreak; 2) results from epidemiological studies conducted; 3) specimen and water sample testing; and 4) factors contributing to the outbreak, including environmental factors, water distribution, and disinfection concerns. Each year, CDC requests reports from state and territorial epidemiologists or from persons designated as WBDO surveillance coordinators.

Additional information regarding water quality and treatment is obtained from the state's drinking water agency as needed. Numerical and text data are abstracted from the outbreak form and supporting documents and are entered into a database before analysis.

Definitions[§]
The unit of analysis for the WBDO surveillance system is an outbreak, not an individual case of a waterborne disease. Two criteria must be met for an event to be defined as a WBDO. First, ≥2 persons must have experienced a similar illness after either ingestion of drinking water or exposure to water encountered in recreational or occupational settings. This criterion is waived for single cases of laboratory-confirmed primary amebic meningoencephalitis and for single cases of chemical poisoning if water-quality data indicate contamination by the chemical. Second, epidemiologic evidence (Table 1) must implicate water as the probable source of the illness.

For drinking water, reported outbreaks caused by contaminated water or ice at the point of use (e.g., a contaminated water faucet or serving container) are not classified as WBDOs.

If primary cases (i.e., among persons exposed to contaminated water) and secondary cases (i.e., among persons who became ill after contact with primary persons) are distinguished on the outbreak report form, only primary cases are included in the total number of cases. If both actual and estimated case counts are included on the outbreak report form, the estimated case count can be used if the population was sampled randomly or the estimated count was calculated by applying the attack rate to a standardized population.

Public water systems, which are classified as either community or noncommunity (Glossary), are regulated under SDWA. Of the approximately 170,000 public water systems in the United States, 113,000 (66.5 %) are noncommunity systems, of which 93,000 are transient systems (i.e., public water systems that regularly serve ≥25 of the same persons for ≥6 months/year [e.g., highway rest stations, restaurants, and parks with their own public water systems]) and 20,000 are nontransient systems (Glossary). A total of 54,000 systems (31.8%) are community systems. Community water systems serve approximately 264 million persons in the United States (96.0% of the U.S. population).

Approximately 11 million persons (4.0%) rely on private or individual water systems (*24,25*) (Glossary). These statistics exclude outbreaks associated with these sources

because they are not intended for drinking and are not considered to be public water systems. Also excluded from these statistics are the millions of persons who use noncommunity systems while traveling or working.

In this surveillance system, outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water and other nonpotable sources with or without taps) are also classified as individual systems (Glossary). Sources used for bottled water are also classified as individual systems; bottled water is not regulated by the EPA but is subject to regulation by the Food and Drug Administration (FDA). Each drinking water system associated with a WBDO is classified as having one of the deficiencies in the following list. If >1 deficiency is noted on the outbreak report form, the deficiency that most likely caused the outbreak is noted.
Deficiency classifications are as follows:
1: untreated surface water;
2: untreated groundwater;
3: treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, or inadequate or no filtration);
4: distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, or contamination of a storage facility); and
5: unknown or miscellaneous deficiency (e.g., contaminated bottled water) or water source not intended for drinking (e.g., irrigation water tap).

Recreational waters include swimming pools, wading pools, whirlpools, hot tubs, spas, water parks, interactive fountains, and fresh and marine surface waters. Although the WBDO surveillance system includes whirlpool- and hot tub-associated outbreaks of dermatitis caused by *Pseudomonas aeruginosa*, wound infections resulting from waterborne organisms are not included.

Outbreak Classification ***All related tables and figures are in the rear of this section***
WBDOs reported to the surveillance system are classified according to the strength of the evidence implicating water as the vehicle of transmission (Table 1). The classification scheme (i.e., Classes I--IV) is based on the epidemiologic and water-quality data provided with the outbreak report form. Epidemiologic data are weighted more than water-quality data. Although outbreaks without water-quality data might be included in this summary, reports that lack epidemiologic data were excluded. Outbreaks of dermatitis and single cases of either primary amebic meningoencephalitis or illness resulting from chemical poisoning were not classified according to this scheme. Weighting of epidemiologic data does not preclude the relative importance of both types of data. The purpose of the outbreak system is not only to implicate water as the vehicle for the outbreak, but also to understand the circumstances that led to the outbreak. A classification of I indicates that adequate epidemiologic and water-quality data were reported (Table 1); however, the classification does not necessarily imply whether an investigation was optimally conducted. Likewise, a classification of II, III, or IV should not be interpreted to mean that the investigations were inadequate or incomplete. Outbreaks and the resulting investigations occur under various circumstances, and not all outbreaks can or should be rigorously investigated. In addition, outbreaks that affect fewer persons are more likely to receive a classification of III, rather than I, on the basis of the relatively limited sample size available for analysis.

Results ***All related tables and figures are in the rear of this section***

Outbreaks Associated with Drinking Water

During 1999--2000, a total of 39 outbreaks associated with drinking water were reported by 25 states (see Appendix A for selected case descriptions). One of the 39 outbreaks was a multistate outbreak of *Salmonella* Bareilly that included cases from 10 states. Of the 39 total drinking water outbreaks, 15 outbreaks were reported for 1999 and 24 for 2000. Florida reported the most outbreaks (15) during this period. These 39 outbreaks caused illness among an estimated 2,068 persons; 122 persons were hospitalized, and two died. The median number of persons affected in an outbreak was 13.5 (range: 2--781). Outbreaks peaked during the summer months (Figure 1), June--August.

Nine of the 39 (23.1%) outbreaks were assigned to Class I on the basis of epidemiologic and water-quality data; three (7.7%) were Class II; 25 (64.1%) were Class III; and 1 was Class IV (Table 1). One of two outbreaks associated with a chemical etiology was not assigned a class because that outbreak was a single case of illness resulting from nitrate poisoning associated with consumption of water from a private well. Outbreaks are listed by state (Tables 2 and 3) and are tabulated by the etiologic agent, the water system type (Table 4), and by the type of deficiency and type of water system type (Table 5).

Etiologic Agents
Twenty (51.3%) of the 39 outbreaks were of known infectious etiology; 17 (43.6%) were of unknown etiology; and two (5.1%) were attributed to chemical poisoning. Of the 20 outbreaks with known infectious etiology, seven (35.0%) were caused by parasites; nine (45.0%) were caused by bacteria; and four (20.0%) were caused by viruses (Figure 2) (Appendix A).

Parasites. Seven outbreaks affecting 57 persons were attributed to parasitic infection: six *Giardia* outbreaks and one *Cryptosporidium* outbreak. Six outbreaks of *Giardia* associated with drinking water affected 52 persons from five states: Florida (two outbreaks), New Mexico (one), New Hampshire (one), Minnesota (one), and Colorado (one). These outbreaks occurred in January (one), June (one), July (one), August (one), and September (two). Four outbreaks were associated with well water systems, and two were associated with surface water systems. Two outbreaks caused by *G. intestinalis* involved possible contamination of wells by animal feces. *G. intestinalis* can infect mammalian hosts, which in turn, can serve as reservoirs for human infection. Water treatment failure was a factor in two other outbreaks of *Giardia*.

Bacteria. Nine outbreaks affecting an estimated 1,166 persons were attributed to bacterial infection: four *Es. coli* O157: H7 outbreaks, one *Campylobacter jejuni*, one *Salmonella* Typhimurium, one *Sa.* Bareilly, and two mixed *Ca. jejuni* and shiga toxin-producing *Es. coli* (O157:H7 or O111) outbreaks. The two outbreaks with multiple pathogens caused the two largest bacterial drinking water outbreaks reported during this study period.

Viruses. During this period, four outbreaks involving viral gastroenteritis were reported. A total of 426 persons reported illness; no hospitalizations or deaths were reported in association with these four viral outbreaks. Three of the four outbreaks occurred in camp facilities in California, New Mexico, and West Virginia. All three water sources were noncommunity groundwater sources.

Chemicals. During 1999, two outbreaks involving chemical contamination were reported. A total of three persons were affected by contamination of drinking water from nitrate and sodium hydroxide.

Unidentified Etiologic Agents. Seventeen outbreaks involving gastroenteritis of unknown etiology were reported from four states, affecting an estimated 416 persons and resulting in five hospitalizations. Testing for certain enteric pathogens (including ova and parasite testing) was attempted in five of the 17 outbreaks. In a June 2000 outbreak affecting 2 persons, stool specimens collected from one person tested negative for *G. intestinalis* but positive for *Blastocystis hominis*. However, whether *B. hominis* was the cause of the reported illness is unclear, and the pathogenicity of *B. hominis* has been debated in the scientific community (*26*). Stool specimens were negative for parasitic and bacterial enteric pathogens in two outbreaks in Washington (July 1999 and August 1999) and in two Florida outbreaks (March 1999 and April 2000) (Appendix A).

In addition, suspected pathogens were noted in four other outbreak reports submitted. On the basis of symptoms of illness, Norwalk-like virus (NLV) was suspected in an Idaho outbreak among firefighters that caused 65 illnesses and four hospitalizations, but the outbreak was not laboratory-confirmed. *G. intestinalis* was suspected in an April 2000 outbreak in a Florida trailer park affecting 21 persons, on the basis of the incubation period and symptoms reported.

In another outbreak in a Florida trailer park in March 2000 among 19 persons, a bacterial pathogen was suspected as the cause of the outbreak on the basis of the symptoms, which included conjunctivitis and dermatitis in addition to gastroenteritis.

A chemical agent was suspected as the cause of illness among four residents in a Florida apartment building who had a cross-connection between their drinking water and a toilet flush-valve. The residents of the apartment had noted blue tap water before onset of illness on multiple occasions before an improper flush valve in the toilet tank was discovered.

Four outbreaks of gastroenteritis were associated with consumption of untreated water from private wells. These four outbreaks occurred in Florida and affected 3--4 persons each. In July 2000, flooding was a possible contributor to two outbreaks. Water in each of the homes tested positive for coliforms and did not have adequate disinfection.

Water-Quality Data

Water-quality data (i.e., information regarding the presence of coliform bacteria, pathogens, or chemical contaminants) were available for 35 (89.7%) of the 39 drinking water outbreaks. Two reports of outbreaks of confirmed or suspected infectious etiology and two reports of outbreaks of confirmed or suspected chemical etiology did not provide water-quality data.

Of the 36 reports of outbreaks with a suspected or confirmed infectious etiology, 33 outbreaks provided water-quality data. Twenty-six (78.8%) of the 33 outbreaks with a suspected or confirmed infectious etiology reported a positive coliform, total coliform, or fecal coliform result. Organisms also were detected in the water in two of these outbreaks. In August 2000, *Ca. jejuni* was detected in the water in a mixed *Ca. jejuni*/*Es. coli* O157:H7 outbreak in Utah, although shiga toxins were not detected. *Es. coli* O157:H7 was found in the water in a July 2000 California outbreak. In a 2000 Colorado outbreak, the presence of *G. intestinalis* was demonstrated in a sample from the water holding tank, despite the lack of coliform data.

Of the three outbreaks with either a confirmed or suspected chemical etiology, only one demonstrated that the chemical had been directly in the water. Tap water was tested after the health department was notified that an infant had methemoglobinemia. Both fecal coliforms and 28 mg/L of nitrate were detected in the water. For an outbreak where burns and gastroenteritis were reported and linked to a sodium hydroxide spill, a pH test of the water that could indicate whether NaOH or another basic substance had spilled into the water was not documented. However, the environmental assessment indicated the tank contents had emptied into the water. A third suspected chemical outbreak involving a cross-connection between a toilet flush-valve and the drinking water system did not have water-quality data available.

In 11 of the 35 outbreaks, water was not sampled for coliforms until >1 month after the first case associated with the outbreak was reported (range: 5--16 weeks). In four of these 11 outbreaks, the water samples did not test positive for coliforms (fecal or total), chemicals, or pathogens. Instead, these were confirmed as outbreaks by epidemiologic data or by reports that treatment deficiencies had occurred.

Water Systems and Water Sources

Eleven (28.2%) of the 39 drinking water outbreaks were associated with community systems, 11 (28.2%) with noncommunity systems, and 17 (43.6%) with individual water systems (Tables 4 and 5). Ten (25.6%) of the 39 drinking water outbreaks were associated with surface water, including three outbreaks that implicated irrigation water not intended for consumption. Twenty-nine (74.4%) of the 39 drinking water outbreaks, including the outbreak associated with bottled water, were associated with groundwater sources (wells and springs).

Five (45.5%) of the 11 outbreaks associated with community water systems were caused by treatment deficiencies; one (9.0%) outbreak was related to contaminated, untreated groundwater, and five (45.5%) outbreaks were related to problems in the water distribution system. Two of the five distribution system problems were related to cross-connections between the distribution system and an irrigation well.

The third outbreak related to a community water source had a household cross-connection between the toilet water and main kitchen tap. One outbreak of *Cr. parvum* (Florida, December 2000) was related to a repeated history of water main breaks. In another outbreak in Ohio in August 2000, deficiencies in the distribution system of a fairgrounds might have allowed back-siphonage of animal manure into the water used by food and beverage vendors.

Ten (90.1%) of 11 outbreaks associated with noncommunity water systems occurred in groundwater systems. Seven of the 10 groundwater outbreaks were linked to untreated wells, and one of the 10 involved consumption of untreated spring water. Two of the 10 outbreaks were related to treatment deficiencies in water taken from wells or a spring and were associated with outbreaks of NLV and a small round-structured virus. An outbreak associated with *G. intestinalis* related to consumption of surface water occurred when a pump failure and a defective filter cartridge resulted in river water entering the drinking water holding tank without filtration. No information concerning chlorine levels from water samples was provided.

Nine (52.9%) of 17 outbreaks associated with individual water systems occurred in groundwater systems. Eight of these groundwater systems were wells that were not treated routinely; one outbreak of giardiasis occurred when the filtration system for a well was inadvertently turned off. Five (31.3%) of the 16 outbreaks occurred when persons drank water not intended for direct consumption from irrigation systems or when they consumed surface water that had been ineffectively or improperly treated. One (6.3%) of the 16 outbreaks in a system occurred in a home where creek water on the property was directly consumed without treatment.

Of the nine bacterial outbreaks, four occurred in groundwater systems (one was associated with a deficiency in the distribution system, one with a treatment deficiency, and two occurred in untreated systems). Six of seven parasitic outbreaks occurred in groundwater systems: three occurred in untreated systems; two involved problems in the distribution system; and one was related to a treatment deficiency. All four viral outbreaks occurred in noncommunity groundwater systems. Two occurred in untreated wells, and two were related to treatment deficiencies in a spring and well. Two chemical outbreaks were related to treatment deficiencies in well water. Fourteen of the 17 outbreaks of unknown etiology were linked to groundwater systems. Ten of these 14 outbreaks occurred in untreated systems; two were related to distribution system problems, and two were related to treatment deficiencies.

Outbreaks Associated with Recreational Water

During 1999--2000, a total of 23 states reported 59 outbreaks associated with recreational water (Tables 6--9) (see Appendix B for selected case descriptions).

Twenty-three outbreaks were reported for 1999, and 36 for 2000. The states that reported the largest number of outbreaks were Florida (14 outbreaks) and Minnesota (eight outbreaks). These 59 outbreaks affected 2,093 persons and resulted in 25 hospitalizations and four deaths. The median size of the outbreak was 10 persons (range: 1--700).

Of the 59 outbreaks, 36 were outbreaks of gastroenteritis (Tables 6 and 7); 15 were outbreaks of dermatitis (Table 9); four were cases of meningoencephalitis; and the remaining four outbreaks were of leptospirosis, chemical keratitis, acute respiratory

infection of unknown etiology, and Pontiac fever (Table 8). Thirty-one (86.1%) of the 36 outbreaks involving gastroenteritis occurred during the summer months (i.e., June--August) (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) (*2*,*3*).

Changes in surveillance and reporting of outbreaks might have improved detection of outbreaks affecting limited, private systems that in turn, affect a relatively limited number of persons. However, the increase in outbreaks that affect persons in limited, private systems merits further investigation by public health and water-quality agencies.

Certain states reported drinking water outbreaks for the first time in >10 years (e.g., Connecticut since 1976 or Utah since 1986). California reported multiple drinking water outbreaks after reporting no outbreaks in 1997 and 1998, and compared with other years during 1990--2000, the number of reported outbreaks in California increased slightly.

The number of outbreaks reported by Florida also increased. Although the numbers of reported outbreaks increased overall, the seasonality of the drinking water outbreaks is consistent with previous years, with the number peaking during the summer months. The observed increase in the number of outbreaks is associated with an increase in outbreaks associated with consumption of untreated water from both surface and groundwater sources, but specifically private wells.

The percentage of drinking water outbreaks associated with surface water during 1999--2000 was 17.9% (i.e., seven outbreaks) (Figure 2). This percentage is higher than the 11.8% reported during 1997--1998 period (i.e., two outbreaks). However, three of the seven surface water outbreaks reported during 1999--2000 were associated with the direct ingestion of surface water without any treatment or with inadequate individual treatment. Two of these outbreaks were associated with consumption of water during outdoor excursions where point-of-use treatment (e.g., filtration or disinfection) might have been attempted and was either inadequate to protect health or was inconsistently or incorrectly applied. The third outbreak occurred after a household had run out of potable water and instead served untreated creek water to their guests.

These three outbreaks illustrate that the public might be unaware that surface water, despite its clarity, is prone to contamination by organisms. Surface waters should not be directly consumed without being treated at the point of use or boiled. Manufacturers of point-of-use devices and the National Sanitation Foundation (NSF) provide information regarding different devices, instructions for use, and their ability to make water safe for human consumption.

The remaining four outbreaks comprise approximately 11% of all drinking water outbreaks, an equivalent percentage to that reported in 1997--1998. These four outbreaks were associated with systems that routinely received treatment. One outbreak of giardiasis occurred at a resort (Colorado, August 2000) served by a noncommunity system. The increased demand for water during the summer, coupled with multiple treatment failures, resulted in the delivery of unfiltered and non-disinfected water to the resort.

Cross-connections

These multiple failures illustrate the importance of routine maintenance, specifically among noncommunity systems, which do not have consistent demand for water year-round. Two outbreaks (Florida, March 1999 and August 1999) were associated with cross-connections: one to an irrigation well and another to a toilet. Another surface water outbreak (Ohio, August 2000) at a fairgrounds was suspected to have resulted from back-siphonage into the drinking water from an animal manure site. These outbreaks indicate that even when treatment of water at the source is adequate, deficiencies in the distribution system or at the home can result in illness. Such deficiencies are preventable, and the public should be informed of how to detect and avoid creating cross-connections.

Twenty-eight (71.8%) of the 39 outbreaks related to drinking water were associated with groundwater sources. This number is an 87% increase from the number reported in the previous period (i.e., 15). Seventeen of the 28 outbreaks (60.7%) were linked to consumption of untreated groundwater; eight of 28 (28.6%) outbreaks were associated with treatment deficiencies; and three (10.7%) were linked to deficiencies in the distribution system. The observed pattern of deficiencies is contrary to what was observed in the previous reporting period, where the majority of groundwater outbreaks were associated with treatment or distribution system problems.

This pattern indicates that untreated groundwater systems are increasingly associated with outbreaks of illness. Groundwater systems, with the exception of systems influenced by surface water, are not routinely required to use filtration or treatment that would be expected to reduce the number of pathogens in the water. The EPA's pending GWR** is expected to establish multiple barriers in groundwater systems to protect against bacteria and viruses in drinking water from groundwater sources and should establish a targeted strategy to identify groundwater systems at high risk for fecal contamination.

Twenty-six of these 28 groundwater outbreaks had a well as the implicated water source, and two were linked to a spring. The percentages of outbreaks associated with wells and springs were similar during this reporting period to the 1997--1998 period. Although GWR is expected to have public health benefits, these protections extend primarily to community groundwater systems. Of the 26 well-related outbreaks that occurred during the 1999--2000 period, only eight of 26 were associated with community wells. Ten were associated with individual private wells, and eight were associated with noncommunity wells.

These systems would not necessarily benefit from the promulgation of GWR, and therefore, the quality of water in wells remains a public health concern. Approximately 14--15 million households in the United States rely on a private, household well for drinking water each year, and >90,000 new wells are drilled throughout the United

States each year (*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 (*35*). Investigators are also encouraged to contact CDC and EPA regarding testing of water samples.

Only two outbreaks of chemical origin were identified during this surveillance period, the same number as was reported during the 1997--1998 period. One outbreak related to a spill of sodium hydroxide at a community water treatment plant demonstrated the need for safe water treatment practices.

The other outbreak was a single case of methemoglobinemia in an infant who required hospitalization after having been fed boiled water taken from a private well.

Coordination of public health messages is critical; an intervention that was intended to reduce the transmission of infections agents concentrated the chemicals present in the water. These figures, as in the past, probably under represent the actual waterborne chemical poisonings that occur.

Multiple factors can explain the low reporting rate, including the likelihood that:
1) the majority of waterborne chemical poisonings typically occur in private residences and affect a relatively limited number of persons;
2) exposures to chemicals through drinking water might cause illness that is difficult to link to a chemical exposure;
3) the mechanisms for reporting waterborne chemical poisonings to the WBDO surveillance system are not as established for chemicals as they are for WBDOs attributed to infectious agents; and 4) health-care providers and those affected might not as easily recognize chemical poisonings. As a result of these factors, WBDOs of chemical poisonings are less likely to be reported to public health officials.

We Need To Do More
Strengthening the capacity of local and state public health epidemiologists and environmental health specialists to detect and investigate outbreaks remains a priority at CDC and the EPA. As part of that effort, CDC and the EPA should partner with the states, CSTE, and the Association of Public Health Laboratories to develop training materials and online resources that would be useful and easily accessible to local and state public health personnel.

Although no federal regulation exists for monitoring private wells, developing educational materials targeted towards the general public, informing them of ways to maintain the safety and water quality of their wells would be valuable. In addition, health messages regarding the consumption of nonpotable water and appropriate point-of-use treatment should be developed and distributed to the public.

Outbreaks Associated with Recreational Water
Of the 59 recreational WBDOs, those involving gastroenteritis were most frequently reported (n = 36). The 15 outbreaks reported in 1999 and 21 outbreaks reported in 2000 equal or surpass the number reported in 1998, which previously was the highest number of outbreaks involving recreational water-related gastroenteritis reported in one year since the inception of the surveillance system. Together, the outbreaks involving gastroenteritis reported during the 1999--2000 period are higher than the 18 outbreaks documented in the previous reporting period (Figures 7 and 8). Since 1989, the number of gastroenteritis-related outbreaks has been gradually increasing, and this increase is statistically significant ($p = 0.01$).

Because swimming is essentially a shared water activity or communal bathing, rinsing of soiled bodies and overt fecal accidents cause contamination of the water. Unintentional ingestion of recreational water contaminated with pathogens can then lead to gastrointestinal illness, even in non-outbreak settings (*36,37*). Fresh and marine waters are also subject to other modes of contamination from point sources (i.e., sewage releases), watersheds (i.e., runoff from agriculture and residential areas), and floods.

Outbreaks involving gastroenteritis are more frequently observed during the swimming season, which usually starts on Memorial Day weekend (the last weekend in May) and ends Labor Day weekend (the first weekend in September). However, swimming also occurs year-round in indoor venues and in states with more temperate climates. Outbreaks of illness by month (Figure 3) include two outbreaks that occurred noticeably outside the summer months: one outbreak in a Florida pool in March and another outbreak in an indoor pool in Wisconsin in January.

As during the previous reporting period, *Cr. parvum* accounted for the largest percentage of outbreaks involving gastroenteritis (44.4%), followed by *Es. coli* O157:H7 (11.1%), NLV (8.3%), and *Shigella* (8.3%). An outbreak of *G. intestinalis* was also reported in 1999. The last reported recreational water outbreak of *Giardia* occurred in 1996. Outbreaks of *Ca. jejuni*, *Es. coli* O121:H19, and a mixed *Sh. sonnei*/*Cr. parvum* outbreak were also reported for the first time to the surveillance system. Outbreaks of unknown etiology comprised 16.7% of the recreational water outbreaks involving gastroenteritis.

Twenty-two (61.1%) of the 36 outbreaks of gastroenteritis occurred in treated systems (i.e., pools) that would usually be expected to be chlorinated or disinfected to prevent transmission of infectious agents after unintentional ingestion. However, the term *treated system* might pertain to systems not routinely treated, including wading pools, interactive fountains, and in one case, an untreated pool that was served by a natural hot springs source.

Multiple interrelated factors can impede disinfection in treated venues, including an increased bather load in a pool, high levels of organic material (e.g., fecal material or environmental or skin debris) and ultraviolet light, all of which deplete chlorine residuals that usually maintain protection in the system. In certain outbreaks, fecal material was indicated on the report as a contributing factor to the outbreak; the majority of fecal accidents were attributed to young children who were in or near the water at the time the accident occurred.

Unlike previous years, a substantial number of different bacterial and viral organisms were reported as causing gastrointestinal illness in these treated recreational water venues (Figure 9). Nevertheless, >66% of these outbreaks were attributed to *Cr. parvum* (Figure 4). Unlike other organisms, which are more susceptible to the levels of chlorine typically found in a pool, *Cr. parvum* is highly chlorine-resistant and requires increased levels of chlorine and longer contact times with chlorine for inactivation. *Cr. parvum* can survive for days in public health-mandated chlorine concentrations required for pools. In addition, its relatively limited size (4--6 µm) can allow it to pass through particulate filtration systems during recirculation of water in the pool.

Because a low number of oocysts might cause illness in a person, even ingestion of a limited amount of water can cause infection. Although the number of *Cr. parvum* outbreaks has been steadily increasing during 1990--2000, multiple explanations could exist for the increase. The properties of the organism, coupled with the popularity of swimming and the tendency of persons to aggregate in larger water venues, increases the likelihood that swimming water can become contaminated and that swimmers will ingest the water and become infected.

However, the increases in outbreaks could be explained by a higher awareness of *Cr. parvum* as a potential cause of illness among swimmers by the public health community and the recreational water industry and, as a result, are more likely to be detected.

The majority of these *Cr. parvum* outbreak investigations noted inadequate pool maintenance. Although low chlorine levels are unlikely to have been the cause of the outbreaks, the frequent reporting of low chlorine levels in these outbreaks indicates a disturbing lack of awareness concerning the role of chlorine and pH control as the major protective barrier against infectious disease transmission in pools.

Inadequate disinfectant levels in any pool increases the risk for transmission of chlorine-sensitive pathogens (e.g., *Es. coli* O157:H7 or *Shigella* species) if an infected swimmer contaminates the pool. Pool operators and staff should be appropriately trained regarding the spread of recreational water illnesses and the critical role of pool maintenance (i.e., disinfection, pH control, and filtration) in preventing WBDOs.§§

Gastroenteritis
Fourteen outbreaks involving gastroenteritis after freshwater exposure were reported during 1999--2000, compared with eight during 1997--1998. *Es. coli* O157:H7 accounted for the most outbreaks of known etiology (three), followed by NLV (two), *Shigella* species (two), *Es. coli* O121:H19 (one), *G. intestinalis* (one), and *Cr. parvum* (one). Four outbreaks were of unknown etiology. Certain outbreaks occurred in beach areas that had substantial numbers of families bathing and swimming in the water. Again, a common element noted in these reports was the presence of diaper-aged children in the water, diaper-changing on the beach, and even washing off young children in the water. One incident involved persons who swam in a lake that was had posted signs indicating that the lake was unsafe for swimming.

Reports of infants and children swimming when they have diarrhea is a problem common to both freshwater systems and treated venues. Although health communication messages have been targeted in the past for treated venues, similar messages should be provided to those who swimming in freshwater venues. EPA, as part of the Beaches Action Plan, is developing guidelines and information for users of freshwaters.¶¶

Geothermal Pools and Hot Springs
Geothermal pools and hot springs should be examined closely. In one outbreak, pools in a complex were exempt from public health regulation because they were naturally occurring hot springs and mineral waters. Hot springs, which feature high levels of minerals and elevated temperatures, are potentially ideal venues for microbial growth or contamination. These springs and geothermal pools pose an increased risk to swimmers, compared with treated pools because of their lack of disinfection and filtration. Improved consumer and staff education and supplementary treatment might be necessary to prevent future outbreaks in these enclosed freshwater pools.

Twelve of the 15 outbreaks of dermatitis were associated with hot tub or pool use. The majority of these reports of dermatitis are associated with deficient maintenance and inadequate disinfection of the water. The higher temperatures commonly found in hot tubs deplete disinfectant levels at a more rapid rate; hot tub operators should be encouraged to actively check and maintain adequate disinfectant levels. In addition to rashes, reports have been received of other symptoms.

In Alaska, three of 29 persons reported nausea. In the two Maine outbreaks, persons also reported headache, fatigue, and other symptoms. The Colorado outbreak was notable for its severe symptomatology and an extended duration of illness. Extended and painful rashes associated with *P. aeruginosa* outbreaks are unusual but have been documented (*38,39*).

One report (*39*) 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.

Table 1

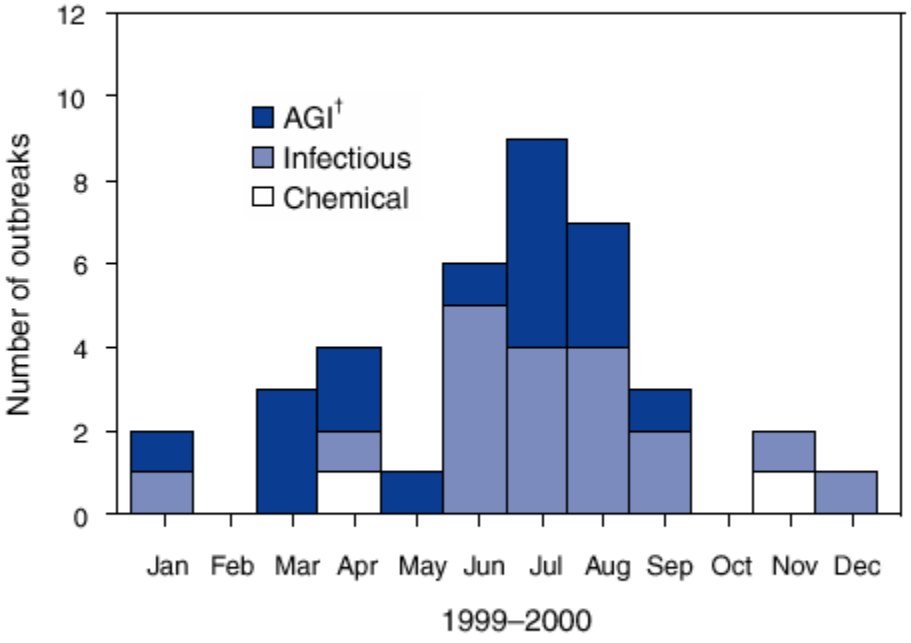
TABLE 1. Classification of investigations of waterborne-disease outbreaks — United States*

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.

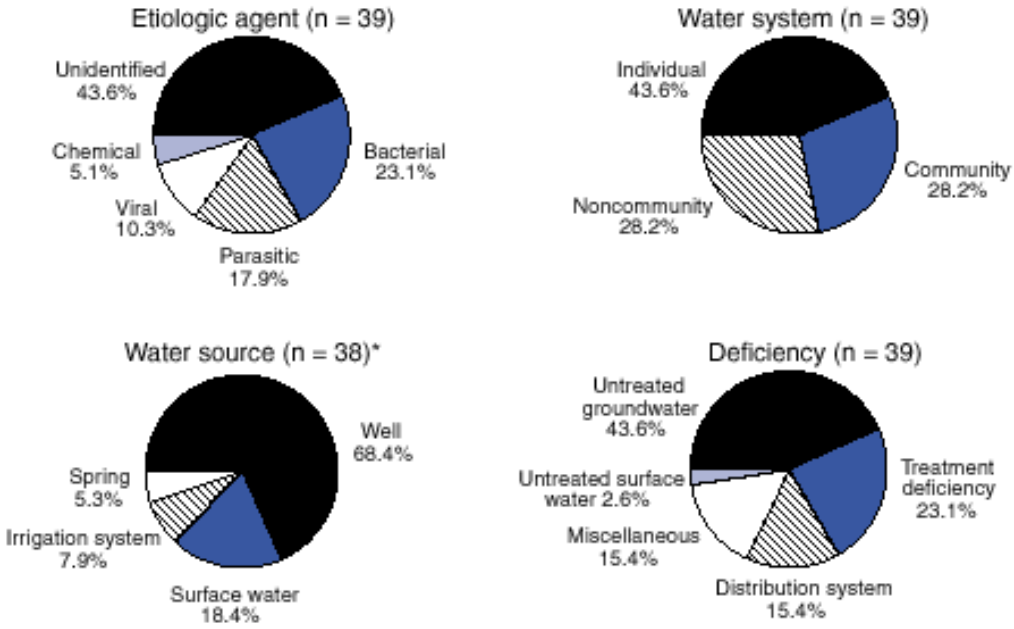
§§ Three persons had stool specimens that tested positive for small round-structured virus, and one person's stool specimen tested positive for *Ca. jejuni*.

¶¶ 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*.

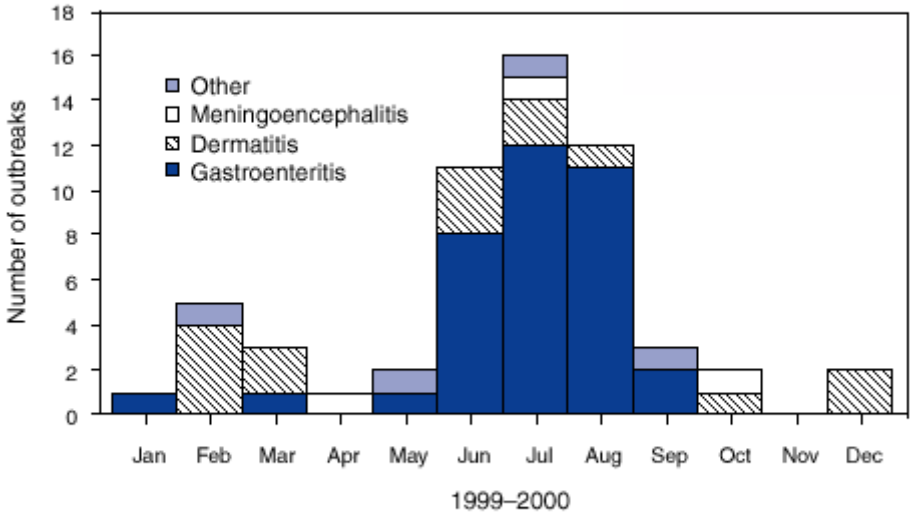
*** Type of water was not specified on report form.

††† 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

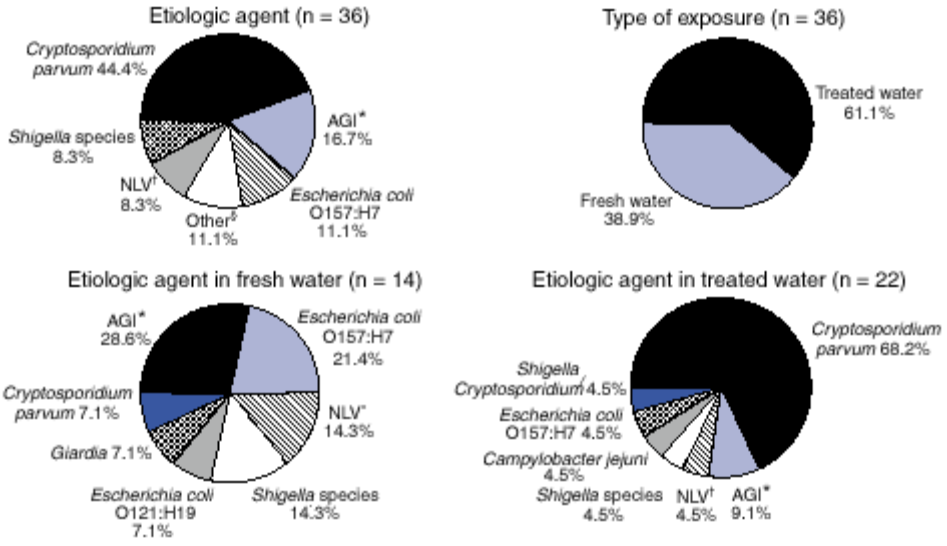
TABLE 4. Waterborne-disease outbreaks associated with drinking water, by etiologic agent and type of water system — United States, 1999–2000 (n = 39)

	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)

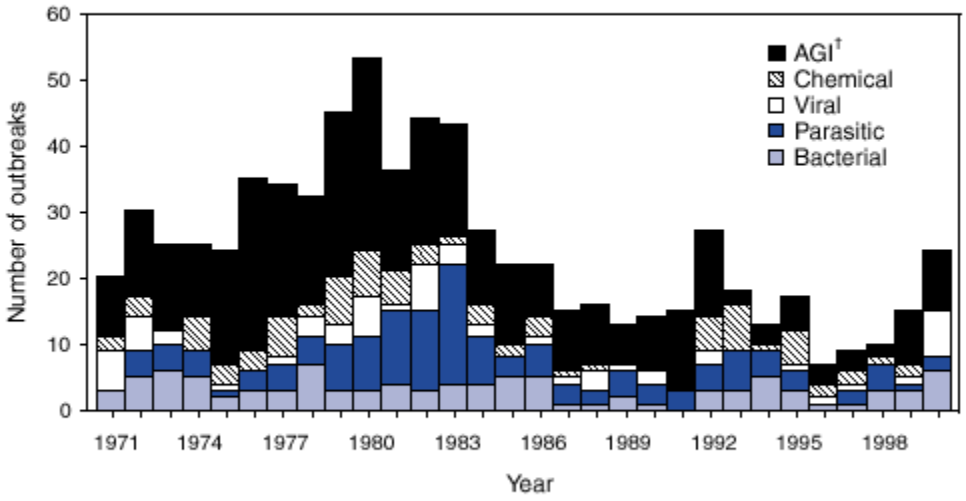
	Type of water system*							
	Community		Noncommunity		Individual		Total	
Type of deficiency†	Outbreaks	%	Outbreaks	%	Outbreaks	%	Outbreaks	%
Untreated surface water	0	0	0	0	1	5.9	**1**	**2.6**
Untreated groundwater	1	9.0	8	72.7	8	47.0	**17**	**43.6**
Inadequate treatment	5	45.5	3	27.3	1	5.9	**9**	**23.1**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

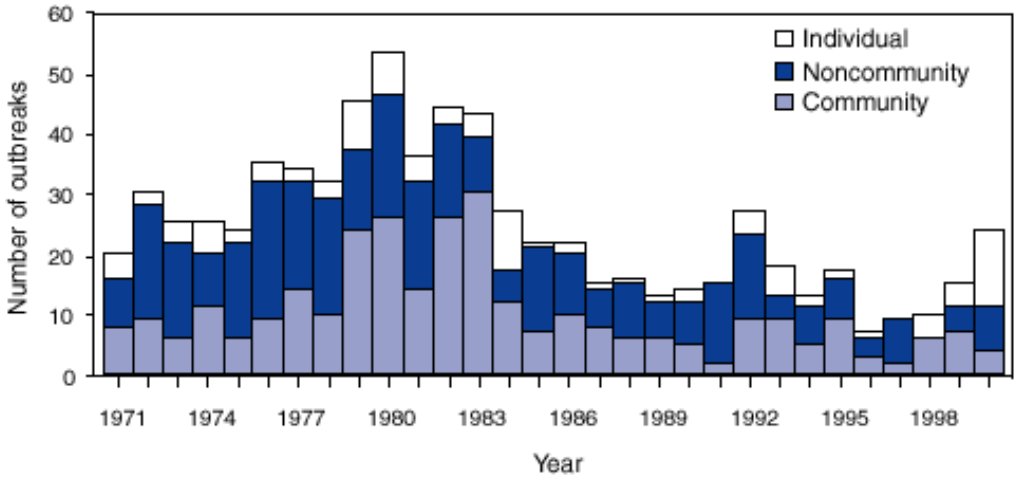
TABLE 6. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 1999 (n = 15)

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.

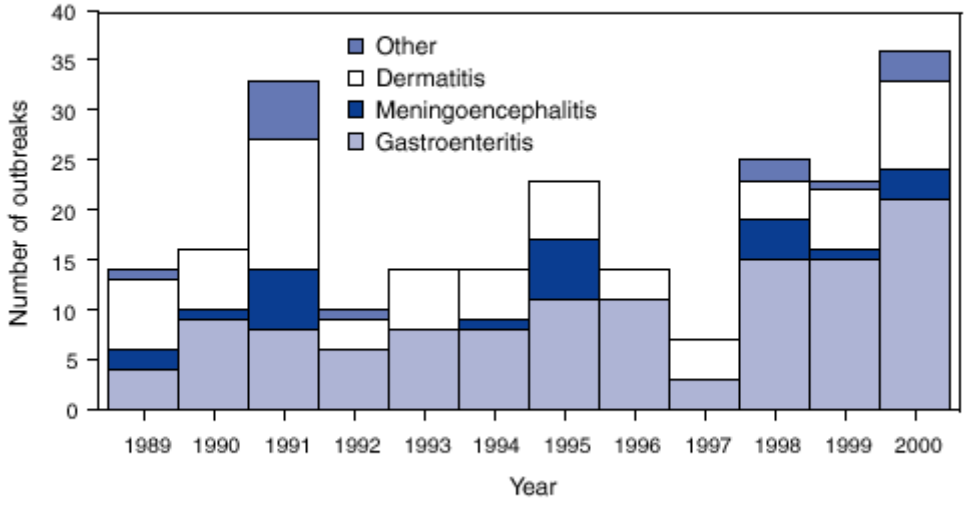
§ Persons swam in a community pool and an inflatable pool.

¶ 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)

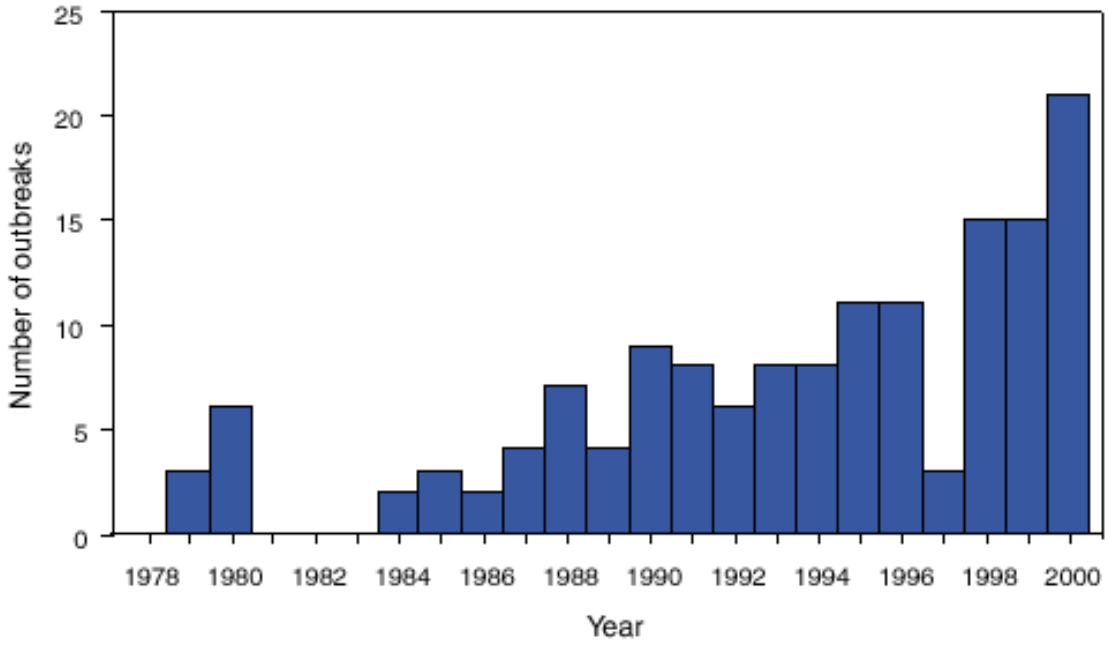


Table 9

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)

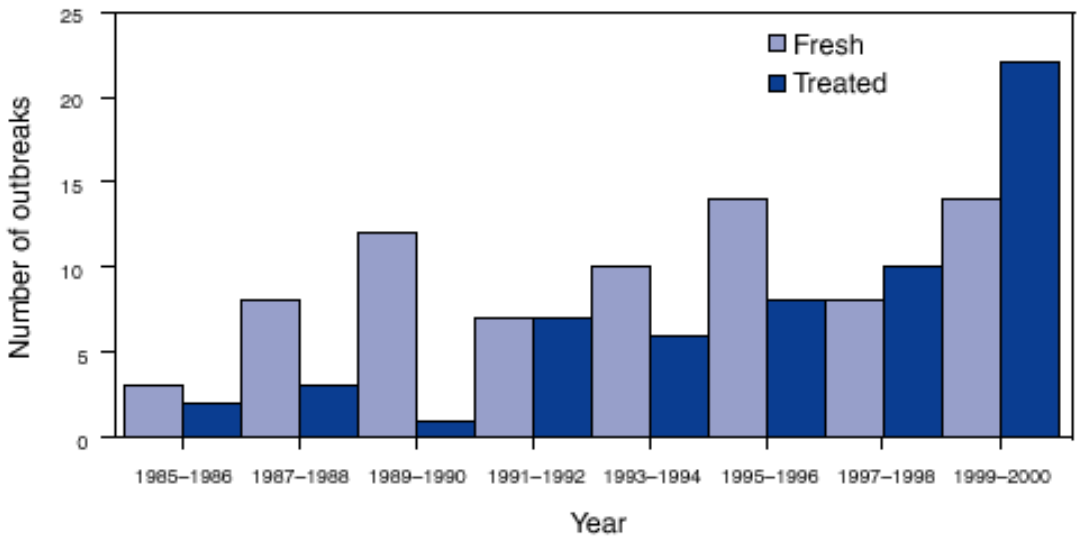


Table 10

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).

Drinking Water Rules and Disease Chapter 8 Review

1. The first list was called the drinking water _____. CCL contained 60 contaminants/contaminant groups, included 10 pathogens, and was published in the Federal Register on March 2, 1998. A decision concerning whether to regulate ≥5 contaminants from CCL was required by August 2001.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

2. Public water systems are regulated under the _____and its subsequent 1986 and 1996 amendments. Under it, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

3. The _____ require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

4. Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the _____ of 1989.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

5. _____ covers all water systems that use surface water or groundwater under the direct influence of surface water.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

6. _____ is intended to protect against exposure to Giardia intestinalis, viruses, and Legionella, as well as selected other pathogens.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

7. In 1998, the EPA promulgated the _____, which provides additional protection against Cryptosporidium and other waterborne pathogens for systems that serve ≥10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

8. In 2002, the EPA finalized the _____ for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

9. _____ was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2001.
A. Total Coliform Rule (TCR)
B. Filter Backwash Recycling Rule (FBRR)
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

10. The _____ require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; EPA has proposed the Ground Water Rule (GWR) to meet this mandate.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

11. _____ specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)

12. This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. _____ does not apply to privately owned wells.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

13. To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the _____in 1996, which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

14. _____ are also required to monitor for the presence of Cryptosporidium, Giardia, total culturable viruses, and total* and fecal coliforms or Escherichia coli ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

15. The EPA also made minor changes in 2000 to the _____ to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same.
A. Information Collection Rule
B. Lead and Copper Rule
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

1.D, 2.C, 3.B, 4.E, 5.E, 6.E, 7.C, 8.D, 9.C, 10.B, 11.D, 12.D, 13.A, 14.E, 15.B

Water Sampling and Laboratory Procedures Chapter 9

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

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

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

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

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

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

Bacteriological Monitoring, more detailed information in Lab Section

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

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

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

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

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

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

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

Standard Sample Coliform Bacteria Bac-T

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

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

Laboratory Procedures, more detailed information in the next section

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

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

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

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

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

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

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

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

Table 3 Number of Samples per System Population

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

Repeat Sampling

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

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

The follow-up for repeat sampling is:

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

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

4. If the system has only one service connection, the repeat samples must be collected from the same sampling location over a four-day period or on the same day.
5. All repeat samples are included in the MCL compliance calculation.
6. If a system which normally collects fewer than five (5) routine samples per month has a coliform present sample, it must collect five (5) routine samples the following month or quarter regardless of whether an MCL violation occurred or if repeat sampling was coliform absent. **(*Check with your governmental environmental or health agency for more information*).**

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. Most of us have had this problem. It can be stressful, and people have lost their jobs over these problems. The best option is to have a SOP in place because positive samples or false positives will come to your section.

Assistance

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

Some examples of typical corrective measures to coliform problems are:

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

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

Maximum Contaminant Levels (MCLS)

State and federal laws establish standards for drinking water quality. Under normal circumstances when these standards are being met, the water is safe to drink with no threat to human health. These standards are known as maximum contaminant levels (**MCL**). When a particular contaminant exceeds its MCL a potential health threat may occur.

The MCLs are based on extensive research of the toxicological properties of the contaminants, risk assessments and factors, short term (**acute**) exposure and long term (chronic) exposure. You conduct the monitoring to make sure your water is in compliance with the MCL. There are two types of MCL violations for coliform bacteria. The first is for total coliform; the second is an acute risk to health violation characterized by the confirmed presence of fecal coliform or E. coli.

(*Check with your governmental environmental or health agency for more information*)

Common Sample bottles, *Radiochems, VOCs, (Volatile Organic Compounds), TTHMs, Total Trihalomethanes), Nitrate, Nitrite.*

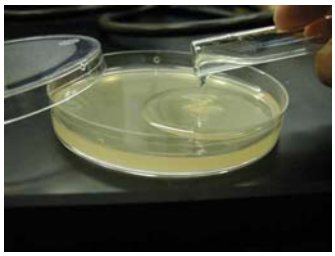
Most of these sample bottles will come with the preservative already inside the bottle. Some bottles will come with a separate preservative (acid) for the field preservation. Slowly add the acid or other preservative to the water sample; not water to the acid or preservative. Put a new label on the preservative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

Heterotrophic Plate Count, more detailed information in the next section

Heterotrophic Plate Count (**HPC**) --- formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria and measuring changes during water treatment and distribution in water or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term "***colony-forming units***" (**CFU**).

Method:

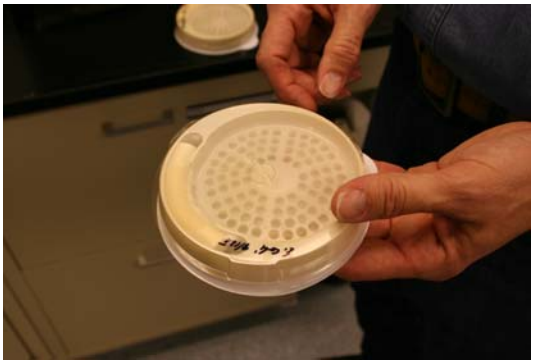
There are three methods for standard plate count:



1. **Pour Plate Method**
The colonies produced are relatively small and compact, showing less tendency to encroach on each other than those produced by surface growth. On the other hand, submerged colonies often are slower growing and are difficult to transfer.

2. Spread Plate Method
All colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Colonies can be transferred quickly, and colony morphology easily can be discerned and compared to published descriptions.

3. Membrane Filter Method
This method permits testing large volumes of low-turbidity water and is the method of choice for low-count waters.



Material:

i) Apparatus
- Glass rod
- Erlenmeyer flask
- Graduated Cylinder
- Pipette
- Petri dish
- Incubator

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

Procedure*

1.Boil mixture of nutrient agar and nutrient broth for 15 minutes, then cool for about 20 minutes.
2.Pour approximately 15 ml of medium in each Petri dish, let medium solidify.
3.Pipet 0.1 ml of each dilution onto surface of pre-dried plate, starting with the highest dilution.
4.Distribute inoculum over surface of the medium using a sterile bent glass rod.
5.Incubate plates at 35^{o}C for 48h.

6.Count all colonies on selected plates promptly after incubation, consider only plates having 30 to 300 colonies in determining the plate count.
*Duplicate samples

Computing and Reporting: Compute bacterial count per milliliter by the following equation:
CFU/ml = colonies counted / actual volume of sample in dish
a) If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. (*Check with your governmental environmental or health agency for more information*)
b) If plates from all dilutions of any sample have no colony, report the count as less than 1/actual volume of sample in dish estimated CFU/ml.
c) Avoid creating fictitious precision and accuracy when computing CFU by recording only the first two left-hand digits.

Heterotrophic Plate Count
(Spread Plate Method)
Heterotrophic organisms utilize organic compounds as their carbon source (food or substrate). In contrast, autotrophic organisms use inorganic carbon sources. The Heterotrophic Plate Count provides a technique to quantify the bacteriological activity of a sample. The R2A agar provides a medium that will support a large variety of heterotrophic bacteria. After an incubation period, a bacteriological colony count provides an estimate of the concentration of heterotrophs in the sample of interest.

Required Laboratory Equipment
100 x 15 Petri Dishes
Turntable
Glass Rods: Bend fire polished glass rod 45 degrees about 40 mm from one end. Sterilize before using.
Pipette: Glass, 1.1 mL. Sterilize before using.
Quebec Colony Counter
Hand Tally Counter

Reagents
1) R2A Agar: Dissolve and dilute 0.5 g of yeast extract, 0.5 g of proteose peptone No. 3, 0.5 g of casamino acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of dipotassium hydrogen phosphate, 0.05 g of magnesium sulfate heptahydrate, 0.3 g of sodium pyruvate, 15.0 g of agar to 1 L. Adjust pH to 7.2 with dipotassium hydrogen phosphate **before adding agar.** Heat to dissolve agar and sterilize at 121 C for 15 minutes.
2) Ethanol: As needed for flame sterilization.

Preparation of Spread Plates
Immediately after agar sterilization, pour 15 mL of R2A agar into sterile 100 x 15 Petri dishes; let agar solidify. Pre-dry plates inverted so that there is a 2 to 3 g water loss overnight with the lids on. Use pre-dried plates immediately or store up to two weeks in sealed plastic bags at 4 degrees C.

Sample Preparation
Mark each plate with sample type, dilution, date and any other information before sample application. Prepare at least duplicate plates for each volume of sample or dilution examined. Thoroughly mix all samples by rapidly making about 25 complete up-and-down movements.

Sample Application
Uncover pre-dried agar plate. Minimize time plate remains uncovered. Pipette 0.1 or 0.5 mL sample onto surface of pre-dried agar plate.

Record volume of sample used. Using a sterile bent glass rod, distribute the sample over the surface of the medium by rotating the dish by hand on a turntable. Let the sample be absorbed completely into the medium before incubating. Put cover back on Petri dish and invert for duration of incubation time. Incubate at 28°C for 7 days. Remove Petri dishes from incubator for counting.

Counting and Recording
After incubation period, promptly count all colonies on the plates. To count, uncover plate and place on Quebec colony counter. Use hand tally counter to maintain count. Count all colonies on the plate, regardless of size. Compute bacterial count per milliliter by the following equation:

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

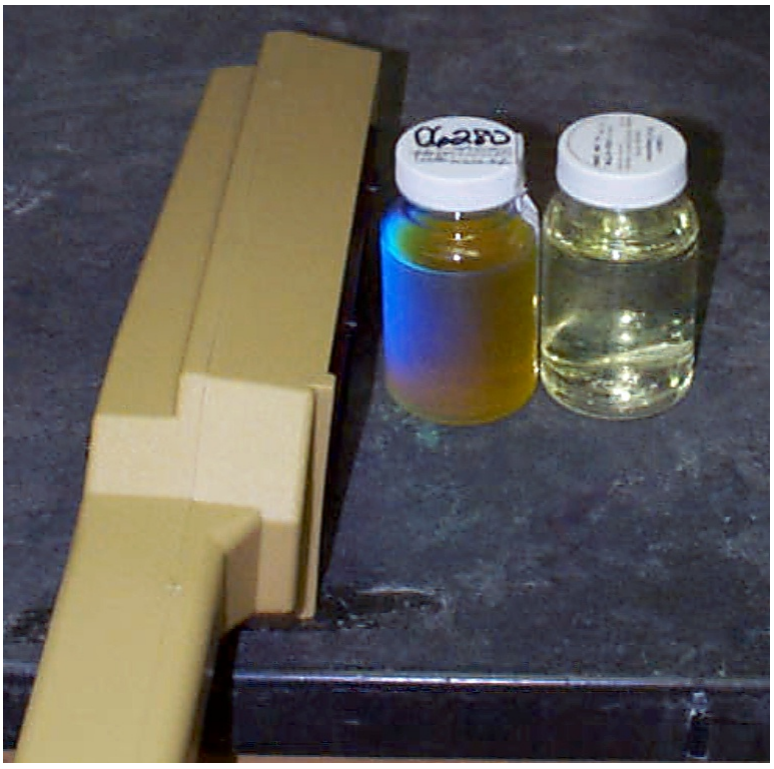
To report counts on a plate with no colonies, report the count as less than one (<1) divided by the sample volume put on that plate (remember to account for any dilution of that sample.)

If plates of all dilutions for a sample have no colonies, report the count as less than one (<1) divided by the largest sample volume used. Example: if 0.1 mL of a 100:1 and 10000:1 dilution of a sample both turned up with no colonies formed, the reported result would be <1 divided by the largest sample volume 0.001 mL (0.1 mL divided by 100). The final reported result for the sample is <1000 CFU per mL.

Assignment
1. Report the number of colony forming units (**CFU**) found on each plate.
2. Calculate the CFU per mL for each plate.
3. The aim of diluting samples is to produce a plate having 30 to 300 colonies, which plates meet these criteria. If no sample produces a plate with a count in this range, use the plate(s) with a count closest to 300. Based on these criteria, use your calculated results to report the CFU per mL for each sample. In the conclusion of your lab report, comment on your final results for each sample type as well as the quality of your application of this analysis technique. Feel free to justify your comments using statistical analysis. Also, comment on the general accuracy of this analytical technique and the factors that affect its accuracy and or applicability.

Data Table for Samples

Sample ID	Volume of Sample, mL	Colonies Counted per plate



You can see the E. Coli or fecal bacteria fluoresce

Total Coliforms, more detailed information in the next section

This MCL is based on the presence of total coliforms and compliance is on a monthly or quarterly basis, depending on your water system type and state rule. For systems which collect *fewer* than 40 samples per month, no more than one sample per month may be positive. In other words, the second positive result (repeat or routine) in a month or quarter results in an MCL violation.

For systems which collect 40 or more samples per month, no more than five (5) percent may be positive. Check with your state drinking water section or health department for further instructions.

Acute Risk to Health (Fecal coliforms and E. coli)
An acute risk to human health violation occurs if either one of the following happen:
1. A routine analysis shows total coliform present and is followed by a repeat analysis which indicates fecal coliform or E. coli present.
2. A routine analysis shows total and fecal coliform or E. coli present and is followed by a repeat analysis which indicates total coliform present. An acute health risk violation requires the water system to provide public notice via radio and television stations in the area. This type of contamination can pose an immediate threat to human health and notice must be given as soon as possible but no later than 72 hours after notification from your laboratory of the test results.

Certain language may be mandatory for both these violations and is included in your state drinking water rule.

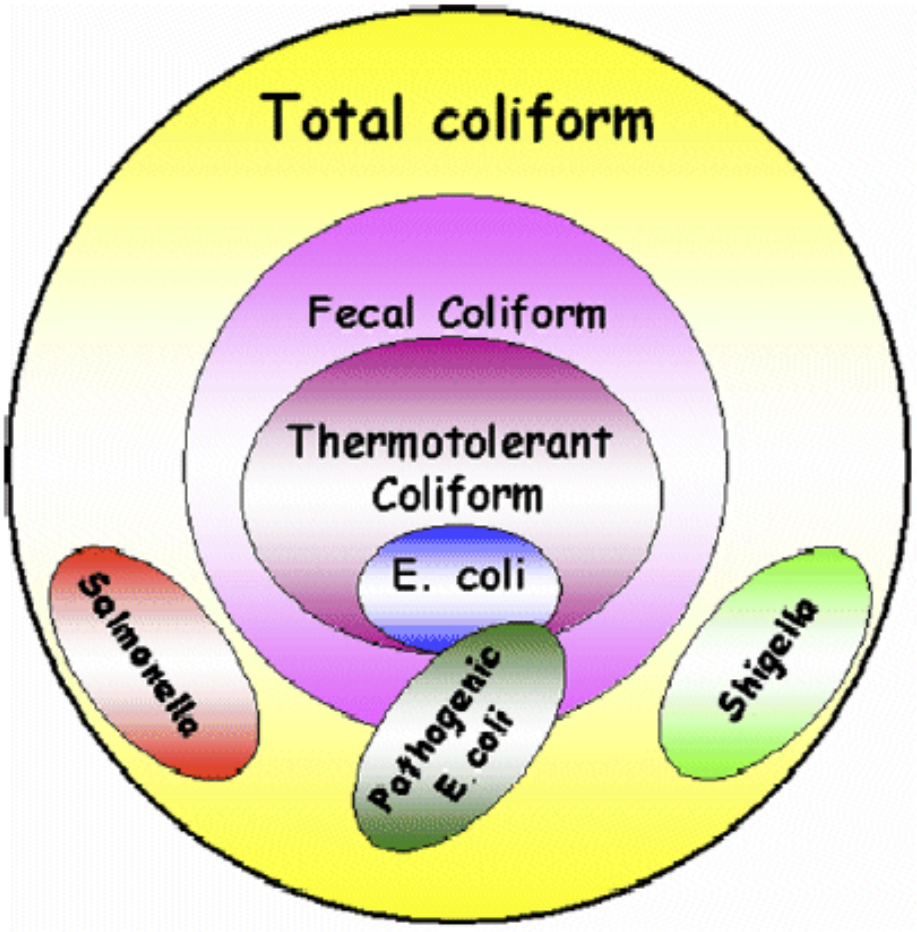
Public Notice
A public notice is required to be issued by a water system whenever it fails to comply with an applicable MCL or treatment technique or fails to comply with the requirements of any scheduled variance or permit. This will inform users when there is a problem with the system and give them information.

A public notice is also required whenever a water system fails to comply with its monitoring and/or reporting requirements or testing procedure. Each public notice must contain certain information, be issued properly and in a timely manner and contain certain mandatory language.

The timing and place of posting of the public notice depends on whether an acute risk is present to users. Check with your state drinking water section or health department for further instructions.

The following are acute violations:
1. Violation of the MCL for nitrate.
2. Any violation of the MCL for total coliforms, when fecal coliforms or E. coli are present in the distribution system.
3. Any outbreak of waterborne disease, as defined by the rules.

(***Check with your governmental environmental or health agency for more information***)


Total coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Pathogenic E. coli
Shigella

Proper Sampling Handling

The proper handling of water quality samples also includes wearing gloves. Gloves not only protect field personnel, but also prevent potential contamination to the water sample. Always wear powderless, disposable gloves. When sampling for inorganics, wear latex gloves. Nitrile gloves are appropriate for organics.

The following will provide a field reference for chain of custody procedures, sampling surface water and ground water, and further provide procedures for measuring field parameters and handling water-quality samples.

Use chain-of-custody procedures when coolers and containers are prepared, sealed and shipped. They will remain sealed until used in the field. When making arrangements with the laboratory, make sure you request enough containers, including those for blank and duplicate samples. Order extra sample bottles to allow for breakage or contamination in the field.

Some samples require low-temperature storage and/or preservation with chemicals to maintain their integrity during shipment and before analysis in the laboratory. The most common preservatives are hydrochloric, nitric, sulfuric and ascorbic acids, sodium hydroxide, sodium thiosulfate, and biocides. Many laboratories provide pre-preserved bottles filled with measured amounts of preservatives.

Although most federal and state agencies allow the use of pre-preserved sample containers, some may require either cool temperatures or added preservatives in the field.

When the containers and preservatives are received from the laboratory, check to see that none have leaked. Be aware that many preservatives can burn eyes and skin, and must be handled carefully.

Sampling bottles should be labeled with type of preservative used, type of analysis to be done and be accompanied by A Material Safety Data Sheet (**MSDS**). Make sure you can tell which containers are pre-preserved, because extra care must be taken not to overfill them when collecting samples in the field. Check with the laboratory about quality control procedures when using pre-preserved bottles.

Coolers used for sample shipment must be large enough to store containers, packing materials and ice. Obtain extra coolers, if necessary. Never store coolers and containers near solvents, fuels or other sources of contamination or combustion. In warm weather, keep coolers and samples in the shade.

Field Parameters
Measure and record the field parameters of temperature, electrical conductivity, pH and dissolved oxygen in an undisturbed section of streamflow. Other parameters may be measured, if desired.

(*Check with your governmental environmental or health agency for more information*).

QA/QC Measures

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

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

If protective gloves are used, they shall be clean, new and disposable. These should be changed upon arrival at a new sampling point.

Highly contaminated samples shall never be placed in the same ice chest as environmental samples. It is good practice to enclose highly contaminated samples in a plastic bag before placing them in ice chests. The same is true for wastewater and drinking water samples.

Ice chests or shipping containers with samples suspected of being highly contaminated shall be lined with new, clean, plastic bags.

If possible, one member of the field team should take all the notes, fill out labels, etc., while the other member does all of the sampling.

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

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

- Follow the preservative solution preparation instructions.
- Always use strong safety precautions diluting the acid.
- Put a new label on the dispensing bottle with the current date.
- Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.
- Wait 3-4 hours for the preservative to cool most samples down to 4°C.
- Most preservatives have a shelf life of one year from the preparation date.

When samples are analyzed for TKN, TP, NH_4 and NOx, 1 mL of 50% Trace Metal grade sulfuric acid is added to the each discrete auto sampler bottles/bags in the field lab before sampling collection. The preservative maintains the sample at 1.5<pH<2 after collection. To meet maximum holding time for these preserved samples (28 days), pull and ship samples every 14 days. Narrow range pH paper (test strips) can be used to test an aliquot of the preserved sample. Place the pH paper into the container and compare the color with the manufacturer's color chart.

Collection of Surface Water Samples

Representative samples may be collected from rivers, streams and lakes if certain rules are followed:

1. Watch out for flash floods! If a flooding event is likely and samples must be obtained, always go in two-person teams for safety. Look for an easy route of escape;

2. Select a sampling location at or near a gauging station so that stream discharge can be related to water-quality loading. If no gauging station exists, then measure the flow rate at the time of sampling, using the streamflow method described below;

3. Locate a straight and uniform channel for sampling;

4. Unless specified in the sampling plan, avoid sampling locations next to confluences or point sources of contamination;

5. Use bridges or boats for deep rivers and lakes where wading is dangerous or impractical;

6. Do not collect samples along a bank as they may not be representative of the surface water body as a whole; and

7. Use appropriate gloves when collecting the sample.

Streamflow Measurement

Before collecting water quality samples, record the stream's flow rate at the selected station. The flow rate measurement is important for estimating contaminant loading and other impacts. The first step in streamflow measurement is selecting a cross-section. Select a straight reach where the stream bed is uniform and relatively free of boulders and aquatic growth. Be certain that the flow is uniform and free of eddies, slack water and excessive turbulence. After the cross-section has been selected, determine the width of the stream by stringing a measuring tape from bank-to-bank at right angles to the direction of flow. Next, determine the spacing of the verticals. Space the verticals so that no partial section has more than 5 percent of the total discharge within it.

At the first vertical, face upstream and lower the velocity meter to the channel bottom, record its depth, then raise the meter to 0.8 and 0.2 of the distance from the stream surface, measure the water velocities at each level, and average them.

Move to the next vertical and repeat the procedure until you reach the opposite bank. Once the velocity, depth and distance of the cross-section have been determined, the mid-section method can be used for determining discharge. Calculate the discharge in each increment by multiplying the averaged velocity in each increment by the increment width and averaged depth.

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

(*Check with your governmental environmental or health agency for more information*)

River and Stream Sampling

Collection of samples from rivers and stream involves transporting all necessary items to the water-quality station and setting up field notes, instrumentation, filtration equipment (if not performed elsewhere as with microbiologicals), sample containers and decontamination washes near the channel. The first step is to measure all field parameters and then measure streamflow. After collecting and preserving the samples, equipment storage and decontamination will follow. Avoid spills when decontaminating equipment. For remote sites, extra collections equipment may be used to eliminate the need for field decontamination. Your governmental agencies have written procedures covering all aspects of surface-water characterization and sampling.

Composite Sampling
Composite sampling is intended to produce a water quality sample representative of the total stream discharge at the sampling station. If your sampling plan calls for composite sampling, use an automatic type sampler.

River or Channel Grab Sampling
Grab sampling is performed when uniform mixing in the river or stream channel makes composite sampling unnecessary, when point samples are desired, when sample degassing may occur, or when the water is too shallow for composite sampling. Record any decision to use grab sampling in the sampling plan. For streams at least 4 inches (10 cm) deep, collect grab samples in the middle of the channel using a laboratory cleaned or decontaminated glass or plastic container, and add the required preservatives.

An automatic refrigerator sampler with a Pickle Jar, this automatic sampler can also do grab type samples.

Laboratory
123 W. Main St
Sun City, Arizona 85541

LAB I.D. NUMBER

Sampler:

DATE:

PAGE 1 OF 1

Company:
Department:
Address:
Contact:
Telephone:

Sample Identification	Date	Time	Matrix	Lab ID	Metals* See Attached	TSS	Lead/Copper	BOD/COD	Nitrate	Nitrate + Nitrite	TKN / Ammonia	VOC / THM's	Semi Volital Organics (625)	Chloride		Cyanide	Florida	Surfactants (MBAS)	Tot. Coliform MPN	Fecal Coliform MPN-HPC	Organo-Phosphorus Pest. (8141)	Sulfate	EC Conductivity	Number/Containers

Project Name

Project Number

Field Measurements:

pH:

Temp:

Sample Receipt

No. Containers: _____

Custody Seals: Yes No

Received Intact: Yes No

Received Cold: Yes No

Temperature:

PRIORITY:

RELINQUISHED BY:

Signature: Time:

Printed Name: Date:

Company

RELINQUISHED BY:

Signature: Time:

Printed Name: Date:

Company

SAMPLED RECEIVED BY:

Signature: Time:

Printed Name: Date:

Company

SAMPLED RECEIVED BY:

Signature: Time:

Printed Name: Date:

Company

Chain-of-Custody Report Example

Chain of Custody Procedures

Because a sample is physical evidence, chain of custody procedures are used to maintain and document sample possession from the time the sample is collected until it is introduced as evidence. Chain of custody requirements will vary from agency to agency. However, these procedures are similar and the chain of custody outlined in this manual is only a guideline. Consult your project manager for specific requirements.

If you have physical possession of a sample, have it in view, or have it physically secured to prevent tampering, then it is defined as being in "***custody***." A chain of custody record, therefore, begins when the sample containers are obtained from the laboratory. From this point on, a chain of custody record will accompany the sample containers.

Handle the samples as little as possible in the field. Each custody sample requires a chain of custody record and may require a seal. If you do not seal individual samples, then seal the containers in which the samples are shipped.

When the samples transfer possession, both parties involved in the transfer must sign, date and note the time on the chain of custody record. If a shipper refuses to sign, you must seal the samples and chain of custody documents inside a box or cooler with bottle seals or evidence tape. The recipient will then attach the shipping invoices showing the transfer dates and times to the custody sheets.

If the samples are split and sent to more than one laboratory, prepare a separate chain of custody record for each sample. If the samples are delivered to after hours night drop-off boxes, the custody record should note such a transfer and be locked with the sealed samples inside sealed boxes.

Laboratory Analysis Section

Although development of an acceptable immunomagnetic separation system for *Giardia* lagged behind development of an acceptable system for *Cryptosporidium*, an acceptable system was identified in October 1998, and EPA validated a method for simultaneous detection of *Cryptosporidium* and *Giardia* in February 1999 and developed quality control (QC) acceptance criteria for the method based on this validation study. To avoid confusion with Method 1622, which already had been validated and was in use both domestically and internationally as a stand-alone *Cryptosporidium*-only detection method, EPA designated the new combined procedure EPA Method 1623.

The interlaboratory validated versions of Method 1622 (January 1999; EPA-821-R-99-001) and Method 1623 (April 1999; EPA-821-R-99-006) were used to analyze approximately 3,000 field and QC samples during the Information Collection Rule Supplemental Surveys (ICRSS) between March 1999 and February 2000. Method 1622 was used to analyze samples from March 1999 to mid-July 1999; Method 1623 was used from mid-July 1999 to February 2000. The April 2001 revision of both methods include updated QC acceptance criteria based on analysis of the QC samples analyzed during the ICRSS.

EPA Method 1623 is a performance-based method applicable to the determination of *Cryptosporidium* and *Giardia* in aqueous matrices. EPA Method 1623 requires filtration, immunomagnetic separation of the oocysts and cysts from the material captured, and an immunofluorescence assay for determination of oocyst and cyst concentrations, with confirmation through vital dye staining and differential interference contrast microscopy.

The interlaboratory validation of EPA Method 1623 conducted by the EPA used the Pall Gelman capsule filtration procedure, Dynal immunomagnetic separation (IMS) procedure, and Meridian sample staining procedure are described in this document. Alternate procedures are allowed, provided that required quality control tests are performed and all quality control acceptance criteria in this method are met.

Since the interlaboratory validation of EPA Method 1623, interlaboratory validation studies have been performed to demonstrate the equivalency of modified versions of the method using the following components:
- Whatman Nuclepore CryptTest™ filter
- IDEXX Filta-Max™ filter
- Waterborne Aqua-Glo™ G/C Direct FL antibody stain
- Waterborne Crypt-a-Glo™ and Giardi-a-Glo™ antibody stains

The validation studies for these modified versions of the method met EPA performance-based measurement system Tier 2 validation for nationwide use (see Section 9.1.2 for details), and have been accepted by the EPA as equivalent in performance to the original version of the method validated by the EPA.

The equipment and reagents used in these modified versions of the method are noted in Sections 6 and 7 of the method; the procedures for using these equipment and reagent options are available from the manufacturers.

Because this is a performance-based method, other alternative components not listed in the method may be available for evaluation and use by the laboratory. Confirming the acceptable performance of the modified version of the method using alternate components in a single laboratory does not require an interlaboratory validation study be conducted.

However, method modifications validated only in a single laboratory have not undergone sufficient testing to merit inclusion in the method.

Only those modified versions of the method that have been demonstrated as equivalent at multiple laboratories and multiple water sources through a Tier 2 interlaboratory study will be cited in the method.

The EPA initiated an effort in 1996 to identify new and innovative technologies for protozoan monitoring and analysis. After evaluating potential alternatives to the then-current method through literature searches, discussions with research and commercial laboratories, and meetings with experts in the field, the Engineering and Analysis Division within the Office of Science and Technology within the EPA's Office of Water developed draft Method 1622 for *Cryptosporidium* detection in December 1996.

This *Cryptosporidium*-only method was validated through an interlaboratory study in August 1998, and was revised as a final, valid method for detecting *Cryptosporidium* in water in January 1999.

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 material safety data sheets should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 20.2 through 20.5.

5.3 Samples may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves and opened in a biological safety cabinet to prevent exposure. Reference materials and standards containing oocysts and cysts must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Do not mouth-pipette.

5.4 Laboratory personnel must change gloves after handling filters and other contaminant-prone equipment and reagents. Gloves must be removed or changed before touching any other laboratory surfaces or equipment.

5.5 Centers for Disease Control (CDC) regulations (42 CFR 72) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. Unless the sample is known or suspected to contain *Cryptosporidium*, *Giardia*, or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by CDC or state regulations, the sample should be shipped in accordance with these regulations.

6.0 Equipment and Supplies

NOTE*: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection equipment for shipment of bulk water samples for laboratory filtration. Collapsible LDPE cubitainer for collection of 10-L bulk sample(s)—Cole Parmer cat. no. U-06100-30 or equivalent. Fill completely to ensure collection of a full 10-L sample. Discard after one use.

6.2 Equipment for sample filtration. Three options have been demonstrated to be acceptable for use with Method 1623. Other options may be used if their acceptability is demonstrated according to the procedures outlined in Section 9.1.2.

6.2.1 Cubitainer spigot to facilitate laboratory filtration of sample (for use with any filtration option)—Cole Parmer cat. no. U-06061-01, or equivalent.

6.2.2 Envirochek™ sampling capsule equipment requirements for use with the procedure described in Section 12.0. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

6.2.2.1 Sampling capsule—Envirochek™, Pall Gelman Laboratory, Ann Arbor, MI, product 12110

6.2.2.2 Laboratory shaker with arms for agitation of sampling capsules

6.2.2.2.1 Laboratory shaker—Lab-Line model 3589, VWR Scientific cat. no. 57039-055, Fisher cat. no. 14260-11, or equivalent

6.2.2.2.2 Side arms for laboratory shaker—Lab-Line Model 3587-4, VWR Scientific cat. no. 57039-045, Fisher cat. no. 14260-13, or equivalent

6.2.3 CrypTest™ capsule filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.3.1 Capsule filter—CrypTest™, Whatman Inc, Clifton, NJ, product no. 610064

6.2.3.2 Cartridge housing—Ametek 5-in. clear polycarbonate, Whatman cat. no. 71503, or equivalent

6.2.3.3 Ultrasonic bath—VWR Model 75T#21811-808, or equivalent

6.2.3.4 Laboratory tubing—Tygon formula R-3603, or equivalent

6.2.4 Filta-Max™ foam filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 50-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.4.1 Foam filter—Filta-Max™, IDEXX, Westbrook, ME. Filter module and membrane: product code FMC 10601; filter membranes (100 pack), product code FMC 10800

NOTE: Check at least one filter per batch to ensure that the filters have not been affected by improper storage or other factors that could result in brittleness or other problems. At a minimum confirm that the test filter expands properly in water before using the batch or shipping filters to the field.

6.2.4.2 Filter processing equipment—Filta-Max starter kit, IDEXX, Westbrook, ME, cat. no. FMC 11002. Includes all equipment required to run and process Filta-Max filter modules (manual wash station (FMC 10102) including plunger head (FMC 12001), elution tubing set (FMC 10301), vacuum set (FMC 10401), filter housing (FMC 10501), and magnetic stirrer (FMC 10901).

6.3 Ancillary sampling equipment

6.3.1 Tubing—Glass, polytetrafluoroethylene (PTFE), high-density polyethylene (HDPE), or other tubing to which oocysts and cysts will not easily adhere—Tygon formula R-3603, or equivalent. If rigid tubing (glass, PTFE, HDPE) is used and the sampling system uses a peristaltic pump, a minimum length of compressible tubing may be used in the pump. Before use, the tubing must be autoclaved, thoroughly rinsed with detergent solution, followed by repeated rinsing with reagent water to minimize sample contamination. Alternately, decontaminate using hypochlorite solution, sodium thiosulfate, and multiple reagent water rinses; dispose of tubing when wear is evident. Dispose of tubing after one use whenever possible.

6.3.2 Flow control valve—0.5 gpm (0.03 L/s), Bertram Controls, Plast-O-Matic cat. no. FC050B½-PV, or equivalent; or 0.4- to 4-Lpm flow meter with valve—Alamo Water Treatment, San Antonio, TX, cat. no. R5310, or equivalent.

6.3.3 Centrifugal pump—Grainger, Springfield, VA, cat. no. 2P613, or equivalent

6.3.4 Flow meter—Sameco cold water totalizer, E. Clark and Associates, Northboro, MA, product no. WFU 10.110, or equivalent.

6.4 Equipment for spiking samples in the laboratory

6.4.1 10-L carboy with bottom delivery port (½")—Cole-Palmer cat. no. 06080-42, or equivalent; calibrate to 10.0 L and mark level with waterproof marker.

6.4.2 Stir bar—Fisher cat. no. 14-511-93, or equivalent.

6.4.3 Stir plate—Fisher cat. no. 14-493-120S, or equivalent.

6.4.4 Hemacytometer—Neubauer type, Hauser Scientific, Horsham, PA, cat. no. 3200 or 1475, or equivalent.

6.4.5 Hemacytometer coverslip—Hauser Scientific, cat. no. 5000 (for hemacytometer cat. no. 3200) or 1461 (for hemacytometer cat. no 1475), or equivalent.

6.4.6 Lens paper without silicone—Fisher cat. no. 11-995, or equivalent.

6.4.7 Polystyrene or polypropylene conical tubes with screw caps—15- and 50-mL.

6.4.8 Equipment required for enumeration of spiking suspensions using membrane filters.

6.4.8.1 Glass microanalysis filter holder—25-mm-diameter, with fritted glass support, Fisher cat. no. 09-753E, or equivalent. Replace stopper with size 8, one-hole rubber stopper, Fisher Cat. No. 14-135M, or equivalent.

6.4.8.2 Three-port vacuum filtration manifold and vacuum source—Fisher Cat. No. 09-753-39A, or equivalent.

6.4.8.3 Cellulose acetate support membrane—1.2-µm-pore-size, 25-mm-diameter, Fisher cat. no. A12SP02500, or equivalent.

6.4.8.4 Polycarbonate track-etch hydrophilic membrane filter—1-µm-pore-size, 25-mm-diameter, Fisher cat. no. K10CP02500, or equivalent.

6.4.8.5 100 × 15 mm polystyrene Petri dishes (bottoms only).

6.4.8.6 60 × 15 mm polystyrene Petri dishes.

6.4.8.7 Glass microscope slides—1 in. × 3 in or 2 in. × 3 in.

6.4.8.8 Coverslips—25 mm^2

6.5 Immunomagnetic separation (IMS) apparatus

6.5.1 Sample mixer—Dynal Inc., Lake Success, NY, cat. no. 947.01, or equivalent.

6.5.2 Magnetic particle concentrator for 10-mL test tubes—Dynal MPC-1® , cat. no. 120.01, or equivalent.

6.5.3 Magnetic particle concentrator for microcentrifuge tubes—Dynal MPC-M®, cat. no. 120.09, or equivalent.

6.5.4 Flat-sided sample tubes—16 × 125 mm Leighton-type tubes with 60 × 10 mm flat-sided magnetic capture area, Dynal L10, cat. no. 740.03, or equivalent.

6.6 Powder-free latex gloves—Fisher cat no. 113945B, or equivalent.

6.7 Graduated cylinders, autoclavable—10-, 100-, and 1000-mL.

6.8 Centrifuges

6.8.1 Centrifuge capable of accepting 15- to 250-mL conical centrifuge tubes and achieving 1500 × G—International Equipment Company, Needham Heights, MA, Centrifuge Size 2, Model K with swinging bucket, or equivalent.

6.8.2 Centrifuge tubes—Conical, graduated, 1.5-, 50-, and 250-mL.

6.9 Microscope

6.9.1 Epifluorescence/differential interference contrast (DIC) with stage and ocular micrometers and 20X (N.A.=0.4) to 100X (N.A.=1.3) objectives—Zeiss™ Axioskop, Olympus™ BH, or equivalent.

6.9.2 Excitation/band-pass filters for immunofluorescence assay (FA)—Zeiss™ 487909 or equivalent, including, 450- to 490-nm exciter filter, 510-nm dicroic beam-splitting mirror, and 515- to 520-nm barrier or suppression filter.

6.9.3 Excitation/band-pass filters for DAPI—Filters cited below (Chroma Technology, Brattleboro, VT), or equivalent.

Microscope model	Fluoro-chrome	Excitation filter (nm)	Dichroic beam-splitting mirror (nm)	Barrier or suppression filter (nm)	Chroma catalog number
Zeiss™ - Axioskop	DAPI (UV)	340-380	400	420	CZ902
Zeiss™ -IM35	DAPI (UV)	340-380	400	420	CZ702
Olympus™ BH	DAPI (UV)	340-380	400	420	11000
			Filter holder		91002
Olympus™ BX	DAPI (UV)	340-380	400	420	11000
			Filter holder		91008
Olympus™ IMT2	DAPI (UV)	340-380	400	420	11000
			Filter holder		91003

6.10 Ancillary equipment for microscopy

6.10.1 Well slides— Spot-On well slides, Dynal cat. no. 740.04; treated, 12-mm diameter well slides, Meridian Diagnostics Inc., Cincinnati, OH, cat. no. R2206; or equivalent.

6.10.2 Glass coverslips—22 × 50 mm.

6.10.3 Nonfluorescing immersion oil.

6.10.4 Micropipette, adjustable: 0- to 10-µL with 0- to 10-µL tips 10- to 100-µL, with 10- to 200-µL tips 100- to 1000-µL with 100- to 1000-µL tips

6.10.5 Forceps—Splinter, fine tip.

6.10.6 Forceps—Blunt-end.

6.10.7 Desiccant—Drierite™ Absorbent, Fisher cat. no. 07-577-1A, or equivalent

6.10.8 Humid chamber—A tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

6.11 Pipettes—Glass or plastic

6.11.1 5-, 10-, and 25-mL.

6.11.2 Pasteur, disposable.

6.12 Balances

6.12.1 Analytical—Capable of weighing 0.1 mg.

6.12.2 Top loading—Capable of weighing 10 mg.

6.13 pH meter

6.14 Incubator—Fisher Scientific Isotemp™, or equivalent.

6.15 Vortex mixer—Fisons Whirlmixer, or equivalent.

6.16 Vacuum source—Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.17 Miscellaneous labware and supplies

6.17.1 Test tubes and rack.

6.17.2 Flasks—Suction, Erlenmeyer, and volumetric, various sizes.

6.17.3 Beakers—Glass or plastic, 5-, 10-, 50-, 100-, 500-, 1000-, and 2000-mL.

6.17.4 Lint-free tissues.

6.18 10- to 15-L graduated container—Fisher cat. no. 02-961-50B, or equivalent; calibrate to 9.0, 9.5, 10.0, 10.5, and 11.0 L and mark levels with waterproof marker.

6.19 Filters for filter-sterilizing reagents—Sterile Acrodisc, 0.45 µm , Gelman Sciences cat no. 4184, or equivalent.

7.0 Reagents and Standards

7.1 Reagents for adjusting pH

7.1.1 Sodium hydroxide (NaOH)—ACS reagent grade, 6.0 N and 1.0 N in reagent water

7.1.2 Hydrochloric acid (HCl)—ACS reagent grade, 6.0 N, 1.0 N, and 0.1 N in reagent water.

NOTE: Due to the low volumes of pH-adjusting reagents used in this method, and the impact that changes in pH have on the immunofluorescence assay, the laboratory should purchase standards at the required normality directly from a vendor. Normality should not be adjusted by the laboratory.

7.2 Solvents—Acetone, glycerol, ethanol, and methanol, ACS reagent grade

7.3 Reagent water—Water in which oocysts and cysts and interfering materials and substances, including magnetic minerals, are not detected by this method.

7.4 Reagents for eluting filters

7.4.1 Reagents for eluting Envirochek™ sampling capsules (Section 6.2.2)

7.4.1.1 Laureth-12—PPG Industries, Gurnee, IL, cat. no. 06194, or equivalent. Store Laureth-12 as a 10% solution in reagent water. Weigh 10 g of Laureth-12 and dissolve using a microwave or hot plate in 90 mL of reagent water. Dispense 10-mL aliquots into sterile vials and store at room temperature for up to 2 months, or in the freezer for up to a year.

7.4.1.2 1 M Tris, pH 7.4—Dissolve 121.1 g Tris (Fisher cat. no. BP152) in 700 mL of reagent water and adjust pH to 7.4 with 1 N HCl or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize through a 0.2-µm membrane into a sterile plastic container and store at room temperature.

7.4.1.3 0.5 M EDTA, 2 Na, pH 8.0—Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate (Fisher cat. no. S311) in 800 mL and adjust pH to 8.0 with 6.0 N HCl or NaOH. Dilute to a final volume of 1000 mL with reagent water and adjust to pH 8.0 with 1.0 N HCl or NaOH.

7.4.1.4 Antifoam A—Sigma Chemical Co. cat. no. A5758, or equivalent

7.4.1.5 Preparation of elution buffer solution—Add the contents of a pre-prepared Laureth-12 vial (Section 7.4.1.1) to a 1000-mL graduated cylinder. Rinse the vial several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution (Section 7.4.1.2), 2 mL of EDTA solution (Section 7.4.1.3), and 150 µL Antifoam A (Section 7.4.1.4). Dilute to 1000 mL with reagent water.

7.4.2 Reagents for eluting CrypTest™ capsule filters (Section 6.2.3). To 900 mL of reagent water add 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 ($12H_2O$) 0.2 g KCl, 0.2 g sodium lauryl sulfate (SDS), 0.2 mL Tween 80, and 0.02 mL Antifoam A (Sigma Chemical Co. cat. no. A5758, or equivalent). Adjust volume to 1 L with reagent water and adjust pH to 7.4 with 1 N NaOH or HCl.

7.4.3 Reagents for eluting Filta-Max™ foam filters (Section 6.2.4)

7.4.3.1 Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4.

7.4.3.2 Tween 20—Sigma Chemical Co. cat. no. P-7949, or equivalent.

7.4.3.3 High-vacuum grease—BDH/Merck. cat. no. 636082B, or equivalent.

7.4.3.4 Preparation of PBST elution buffer. Add the contents of one sachet of PBS to 1.0 L of reagent water. Dissolve by stirring for 30 minutes. Add 100 µL of Tween 20. Mix by stirring for 5 minutes.

7.5 Reagents for immunomagnetic separation (IMS)—Dynabeads® GC-Combo, Dynal cat. nos. 730.02, 730.12, or equivalent.

7.6 Direct antibody labeling reagents for detection of oocysts and cysts. Store reagents at 0 °C to 8 °C and return promptly to this temperature after each use. Do not allow any of the reagents to freeze. The reagents should be protected from exposure to light. Diluted, unused working reagents should be discarded after 48 hours. Discard reagents after the expiration date is reached. The labeling reagents in Sections 7.6.1-7.6.3 have been approved for use with this method.

7.6.1 Merifluor Cryptosporidium/Giardia, Meridian Diagnostics cat. no. 250050, Cincinnati, OH, or equivalent.

7.6.2 Aqua-Glo™ G/C Direct FL, Waterborne cat. no. A100FLR, New Orleans, LA, or equivalent.

7.6.3 Crypt-a-Glo™ and Giardi-a-Glo™, Waterborne cat. nos. A400FLR and A300FLR, respectively, New Orleans, LA, or equivalent.

> **NOTE**: If a laboratory will use multiple types of labeling reagents, the laboratory must demonstrate acceptable performance through an initial precision and recovery test (Section 9.4) for each type, and must perform positive and negative staining controls for each batch of slides stained using each product. However, the laboratory is not required to analyze additional ongoing precision and recovery samples or method blank samples for each type.

7.6.4 Diluent for labeling reagents—Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4. Filter-sterilize (Section 6.19) or autoclave. Discard if growth is detected or after 6 months, whichever comes first.

7.7 4',6-diamidino-2-phenylindole (DAPI) stain—Sigma Chemical Co. cat. no. A5758, or equivalent.

7.7.1 Stock solution—Dissolve 2 mg/mL DAPI in absolute methanol. Prepare volume consistent with minimum use. Store at 0 $^{\circ}$C to 8 $^{\circ}$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 $^{\circ}$C to 8 $^{\circ}$C in the dark except when staining. Do not allow to freeze. The solution concentration may be increased up to 1µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.

7.8 Mounting medium

7.8.1 DABCO/glycerol mounting medium (2%)—Dissolve 2 g of DABCO (Sigma Chemical Co. cat no. D-2522, or equivalent) in 95 mL of warm glycerol/PBS (60% glycerol, 40% PBS [Section 7.6.4]). After the DABCO has dissolved completely, adjust the solution volume to 100 mL by adding an appropriate volume of glycerol/PBS solution. Alternately, dissolve the DABCO in 40 mL of PBS, then add azide (1 mL of 100X, or 10% solution), then 60 mL of glycerol.

7.8.2 Mounting medium supplied with Merifluor direct labeling kit (Section 7.6.1)

7.9 Clear fingernail polish or clear fixative, PGC Scientifics, Gaithersburg, MD, cat. no. 60-4890, or equivalent.

7.10 Oocyst and cyst suspensions for spiking

7.10.1 Enumerated spiking suspensions prepared by flow cytometer—not heat-fixed or formalin fixed: Wisconsin State Laboratory of Hygiene Flow Cytometry Unit or equivalent

7.10.2 Materials for manual enumeration of spiking suspensions

7.10.2.1 Purified Cryptosporidium oocyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Sterling Parasitology Laboratory, University of Arizona, Tucson, or equivalent

7.10.2.2 Purified Giardia cyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Waterborne, Inc., New Orleans, LA; Hyperion Research, Medicine Hat, Alberta, Canada; or equivalent

7.10.2.3 Tween-20, 0.01%—Dissolve 1.0 mL of a 10% solution of Tween-20 in 1 L of reagent water

7.10.2.4 Storage procedure—Store oocyst and cyst suspensions at 0 °C to 8 °C, until ready to use; do not allow to freeze

7.11 Additional reagents for enumeration of spiking suspensions using membrane filtration (Section 11.3.6)—Sigmacote® Sigma Company Product No. SL-2, or equivalent

8.0 Sample Collection and Storage

8.1 Samples are collected as bulk samples and shipped to the laboratory for processing through the entire method, or are filtered in the field and shipped to the laboratory for processing from elution (Section 12.2.6) onward. Samples must be shipped via overnight service on the day they are collected. Chill samples as much as possible between collection and shipment by storing in a refrigerator or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment. Samples should be shipped at 0 °C to 8 °C, unless the time required to chill the sample to 8 °C would prevent the sample from being shipped overnight for receipt at the laboratory the day after collection. Samples must not be allowed to freeze. Upon receipt, the laboratory should record the temperature of the samples and store them refrigerated at 0 °C to 8 °C until processed. Results from samples shipped overnight to the laboratory and received at >8 °C should be qualified by the laboratory.

NOTE: See transportation precautions in Section 5.5.

8.2 Sample holding times. Sample processing should be completed as soon as possible by the laboratory. The laboratory should complete sample filtration, elution, concentration, purification, and staining the day the sample is received wherever possible. However, the laboratory is permitted to split up the sample processing steps if processing a sample completely in one day is not possible. If this is necessary, sample processing can be halted after filtration, application of the purified sample onto the slide, or staining. Table 1, in Section 21.0 provides a breakdown of the holding times for each set of steps. Sections 8.2.1 through 8.2.4 provide descriptions of these holding times.

8.2.1 Sample collection and filtration. Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

8.2.2 Sample elution, concentration, and purification. The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.
8.2.3 Staining. The sample must be stained within 72 hours of application of the purified sample to the slide.
8.2.4 Examination. Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.
8.5 Spiking suspension enumeration holding times. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometersorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance (QA) program (Reference 20.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability through performance of the initial precision and recovery (IPR) test (Section 9.4), analysis of spiked samples to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
9.1.1 A test of the microscope used for detection of oocysts and cysts is performed prior to examination of slides. This test is described in Section 10.0.
9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to modify certain method procedures to improve recovery or lower the costs of measurements, provided that all required quality control (QC) tests are performed and all QC acceptance criteria are met. Method procedures that can be modified include front-end techniques, such as filtration or immunomagnetic separation (IMS). The laboratory is not permitted to use an alternate determinative technique to replace immunofluorescence assay in this method (the use of different determinative techniques are considered to be different methods, rather than modified version of this method). However, the laboratory is permitted to modify the immunofluorescence assay procedure, provided that all required QC tests are performed (Section 9.1.2.1) and all QC acceptance criteria are met (see guidance on the use of multiple labeling reagents in Section 7.6).

9.1.2.1 Method modification validation/equivalency demonstration requirements.
9.1.2.1.1 Method modifications at a single laboratory. Each time a modification is made to this method for use in a single laboratory, the laboratory is required to validate the modification according to Tier 1 of EPA's performance-based measurement system (PBMS) (Table 2 and Reference 20.7) to demonstrate that the modification produces results equivalent or superior to results produced by this method as written. Briefly, each time a modification is made to this method, the laboratory is required to demonstrate acceptable modified method performance through the IPR test (Section 9.4). IPR results must meet the QC acceptance criteria in Tables 3 and 4 in Section 21.0, and should be comparable to previous results using the unmodified procedure. Although not required, the laboratory also should perform a matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate the performance of the modified method in at least one real-world matrix before analyzing field samples using the modified method. The laboratory is required to perform MS samples using the modified method at the frequency noted in Section 9.1.8.
9.1.2.1.2 Method modifications for nationwide approval. If the laboratory or a manufacturer seeks EPA approval of a method modification for nationwide use, the laboratory or manufacturer must validate the modification according to Tier 2 of EPA's PBMS (Table 2 and Reference 20.7). Briefly, at least three laboratories must perform IPR tests (Section 9.4) and MS/MSD (Section 9.5) tests using the modified method, and all tests must meet the QC acceptance criteria specified in Tables 3 and 4 in Section 21.0. Upon nationwide approval, laboratories electing to use the modified method still must demonstrate acceptable performance in their own laboratory according to the requirements in Section 9.1.2.1.1.
9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
9.1.2.2.2 A listing of the analyte(s) measured (Cryptosporidium and Giardia).
9.1.2.2.3 9.1.2.2.4 A narrative stating reason(s) for the modification.
9.1.2.2.5 Results from all QC tests comparing the modified method to this method, including: (a) IPR (Section 9.4) (b) MS/MSD (Section 9.5) (c) Analysis of method blanks (Section 9.6) Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:
(a) Sample numbers and other identifiers
(b) Source of spiking suspensions, as well as lot number and date received (Section 7.10)
(c) Spike enumeration date and time
(d) All spiking suspension enumeration counts and calculations (Section 11.0)
(e) Sample spiking dates and times
(f) Volume filtered (Section 12.2.5.2)
(g) Filtration and elution dates and times
(h) Pellet volume, resuspended concentrate volume, resuspended concentrate volume transferred to IMS, and all calculations required to verify the percent of concentrate examined (Section 13.2)
(i) Purification completion dates and times (Section 3.3.3.11)
(j) Staining completion dates and times (Section 14.10)
(k) Staining control results (Section 15.2.1)

(l) All required examination information (Section 15.2.2)
(m) Examination completion dates and times (Section 15.2.4)
(n) Analysis sequence/run chronology
(o) Lot numbers of elution, IMS, and staining reagents
(p) Copies of bench sheets, logbooks, and other recordings of raw data
(q) Data system outputs, and other data to link the raw data to the results reported

9.1.3 The laboratory shall spike a separate sample aliquot from the same source to monitor method performance. This MS test is described in Section 9.5.1.

9.1.4 Analysis of method blanks is required to demonstrate freedom from contamination. The procedures and criteria for analysis of a method blank are described in Section 9.6.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample that the analysis system is in control. These procedures are described in Section 9.7.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Development of accuracy statements is described in Sections 9.5.1.4 and 9.7.3.

9.1.7 The laboratory shall analyze one method blank (Section 9.6) and one OPR sample (Section 9.7) each week during which samples are analyzed if 20 or fewer field samples are analyzed during this period. The laboratory shall analyze one laboratory blank and one OPR sample for every 20 samples if more than 20 samples are analyzed in a week.

9.1.8 The laboratory shall analyze one MS sample (Section 9.5.1) when samples are first received from a utility for which the laboratory has never before analyzed samples. The MS analysis is performed on an additional (second) sample sent from the utility. If the laboratory routinely analyzes samples from 1 or more utilities, 1 MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a given site, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 21st sample from this site, a separate aliquot of this 21st sample must be collected and spiked.

9.2 Micropipette calibration

9.2.1 Micropipettes must be sent to the manufacturer for calibration annually. Alternately, a qualified independent technician specializing in micropipette calibration can be used. Documentation on the precision of the recalibrated micropipette must be obtained from the manufacturer or technician.

9.2.2 Internal and external calibration records must be kept on file in the laboratory's QA logbook.

9.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. Ten replicates should be performed at each weight. Record the weight of the water (assume that 1.00 mL of reagent water weighs 1.00 g) and calculate the relative standard deviation (RSD) for each. If the weight of the reagent water is within 1% of the desired weight (mL) and the RSD of the replicates at each weight is within 1%, then the pipette remains acceptable for use.

9.2.4 If the weight of the reagent water is outside the acceptable limits, consult the manufacturer's instruction manual troubleshooting section and repeat steps described in Section 9.2.3. If problems with the pipette persist, the laboratory must send the pipette to the manufacturer for recalibration.

9.3 Microscope adjustment and certification: Adjust the microscope as specified in Section 10.0. All of the requirements in Section 10.0 must be met prior to analysis of IPRs, blanks, OPRs, field samples, and MS/MSDs.

9.4 Initial precision and recovery (IPR)—To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.4.1 Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine four reagent water samples spiked with 100 to 500 oocysts and 100 to 500 cysts. If more than one process will be used for filtration and/or separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE: IPR tests must be accompanied by analysis of a method blank (Section 9.6).

9.4.2 Using results of the four analyses, calculate the average percent recovery and the relative standard deviation (RSD) of the recoveries for Cryptosporidium and for Giardia. The RSD is the standard deviation divided by the mean times 100.

9.4.3 Compare RSD and the mean with the corresponding limits for initial precision and recovery in Tables 3 and 4 in Section 21.0. If the RSD and the mean meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If the RSD or the mean falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test (Section 9.4.1).

9.5 Matrix spike (MS) and matrix spike duplicate (MSD):

9.5.1 Matrix spike—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's oocyst and cyst recovery. The MS shall be analyzed according to the frequency in Section 9.1.8.

9.5.1.1 Analyze an unspiked field sample according to the procedures in Sections 12.0 to 15.0. Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine a second field sample aliquot with the number of organisms used in the IPR or OPR tests (Sections 9.4 and 9.7).

9.5.1.2 For each organism, calculate the percent recovery (R) using the following equation.

$$R = 100 \times \frac{Nsp - Ns}{T}$$

where
R is the percent recovery
N_{sp} is the number of oocysts or cysts detected in the spiked sample

N_s is the number of oocysts or cysts detected in the unspiked sample T is the true value of the oocysts or cysts spiked

9.5.1.3 Compare the recovery for each organism with the corresponding limits in Tables 3 and 4 in Section 21.0.

NOTE: Some sample matrices may prevent the acceptance criteria in Tables 3 and 4 from being met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.
9.5.1.4 As part of the QA program for the laboratory, method precision for samples should be assessed and records maintained. After the analysis of five samples for which the spike recovery for each organism passes the tests in Section 9.5.1.3, the laboratory should calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_r). Express the precision assessment as a percent recovery interval from P $^-$2 s_r to P + 2 s_r for each matrix. For example, if P = 80% and s_r = 30%, the accuracy interval is expressed as 20% to 140%. The precision assessment should be updated regularly across all MS samples and stratified by MS samples for each source.
9.5.2 Matrix spike duplicate—MSD analysis is required as part of nationwide approval of a modified version of this method to demonstrate that the modified version of this method produces results equal or superior to results produced by the method as written (Section 9.1.2.1.2). At the same time the laboratory spikes and analyzes the second field sample aliquot in Section 9.5.1.1, the laboratory shall spike and analyze a third, identical field sample aliquot.

NOTE: Matrix spike duplicate samples are only required for Tier 2 validation studies. They are recommended for Tier 1 validation, but not required.

9.5.2.1 For each organism, calculate the percent recovery (R) using the equation in Section 9.5.1.2.

9.5.2.2 Calculate the mean of the number of oocysts or cysts in the MS and MSD (X_{mean}) (= [MS+MSD]/2).

9.5.2.3 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \frac{| NMS - NMSD |}{Xmean}$$

where
RPD is the relative percent difference
N_{MS} is the number of oocysts or cysts detected in the MS
N_{MSD} is the number of oocysts or cysts detected in the MSD
X_{mean} is the mean number of oocysts or cysts detected in the MS and MSD

9.5.2.4 Compare the mean MS/MSD recovery and RPD with the corresponding limits in Tables 3 and 4 in Section 21.0 for each organism.

9.6 Method blank (negative control sample, laboratory blank): Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (Section 9.4) and OPR test (Section 9.7) and prior to analysis of samples for the week to demonstrate freedom from contamination.
9.6.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week (Section 9.1.7) according to the procedures in Sections 12.0 to 15.0. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.
9.6.2 If *Cryptosporidium* oocysts, *Giardia* cysts, or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more oocysts or cysts is assumed to be contaminated and should be recollected, if possible. Any method blank in which oocysts or cysts are not detected is assumed to be uncontaminated and may be reported.

9.7 Ongoing precision and recovery ([OPR]; positive control sample; laboratory control sample): Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water sample spiked with 100 to 500 oocysts and 100 to 500 cysts each week to verify all performance criteria. The laboratory must analyze one OPR sample for every 20 samples if more than 20 samples are analyzed in a week. If multiple method variations are used, separate OPR samples must be prepared for each method variation. Adjustment and/or recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.
9.7.1 Examine the slide from the OPR prior to analysis of samples from the same batch.
9.7.1.1 Using 200X to 400X magnification, more than 50% of the oocysts or cysts must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the organisms. Determine the step or reagent that is causing damage to the organisms. Correct the problem and repeat the OPR test.

9.7.1.2 Identify and enumerate each organism using epifluorescence microscopy. The first three presumptive *Cryptosporidium* oocysts and three *Giardia* cysts identified in the OPR sample must be examined using FITC, DAPI, and DIC, as per Section 15.2, and the detailed characteristics (size, shape, DAPI category, and DIC category) reported on the *Cryptosporidium* and *Giardia* report form, as well as any additional comments on organism appearance, if notable.

9.7.2 For each organism, calculate the percent recovery (R) using the following equation:

$$R = 100 \times \frac{N}{T}$$

where:
R = the percent recovery
N = the number of oocysts or cysts detected
T = the number of oocysts or cysts spiked

9.7.3 Compare the recovery with the limits for ongoing precision and recovery in Tables 3 and 4 in Section 21.0. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, system performance is unacceptable. In this event, there may be a problem with the microscope or with the filtration or separation systems. Troubleshoot the problem using the procedures at Section 9.7.4 as a guide. After assessing the issue, reanalyze the OPR sample. All samples must be associated with an OPR that passes the criteria in Section 21.0. Samples that are not associated with an acceptable OPR must be flagged accordingly.

9.7.4 Troubleshooting. If an OPR sample has failed, and the cause of the failure is not known, the laboratory generally should identify the problem working backward in the analytical process from the microscopic examination to filtration.
9.7.4.1 Microscope system and antibody stain: To determine if the failure of the OPR test is due to changes in the microscope or problems with the antibody stain, re-examine the positive staining control (Section 15.2.1), check Köhler illumination, and check the fluorescence of the fluorescein-labeled monoclonal antibodies (Mabs) and 4',6-diamidino-2-phenylindole (DAPI). If results are unacceptable, re-examine the previously-prepared positive staining control to determine whether the problem is associated with the microscope or the antibody stain.
9.7.4.2 Separation (purification) system: To determine if the failure of the OPR test is attributable to the separation system, check system performance by spiking a 10-mL volume of reagent water with 100 - 500 oocysts and cysts and processing the sample through the IMS, staining, and examination procedures in Sections 13.3 through 15.0.
9.7.4.3 Filtration/elution/concentration system: If the failure of the OPR test is attributable to the filtration/elution/concentration system, check system performance by processing spiked reagent water according to the procedures in Section 12.2 through 13.2.2.1, and filter, stain, and examine the sample concentrate according to Section 11.3.6.

9.7.5 The laboratory should add results that pass the specifications in Section 9.7.3 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r).

Express the accuracy as a recovery interval from R ¯2 s_r to R + 2 s_r. For example, if R = 95% and s_r = 25%, the accuracy is 45% to 145%.

9.8 The laboratory should periodically analyze an external QC sample, such as a performance evaluation or standard reference material, when available.

The laboratory also should periodically participate in interlaboratory comparison studies using the method.

9.9 The specifications contained in this method can be met if the analytical system is under control. The standards used for initial (Section 9.4) and ongoing (Section 9.7) precision and recovery should be identical, so that the most precise results will be obtained. The microscope in particular will provide the most reproducible results if dedicated to the settings and conditions required for the determination of Cryptosporidium and Giardia by this method.

9.10 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and duplicate spiked samples may be required to determine the precision of the analysis.

10.0 Microscope Calibration and Analyst Verification

10.1 In a room capable of being darkened to near-complete darkness, assemble the microscope, all filters, and attachments. The microscope should be placed on a solid surface free from vibration. Adequate workspace should be provided on either side of the microscope for taking notes and placement of slides and ancillary materials.

10.2 Using the manuals provided with the microscope, all analysts must familiarize themselves with operation of the microscope.

10.3 Microscope adjustment and calibration (adapted from Reference 20.6)

10.3.1 Preparations for adjustment

10.3.1.1 The microscopy portion of this procedure depends upon proper alignment and adjustment of very sophisticated optics. Without proper alignment and adjustment, the microscope will not function at maximal efficiency, and reliable identification and enumeration of oocysts and cysts will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.

10.3.1.2 While microscopes from various vendors are configured somewhat differently, they all operate on the same general physical principles. Therefore, slight deviations or adjustments may be required to make the procedures below work for a particular instrument.

10.3.1.3 The sections below assume that the mercury bulb has not exceeded time limits of operation, that the lamp socket is connected to the lamp house, and that the condenser is adjusted to produce Köhler illumination.

10.3.1.4 Persons with astigmatism should always wear contact lenses or glasses when using the microscope.

CAUTION: In the procedures below, do not touch the quartz portion of the mercury bulb with your bare fingers. Finger oils can cause rapid degradation of the quartz and premature failure of the bulb.
WARNING: Never look at the ultraviolet (UV) light from the mercury lamp, lamp house, or the UV image without a barrier filter in place. UV radiation can cause serious eye damage.

10.3.2 Epifluorescent mercury bulb adjustment: The purpose of this procedure is to ensure even field illumination. This procedure must be followed when the microscope is first used, when replacing bulbs, and if problems such as diminished fluorescence or uneven field illumination are experienced.
10.3.2.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.2.2 Using a prepared microscope slide, adjust the focus so the image in the oculars is sharply defined.
10.3.2.3 Replace the slide with a business card or a piece of lens paper.
10.3.2.4 Close the field diaphragm (iris diaphragm in the microscope base) so only a small point of light is visible on the card. This dot of light indicates the location of the center of the field of view.
10.3.2.5 Mount the mercury lamp house on the microscope without the UV diffuser lens in place and turn on the mercury bulb.
10.3.2.6 Remove the objective in the light path from the nosepiece. A primary (brighter) and secondary image (dimmer) of the mercury bulb arc should appear on the card after focusing the image with the appropriate adjustment.
10.3.2.7 Using the lamp house adjustments, adjust the primary and secondary mercury bulb images so they are side by side (parallel to each other) with the transmitted light dot in between them.
10.3.2.8 Reattach the objective to the nosepiece.
10.3.2.9 Insert the diffuser lens into the light path between the mercury lamp house and the microscope.
10.3.2.10 Turn off the transmitted light and replace the card with a slide of fluorescent material. Check the field for even fluorescent illumination. Adjustment of the diffuser lens probably will be required. Additional slight adjustments as in Section 10.3.2.7 above may be required.
10.3.2.11 Maintain a log of the number of hours the UV bulb has been used. Never use the bulb for longer than it has been rated. Fifty-watt bulbs should not be used longer than 100 hours; 100-watt bulbs should not be used longer than 200 hours.

10.3.3 Transmitted bulb adjustment: The purpose of this procedure is to center the filament and ensure even field illumination. This procedure must be followed when the bulb is changed.
10.3.3.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.3.2 Using a prepared microscope slide and a 40X (or similar) objective, adjust the focus so the image in the oculars is sharply defined.

10.3.3.3 Without the ocular or Bertrand optics in place, view the pupil and filament image at the bottom of the tube.
10.3.3.4 Focus the lamp filament image with the appropriate adjustment on the lamp house.
10.3.3.5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on the lamp house.
10.3.3.6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

10.3.4 Adjustment of the interpupillary distance and oculars for each eye: These adjustments are necessary so that eye strain is reduced to a minimum, and must be made for each individual using the microscope. Section 10.3.4.2 assumes use of a microscope with both oculars adjustable; Section 10.3.4.3 assumes use of a microscope with a single adjustable ocular. The procedure must be followed each time an analyst uses the microscope.

10.3.4.1 Interpupillary distance

10.3.4.1.1 Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.4.1.2 Using both hands, move the oculars closer together or farther apart until a single circle of light is observed while looking through the oculars with both eyes. Note interpupillary distance.

10.3.4.2 Ocular adjustment for microscopes capable of viewing a photographic frame through the viewing binoculars: This procedure assumes both oculars are adjustable.

10.3.4.2.1 Place a card between the right ocular and eye keeping both eyes open. Adjust the correction (focusing) collar on the left ocular by focusing the left ocular until it reads the same as the interpupillary distance. Bring an image located in the center of the field of view into as sharp a focus as possible.

10.3.4.2.2 Transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the same image into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

10.3.4.3 Ocular adjustment for microscopes without binocular capability: This procedure assumes a single focusing ocular. The following procedure assumes that only the right ocular is capable of adjustment.

10.3.4.3.1 Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.

10.3.4.3.2 Transfer the card to between the left eye and ocular. Keeping both eyes open, bring the image for the right eye into sharp focus by adjusting the ocular collar at the top of the ocular without touching the coarse or fine adjustment.

10.3.5 Calibration of an ocular micrometer: This section assumes that a reticle has been installed in one of the oculars by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed, the ocular reticle should be left in place. The more an ocular is manipulated the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is a top lens on the microscope, the calibration procedure must be done for the respective objective at each top lens setting. The procedure must be followed when the microscope is first used and each time the objective is changed.

10.3.5.1 Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.

10.3.5.2 Adjust the stage and ocular with the micrometer so the 0 line on the ocular micrometer is exactly superimposed on the 0 line on the stage micrometer.

10.3.5.3 Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.

10.3.5.4 Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition. For example: Suppose 48 ocular micrometer spaces equal 0.6 mm.

10.3.5.5 Calculate the number of mm/ocular micrometer space. For example:

0.6 *mm* 0.0125 *mm* = 48 *ocular micrometer spaces ocular micrometer space*

10.3.5.6 Because most measurements of microorganisms are given in µm rather than mm, the value calculated above must be converted to µm by multiplying it by 1000 µm /mm. For example:

0.0125 mm 1,000 µm 12.5 µm x =
ocular micrometer space mm ocular micrometer space

10.3.5.7 Follow the procedure below for each objective. Record the information as shown in the example below and keep the information available at the microscope.

Item no.	Objective power	Description	No. of ocular micrometer spaces	No. of stage micrometer mm[1]	µm/ocular micrometer space[2]
1		10X		N.A.[3]=	
2		20X		N.A.=	
3		40X		N.A.=	
4		100X		N.A.=	

[1]100 µm /mm [2](Stage micrometer length in mm × (1000 µm /mm)) ÷ no. ocular micrometer spaces [3]N.A. refers to numerical aperature. The numerical aperature value is engraved on the barrel of the objective.

10.3.6 Köhler illumination: This section assumes that Köhler illumination will be established for only the 100X oil DIC objective that will be used to identify internal morphological characteristics in Cryptosporidium oocysts and Giardia cysts. If more than one objective is to be used for DIC, then each time the objective is changed, Köhler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Köhler illumination is not properly established, then DIC will not work to its maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore. The procedure must be followed each time an analyst uses the microscope and each time the objective is changed.

10.3.6.1 Place a prepared slide on the microscope stage, place oil on the slide, move the 100X oil objective into place, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.6.2 At this point both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.

10.3.6.3 Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the field to the center of the visual field.

10.3.6.4 Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up and down with the condenser focusing knob while you are looking through the binoculars. Once you have accomplished the precise focusing of the radiant field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

10.3.6.5 The aperture diaphragm of the condenser is now adjusted to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

10.3.6.6 After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish DIC

10.4 Protozoa libraries: Each laboratory is encouraged to develop libraries of photographs and drawings for identification of protozoa.

10.4.1 Take color photographs of Cryptosporidium oocysts and Giardia cysts by FA and 4',6-diamidino-2-phenylindole (DAPI) that the analysts (Section 22.2) determine are accurate (Section 15.2).

10.4.2 Similarly, take color photographs of interfering organisms and materials by FA and DAPI that the analysts believe are not Cryptosporidium oocysts or Giardia cysts. Quantify the size, shape, microscope settings, and other characteristics that can be used to differentiate oocysts and cysts from interfering debris and that will result in positive identification of DAPI positive or negative organisms.

10.5 Verification of performance: Until standard reference materials, such as National Institute of Standards and Technology standard reference materials, are available that contain a reliable number of DAPI positive or negative oocysts and cysts, this method shall rely upon the ability of the analyst for identification and enumeration of oocysts and cysts.

10.5.1 At least monthly when microscopic examinations are being performed, the laboratory shall prepare a slide containing 40 to 100 oocysts and 40 to 100 cysts. More than 50% of the oocysts and cysts must be DAPI positive.

10.5.2 Each analyst shall determine the total number of oocysts and cysts and the number that are DAPI positive or negative using the slide prepared in Section 10.5.1.

10.5.3 The total number and the number of DAPI positive or negative oocysts and cysts determined by each analyst (Section 10.5.2.) must be within ±10% of each other. If the number is not within this range, the analysts must identify the source of any variability between analysts' examination criteria, prepare a new slide, and repeat the performance verification (Sections 10.5.1 to 10.5.2).

10.5.4 Document the date, name(s) of analyst(s), number of total, DAPI positive or negative oocysts and cysts determined by the analyst(s), whether the test was passed/failed and the results of attempts before the test was passed.

10.5.5 Only after an analyst has passed the criteria in Section 10.5.3, may oocysts and cysts in QC samples and field samples be identified and enumerated.

11.0 Oocyst and Cyst Suspension Enumeration and Spiking

11.1 This method requires routine analysis of spiked QC samples to demonstrate acceptable initial and ongoing laboratory and method performance (initial precision and recovery samples [Section 9.4], matrix spike and matrix spike duplicate samples [Section 9.5], and ongoing precision and recovery samples [Section 9.7]). The organisms used for these samples must be enumerated to calculate recoveries and precision. EPA recommends that flow cytometry be used for this enumeration, rather than manual techniques. Flow cytometer–sorted spikes generally are characterized by a relative standard deviation of ≤ 2.5%, versus greater variability for manual enumeration techniques (Reference 20.8). Guidance on preparing spiking suspensions using a flow cytometer is provided in Section 11.2. Manual enumeration procedures are provided in Section 11.3. The procedure for spiking bulk samples in the laboratory is provided in Section 11.4.

11.2 Flow cytometry enumeration guidelines. Although it is unlikely that many laboratories performing Method 1623 will have direct access to a flow cytometer for preparing spiking suspensions, flow-sorted suspensions are available from commercial vendors and other sources (Section 7.10.1). The information provided in Sections 11.2.1 through 11.2.4 is simply meant as a guideline for preparing spiking suspensions using a flow cytometer. Laboratories performing flow cytometry must develop and implement detailed standardized protocols for calibration and operation of the flow cytometer.

11.2.1 Spiking suspensions should be prepared using unstained organisms that have not been heat-fixed or formalin-fixed.

11.2.2 Spiking suspensions should be prepared using Cryptosporidium parvum oocysts <3 months old, and Giardia intestinalis cysts <2 weeks old.

11.2.3 Initial calibration. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The oocyst and cyst levels used for the initial calibration should be the same as the levels used for the spiking suspensions. Each initial calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the laboratory should perform the initial calibration again, until the RSD of the 10 counts is ≤ 2.5%. In addition to counting the organisms, the laboratory also should evaluate the quality of the organisms using DAPI and DIC to confirm that the organisms are in good condition.

11.2.4 Ongoing calibration. When sorting the spiking suspensions for use in QC samples, the laboratory should perform ongoing calibration samples at a 10% frequency, at a minimum. The laboratory should sort the first run and every eleventh sample directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the laboratory should discard the batch.

11.2.5 Method blanks. Depending on the operation of the flow cytometer, method blanks should be prepared and examined at the same frequency as the ongoing calibration samples (Section 11.2.4).

11.2.6 Holding time criteria. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometer-sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory.

11.3 Manual enumeration procedures. Two sets of manual enumerations are required per organism before purified Cryptosporidium oocyst and Giardia cyst stock suspensions (Sections 7.9.2.1 and 7.9.2.2) received from suppliers can be used to spike samples in the laboratory. First, the stock suspension must be diluted and enumerated (Section 11.3.3) to yield a suspension at the appropriate oocyst or cyst concentration for spiking (spiking suspension). Then, 10 aliquots of spiking suspension must be enumerated to calculate a mean spike dose. Spiking suspensions can be enumerated using hemacytometer chamber counting (Section 11.3.4), well slide counting (Section 11.3.5), or membrane filter counting (Section 11.3.6).

11.3.1 Precision criteria. The relative standard deviation (RSD) of the calculated mean spike dose for manually enumerated spiking suspensions must be ≤16% for *Cryptosporidium* and ≤19% for *Giardia* before proceeding (these criteria are based on the pooled RSDs of 105 manual *Cryptosporidium* enumerations and 104 manual *Giardia* enumerations submitted by 20 different laboratories under the EPA Protozoa Performance Evaluation Program).

11.3.2 Holding time criteria. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension or membrane filter to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

11.3.3 Enumerating and diluting stock suspensions

11.3.3.1 Purified, concentrated stock suspensions (Sections 7.10.2.1 and 7.10.2.2) must be diluted and enumerated before the diluted suspensions are used to spike samples in the laboratory. Stock suspensions should be diluted with reagent water/Tween-20, 0.01% (Section 7.10.2.3), to a concentration of 20 to 50 organisms per large hemacytometer square before proceeding to Section 11.3.3.2.

11.3.3.2 Apply a clean hemacytometer coverslip (Section 6.4.5) to the hemacytometer and load the hemacytometer chamber with 10 µL of vortexed suspension per chamber. If this operation has been properly executed, the liquid should amply fill the entire chamber without bubbles or overflowing into the surrounding moats. Repeat this step with a clean, dry hemacytometer and coverslip if loading has been incorrectly performed. See Section 11.3.3.13, below, for the hemacytometer cleaning procedure.

11.3.3.3 Place the hemacytometer on the microscope stage and allow the oocysts or cysts to settle for 2 minutes Do not attempt to adjust the coverslip, apply clips, or in any way disturb the chamber after it has been filled.

11.3.3.4 Use 200X magnification.

11.3.3.5 Move the chamber so the ruled area is centered underneath it.

11.3.3.6 Move the objective close to the coverslip while watching it from the side of the microscope, rather than through the microscope.

11.3.3.7 Focus up from the coverslip until the hemacytometer ruling appears.

11.3.3.8 At each of the four corners of the chamber is a 1-square-mm area divided into 16 squares in which organisms are to be counted (Figure 1). Beginning with the top row of four squares, count with a hand-tally counter in the directions indicated in Figure 2. Avoid counting organisms twice by counting only those touching the top and left boundary lines. Count each square millimeter in this fashion.

11.3.3.9 Use the following formula to determine the number of organisms per mL of suspension:

11.3.3.10 Record the result on a hemacytometer data sheet.

11.3.3.11 A total of six different hemacytometer chambers must be loaded, counted, and averaged for each suspension to achieve optimal counting accuracy.

11.3.3.12 Based on the hemacytometer counts, the stock suspension should be diluted to a final concentration of between 8000 and 12,000 organisms per mL (80 to 120 organisms per 10 $_{\mu L}$); however, ranges as great as 5000 to 15,000 organisms per mL (50 to 150 organisms per 10 µL) can be used.

NOTE: If the diluted stock suspensions (the spiking suspensions) will be enumerated using hemacytometer chamber counts (Section 11.3.4) or membrane filter counts (Section 11.3.6), then the stock suspensions should be diluted with 0.01% Tween-20. If the spiking suspensions will be enumerated using well slide counts (Section 11.3.3), then the stock suspensions should be diluted in reagent water.

To calculate the volume (in µL) of stock suspension required per mL of reagent water (or reagent water/Tween-20, 0.01%), use the following formula:

required number of organisms x 1000 µLvolume of stock suspension (µL)
required = number of organisms/mL of Stock suspension

If the volume is less than 10 µL , an additional dilution of the stock suspension is recommended before proceeding.

To calculate the dilution factor needed to achieve the required number of organisms per 10 µL , use the following formula:
Total volume (µL) number of organisms required x 10 µL
predicted number of organisms per 10 µL (80 to 120)

To calculate the volume of reagent water (or reagent water/Tween-20, 0.01%) needed, use the following formula:
reagent water volume (µL) = total volume (µL) -stock suspension volume required (µL)

11.3.3.13 After each use, the hemacytometer and coverslip must be cleaned immediately to prevent the organisms and debris from drying on it. Since this apparatus is precisely machined, abrasives cannot be used to clean it, as they will disturb the flooding and volume relationships.

11.3.3.13.1 Rinse the hemacytometer and cover glass first with tap water, then 70% ethanol, and finally with acetone.

11.3.3.13.2 Dry and polish the hemacytometer chamber and cover glass with lens paper. Store it in a secure place.

11.3.3.14 Several factors are known to introduce errors into hemacytometer counts, including:

• Inadequate mixing of suspension before flooding the chamber.
• Irregular filling of the chamber, trapped air bubbles, dust, or oil on the chamber or coverslip.
• Total number of organisms counted is too low to provide statistical confidence in the result
• Error in recording tally.
• Calculation error; failure to consider dilution factor, or area counted.
• Inadequate cleaning and removal of organisms from the previous count.
• Allowing filled chamber to sit too long, so that the chamber suspension dries and concentrates.

11.3.4 Enumerating spiking suspensions using a hemacytometer chamber

NOTE: Spiking suspensions enumerated using a hemacytometer chamber must be used within 24 hours of enumeration.

11.3.4.1 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.4.2 To an appropriate-size beaker containing a stir bar, add enough spiking suspension to perform all spike testing and the enumeration as described. The liquid volume and beaker relationship should be such that a spinning stir bar does not splash the sides of the beaker, the stir bar has unimpeded rotation, and there is enough room to draw sample from the beaker with a 10-µL micropipette without touching the stir bar. Cover the beaker with a watch glass or Petri dish to prevent evaporation between sample withdrawals.

11.3.4.3 Allow the beaker contents to stir for a minimum of 30 minutes before beginning enumeration.

11.3.4.4 While the stir bar is still spinning, remove a 10-µL aliquot and carefully load one side of the hemacytometer. Count all organisms on the platform, at 200X magnification using phase-contrast or darkfield microscopy. The count must include the entire area under the hemacytometer, not just the four outer 1-mm^2 squares. Repeat this procedure nine times. This step allows confirmation of the number of organisms per 10 µL (Section 11.3.3.12). Based on the 10 counts, calculate the mean, standard deviation, and RSD of the counts. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts. Refer to Section 11.3.3.14 for factors that may introduce errors.

Enumerating spiking suspensions using well slides

NOTE: Spiking suspensions enumerated using well slides must be used within 24 hours of application of the spiking suspension to the slides.

11.3.5.1 Remove well slides from cold storage and lay the slides on a flat surface for 15 minutes to allow them to warm to room temperature.

11.3.5.2 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.5.3 Remove a 10-µL aliquot from the spiking suspension and apply it to the center of a well.

11.3.5.4 Before removing subsequent aliquots, cap the tube and gently invert it three times to ensure that the oocysts or cysts are in suspension.

11.3.5.5 Ten wells must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Air-dry the well slides. Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

11.3.5.6 Positive and negative controls must be prepared.

11.3.5.6.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts or cysts to the center of a well and distribute evenly over the well area.

11.3.5.6.2 For the negative control, pipette 50 µL of PBS onto the center of a well and spread it over the well area with a pipette tip.
11.3.5.6.3 Air-dry the control slides.

11.3.5.7 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

11.3.5.8 Follow the manufacturer's instructions (Section 7.6) in applying the stain to the slide.

11.3.5.9 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

11.3.5.10 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with a paper towel or other absorbent material. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

11.3.5.11 Add mounting medium (Section 7.8) to each well.

11.3.5.12 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.

11.3.5.13 Record the date and time that staining was completed. If slides will not be read immediately, store in a humid chamber in the dark at 0 °C to 8 °C until ready for examination.

11.3.5.14 After examination of the 10 wells, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6 Enumeration of spiking suspensions using membrane filters

NOTE: Spiking suspensions enumerated using membrane filters must be used within 24 hours of application of the filters to the slides.

11.3.6.1 Pre-coat the glass funnels with Sigmacote® by placing the funnel in a large Petri dish and applying 5-mL of Sigmacoat® to the funnel opening using a pipette and allowing it to run down the inside of the funnel. Repeat for all funnels to be used. The pooled Sigmacoat® may be returned to the bottle for re-use. Place the funnels at 35 °C or 41 °C for approximately 5 minutes to dry.

11.3.6.2 Place foil around the bottoms of the 100 × 15 mm Petri dishes.

11.3.6.3 Filter-sterilize (Section 6.19) approximately 10 mL of PBS pH

7.2 (Section 7. 9. 4). Dilute detection reagent (Section 7.7) as per manufacturer's instructions using sterile PBS. Multiply the anticipated number of filters to be stained by 100 mL to calculate total volume of stain required. Divide the total volume required by 5 to obtain the microliters of antibody necessary. Subtract the volume of antibody from the total stain volume to obtain the required microliters of sterile PBS to add to the antibody.

11.3.6.4 Label the tops of foil-covered, 60 × 15 mm Petri dishes for 10 spiking suspensions plus positive and negative staining controls and multiple filter blanks controls (one negative control, plus a blank after every five sample filters to control for carry-over). Create a humid chamber by laying damp paper towels on the bottom of a stain tray (the inverted foil-lined Petri dishes will protect filters from light and prevent evaporation during incubation).

11.3.6.5 Place a decontaminated and cleaned filter holder base (Section 6.4.8.1) into each of the three ports of the vacuum manifold (Section 6.4.8.2).

11.3.6.6 Pour approximately 10 mL of 0.01% Tween 20 into a 60 × 15 mm Petri dish.

11.3.6.7 Using forceps, moisten a 1.2-µm cellulose-acetate support membrane (Section 6.4.8.3) in the 0.01% Tween 20 and place it on the fritted glass support of one of the filter bases. Moisten a polycarbonate filter (Section

6.4.8.4) the same way and position it on top of the cellulose-acetate support membrane. Carefully clamp the glass funnel to the loaded filter support. Repeat for the other two filters.

11.3.6.8 Add 5 mL of 0.01% Tween 20 to each of the three filtration units and allow to stand.

11.3.6.9 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.6.10 Using a micropipettor, sequentially remove two, 10-µL aliquots from the spiking suspension and pipet into the 5 mL of 0.01% Tween 20 standing in the unit. Rinse the pipet tip twice after each addition. Apply 10 µL of 0.01% Tween 20 to the third unit to serve as the negative control. Apply vacuum at 2" Hg and allow liquid to drain to miniscus, then close off vacuum. Pipet 10 mL of reagent water into each funnel and drain to miniscus, closing off the vacuum. Repeat the rinse and drain all fluid, close off the vacuum.

11.3.6.11 Pipet 100 mL of diluted antibody to the center of the bottom of a 60 × 15 mm Petri dish for each sample.

11.3.6.12 Unclamp the top funnel and transfer each cellulose acetate support membrane/ polycarbonate filter combination onto the drop of stain using forceps (apply each membrane/filter combination to a different Petri dish containing stain). Roll the filter into the drop to exclude air. Place the small Petri dish containing the filter onto the damp towel and cover with the corresponding labeled foil-covered top. Incubate for approximately 45 minutes at room temperature.

11.3.6.13 Reclamp the top funnels, apply vacuum and rinse each three times, each time with 20 mL of reagent water.

11.3.6.14 Repeat Sections 11.3.6.4 through 11.3.6.10 for the next three samples (if that the diluted spiking suspension has sat less than 15 minutes, reduce the suspension vortex time to 60 seconds). Ten, 10-µL spiking suspension aliquots must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose.

Include a filter blank sample at a frequency of every five samples; rotate the position of filter blank to eventually include all three filter placements.
11.3.6.15 Repeat Sections 11.3.6.4 through 11.3.6.10 until the 10-µL spiking suspensions have been filtered. The last batch should include a 10-µL 0.01 Tween 20 blank control and 20 µL of positive control antigen as a positive staining control.
11.3.6.16 Label slides. After incubation is complete, for each sample, transfer the cellulose acetate filter support and polycarbonate filter from drop of stain and place on fritted glass support. Cycle vacuum on and off briefly to remove excess fluid. Peel the top polycarbonate filter off the supporting filter and place on labeled slide. Discard cellulose acetate filter support. Mount and apply coverslips to the filters immediately to avoid drying.
11.3.6.17 To each slide, add 20 µL of mounting medium (Section 7.8).
11.3.6.18 Apply a coverslip. Seal the edges of the coverslip onto the slide using clear nail polish. (Sealing may be delayed until cover slips are applied to all slides.)

11.3.6.19 Record the date and time that staining was completed. If slides will not be read immediately, store sealed slides in a closed container in the dark at 0 $^{\circ}$C to 8 $^{\circ}$C until ready for examination.

11.3.6.20 After examination of the 10 slides, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6.21 If oocysts or cysts are detected on the filter blanks, modify the rinse procedure to ensure that no carryover occurs and repeat enumeration.

11.4 Procedure for spiking samples in the laboratory with enumerated spiking suspensions.

11.4.1 Arrange a bottom-dispensing container to feed the filter.

11.4.2 For initial precision and recovery (Section 9.4) and ongoing precision and recovery (Section 9.7) samples, fill the container with a volume of reagent water equal to the volume of the field samples analyzed in the analytical batch. For matrix spike samples (Section 9.5), fill the container with the field sample to be spiked. Continuously mix the sample (using a stir bar and stir plate for smaller-volume samples and alternate means for larger-volume samples).

11.4.3 Vortex the spiking suspension(s) (Section 11.2 or Section 11.3) for a minimum of 2 minutes.

11.4.3.1 For flow cytometer–enumerated suspensions (where the entire volume of a spiking suspension tube will be used):

11.4.3.1.1 Add 500 µL of the diluted antifoam to the tube containing the spiking suspension and vortex for 2 minutes.
11.4.3.1.2 Pour the suspension into the sample container.
11.4.3.1.3 Add 20 mL of reagent water to the empty tube, cap, vortex 10 seconds to rinse, and add the rinsate to the carboy.
11.4.3.1.4 Repeat this rinse using another 20 mL of reagent water.
11.4.3.1.5 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet.

11.4.3.1.6 Proceed to Section 11.4.4.

11.4.3.2 For manually enumerated spiking suspensions:

11.4.3.2.1 Rinse a pipette tip with 0.01% Tween-20 once, then rinse with the well-mixed spiking suspension a minimum of five times before pulling an aliquot to be used to spike the container.

11.4.3.2.2 Add the spiking suspension(s) to the carboy, delivering the aliquot below the surface of the water.

11.4.3.2.3 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet. Proceed to Section 11.4.4

11.4.4 Allow the spiking suspensions to mix for approximately 1 minute in the container.

11.4.5 Turn on the pump and allow the flow rate to stabilize. Set flow at the rate designated for the filter being used. As the carboy is depleted, check the flow rate and adjust if necessary.

11.4.6 When the water level approaches the discharge port of the carboy, tilt the container so that it is completely emptied. At that time, turn off the pump and add sufficient reagent water to the container to rinse. Swirl the contents to rinse down the sides.

11.4.7 Turn on the pump. Allow all of the water to flow through the filter and turn off the pump.

12.0 Sample Filtration and Elution

12.1 A water sample is filtered according to the procedures in Section 12.2. Alternate procedures may be used if the laboratory first demonstrates that the alternate procedure provides equivalent or superior performance per Section 9.1.2.

NOTE: Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

12.2 Capsule filtration (adapted from Reference 20.9). This procedure was validated using 10-L sample volumes. Alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

NOTE: The filtration procedures specified in Section 12.2.1 - 12.2.5.3 are specific to laboratory filtration of a bulk sample, and reflect the procedures used during the interlaboratory validation of this method (Reference 20.10). These procedures may require modification if samples will be filtered in the field.

12.2.1 Flow rate adjustment

12.2.1.1 Connect the sampling system, minus the capsule, to a carboy filled with reagent water (Figure 3).

12.2.1.2 Turn on the pump and adjust the flow rate to 2.0 L/min.

12.2.1.3 Allow 2 to 10 L of reagent water to flush the system. Adjust the pump speed as required during this period. Turn off the pump when the flow rate has been adjusted.

12.2.2 Install the capsule filter in the line, securing the inlet and outlet ends with the appropriate clamps/fittings.

12.2.3 Record the sample number, sample turbidity (if not provided with the field sample), sample type, and sample filtration start date and time on a bench sheet.

12.2.4 Filtration

12.2.4.1 Connect the sampling system to the field carboy of sample water, or transfer the sample water to the laboratory carboy used in Section
12.2.1.1. If the sample will be filtered from a field carboy, a spigot (Section 6.2.1) can be used with the carboy to facilitate sample filtration.

NOTE: If the bulk field sample is transferred to a laboratory carboy, the laboratory carboy must be cleaned and disinfected before it is used with another field sample.

12.2.4.2 Place the drain end of the sampling system tubing into an empty graduated container with a capacity of 10 to 15 L, calibrated at 9.0, 9.5, 10.0, 10.5, and 11.0 L (Section 6.18). This container will be used to determine the sample volume filtered. Alternately, connect a flow meter (Section 6.3.4) downstream of the filter, and record the initial meter reading.

12.2.4.3 Allow the carboy discharge tube and capsule to fill with sample water. Vent residual air using the bleed valve/vent port, gently shaking or tapping the capsule, if necessary. Turn on the pump to start water flowing through the filter. Verify that the flow rate is 2 L/min.

12.2.4.4 After all of the sample has passed through the filter, turn off the pump. Allow the pressure to decrease until flow stops. (If the sample was filtered in the field, and excess sample remains in the filter upon receipt in the laboratory, pull the remaining sample volume through the filter before eluting the filter [Section 12.2.6].)

12.2.5 Disassembly

12.2.5.1 Disconnect the inlet end of the capsule filter assembly while maintaining the level of the inlet fitting above the level of the outlet fitting to prevent backwashing and the loss of oocysts and cysts from the filter. Restart the pump and allow as much water to drain as possible. Turn off the pump.

12.2.5.2 Based on the water level in the graduated container or meter reading, record the volume filtered on the bench sheet to the nearest quarter liter. Discard the contents of the graduated container.

12.2.5.3 Loosen the outlet fitting, then cap the inlet and outlet fittings.

12.2.6 Elution

NOTE: The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.

12.2.6.1 Setup

12.2.6.1.1 Assemble the laboratory shaker with the clamps aligned vertically so that the filters will be aligned horizontally. Extend the clamp arms to their maximum distance from the horizontal shaker rods to maximize the shaking action.

12.2.6.1.2 Prepare sufficient elution buffer so that all samples to be eluted that day can be eluted with the same batch of buffer. Elution may require up to 275 mL of buffer per sample.

12.2.6.1.3 Designate at least one 250-mL conical centrifuge tube for each sample and label with the sample number.

12.2.6.2 Elution

12.2.6.2.1 Record the elution date and time on the bench sheet. Using a ring stand or other means, clamp each capsule in a vertical position with the inlet end up. Remove the inlet cap and allow the liquid level to stabilize.

12.2.6.2.2 Pour elution buffer through the inlet fitting. Sufficient elution buffer must be added to cover the pleated white membrane with buffer solution. Replace the inlet cap and clamp the cap in place.

12.2.6.2.3 Securely clamp the capsule in one of the clamps on the laboratory shaker with the bleed valve positioned at the top on a vertical axis (in the 12 o'clock position). Turn on the shaker and set the speed to maximum (approximately 900 rpm). Agitate the capsule for approximately 5 minutes. Time the agitation using a lab timer, rather than the timer on the shaker to ensure accurate time measurement.

12.2.6.2.4 Remove the filter from the shaker, remove the inlet cap, and pour the contents of the capsule into the 250-mL conical centrifuge tube.

12.2.6.2.5 Clamp the capsule vertically with the inlet end up and add sufficient volume of elution buffer through the inlet fitting to cover the pleated membrane. Replace the inlet cap.

12.2.6.2.6 Return the capsule to the shaker with the bleed valve positioned at the 4 o'clock position. Turn on the shaker and agitate the capsule for approximately 5 minutes.

12.2.6.2.7 Remove the filter from the shaker, but leave the elution buffer in the capsule. Re-clamp the capsule to the shaker at the 8 o'clock position. Turn on the shaker and agitate the capsule for a final 5 minutes.

12.2.6.2.8 Remove the filter from the shaker and pour the contents into the 250-mL centrifuge tube. Rinse down the inside of the capsule filter walls with reagent water or elution buffer using a squirt bottle inserted in the inlet end of the capsule. Invert the capsule filter over the centrifuge tube and ensure that as much of the eluate as possible has been transferred.

12.2.7 Proceed to Section 13.0 for concentration and separation (purification).

13.0 Sample Concentration and Separation (Purification)

13.1 During concentration and separation, the filter eluate is concentrated through centrifugation, and the oocysts and cysts in the sample are separated from other particulates through immunomagnetic separation (IMS). Alternate procedures and products may be used if the laboratory first demonstrates equivalent or superior performance as per Section 9.1.2.

13.2 Adjustment of pellet volume

13.2.1 Centrifuge the 250-mL centrifuge tube containing the capsule filter eluate at 1500 × G for 15 minutes. Allow the centrifuge to coast to a stop—do not use the brake. Record the pellet volume (volume of solids) on the bench sheet.

NOTE: Recoveries may be improved if centrifugation force is increased to 2000 × G. However, do not use this higher force if the sample contains sand or other gritty material that may degrade the condition of any oocysts and/or cysts in the sample.

13.2.2 Using a Pasteur pipette, carefully aspirate the supernatant to 5 mL above the pellet. Extra care must be taken to avoid aspirating oocysts and cysts during this step, particularly if the sample is reagent water (e.g. initial or ongoing precision and recovery sample).
13.2.3 If the packed pellet volume is ≤ 0.5 mL, vortex the tube vigorously until pellet is completely resuspended. Swirl the centrifuge tube gently to reduce any foaming after vortexing. Record the resuspended pellet volume on the bench sheet. Proceed to Section 13.3.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts and/or cysts in the sample is not compromised.

13.2.4 If the packed pellet volume is > 0.5 mL, the concentrate needs to be separated into multiple subsamples (a subsample is equivalent to no greater than 0.5 mL of packed pellet material, the recommended maximum amount of particulate material to process through the subsequent purification and examination steps in the method). Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples:

$$\text{total volume (mL) required} = \frac{\text{pellet volume}}{0.5\ \text{mL}} \times 5\ \text{mL}$$

(For example, if the packed pellet volume is 1.2 mL, the total volume required is 12 mL.) Add reagent water to the centrifuge tube to bring the total volume to the level calculated above. Vortex the tube vigorously for 10 to 15 seconds to completely resuspend the pellet. Record the resuspended pellet volume on the bench sheet.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts in the sample is not compromised.

13.2.4.1 Analysis of entire sample. If analysis of the entire sample is required, determine the number of subsamples to be processed independently through the remainder of the method:

13.2.4.1.1 Calculate number of subsamples: Divide the total volume in the centrifuge tube by 5 mL and round up to the nearest integer (for example, if the resuspended volume in Section 13.2.4 is 12 mL, then the number of subsamples would be 12 mL / 5 mL = 2.4, rounded = 3 subsamples).

13.2.4.1.2 Determine volume of resuspended concentrate per subsample. Divide the total volume in the centrifuge tube by the calculated number of subsamples (for 13.2.4.1.3 example, if the resuspended volume in Section 13.2.4 is 12 mL, then the volume to use for each subsample = 12 mL / 3 subsamples = 4 mL).

Process sub-samples through IMS. Proceed to Section 13.3, and transfer aliquots of the resuspended concentrate equivalent to the volume in the previous step to multiple, flat-sided sample tubes in Section 13.3.2.1. Process the sample as multiple, independent subsamples from Section 13.3 onward, including the preparation and examination of separate slides for each aliquot. Record the volume of resuspended concentrate transferred to IMS on the bench sheet (this will be equal to the volume recorded in Section 13.2.4). Also record the number of subsamples processed independently through the method on the bench sheet.

13.2.4.2 Analysis of partial sample. If not all of the concentrate will be examined, proceed to Section 13.3, and transfer one or more 5-mL aliquots of the resuspended concentrate to one or more flat-sided sample tubes in Section 13.3.2.1. Record the volume of resuspended concentrate transferred to IMS on the bench sheet. To determine the volume analyzed, calculate the percent of the concentrate examined using the following formula:

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS}}{\text{total volume of resuspended concentrate in Section 13.2.4}} \times 100\%$$

Then multiply the volume filtered (Section 12.2.5.2) by this percentage to determine the volume analyzed.

13.3 IMS procedure (adapted from Reference 20.11)

NOTE: The IMS procedure should be performed on a bench top with all materials at room temperature, ranging from 15 ^{o}C to 25 ^{o}C.

13.3.1 Preparation and addition of reagents

13.3.1.1 Prepare a 1X dilution of SL-buffer-A from the 10X SL-buffer-A (clear, colorless solution) supplied. Use reagent water (demineralized; Section 7.3) as the diluent. For every 1 mL of 1X SL-buffer-A required, take 100 µL of 10X SL-buffer-A and make up to 1 mL with the diluent water. A volume of 1.5 mL of 1X SL-buffer-A will be required per sample or subsample on which the Dynal IMS procedure is performed.

13.3.1.2 For each sample or subsample (Section 13.2) to be processed through IMS, add 1 mL of the 10X SL-buffer-A (supplied—not the diluted 1X SL-buffer-A) to a flat-sided tube (Section 6.5.4).

13.3.1.3 For each subsample, add 1 mL of the 10X SL-buffer-B (supplied— magenta solution) to the flat-sided tube containing the 10X SL-buffer-A.

13.3.2 Oocyst and cyst capture

13.3.2.1 Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample concentrate from Section 13.2 to the flat-sided tube(s) containing the SL-buffer. If all of the concentrate is used, rinse the centrifuge tube twice with reagent water and add the rinsate to the flat-sided tube containing the concentrate (or to the tube containing the first subsample, if multiple subsamples will be processed). Each of the two rinses should be half the volume needed to bring the total volume in the flat-sided sample tube to 10 mL. (For example, if 5 mL was

transferred after resuspension of the pellet, the centrifuge tube would be rinsed twice with 2.5 mL of reagent water to bring the total volume in the flat-sided tube to 10 mL.) Visually inspect the centrifuge tube after completing the transfer to ensure that no concentrate remains. If multiple subsamples will be processed, bring the volume in the remaining flat-sided tubes to 10 mL with reagent water. Label the flat-sided tube(s) with the sample number (and subsample letters).

13.3.2.2 Vortex the Dynabeads®Crypto-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the sample tube and making sure that there is no residual pellet at the bottom.

13.3.2.3 Add 100 µL of the resuspended Dynabeads®Crypto-Combo (Section 13.3.2.2) to the sample tube(s) containing the water sample concentrate and SL-buffer.

13.3.2.4 Vortex the Dynabeads®Giardia-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the tube and making sure that there is no residual pellet at the bottom.

13.3.2.5 Add 100 µL of the resuspended Dynabeads®Giardia-Combo (Section 13.3.2.4) to the sample tube(s) containing the water sample concentrate, Dynabeads®Crypto-Combo, and SL-buffer.

13.3.2.6 Affix the sample tube(s) to a rotating mixer and rotate at approximately 18 rpm for 1 hour at room temperature.

13.3.2.7 After rotating for 1 hour, remove each sample tube from the mixer and place the tube in the magnetic particle concentrator (MPC-1) with flat side of the tube toward the magnet.

13.3.2.8 Without removing the sample tube from the MPC-1, place the magnet side of the MPC-1 downwards, so the tube is horizontal and the flat side of the tube is facing down.

13.3.2.9 Gently rock the sample tube by hand end-to-end through approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 minutes with approximately one tilt per second.

13.3.2.10 Ensure that the tilting action is continued throughout this period to prevent binding of low-mass, magnetic or magnetizable material. If the sample in the MPC-1 is allowed to stand motionless for more than 10 seconds, repeat Section 13.3.2.9 before continuing to Section 13.3.2.11.

13.3.2.11 Return the MPC-1 to the upright position, sample tube vertical, with cap at top. Immediately remove the cap and, keeping the flat side of the tube on top, pour off all of the supernatant from the tube held in the MPC-1 into a suitable container. Do not shake the tube and do not remove the tube from MPC-1 during this step.

13.3.2.12 Remove the sample tube from the MPC-1 and resuspend the sample in 1-mL 1X SL-buffer-A (prepared from 10X SL-buffer-A stock—supplied). Mix very gently to resuspend all material in the tube. Do not vortex.

13.3.2.13 Quantitatively transfer (transfer followed by two rinses) all the liquid from the sample tube to a labeled, 1.5-mL microcentrifuge tube. Use 1 mL of 1X SL-buffer-A to perform the first rinse and 0.5 mL of

reagent water for the second rinse. Liberally rinse down the sides of the Leighton tube before transferring. Allow the flat-sided sample tube to sit for a minimum of 1 minute after transfer of the second rinse volume, then use a pipette to collect any residual volume that drips down to the bottom of the tube to ensure that as much sample volume is recovered as possible. Ensure that all of the liquid and beads are transferred.

13.3.2.14 Place the microcentrifuge tube into the second magnetic particle concentrator (MPC-M), with its magnetic strip in place.

13.3.2.15 Without removing the microcentrifuge tube from MPC-M, gently rock/roll the tube through 180° by hand. Continue for approximately 1 minute with approximately one 180° roll/rock per second. At the end of this step, the beads should produce a distinct brown dot at the back of the tube.

13.3.2.16 Immediately aspirate the supernatant from the tube and cap held in the MPC-M. If more than one sample is being processed, conduct three 90° rock/roll actions before removing the supernatant from each tube. Take care not to disturb the material attached to the wall of the tube adjacent to the magnet. Do not shake the tube. Do not remove the tube from MPC-M while conducting these steps.

13.3.3 Dissociation of beads/oocyst/cyst complex

NOTE: Two acid dissociations are required.

13.3.3.1 Remove the magnetic strip from the MPC-M.

13.3.3.2 Add 50 µL of 0.1 N HCl, then vortex at the highest setting for approximately 50 seconds.

NOTE: The laboratory should use 0.1-N standards purchased directly from a vendor, rather than adjusting the normality in-house.

13.3.3.3 Place the tube in the MPC-M without the magnetic strip in place and allow to stand in a vertical position for at least 10 minutes at room temperature.

13.3.3.4 Vortex vigorously for approximately 30 seconds.

13.3.3.5 Ensure that all of the sample is at the base of the tube. Place the microcentrifuge tube in the MPC-M.

13.3.3.6 Replace magnetic strip in MPC-M and allow the tube to stand undisturbed for a minimum of 10 seconds.

13.3.3.7 Prepare a well slide for sample screening and label the slide.

13.3.3.8 Add 5 µL of 1.0 N NaOH to the sample wells of two well slides (add 10 µL to the sample well of one well slide if the volume from the two required dissociations will be added to the same slide).

NOTE: The laboratory should use 1.0-N standards purchased directly from a vendor rather than adjusting the normality in-house.

13.3.3.9 Without removing the microcentrifuge tube from the MPC-M, transfer all of the sample from the microcentrifuge tube in the MPC-M to the sample well with the NaOH. Do not disturb the beads at the back wall of the tube. Ensure that all of the fluid is transferred.

13.3.3.10 Do not discard the beads or microcentrifuge tube after transferring the volume from the first acid dissociation to the well slide. Perform the steps in Sections 13.3.3.1 through 13.3.3.9 a second time. The volume from the second dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.

NOTE: If one slide is used, exert extra care when using Dynal Spot-On slides to ensure that the sample stays within the smaller-diameter wells on these slides.

13.3.3.11 Record the date and time the purified sample was applied to the slide(s).

13.3.3.12 Air-dry the sample on the well slide(s). Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

14.0 Sample Staining

NOTE: The sample must be stained within 72 hours of application of the purified sample to the slide.

14.1 Prepare positive and negative controls.

14.1.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts and 200 to 400 cysts to the center of a well.

14.1.2 For the negative control, pipette 50 µL of 150 mM PBS (Section 7.6.4) into the center of a well and spread it over the well area with a pipette tip.

14.1.3 Air-dry the control slides (see Section 13.3.3.12 for guidance).

14.2 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

14.3 Follow manufacturer's instructions in applying stain to slide.

14.4 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

14.5 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.6 Apply 50 µL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.7.2) to each well. Allow to stand at room temperature for a minimum of 1 minute. (The solution concentration may be increased up to 1 µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.)

14.7 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.8 Add mounting medium (Section 7.8) to each well.
14.9 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.
14.10 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

15.0 Examination

NOTE: Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.

15.1 Scanning technique: Scan each well in a systematic fashion. An up-and-down or a side-to-side scanning pattern may be used (Figure 4).
15.2 Examination using immunofluorescence assay (FA), 4',6-diamidino-2-phenylindole (DAPI) staining characteristics, and differential interference contrast (DIC) microscopy. The minimum magnification requirements for each type of examination are noted below.
NOTE: All shape and measurements must be determined using 1000X magnification and reported to the nearest 0.5 µm.

Record examination results for Cryptosporidium oocysts on a Cryptosporidium report form; record examination results for Giardia cysts on a Giardia report form. All oocysts and cysts that meet the criteria specified in Sections 15.2.2 and 15.2.3, less atypical organisms specifically identified as non-target organisms by DIC or DAPI (e.g. possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc), must be reported.

15.2.1 Positive and negative staining control.

15.2.1.1 Each analyst must characterize a minimum of three Cryptosporidium oocysts and three Giardia cysts on the positive staining control slide before examining field sample slides. This characterization must be performed by each analyst during each microscope examination session.

FITC examination must be conducted at a minimum of 200X total magnification, DAPI examination must be conducted at a minimum of 400X, and DIC examination must be conducted at a minimum of 1000X. Size, shape, and DIC and DAPI characteristics of the three Cryptosporidium oocysts and Giardia cysts must be recorded by the analyst on a microscope log. The analyst also must indicate on each sample report form whether the positive staining control was acceptable.

15.2.1.2 Examine the negative staining control to confirm that it does not contain any oocysts or cysts (Section 14.1). Indicate on each sample report form whether the negative staining control was acceptable.

15.2.1.3 If the positive staining control contains oocysts and cysts within the expected range and at the appropriate fluorescence for both FA and DAPI, and the negative staining control does not contain any oocysts or cysts (Section 14.1), proceed to Sections 15.2.2 and 15.2.3.

15.2.2 Sample examination—Cryptosporidium

15.2.2.1 FITC examination (the analyst must use a minimum of 200X total magnification). Use epifluorescence to scan the entire well for apple-green fluorescence of oocyst and cyst shapes. When brilliant apple-green fluorescing ovoid or spherical objects 4 to 6 µm in diameter are observed with brightly highlighted edges, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.2.2), then to DIC (Section 15.2.2.3).

15.2.2.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one of the following characteristics: (a) Light blue internal staining (no distinct nuclei) with a green rim (b) Intense blue internal staining (c) Up to four distinct, sky-blue nuclei Record oocysts in category (a) as DAPI negative; record oocysts in categories (b) and (c) as DAPI positive.

15.2.2.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Cryptosporidium oocysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each apple-green fluorescing object as: (a) An empty Cryptosporidium oocyst (b) A Cryptosporidium oocyst with amorphous structure (c) A Cryptosporidium oocyst with internal structure (one to four sporozoites/oocyst)

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of sporozoites (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics. Although not a defining characteristic, surface oocyst folds may be observed in some specimens.

NOTE: All measurements must be made at 1000X magnification.

15.2.3 Sample examination—Giardia

15.2.3.1 FITC examination (the analyst must use a minimum of 200X total magnification). When brilliant apple-green fluorescing round to oval objects (8 - 18 µm long by 5 - 15 µm wide) are observed, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.3.2) then to DIC (Section 15.2.3.3).

15.2.3.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one or more of the following characteristics: (a) Light blue internal staining (no distinct nuclei) and a green rim (b) Intense blue internal staining (c) Two to four sky-blue nuclei Record cysts in category (a) as DAPI negative; record cysts in categories (b) and (c) as DAPI positive.

15.2.3.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Giardia cysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each object meeting the criteria specified in Sections 15.2.3.1 - 15.2.3.3 as one of the following, based on DIC examination: (a) An empty Giardia cyst (b) A Giardia cyst with amorphous structure (c) A Giardia cyst with one type of internal structure (nuclei, median body, or axonemes), or (d) A Giardia cyst with more than one type of internal structure.

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of nuclei and presence of median body or axonemes (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics.

NOTE: All measurements must be made at 1000X magnification.

15.2.4 Record the date and time that sample examination was completed on the report form. 15.2.5 Report Cryptosporidium and Giardia concentrations as oocysts/L and cysts/L.

16.0 Analysis of Complex Samples

16.1 Some samples may contain high levels (>1000/L) of oocysts and cysts and/or interfering organisms, substances, or materials. Some samples may clog the filter (Section 12.0); others will not allow separation of the oocysts and cysts from the retentate or eluate; and others may contain materials that preclude or confuse microscopic examination.

16.2 If the sample holding time has not been exceeded and a full-volume sample cannot be filtered, dilute an aliquot of sample with reagent water and filter this smaller aliquot (Section 12.0). This dilution must be recorded and reported with the results.

16.3 If the holding times for the sample and for microscopic examination of the cleaned up retentate/eluate have been exceeded, the site should be re-sampled. If this is not possible, the results should be qualified accordingly.

17.0 Method Performance

17.1 Method acceptance criteria are shown in Tables 3 and 4 in Section 21.0. The initial and ongoing precision and recovery criteria are based on the results of spiked reagent water samples analyzed during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike and matrix spike duplicate criteria are based on spiked source water data generated during the interlaboratory validation study of Method 1623 involving 11 laboratories and 11 raw surface water matrices across the U.S. (Reference 20.10).

NOTE: Some sample matrices may prevent the MS acceptance criteria in Tables 3 and 4 to be met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

18.0 Pollution Prevention

18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management

19.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of these requirements can be found in the *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

19.2 Samples, reference materials, and equipment known or suspected to have viable oocysts or cysts attached or contained must be sterilized prior to disposal.

19.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

20.0 References

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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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20.11 "Dynabeads® GC-Combo," Dynal Microbiology R&D, P.O. Box 8146 Dep., 0212 Oslo, Norway (September 1998, Revision no. 01).
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).
20.13 Connell, K., J. Scheller, K. Miller, and C.C. Rodgers, 2000. Performance of Methods 1622 and 1623 in the ICR Supplemental Surveys. Proceedings, American Water Works Association Water Quality Technology Conference, November 5 - 9, 2000, Salt Lake City, UT.

21.0 Tables and Figures

Table 1. Method Holding Times (See Section 8.2 for details)

Sample Processing Step	Maximum Allowable Time between Breaks
Collection	
Filtration	
> Up to 96 hours are permitted between sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field) and initiation of elution	
Elution	
	These steps must be completed in 1 working day
Concentration	
Purification	
Application of purified sample to slide	
Drying of sample	
> Up to 72 hours are permitted from application of the purified sample to the slide to staining	
Staining	
> Up to 7 days are permitted between sample staining and examination	
Examination	

Table 2. Tier 1 and Tier 2 Validation/Equivalency Demonstration Requirements

Test	Description	Tier 1 modification(1)	Tier 2 modification(2)
IPR (Section 9.4)	4 replicates of spiked reagent water	Required. Must be accompanied by a method blank.	Required per laboratory
Method blank (Section 9.6)	Unspiked reagent water	Required	Required per laboratory
MS (Section 9.5.1)	Spiked matrix water	Required on each water to which the modification will be applied and on every 20th sample of that water thereafter. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Not required
MS/MSD (Section 9.5)	2 replicates of spiked matrix water	Recommended, but not required. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Required per laboratory. Each laboratory must analyze a different water.

(1) If a modification will be used only in one laboratory, these tests must be performed and the results must meet all of the QC acceptance criteria in the method (these tests also are required the first time a laboratory uses the validated version of the method).
(2) If nationwide approval of a modification is sought for one type of water matrix (such as surface water), a minimum of 3 laboratories must perform the tests and the results from each lab individually must meet all QC acceptance criteria in the method. If more than 3 laboratories are used in a study, a minimum of 75% of the laboratories must meet all QC acceptance criteria.

NOTE: The initial precision and recovery and ongoing precision and recovery (OPR) acceptance criteria listed in Tables 3 and 4 are based on results from 293 Cryptosporidium OPR samples and 186 Giardia OPR samples analyzed by six laboratories during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike acceptance criteria are based on data generated through interlaboratory validation of Method 1623 (Reference 20.10).

Table 3. Quality Control Acceptance Criteria for *Cryptosporidium*

Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 55
Ongoing precision and recovery (percent)	9.7	11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery[1, 2](as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	13 - 111 61

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries from multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 4.
Quality Control Acceptance Criteria for *Giardia*

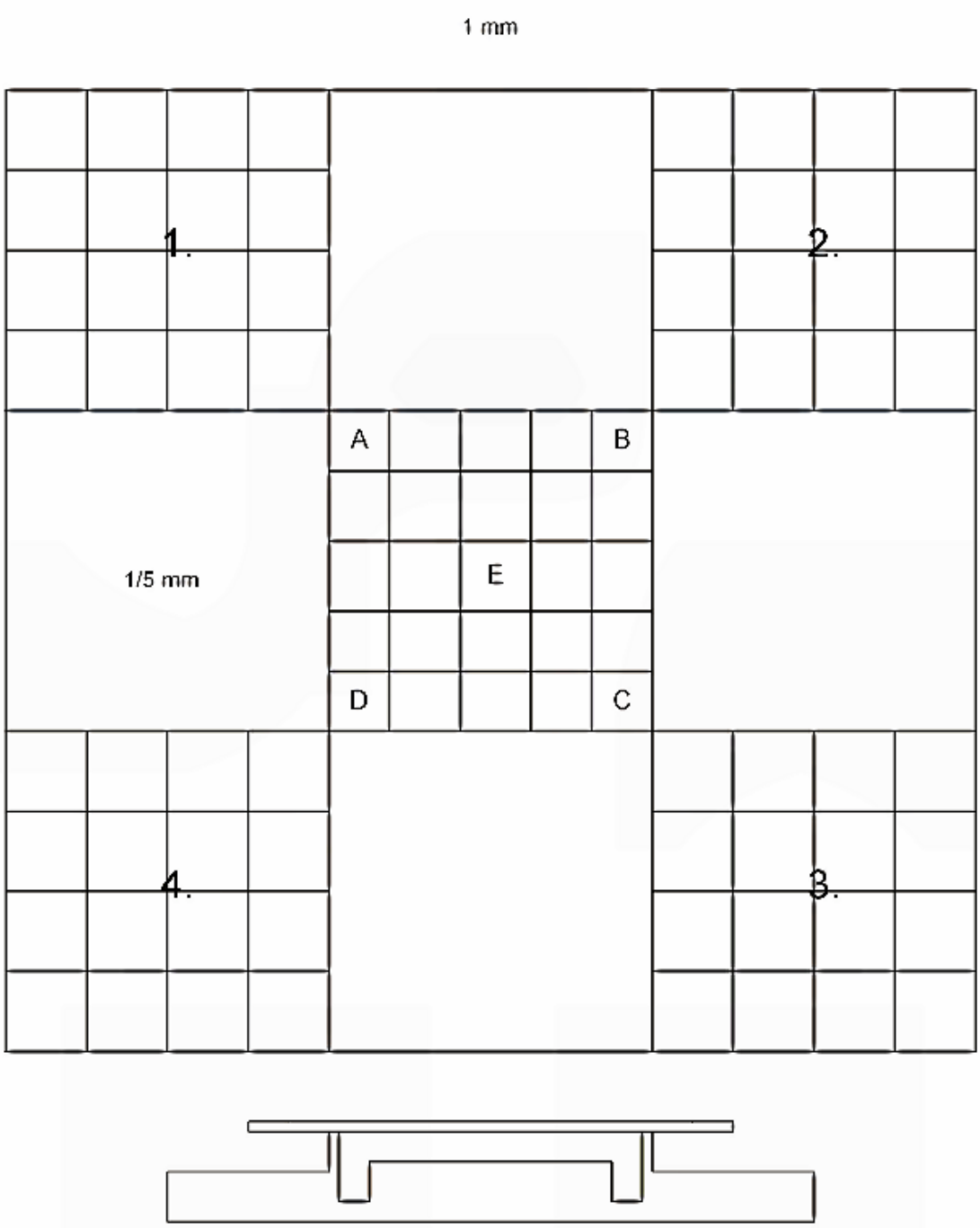
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 49
Ongoing precision and recovery (percent)	9.7 9.5 9.5.2 9.5.2	14 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery* (as percent) Precision (as maximum relative percent difference)		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)

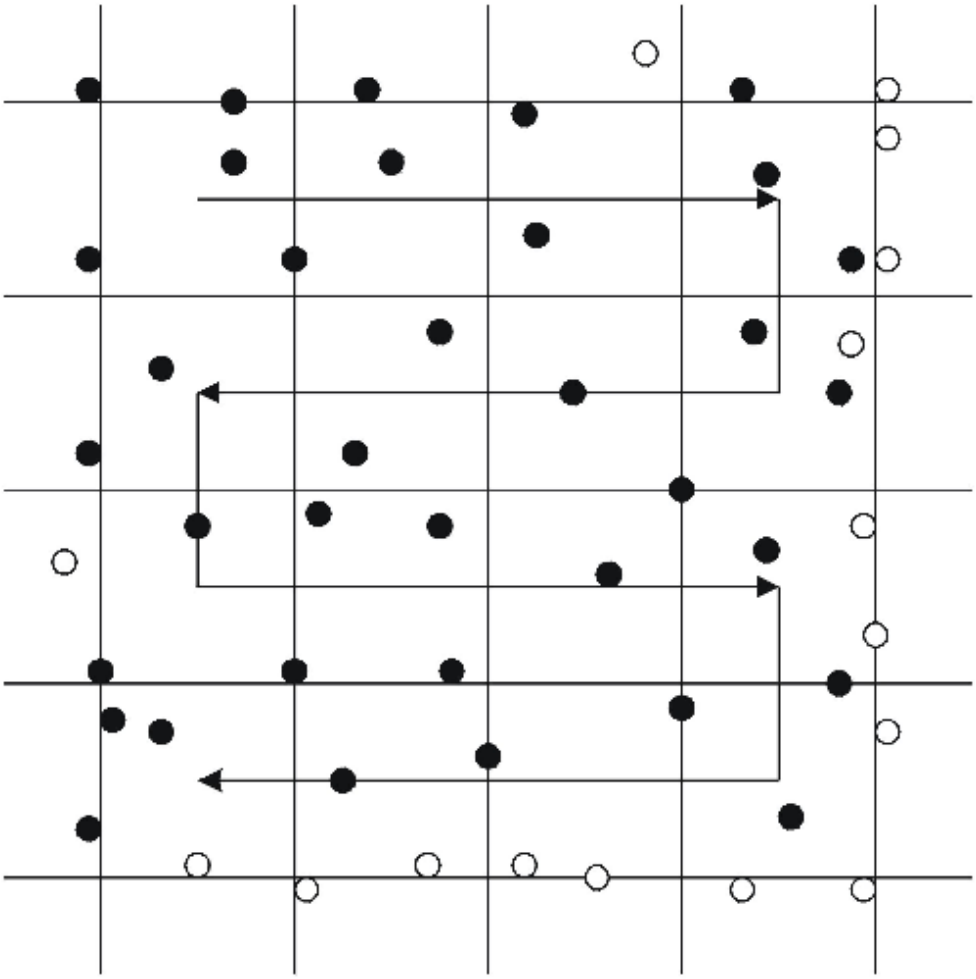
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium*MS Samples in Recovery Range	Percent of 270 *Giardia*MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 50%	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

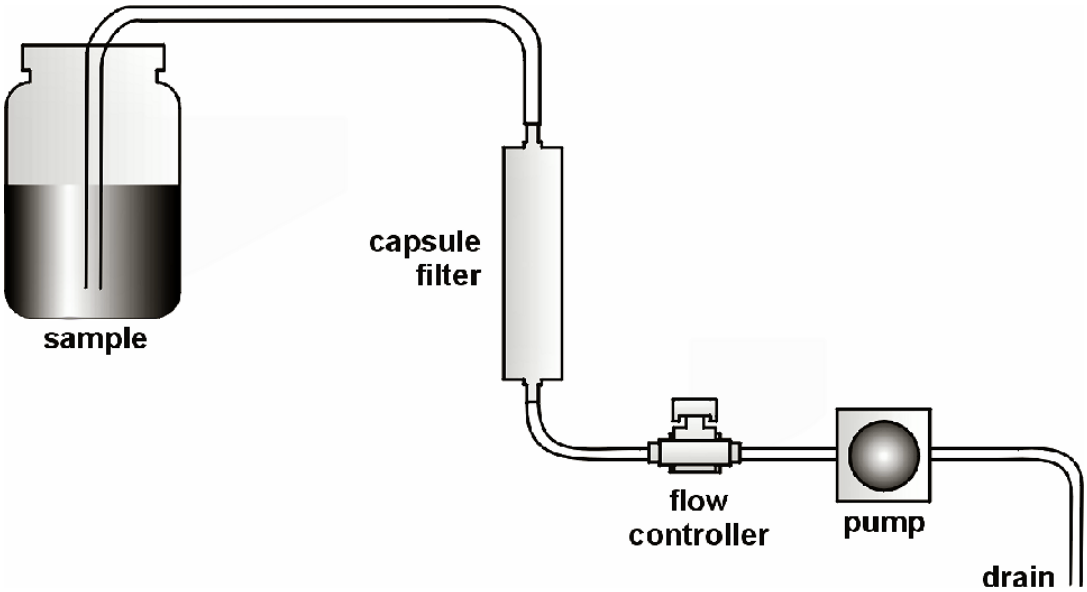
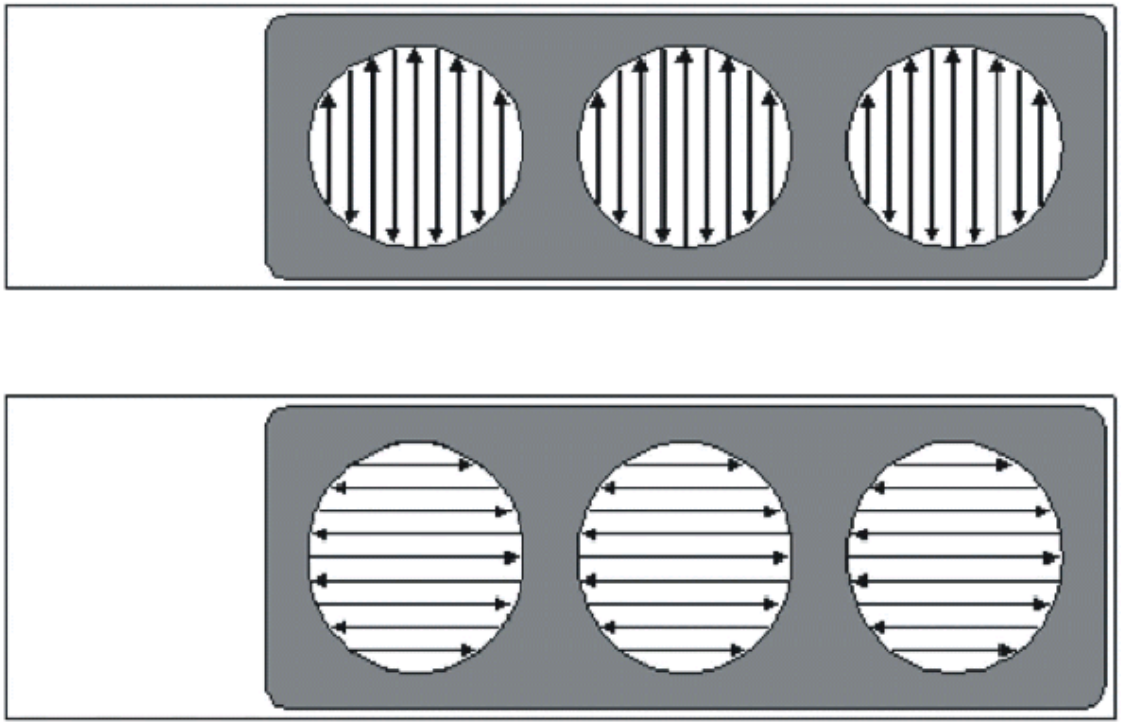


Figure 4. Methods for Scanning a Well Slide



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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16.13 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.

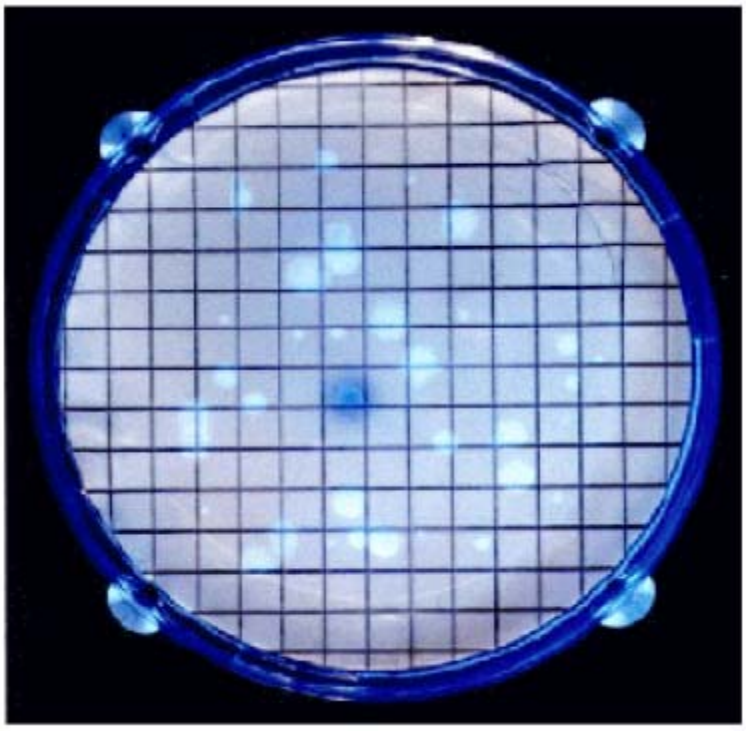

Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

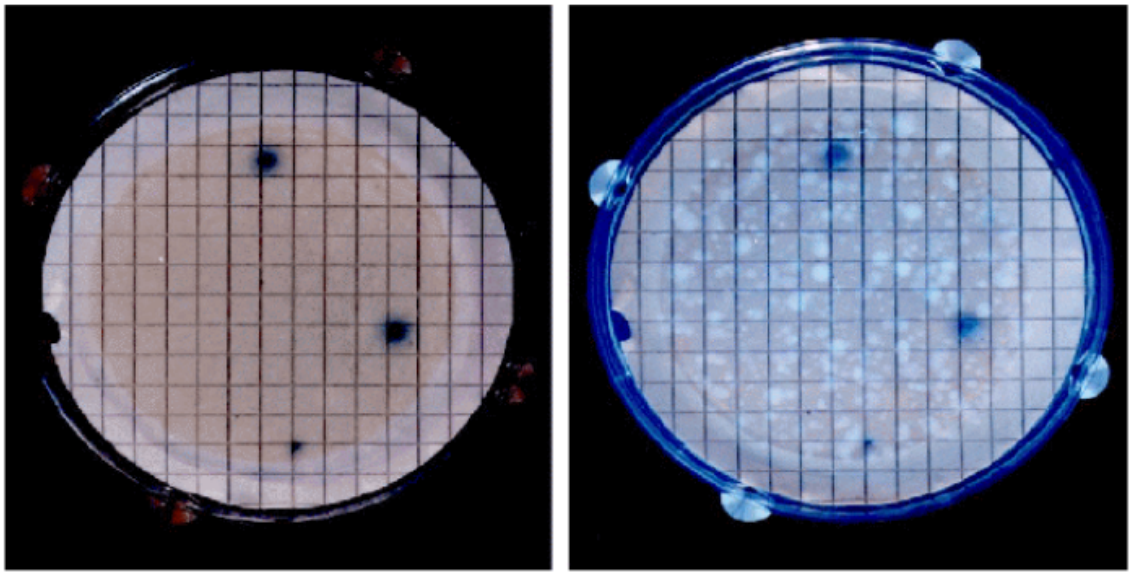

Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other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

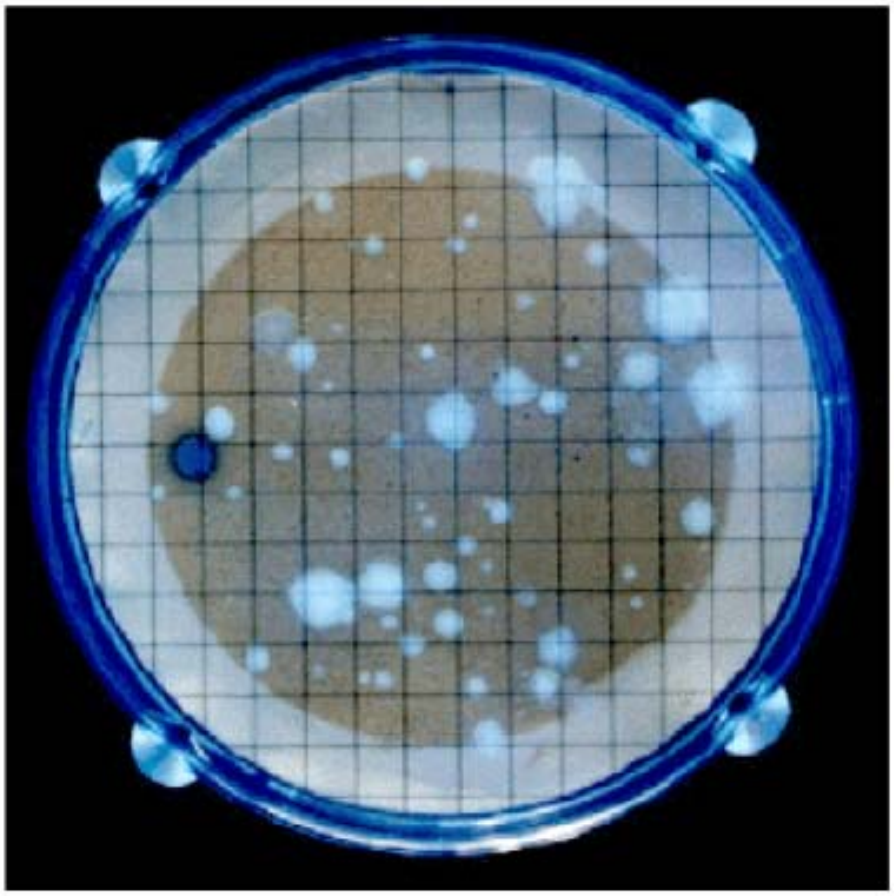

Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

Disclaimer

This method has been validated by the U.S. Environmental Protection Agency through an interlaboratory validation study, and will be proposed for use in drinking water monitoring in the *Federal Register*. This method is not an EPA-approved method until it is promulgated as an approved method in the *Federal Register*.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

Aeromonas is a common genus of bacteria indigenous to surface waters, and may be found in non-chlorinated or low-flow parts of chlorinated water distribution systems. Monitoring their presence in distribution systems is desirable because some aeromonads may be pathogenic and pose a potential human health risk. Method 1605 describes a membrane filtration technique for the detection and enumeration of *Aeromonas* species. This method uses a selective medium that partially inhibits the growth of non-target bacterial species while allowing most species of *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation and the presence of yellow colonies on ampicillin-dextrin agar medium with vancomycin (ADA-V). Yellow colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Safe Drinking Water Act.

Questions concerning this method or its application should be addressed to:

Mary Ann Feige
U.S. EPA Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
26 West Martin Luther King Drive
Cincinnati, OH 45268-1320

Method 1605: *Aeromonas* in Finished Water by Membrane
Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Aeromonas* species in finished water samples. *Aeromonas* is a common genus of bacteria indigenous to surface waters. Its numbers are more likely to be greater during periods of warmer weather and when increased concentrations of organic nutrients are present. It is also more likely to be found in non-chlorinated water distribution systems or low-flow parts of chlorinated systems. Some *Aeromonas* species are opportunistic pathogens.

1.2 This method is adapted from Havelaar et al. (1987) for the enumeration of *Aeromonas* species in finished water by membrane filtration (Reference 15.1). It is a quantitative assay that uses a selective medium which partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation producing yellow colonies. Presumptively positive colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

1.3 This method is designed to meet the finished water monitoring requirements of the U.S. Environmental Protection Agency. *Aeromonas* was included on the Contaminant Candidate List (CCL) (Mar. 2, 1998, 63 *FR* 10274) and in the Revisions to the Unregulated Contaminant Monitoring Proposed Rule (UCMR) (September 17, 1999, 64 FR 50556). Contaminants listed in the UCMR are candidates for future regulation and may be included in a monitoring program for unregulated contaminants. Unregulated contaminant monitoring would be required for large systems and a representative sample of small and medium sized water distribution systems.

1.4 This method was subjected to an interlaboratory validation study involving 11 laboratories and 11 finished drinking water matrices. This method was not validated for other water types. Use of this method and appropriate validation for other water types is the responsibility of the user.

2.0 Summary of Method

2.1 The method provides a direct count of *Aeromonas* species in water based on the growth of yellow colonies on the surface of the membrane filter using a selective medium. A water sample is filtered through 0.45-Fm-pore-size membrane filter. The filter is placed on ampicillin-dextrin agar with vancomycin (ADA-V) and incubated at 35EC ± 0.5EC for 24 ± 2 hours. This medium uses ampicillin and vancomycin to inhibit non-*Aeromonas* species, while allowing most *Aeromonas* species to grow. The medium uses dextrin as a fermentable carbohydrate, and bromothymol blue as an indicator of acidity produced by the fermentation of dextrin. Presumptively identified yellow colonies are counted and confirmed by testing for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose and produce indole.

2.2 The membrane filtration procedure provides a direct count of culturable Aeromonas in water samples that is based on the growth of bacterial colonies on the surface of the membrane filter placed on a selective medium.

2.3 Aeromonas isolates may be archived for further analysis to determine species or hybridization group by inoculating a nutrient agar slant for short term use or shipping, or nutrient broth for freezing.

3.0 Definitions

3.1 Aeromonas are bacteria that are facultative anaerobes, Gram-negative, oxidase-positive, polarly flagellated, and rod shaped. They are classified as members of the family Aeromonadaceae. Demarta et al. (1999) reported 15 Aeromonas species based on 16S rDNA sequences though not all are officially recognized. Some species have been associated with human disease. In this method, Aeromonas are those bacteria that grow on ampicillin-dextrin agar with vancomycin (ADA-V), produce yellow colonies, are oxidase-positive, and have the ability to ferment trehalose and produce indole.

3.2 Definitions for other terms are provided in the glossary at the end of the method (Section 17.3).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material may clog the membrane filter and prevent filtration or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.2 Other ampicillin/vancomycin resistant bacteria that are not aeromonads may be able to grow on this medium. Some of these bacteria may also produce yellow colonies if they are able to produce acid byproducts from the fermentation of dextrin or some other media component, or if they produce a yellow pigment. Enterococcus are reported to produce pinpoint-size yellow colonies on ADA. Confirmation of presumptive Aeromonas colonies is necessary to mitigate false positives.

5.0 Safety

5.1 Some strains of Aeromonas are opportunistic pathogens. Sample containers and waste materials should be autoclaved prior to cleaning or disposal.

5.2 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and other materials.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Equipment for collection and transport of samples to laboratory

- **6.1.1** Autoclavable sample container—Use sterile, non-toxic, glass or plastic containers with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis.
- **6.1.2** Ice chest
- **6.1.3** Ice packs

6.2 Autoclavable dilution bottles—125-mL marked at 99 mL or 90 mL; commercially produced dilution bottles may be used.

6.3 Rinse water bottles

6.4 Sterile plastic or autoclavable glass pipettes with a 2.5% tolerance—to deliver (TD), 1- and 10-mL

6.5 Pipette bulbs or automatic pipetter.
6.6 Autoclavable pipette container (if using glass pipettes).
6.7 Thermometer—with 0.5EC gradations checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
6.8 Inoculating loop—Sterile metal, plastic, or wooden applicator sticks.
6.9 Burner—Flame or electric incinerator for sterilizing metal inoculating loops and forceps.
6.10 Colony counting device—Mechanical, electric or hand tally.
6.11 Hotplate stirrer
6.12 Magnetic stir bar
6.13 Graduated cylinders—100 mL, 500 mL and 1 L, sterile, polypropylene or glass
6.14 Balance—Capable of weighing samples up to 200 g, with a readability of 0.1 g
6.15 Weigh boats
6.16 pH meter
6.17 Turbidimeter (optional)
6.18 Equipment for membrane filter procedure

6.18.1 Incubator—Hot air or water-jacketed microbiological type to maintain a temperature of 35EC ± 0.5EC
6.18.2 Petri dishes—sterile, 50 × 9 mm or other appropriate size
6.18.3 Membrane filtration units (filter base and funnel made of glass, plastic, or stainless steel), wrapped with aluminum foil or Kraft paper, and sterilized by autoclaving.
6.18.4 Vacuum source
6.18.5 Flasks—1-L vacuum filter with appropriate tubing; a filter manifold to hold a number of units is optional
6.18.6 Side-arm flask to place between vacuum source and filtration devices or filter manifold
6.18.7 Membrane filters—Sterile, cellulose ester, white, gridded, 47-mm-diameter with 0.45-Fm pore size (Gelman E04WG04700 or equivalent)
6.18.8 Forceps—Sterile, straight or curved, with smooth tips to handle filters without causing damage
6.18.9 Ethanol or other alcohol in a container to sterilize forceps
6.18.10 Test tubes—125 × 16 mm sterile, screw-cap tube

6.19 Dissecting microscope—Low power (10X to 15X), binocular, illuminated
6.20 Autoclave—Capable of 121EC at 15 psi. Must meet requirements set forth in the Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition. (Reference 15.5)
6.21 Membrane filters (for sterilization purposes)—Sterile with 0.22-Fm pore size (Gelman Acrodisc No. 4192 or equivalent)

7.0 Reagents and Standards
7.1 Purity of reagents and culture media—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents and culture media shall conform to the specifications in Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9050 (Reference 15.2). The agar used in preparation of culture media must be of microbiological grade.
7.2 Purity of water—Reagent-grade water conforming to specifications in Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition (Reference 15.5) or

Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9020 (Reference 15.2).

7.3 Phosphate buffered dilution water

7.3.1 Concentrated stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water. Adjust the pH to 7.2 ± 0.5 with 1N sodium hydroxide (NaOH) and dilute to 1 L with reagent-grade water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.2 Magnesium chloride solution—Dissolve 81.1 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$) in reagent-grade water and dilute to 1 L. Autoclave or filter sterilize through a 0.22-Fm-pore-size filter.

7.3.3 Prepare phosphate buffered dilution water by adding 1.25 mL of concentrated stock phosphate buffer solution (Section 7.3.1) and 5.0 mL of magnesium chloride solution (Section 7.3.2) to a 1-L graduated cylinder and adjust final volume to 1 L with reagent-grade water. Prepare a portion of buffered dilution water in 1-L bottles for rinse water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.4 Stored phosphate buffered dilution water should be free from turbidity.

7.4 Ampicillin-dextrin agar with vancomycin (ADA-V)

7.4.1 Preparation of dextrin agar—EPA highly recommends the use of commercial ADA (m-Aeromonas Selective Agar Base [Havelaar]), Section **7.4.1.1**. However, ADA may be prepared by the laboratory (Section 7.4.1.2)

7.4.1.1 Commercial dextrin agar—Tech Pac (distributor, tech@fuse.net), Cincinnati, Ohio; Biolife (www.biolifeit.com) Italiana Srl, 272 Viale Monza, Milan, Italy, Cat. No. 401019 or equivalent. Prepare 1-L of media, according to manufacturer's instructions. Cool to room temperature, and adjust pH to 8.0 using 1N NaOH or 1N HCl. Autoclave for 15 min, cool to 50EC.

7.4.1.2 Laboratory-prepared dextrin agar.

7.4.1.2.1 5.0 g tryptose—Difco cat. no. 0124-17, or equivalent

7.4.1.2.2 11.4 g dextrin—Difco cat. no. 0161-17, or equivalent

7.4.1.2.3 2.0 g yeast extract—Difco cat. no. 0127-17, or equivalent

7.4.1.2.4 3.0 g sodium chloride (NaCl)—Baker cat. no. 3624, or equivalent

7.4.1.2.5 2.0 g potassium chloride (KCl)—Fisher cat. no. P217-500, or equivalent

7.4.1.2.6 0.1 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)—Fishercat. no. M63-500, or equivalent

7.4.1.2.7 0.06 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)—Sigma cat. no. F-2877, or equivalent

7.4.1.2.8 0.08 g bromothymol blue—Baker cat. no. D470, or equivalent

7.4.1.2.9 Sodium deoxycholate—Sigma cat. no. D-6750, or equivalent. Add 100 mg of sodium deoxycholate to 10 mL of reagent water.

7.4.1.2.10 13.0 g agar, bacteriological grade—Fisher cat. no. BP1423-500, or equivalent.

7.4.1.2.11 Add reagents in Sections 7.4.1.2.1 through 7.4.1.2.8 to 1-L of reagent-grade water, stir to dissolve and adjust pH to 8.0 using1N NaOH or 1N HCl. After the pH has been adjusted, add sodium deoxycholate (Section 7.4.1.2.9) and agar (Section7.4.1.2.10) and heat to dissolve. Autoclave for 15 min, cool to 50°C.

7.4.2 Ampicillin, sodium salt—Sigma cat. no. A0166, or equivalent. Add 10 mg of ampicillin, sodium salt to 10 mL reagent water. Prepare on the same day that medium is prepared and filter sterilize through a 0.22-Fm-pore-size filter. Alternatively, use Biolife cat. no. 4240012 prepared according to manufacturer's instructions, taking care to use an appropriate amount of ampicillin for the volume of media being prepared (for example, use two vials for a 1-L batch of ADA-V). Follow manufacturer's instructions for appropriate storage temperature and shelf-life. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.3 Vancomycin hydrochloride—Sigma cat. no. V2002, or equivalent. Add 2 mg of vancomycin hydrochloride to 10 mL of reagent water. Filter sterilize through a 0.22-Fm-pore-size filter. Follow manufacturer's instructions for appropriate storage temperature and time. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.4 After dextrin agar (Section 7.4.1) has been autoclaved and cooled to 50EC, add the sterile ampicillin (Section 7.4.2) and sterile vancomycin hydrochloride solutions (Section 7.4.3).

7.4.5 Add approximately 5 mL of ADA-V per 50 × 9 mm Petri dish and allow to solidify. For larger plates, adjust volume appropriately. ADA-V plates should be stored in a tight fitting container (i.e. sealed plastic bag) at a temperature of 1EC to 5EC for no longer than 14 days.

7.5 Pentahydrate ACS Reagent grade sodium thiosulfate—Fisher cat. no. S445-500, or equivalent. Prepare a 3% stock solution by adding 3 g sodium thiosulfate to 100 mL reagent-grade water.

7.6 Disodium salt of ethylenediaminetetraacetic acid (EDTA)—Sigma cat. no. E 4884, or equivalent. EDTA should only be added to samples if metals in water samples exceed 1.0 mg/L. To prepare stock solution, add 12.4 g EDTA to 80 mL of reagent-grade water. Adjust pH to 8.0 using 10N NaOH. After the pH has been adjusted, bring the volume up to 100 mL with reagent-grade water.

7.7 Positive control culture—Aeromonas hydrophila ATCC #7966; obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, 20110-2209; http://www.atcc.org).

7.8 Negative culture control—Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-Aeromonas bacteria that may grow on ADA-V and to ensure that confirmation test results are appropriate. E. coli (ATCC #25922) is the negative culture control for oxidase, Pseudomonas aeruginosa (ATCC #27853) is the negative culture control for trehalose fermentation, and Bacillus cereus (ATCC #11778) is the negative culture control for indole.

7.9 Nutrient agar—Difco cat. no. 0001-17 or equivalent. Prepare according to manufacturers instructions.

7.10 Oxidase reagents—Dry Slide BBL cat. no. 231746 or equivalent.

7.11 0.5% Trehalose confirmation reagent

7.11.1 Add 5 g trehalose (Sigma cat. no. T0167, or equivalent) to 100 mL water and filter sterilize solution through a filter with 0.22-Fm-pore-size.

7.11.2 Prepare 900 mL purple broth base (Difco cat. no. 0222-17, or equivalent) according to manufacturer's instructions and autoclave.

7.11.3 Aseptically add 100 mL trehalose solution to the cooled 900 mL of purple broth base.

7.11.4 Dispense into 6 mL or larger size tubes and fill approximately half full. Store in refrigerator.

Note: *Alternatively, prepare purple broth base according to manufacturers instructions, add 5 g trehalose per liter, and filter sterilize through a filter with 0.22-*F*m-pore-size.*

7.12 Tryptone broth—Oxoid cat. no. CM0087B, or equivalent. Alternatively, the laboratory may prepare tryptone broth by adding 10 g of tryptone (Difco cat. no. 0123-17 or equivalent) and 5 g of NaCl to 1 L of reagent water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.13 Kovac's reagent—Biomeriuex cat. no. V7050, or equivalent

8.0 Sample Collection, Preservation, and Storage

8.1 Adherence to sample preservation procedures and holding time limits specified in *Standard Methods for the examination of Water and Wastewater* (Reference 15.2) is critical to the production of valid data. Sample results will be considered invalid if those conditions are not met.

8.2 Preparation of sample bottles and sample collection—Samples must be representative of the drinking water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

8.2.1 Use sterile, non-toxic, glass or plastic container (Section 6.1.1) with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis. Sampling procedures are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9060 (Reference 15.2).

8.2.2 Add 1 mL of 3% sodium thiosulfate stock (Section 7.5) per L of sample to sample bottles prior to autoclave sterilization. Alternatively, if using presterilized sample bottles, sodium thiosulfate should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size before adding to the sample bottles.

8.2.3 If metals in the sample exceed 1.0 mg/L, add 3 mL of EDTA stock solution (Section 7.6) per L of sample to sample bottles prior to autoclave sterilization. If using presterilized sample bottles, EDTA should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size.

8.2.4 Collect a minimum of 1-L of sample.

8.3 Sample preservation and handling

8.3.1 Immediately following sample collection, tighten the sample container lid(s) and place the sample container(s) upright in an insulated, plastic-lined storage cooler with ice packs or in a refrigerator to chill prior to packing the cooler for shipment. Do not freeze the sample.

8.3.2 Use enough solidly frozen ice packs to ensure that the samples will arrive at a temperature of 1°C to 10°C. Use a minimum of two ice packs per shipment and add extra ice packs for multiple samples. Place one or more ice packs on each side of the container to stabilize samples.

8.3.3 Samples must be maintained at a temperature of 1°C to 10°C during shipment. Samples must not be frozen.

Note: Sample temperature during shipment is critical. Ice packs must be frozen solid immediately prior to shipment.

8.4 Verify and record sample arrival temperature when received in the laboratory. Refrigerate samples at 1°C to 5°C upon receipt at the laboratory and analyze as soon as possible after collection. Samples must be analyzed within 30 hours of sample collection.

9.0 Quality Control

9.1 Each laboratory that uses Method 1605 is required to operate a formal quality assurance (QA) program. The minimum QA requirements consist of the initial demonstration of capability (IDC) test (Section 9.4), ongoing analysis of spiked reagent water (ODC test, Section 9.8) and spiked finished drinking water samples (MS/MSD, Section 9.7), and analysis of negative culture controls (Section 9.6), dilution/rinse water blanks (Section 9.5), and media sterility checks (Section 9.2.6) as tests of continued acceptable performance. Spiked sample results are compared to acceptance criteria for precision, which are based on data generated during the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished water matrices. The more stringent QA requirements in this method, relative to other, currently used methods for bacterial determination, are an effort to improve overall microbiological QA. The specifications contained in this method can be met if the analytical system is maintained under control. Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections 10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

9.2 General QC requirements—Specific quality control (QC) requirements for Method 1605 are provided below. QA and QC criteria for facilities, personnel, and laboratory equipment, instrumentation, and supplies used in microbiological analyses must be followed according to Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141, Reference 15.2) and the U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fourth Edition (March 1997) (Reference 15.5).

9.2.1 Initial demonstration of capability (IDC). The laboratory shall demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples. The procedure for performing the IDC is described in Section 9.4. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.2.2 Dilution/rinse water blanks. The laboratory shall analyze dilution/rinse water blanks to demonstrate freedom from contamination. The procedures for analysis of dilution/rinse water blanks are described in Section 9.5. At a minimum, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series.

9.2.3 Negative culture controls. The laboratory shall analyze negative culture controls (Section 9.6) to ensure that ADA-V and the confirmation procedures are performing properly. Negative culture controls should be run whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples.
9.2.4 Matrix spike/matix spike duplicate (MS/MSD). The laboratory shall analyze one set of MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples (Section 9.7). Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines. When possible, MS/MSD analyses should be conducted on the same day as ODC samples, using the same spiking procedure and volume.
9.2.4.1 Precision. MS/MSD sample results should meet the precision criteria set forth in Section 12, Table 1.
9.2.4.2 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the number of Aeromonas in the spike is unknown. However, each laboratory should control chart the mean number of Aeromonas per MS/MSD set (adjusted for background) and maintain a record of spike preparation procedures and spike volume. The laboratory should compare number of Aeromonas in MS/MSD samples to results of ODC samples (Section 9.2.5 and 9.8) spiked on the same day. This comparison should help the laboratory recognize when a matrix is interfering with method recovery. If the laboratory observes consistent ODC results from week to week, control charting the MS/MSD results by source may also help to recognize fluctuations in recovery from a particular source.

9.2.5 Ongoing demonstration of capability (ODC). The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate, Section 9.8).
9.2.5.1 Frequency. The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently. No more than one set of ODC samples is required per day, provided that the same equipment (i.e., incubators) are being used for all the samples.
9.2.5.2 Precision. ODC sample results must meet the precision criteria set forth in Section 12, Table 1.
9.2.5.3 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the initial spike dose for ODC samples is unknown.
As a result, each laboratory should control chart the mean number of Aeromonas per ODC sample set and maintain a record of spike preparation procedures and ODC spike volume. Maintaining this information will enable the laboratory to recognize when problems arise. Example: A laboratory that prepares spiking suspensions according to Section 9.3, spikes QC samples with 5 mL of dilution D2, and typically recovers approximately 50 Aeromonas per sample, and maintains a control chart of these counts. If the laboratory continues to prepare spiking suspensions the same way, but the number of Aeromonas counted declines noticeably (e.g. 20 Aeromonas per sample), then there may be a problem with the media, reagents, or the spiking suspension.
9.2.6 Media sterility checks. The laboratory shall test media sterility by incubating one unit (tube or plate) from each batch of medium (ADA-V, nutrient agar slant, nutrient agar, streak plate, trehalose, and tryptone) at 35°C ± 0.5°C for 24 ± 2 hours and observing for growth.

9.2.7 Analyst colony counting variability. If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one ODC sample per month (Section 9.9), at a minimum.

9.2.8 Record maintenance. The laboratory shall maintain records to define the quality of data that are generated. The laboratory shall maintain a record of the date and results of all QC sample analyses described in Section 9.2. A record of media sterility check, dilution/rinse water blank, analyst counting variability, IDC, ODC, and MS/MSD sample results must be maintained. Laboratories shall maintain reagent and material lot numbers along with samples analyzed using each of the lots. Laboratories shall also maintain media preparation records.

9.2.9 Performance studies. The laboratory should periodically analyze external QC samples, such as performance evaluation (PE) samples, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

9.2.10 Autoclave sterilization verification. At a minimum, the laboratory shall verify autoclave sterilization according to the procedure in Section 9.10 on a monthly basis.

9.2.11 Culture maintenance. The laboratory should use 24 ± 2 hour-old nutrient agar slant cultures for preparation of IDC, ODC, and MS/MSD spiking suspension dilutions. The laboratory should use 22 to 72 hour-old nutrient agar slant cultures to inoculate ADA-V streak plates for analysis of negative culture controls. With regard to the preparation of subcultures, it is recommended that a maximum of three passages be prepared to help avoid contamination. After three passages, start a new subculture from the frozen stock.

9.2.12 Method modification.

9.2.12.1 Membrane filtration. Because recovery criteria are not available for this method, laboratories are not permitted to modify the membrane filtration procedures (Section 10.1 through Section 10.10.) or ADA-V media.

9.2.12.2 Confirmation procedures. The confirmation procedures in Section 10.11 may be modified, provided that the laboratory demonstrates the ability to generate acceptable performance by performing an IDC test (Section 9.2.1) and the appropriate negative culture control test(s) (Section 9.2.3) before analyzing any field samples using the modified confirmation. 100% of the colonies submitted to confirmation from IDC and negative culture control samples must give the appropriate confirmation response. These tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2) and media sterility checks (Section 9.2.6).

9.3 Preparation of Aeromonas spiking suspension for use in spiking IDC, ODC, and MS/MSD samples—This dilution scheme is adapted from Standard Methods for the Examination of Water and Wastewater, 19th Edition, Section 9020 B (Reference 15.9). This entire process should be performed quickly to avoid loss of viable organisms. See Section 16, Flowchart 1, for an example of this dilution scheme. Please note: Provided that all QC acceptance criteria are met and the recommended target range of 20 - 60 CFU per plate are typically observed, laboratories may prepare QC spiking suspensions using commercial products or other procedures such as growing bacteria in a broth, measuring optical density, and spiking each test sample with an equivalent volume.

9.3.1 Inoculate Aeromonas hydrophila (ATCC #7966) onto the entire surface of several nutrient agar slants with a slope approximately 6.3 cm long in a 125 × 16 mm screw-cap tube. Incubate for 24 ± 2 hours at 35°C ± 0.5°C.

9.3.2 From the slant that has the best growth, prepare serial dilutions using four dilution bottles with 99 mL of sterile buffered dilution water (A, B, C and D below in Sections 9.3.3 and 9.3.4) and one dilution bottle containing 90-mL of sterile buffered dilution water (D2 below in Section 9.3.5).

9.3.3 Pipette 1 mL of buffered dilution water from bottle "A" to one of the slants. Emulsify the growth on the slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar. Pipette the suspension back into dilution bottle "A." Repeat this procedure a second time to remove any remaining growth on the agar slant, without disturbing the agar.

9.3.4 Make serial dilutions as follows:

9.3.4.1 Shake bottle "A" vigorously and pipette 1 mL to bottle "B"

9.3.4.2 Shake bottle "B" vigorously and pipette 1 mL to bottle "C"

9.3.4.3 Shake bottle "C" vigorously and pipette 1 mL to bottle "D"

9.3.4.4 Shake bottle "D" vigorously and pipette 10 mL to bottle "D2"; this should result in a final dilution of approximately 10 CFU / mL.

9.3.5 Filter 1- to 5-mL portions in triplicate from bottles "D" and "D2" according to the procedure in Section 10 to determine the number of CFU in the dilutions. The recommended target dilution and spike volume is one that produces 20 to 60 colonies per ADA-V plate. (It may be difficult to count plates with more than 60 colonies due to crowding.) Dilutions should be stored at 1EC to 5EC and may be used throughout the day they are prepared. However, it should be noted that the QC acceptance criteria were established using dilutions that were prepared immediately prior to spiking samples.

9.3.6 Analysts may practice the dilution scheme by placing filters on nutrient agar plates instead of ADA-V plates. After a growth pattern is determined and the analyst can accurately determine the target concentrations, dilutions from Section 9.3.5 may be used for spiking IDC, ODC, and MS/MSD samples. However, multiple dilutions should be analyzed in replicate when new cultures are received from an outside source to ensure that the analyst can accurately spike target concentrations.

Note: If it is more convenient for your laboratory, an acceptable alternative to the dilution scheme presented in Section 9.3, is to pipette 11 mL of dilution D into a dilution bottle D2, which contains 99 mL of dilution water. There should be approximately 10^{10} Aeromonas hydrophila CFU per slant. Therefore, dilution bottles "A" through "D2" should contain approximately 10^{10}, 10^{8}, 10^{6}, 10^{4}, and 10^{3} CFU per dilution bottle, respectively. Depending on the growing conditions, these numbers may vary. As a result, until experience has been gained, more dilutions may need to be filtered to determine the appropriate dilution.

9.4 Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.4.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.4.2 For each of the four IDC test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.4.3 Process IDC test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per IDC test sample to the confirmation procedures in Section 10.11.

9.4.4 Using all four IDC sample results, compute the relative standard deviation (RSD) of Aeromonas CFU per 100 mL. (See glossary for definition of RSD.) Compare the RSD with the corresponding limits for IDC (Section 12). If the RSD meets the acceptance criteria, the system performance is acceptable and analysis of samples may begin. If the RSD falls outside the range, system performance is unacceptable. In this event, identify and correct the problem and repeat the test.

9.5 Dilution/rinse water blanks—On an ongoing basis, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series. For example, if a laboratory plans to run 30 samples during a filtration series, a dilution/rinse water blank should be processed at the beginning, middle, and end of the filtration series.

9.5.1 Process 100-mL dilution/rinse water blanks according to the procedures in Section 10, as appropriate.

9.5.2 No growth should appear in dilution/rinse water blanks. If growth appears, prepare new dilution/rinse water and reanalyze a 100-mL dilution/rinse water blank. If colonies are present after analyzing the new dilution/rinse water, assess laboratory technique and reagents. If growth in dilution/rinse water blank(s) is presumptively positive, all associated sample results should be discarded and sources re-sampled immediately.

9.6 Negative culture controls— Negative controls should be run whenever a new batch of medium or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples. Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate. *E. coli* is (ATCC #25922) the negative culture control for oxidase, *Pseudomonas aeruginosa* (ATCC #27853) is the negative culture control for trehalose fermentation, and *Bacillus cereus* (ATCC #11778) is the negative culture control for indole.

9.6.1 Using pure cultures obtained from a qualified outside source (Sections 7.7 and 7.8), inoculate negative culture controls onto nutrient agar slants and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Alternatively, nutrient agar slants may be inoculated up to 72 hours in advance. If nutrient agar slants will be incubated for more than 24 ± 2 hours, consider incubation at room temperature to ensure that the slants do not dry out prior to use.

9.6.2 For each negative culture control, place a membrane filter on an ADA-V plate, streak onto the filter, taking care not to break the filter, and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Streaking on a filter will give the laboratory a more realistic example of the appearance of these organisms in field samples. Although not recommended, laboratories may streak directly onto the ADA-V (without the filter).

9.6.3 For each ADA-V negative culture control plate, pick a single colony, streak the colony onto a plate of nutrient agar medium (Section 7.9), and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies. *Please note: Bacillus cereus typically grows only at the point of inoculation on ADA-V or not at all. If Bacillus cereus did not grow on the ADA-V plate, inoculate the streak plate from the nutrient agar slant that was originally used to inoculate the ADA-V plate.*

9.6.4 Negative culture control confirmation procedures

9.6.4.1 Oxidase negative culture control—From the streak plate, submit a single *E. coli* colony to the oxidase confirmation procedure described in Section 10.11.

9.6.4.2 Trehalose negative culture control—From the streak plate, submit a single *Pseudomonas aeruginosa* colony to the trehalose confirmation procedure described in Section 10.11.

9.6.4.3 Indole negative culture control—From the streak plate, submit a single *Bacillus cereus* colony to the indole confirmation procedure described in Section 10.11.

9.6.5 If any of the negative culture controls result in a positive confirmation, prepare, check and/or replace the associated media, reagents, and/or respective control organism and reanalyze the appropriate negative culture control(s). All presumptively positive colonies that have been archived from field samples (10 per sample) should be confirmed using media/reagents that exhibit the appropriate negative culture control response.

9.7 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall analyze MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples. Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines.

9.7.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Sections 9.3.1 through 9.3.4.

9.7.2 For each of the 500-mL MS and MSD test samples, spike enough volume of the appropriate dilution to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.7.3 Process MS/MSD test samples and an unspiked finished drinking water sample according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. (If the filter clogs during filtration, follow the instructions in Section 10, making sure to filter the same volume for both the MS and MSD. The same QC acceptance criteria apply.) Submit 10 colonies per IDC test sample to the confirmation procedures in Section 10.11.

Note: If results exceed the optimum range because of "background" target colonies (as indicated by the results of the unspiked matrix sample), the MS/MSD should be repeated and a smaller volume of sample, for example 200-mL, should be spiked.

9.7.4 For the MS and MSD test samples, calculate the number of confirmed Aeromonas CFU per 100 mL according to Section 11 and adjust based on any background Aeromonas observed in the unspiked sample.

9.7.5 Calculate the relative percent difference (RPD) using the following equation:

$$RPD = 100 \frac{| X_{MS} - X_{MSD} |}{X_{mean}}$$

where **RPD** is the relative percent difference
$\mathbf{X_{MS}}$ is the number of confirmed Aeromonas per 100 mL in the MS sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{MSD}}$ is the number of confirmed Aeromonas per 100 mL in the MSD sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{mean}}$ is the mean number of confirmed Aeromonas per 100 mL in the MS and MSD

9.7.6 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of finished water samples from this source may continue. If the MS/MSD results are unacceptable and the ODC sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. If the MS/MSD results are unacceptable, all associated field data should be flagged.

9.8 Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate). The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently.

9.8.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.8.2 For each of the 500-mL positive control (PC) and positive control duplicate (PC/PCD) test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.8.3 Process PC/PCD test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per PC/PCD test sample to the confirmation procedures in Section 10.11.

9.8.4 Calculate the relative percent difference (RPD) using the following equation:

$$PRD = 100 \frac{| X - XPCD |}{Xmean}$$

where
RPD is the relative percent difference
X_{PC} is the number of confirmed Aeromonas per 100 mL in the PC sample
X_{PCD} is the number of confirmed Aeromonas per 100 mL in the PCD sampleX_{mean} is the mean number of confirmed Aeromonas per 100 mL in the PC and PCD samples

9.8.5 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of samples may continue. If RPD falls outside the range, system performance is unacceptable. Identify and correct the problem and perform another ODC test before continuing with the analysis of field samples.
9.8.6 As part of the QA program for the laboratory, method precision for ODC samples should be charted and records retained.

9.9 Analyst colony counting variability—If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one positive field sample per month. Compare each analyst's count of the target colonies. Counts should fall within 10% between analysts. If counts fail to fall within 10% of each other, analysts should perform additional sets of counts, until the number of target colonies counted fall within 10% between analysts for at least three consecutive samples. If there are no positive samples, an MS, MSD, or ODC sample can be used for this determination (MS or MSD are preferable to ODC samples, since they may have other background growth).

9.10 Autoclave sterilization verification—Verify autoclave sterilization monthly by placing Bacillus stearothermophilus spore suspensions or strips inside glassware. Sterilize at 121°C for 15 minutes. Place in trypticase soy broth tubes and incubate at 55°C for 48 hours. Check for growth to verify that sterilization was adequate. If sterilization was inadequate, determine appropriate time for autoclave sterilization. Filter sterilization may be used provided that these same QC steps are instituted for the filtrate.

10.0 Procedure
10.1 The membrane filter (MF) procedure with ampicillin-dextrin agar with vancomycin (ADA-V) is used to enumerate Aeromonas in finished waters.
10.2 Label each Petri dish with sample identification, preparation date, and analysis start date/time.
10.3 Use a sterile MF unit assembly (Section 6.18.3) at the beginning of each filtration series. The laboratory must sanitize each MF unit between filtrations by using a UV sanitizer, flowing steam, or boiling water for 2 min. A filtration series ends when 30 minutes or more elapse between sample filtrations.

10.4 Sterilize forceps with alcohol. Flame off excess alcohol. Using sterile forceps, place the MF (grid side up) over the sterilized funnel. Carefully place the top half of the filtration unit over the funnel and lock it in place.
10.5 Shake the sample bottle vigorously approximately 25 times to distribute the bacteria uniformly. Using aseptic technique, transfer one, 500-mL aliquot of sample to a single funnel. Use a graduated cylinder with a "to deliver" tolerance of approximately 2.5%.
Note: Laboratories must filter the entire 500-mL sample volume unless the filter clogs. If the filter clogs, a minimum of 100 mL of sample must be filtered, which may require multiple filtrations. If less than 500 mL are filtered and analyzed due to filter clogging, measure the residual, unfiltered volume to determine the volume filtered, and adjust the reporting limit accordingly.
10.6 Filter each sample under partial vacuum through a sterile membrane filter. Rinse the funnel after each sample filtration by filtering three, 30-mL portions of sterile buffered dilution water, being sure to thoroughly rinse the sides of the funnel.
10.7 Upon completion of the final rinse, disengage the vacuum and remove the funnel.
10.8 Using sterile forceps, immediately remove the MF and place it grid-side-up on the ADA-V medium with a rolling motion to avoid trapping air under the filter. Reseat the membrane filter if bubbles occur. Place the inverted Petri dishes in the 35°C ± 0.5°C incubator within 30 minutes of preparation. Sterilize forceps and sanitize the MF unit between the analysis of each sample.
10.9 After 24 ± 2 hours of incubation at 35°C ± 0.5°C, count and record yellow colonies under magnification using a dissecting microscope.
10.10 Isolation of a yellow colony on ampicillin-dextrin agar with vancomycin (ADA-V) should be considered presumptively positive for Aeromonas.
10.11 Confirmation—All presumptive colonies, up to ten per sample, must be submitted to confirmation. In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. If the result for any confirmation procedure is negative, no further confirmation steps are necessary. Slight variations in color and morphology may be present between different Aeromonas species grown on ADA-V medium. The colonies selected for confirmation should be representative of all yellow (presumptively positive) colony morphology types on ADA-V plate. For example, if 30 bright yellow colonies and 20 dull yellow colonies are observed, then 6 bright yellow and 4 dull yellow colonies should be submitted to confirmation.
Note: It is important to record the number of colonies of each presumptively positive morphological type so that the final density of Aeromonas can be reported based on percent confirmation of each morphological type. Also, the laboratory may submit more than ten presumptively positive colonies to the confirmation step.
10.11.1 Nutrient agar streak plate. To confirm as Aeromonas, pick a colony and streak the colony onto a plate of nutrient agar medium (Section 7.9) and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies.
10.11.2 Oxidase confirmation. Apply a very small amount of a discreet colony from the nutrient agar to the oxidase dry slide using a wooden or plastic applicator. Do not use iron or other reactive wire because it may cause false positive reactions. Also, do not transfer any medium with the culture material, as this could lead to inconsistent results. A blue/purple color reaction within 10

seconds is considered a positive oxidase test. For commercially-prepared reagent, adhere to manufacturer's expiration date. Freshly-made solutions should be used within one week. Please note: This method was validated using nutrient agar, if the oxidase reagent is to be dropped directly on colonies, use tryptic soy agar plates because nutrient agar plates give inconsistent results. The use of tryptic soy agar plates for streaking (Section 10.11.1) has not been validated and is considered a method modification and, as a result, the laboratory must demonstrate acceptable performance for the QC analyses described in Section 9.2.12.

Note: Timing of the color reaction is critical, as some Gram-positive bacteria may give false positives after 10 seconds. Also, it is important to put just a small amount of the colony on the oxidase dry slide or saturated pad, as too much bacteria can also cause a false positive oxidase test.

10.11.3 Trehalose confirmation. If the oxidase test is positive, then test for trehalose fermentation. Trehalose fermentation is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of 0.5% trehalose in purple broth base (Section 7.11) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. A change in color of the medium from purple to yellow is considered a positive for trehalose fermentation.

10.11.4 Indole confirmation. If the oxidase and trehalose tests are positive, then test for indole production. (If the laboratory prefers, the indole confirmation procedure may be started on the same day as the trehalose confirmation.) Indole production is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of tryptone broth (Section 7.12) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. After incubation, add 0.2 to 0.3 mL (4 to 6 drops) of Kovac's test reagent (Section 7.13) to each tube, let stand for approximately 10 minutes and observe results. A pink to red color in the surface layer constitutes a positive indole test. The original color of the Kovac's reagent indicates a negative indole test. An orange color probably indicates the presence of skatole, a breakdown product of indole, and is considered a positive result.

10.11.5 If a colony is oxidase, trehalose, and indole positive, report as a confirmed Aeromonas and archive the colony for further identification.

Note: If samples are to be archived for further analysis to determine species or hybridization group, from the nutrient agar plate (Section 10.11.1), inoculate a nutrient agar slant for short term use or shipment to another laboratory.

11.0 Data Analysis and Calculations

11.1 See Standard Methods for the Examination of Water and Wastewater (Reference 15.2) for general counting rules. The density of Aeromonas determined by the membrane filter (MF) procedure is calculated by direct identification and enumeration of yellow colonies by a dissecting microscope (Section 6.19) followed by oxidase, trehalose, and indole confirmation. Bacterial density is recorded as presumptive Aeromonas colony forming units (CFU) per 100 mL of sample and confirmed Aeromonas CFU per 100 mL.

11.2 Counting colonies on ADA-V

11.2.1 Record the number of presumptive Aeromonas CFU/100mL. If there is more than one morphological type that is considered to be presumptively positive, record the number of presumptive positives for each morphological type, as well as the total number of presumptive positives.

11.2.2 If there are more than 200 colonies, including background colonies, report results as too numerous to count (TNTC) and resample. If the filter is TNTC with more background colonies than presumptive aeromonads, split the 500 mL resample between 3 or 4 filters in order to better differentiate the colony morphology types. If the filter is TNTC with mostly aeromonads, a minimum of three dilutions (e.g. 100 mL, 10 mL and 1 mL) should be analyzed.
11.2.3 If the colonies are not discrete and appear to be growing together, report results as confluent growth (CG) and resample.

11.3 Confirmation and calculation of Aeromonas density
11.3.1 In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. For the final density of confirmed Aeromonas, adjust the initial, presumptive count based on the positive confirmation percentage for each presumptively positive morphological type and report as confirmed CFU per 100 mL.
11.3.2 Calculate the number of positive confirmations for each presumptively positive morphological type from all filters of a given sample using the following equation:

$$\left(\frac{\text{Number positively confirmed}}{\text{Number submitted to confirmation}} \text{ X Number of presumptive positives}\right) = A$$

$$A \text{ X } \frac{100}{\text{mL filtered}} = \text{Confirmed Aeromonas /100 mL}$$

11.3.3 Record the number of confirmed Aeromonas per 100 mL for each colony morphology.
11.3.4 Sum the number of confirmed Aeromonas per 100 mL for all presumptively positive colony types (Section 11.3.2) and report as the density of confirmed Aeromonas per 100 mL.
11.3.5 Example 1: In this example, 500 mL of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 1 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Bright yellow, round, opaque	30	6	6	6
Type B: Dull yellow, oval, translucent	20	4	3	3
Total number of confirmed *Aeromonas* per sample:				**9 per 100 mL**

Example 1 results in 9 confirmed *Aeromonas* / 100 mL.
11.3.6 Example 2: In this example, ***200 mL*** of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 2 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Dull yellow, round, opaque	40	5	5	20
Type B: Dull yellow, round, translucent	40	5	3	12
Total number of confirmed *Aeromonas* per sample:				**32 per 100 mL**

Example 2 results in 32 confirmed *Aeromonas* / 100 mL.
11.3.7 If there were no presumptively positive colonies or if none of the presumptive colonies are confirmed, then report the results as less than the detection limit (DL) in CFU per 100 mL based on sample volume filtered. If less than 500 mL are filtered, then adjust the reporting limit per 100 mL accordingly. The DL may be calculated as follows:

DL per 100 mL = 100 / volume filtered CFU per 100mL

11.3.7.1 Example 3: If 500 mL of sample was filtered and there were no confirmed colonies, then report as <0.2 CFU/100 mL.
11.3.7.2 Example 4: If 100 mL of sample was filtered and there were no confirmed colonies, then report as <1.0 CFU/100 mL.

12.0 Method Performance

12.1 Specificity of media **12.1.1** Please refer to Section 16, Table 2, for results of *Aeromonas* growth after 24 hours on ADA at 30EC and 35EC and ADA-V at 35EC .

12.1.2 ADA-V was able to support the growth of the *Aeromonas* species (*hydrophila, caviae,* and *veronii/sobria*) most often associated with human disease.

12.1.3 Efforts continue to identify colonies which give a presumptive positive on the ADA-V media but do not confirm.

12.2 The QC acceptance criteria listed in Table 1, below are based on data generated through the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished drinking water matrices. Detailed method QC procedures applicable to these criteria are discussed in Section 9.

Table 1. QC Acceptance Criteria for Method 1605

QC specification	Maximum acceptable precision
Initial demonstration of capability (IDC): This test will require the analysis of 4 spiked reagent water samples	RSD = 22%
Ongoing demonstration of capability (ODC): This test will require the analysis of 2 spiked reagent water samples	RPD = 37%
Matrix spike/matrix spike duplicate (MS/MSD) precision: This test will require the analysis of 2 spiked finished water (matrix) samples	RPD = 48%

13.0 Pollution Prevention

13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

14.0 Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known or suspected of having bacterial contamination from this work must be sterilized prior to disposal.

14.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both of which are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 References

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16.0 Tables and Flowcharts

Collection #	Hybridization group	*Aeromonas* species	ADA at 30°C	ADA at 35°C	ADA-V at 35°C
ATCC 7966	Group 1	*hydrophila*	+	+	+
ATCC 35654	Group 1	*hydrophila*	+	+	+
AMC 12723-W	Group 1	*hydrophila*	+	+	+
ATCC 51108	Group 2	*bestiarum*	+	+	+
AMC 14228-V	Group 2	*bestiarum*	+	+	+
ATCC 33658[1]	Group 3	*salmonicida/salmonicida*	-	-	NA
AMC 15228-V	Group 3	*salmonicida*	+	+	+
ATCC 15468	Group 4	*caviae*	+	+	+
MML 1685-E	Group 4	*caviae*	+	+	+
ATCC 33907	Group 5	*media*	-	-	NA
AMC Leftwich	Group 5	*media*	-	-	NA
ATCC 23309[1]	Group 6	*eucrenophila*	+	-	NA
ATCC 35993	Group 7	*sobria*	+	+	+
Muldoon SMHC	Group 7	*sobria*	+	+	+
ATCC 9071	Group 8	*veronii/sobria*	+	+	+
AMC 1123-W	Group 8	*veronii/sobria*	+	+	+
ATCC 43700	Group 12	*schubertii*	+[2]	+[5]	+[5]
AMC 1108-W	Group 12	*schubertii*	+	-	NA
ATCC 49657[3]	unknown	*trota*	-	-	NA
NMRI 206	unknown	*trota*	-	-	NA
ATCC 51208	unknown	*allosaccharophila*	+	+	+
ATCC 49568	Group 9	*jandaei*	+	+	+
AS 14	Group 9	*jandaei*	+	+	+
ATCC 35622	Group 10	*veronii/veronii*	+	+	+
WR 4659	Group 10	*veronii/veronii*	+	+	+
CECT 4342	Group 11	*encheleia*	+	-	NA
LMG 17541[4]	unknown	*popoffii*	+	+	+
AMC (ATCC 35941)	unknown	ornithine positive	-	-	NA
AMC (ATCC 43946)	unknown	Group 501	+	+	+
CDC 0434-84	Group 3	Motile Group 3	+	+	+

(1) Respective *Aeromonas* cultures grew on ADA medium when streaked, but not when filtered.

(2) Respective Aeromonas cultures grew when streaked on ADA medium at 30°C, however filtration was not performed with these cultures.(3) Respective Aeromonas cultures did not grow on ADA medium when streaked.(4) Respective Aeromonas cultures grew poorly on ADA medium at both temperatures and on ADA-V at 35°C. The same pattern of poor growth was also observed on non-selective media.(5) Respective Aeromonas cultures grew poorly on ADA and ADA-V medium at 35°C. The same pattern of poor growth was also observed on non-selective media.

Results: A Based on ADA results, it was assumed that the culture would not grow on ADA-V at 35°C.

\+ positive growth

-No growth

ATCC = American Type Culture Collection, Manassas, VA Other cultures were obtained from Amy Carnahan, University of Maryland. Serial dilutions representing approximately 10-200 CFU were filtered and the membrane placed on ADA or ADA-V medium as described in Section 10. Additional membranes representing the same dilution for each of the respective cultures were placed on brain heart infusion agar as a control.

17.3 Definitions

Confirmed colonies—Presumptively positive colonies that test positive for oxidase, ferment trehalose, and produce indole

Dilution/rinse water blank—A 100-mL aliquot of dilution/rinse water that is treated exactly as a sample and carried through all portions of the procedure until determined to be negative or positive. The Dilution/rinse water blank is used to determine if the sample has become contaminated by the introduction of a foreign microorganism through poor technique.

Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples.

Must—This action, activity, or procedural step is required.

Negative culture control—A non-*Aeromonas* bacteria processed to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate.

Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate).

Positive control—A 500-mL reagent water spiked with 20 - 80 CFU of Aeromonas. The positive control is analyzed exactly like a sample. Its purpose is to ensure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Presumptive positive colonies—Colonies that are yellow on ADA-V.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean.

Selective medium—A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of the target bacteria.

Should—This action, activity, or procedural step is suggested but not required.

Symbols and Characters

These symbols and characters are specific to water sampling methods but have been conformed to common usage as much as possible.

Units of weight and measure and their abbreviations
Symbols

°C degrees Celsius
µL microliter
< less than
> greater than
% percent

Alphabetical characters

ACUK Acid chrome violet K
AECL Alternate enhanced coagulant level
AOC Assimilable organic carbon
ASDWA Association of State Drinking Water Administrators
AWWA American Water Works Association
AWWARF AWWA Research Foundation
BAC Biologically active carbon
BAF Biologically active filtration
BAT Best Available Technology
BCAA Bromochloroacetic acid
BDOC Biodegradable organic carbon
BMP Best management practice
BOM Biodegradable Organic Matter (=BDOC + AOC)
Br- Bromide ion
BrO^{2}- Bromite ion
BrO^{3}- Bromate ion
CI Confidence interval
Cl^{2} Chlorine
ClO^{2} Chlorine Dioxide
cm centimeter
CT Concentration-Time
CWS Community Water System
D/DBP Disinfectants/disinfection byproducts
DBP Disinfection byproduct
DBPFP Disinfection byproduct formation potential
DBPP Disinfection byproduct precursors
DBPR Disinfectants/disinfection byproducts rule
DBPRAM DBP Regulatory Assessment Model
DBPs Disinfection byproducts
DOC Dissolved organic carbon
DPD N,N-diethyl-p-phenylenediamine
DWEL Drinking Water Equivalent Level
EBCT Empty bed contact time
EMSL EPA Environmental Monitoring and Support Laboratory (Cincinnati)
EPA United States Environmental Protection Agency
ESWTR Enhanced Surface Water Treatment Rule
FBR Filter Backwash Rule

FY Fiscal year
G acceleration due to gravity
g gram
GAC Granular activated carbon
GWR Ground Water Rule
GWSS Ground Water Supply Survey
H2O2 Hydrogen Peroxide
HAA5 Haloacetic acids (five)
HOBr Hypobromous acid
HOCl Hypochlorous acid
hr hour
IC Ion chromotography
ICR Information Collection Rule
ID inside diameter
IESWTR Interim Enhanced Surface Water Treatment Rule
in. inch
IOA International Ozone Association
IOC Inorganic chemical
KMnO4 Potassium permanganate
L liter
LOAEL Lowest observed adverse effect level
LOQ Limit of quantitation
LT1ESWTR Long Term Stage 1 Enhanced Surface Water Treatment Rule
m meter
MCL Maximum Contaminant Level
MCLG Maximum Contaminant Level Goal
M-DBP Microbial and disinfection byproducts
MDL Method Detection Limit
mg milligram
mg/L Milligrams per liter
mgd Million gallons per day
MIB Methylisoborneol
mL milliliter
mm millimeter
mM millimolar
MRDL Maximum Residual Disinfectant Level (as mg/l)
MRDLG Maximum Residual Disinfectant Level Goal
MRL Minimum Reporting Level
MX 3-chloro-4-(dichloromethyl)-5-hydroxyl-2(5H)-furanone
N normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
NaCl Sodium chloride
NCI National Cancer Institute
ND Not detected
NH^2Cl Monochloramine
NIOSH National Institute for Occupational Safety and Health
NIPDWR National Interim Primary Drinking Water Regulation
NOAEL No Observed Adverse Effect Level
NOM Natural Organic Matter
NOMS National Organic Monitoring Survey
NORS National Organics Reconnaissance Survey for Halogenated Organics

NPDWR National Primary Drinking Water Regulation
NTNCWS Nontransient noncommunity water system
NTP Normal Temperature and Pressure
O^2 Oxygen
O^3 Ozone
OBr- Hypobromite ion
OCl- Hypochlorite ion
PCE Perchloroethylene
PE Performance evaluation
POE Point-of-Entry Technologies
POU Point-of-Use Technologies
ppb Parts per billion
ppm Parts per million
PQL Practical Quantitation Level
PTA Packed Tower Aeration
PWS Public water system
RIA Regulatory Impact Analysis
RMCL Recommended Maximum Contaminant Level
RNDB Regulations Negotiation Data Base
RSC Relative Source Contribution
RSD relative standard deviation
SDWA Safe Drinking Water Act, or the “Act,” as amended in 1996
SM Standard Method
SMCL Secondary Maximum Contaminant Level
SMR Standardized mortality ratios
SOC Synthetic Organic Chemical
s_r standard deviation of recovery
SWTR Surface Water Treatment Rule
TCE Trichloroethylene
THM Trihalomethane
THMFP Trihalomethane formation potential
TMV Tobacco mosaic virus
TOC Total organic carbon
TTHM Total trihalomethanes
TWG Technologies Working Group
UV Ultraviolet
VOC Volatile Organic Chemical
WIDB Water Industry Data Base
WS Water supply
X average percent recovery
XDBPs Halogenated DBPs

Glossary

Abiogenesis: The concept of spontaneous generation (that life can come from non-life). This idea was refuted by Pasteur.

Abiotic: The non-living components of an organism's environment. The term abiotic is also used to denote a process which is not facilitated by living organisms.

Aboral: Pertaining to the region of the body opposite that of the mouth. Normally used to describe radially symmetrical animals.

Abscisic acid (ABA): A plant hormone that generally acts to inhibit growth, promote dormancy, and help the plant withstand stressful conditions.

Absorption spectrum: The range of a material's ability to absorb various wavelengths of light. The absorption spectrum is studied to evaluate the function of photosynthetic pigments.

Accessory pigment: A photosynthetic pigment which absorbs light and transfers energy to chlorophylls during photosynthesis. Because accessory pigments have different absorption optima than chlorophylls, presence of accessory pigments allows photosynthetic systems to absorb light more efficiently than would be possible otherwise.

Acellular: Not within cells. Sometimes used as a synonym for unicellular (but multinucleate). Unicellular also pertains to single: celled organisms.

Acetyl CoA: Acetyl CoenzymeA is the entry compound for the Krebs cycle in cellular respiration; formed from a fragment of pyruvic acid attached to a coenzyme.

Acetylcholine: A neurotransmitter substance that carries information across vertebrate neuromuscular junctions and some other synapses.

Acid rain: Rain that is excessively acidic due to the presence of acid: causing pollutants in the atmosphere. Pollutants include nitrogen and sulfur oxides due to burning of coal and oil.

Acid: A substance that increases the hydrogen ion concentration in a solution.

Acidosis: A condition whereby the hydrogen ion concentration of the tissues is increased (and pH decreased). Respiratory acidosis is due to the retention of CO2; metabolic acidosis by retention of acids due either to kidney failure or diarrhea.

Acoelomate: Lacking a coelom.

Acquired immunity: Results from exposure to foreign substances or microbes (also called natural immunity).

Acrosome: An organelle at the tip of a sperm cell that helps the sperm penetrate the egg.

ACTH (adrenocorticotrophic hormone): A proteineinaceous hormone from the anterior pituitary that stimulates the adrenal cortex. Used to stimulate the production of cortisol.

Actin: A globular protein that links into chains, two of which twist helically about each other, forming microfilaments in muscle and other contractile elements in cells.

Action potential: The stimulus- triggered change in the membrane potential of an excitable cell, caused by selective opening and closing of ion channels.

Action spectrum: A graph which illustrates the relationship between some biological activity and wavelength of light.

Activating enzyme: An enzyme that couples a low-energy compound with ATP to yield a high-energy derivative.

Activation energy: In a chemical reaction, the initial investment required to energize the bonds of the reactants to an unstable transition state that precedes the formation of the products.

Active site: That specific portion of an enzyme that attaches to the substrate by means of weak chemical bonds.

Active transport: The movement of a substance across a biological membrane against its concentration or electrochemical gradient with the help of energy input and specific transport proteins.

Adaptation: Any genetically controlled characteristic that increases an organism's fitness, usually by helping the organism to survive and reproduce in the environment it inhabits.

Adaptive radiation: This refers to the rapid evolution of one or a few forms into many different species that occupy different habitats within a new geographical area.

Adhesion: In chemistry, the phenomenon whereby one substance tends to cling to another substance. Water molecules exhibit adhesion, especially toward charged surfaces.

ADP (Adenosine diphosphate): A doubly phosphorylated organic compound that can be further phosphorylated to form ATP.

Adrenal gland: An endocrine gland located adjacent to the kidney in mammals. It is composed of an outer cortex, and a central medulla, each involved in different hormone: mediated phenomena.

Adrenalin: A hormone produced by the pituitary that stimulates the adrenal cortex.

Adsorb: Hold on a surface.

Advanced: New, unlike the ancestral condition.

Aerobic: The condition of requiring oxygen; an aerobe is an organism which can live and grow only in the presence of oxygen.

Age structure: The relative numbers of individuals of each age in a population.

Agnathan: A member of a jawless class of vertebrates represented today by the lampreys and hagfishes.

Agonistic behavior: A type of behavior involving a contest of some kind that determines which competitor gains access to some resource, such as food or mates.

AIDS (acquired immune deficiency syndrome): A condition in which the body's helper T lymphocytes are destroyed, leaving the victim subject to opportunistic diseases.

Alcohol: Any of a class of organic compounds in which one or more - OH groups are attached to a carbon compound.

Aldehyde: An organic molecule with a carbonyl group located at the end of the carbon skeleton.

Algae: An informal term used to designate photosynthetic, plantlike members of the Kingdom Protista. Formerly, most of these organisms were considered to be plants.

Alkaline: Having a pH of more than 7. Alkaline solutions are also said to be basic.

Allantois: One of the four extraembryonic membranes found associated with developing vertebrates; it serves in gas exchange and as a repository for the embryo's nitrogenous waste. In humans, the allantois is involved in early blood formation and development of the urinary bladder.

Allele: Alternate forms of a gene which may be found at a given location (locus) on members of a homologous set of chromosomes. Structural variations between alleles may lead to different phenotypes for a given trait.

Allometric: The variation in the relative rates of growth of various parts of the body, which helps shape the organism.

Allopatric speciation: A type of speciation which occurs when a population becomes segregated into two populations by some sort of geographic barrier (also called geographic speciation). This phenomenon is presumed to have been the mechanism whereby many species of organisms evolved.

Allopolyploid: A common type of polyploid species resulting from two different species interbreeding and combining their chromosomes.

All-or- none: (event) An action that occurs either completely or not at all, such as the generation of an action potential by a neuron.

Allosteric enzyme: An enzyme that can exist in two or more conformations.

Allosteric site: A receptor on an enzyme molecule which is remote from the active site. Binding of the appropriate molecule to the allosteric site changes the conformation of the active site, making it either more or less receptive to the substrate.

Alpha helix: A spiral shape constituting one form of the secondary structure of proteins, arising from a specific hydrogen-bonding structure.

Alternation of generations: Occurrences of a multicellular diploid form, the sporophyte, with a multicellular haploid form, the gametophyte.

Altruism: The willingness of an individual to sacrifice its fitness for the benefit of another.

Alveolus: One of the dead-end, multilobed air sacs that constitute the gas exchange surface of the lungs.

Amino acid: An organic molecule possessing a carboxyl (COOH) and amino group. Amino acids serve as the monomers of polypeptides and proteins.

Amino group: A functional group consisting of a nitrogen atom bonded to two hydrogens; can act as a base in solution, accepting a hydrogen ion and acquiring a charge of +1.

Aminoacyl: tRNA synthetases- A family of enzymes, at least one for each amino acid, that catalyze the attachment of an amino acid to its specific tRNA molecule.

Amoeboid: (cell) A cell which has the tendency to change shape by protoplasmic flow. (movement) A streaming locomotion characteristic of Amoeba and other protists, as well as some individual cells, such as white blood cells, in animals.

AMP (Adenosine monophosphate): A singly phosphorylated organic compound that can be further phosphorylated to form ADP.

Amylase: A starch-digesting enzyme.

Anabolism: A metabolic pathway of biosynthesis that consumes energy to build a large molecule from simpler ones.

Anaerobic: Without oxygen. An organism which lives in the absence of oxygen is called an anaerobe.

Anagenesis: A pattern of evolutionary change involving the transformation of an entire population, sometimes to a state different enough from the ancestral population to justify renaming it as a separate species; also called phyletic.

Analogous: Characteristics of organisms which are similar in function (and often in structure) but different in embryological and/or evolutionary origins.

Analyst: The analyst must have at least 2 years of college lecture and laboratory course work in microbiology or a closely related field. The analyst also must have at least 6 months of continuous bench experience with environmental protozoa detection

techniques and IFA microscopy, and must have successfully analyzed at least 50 water and/or wastewater samples for *Cryptosporidium* and *Giardia*. Six months of additional experience in the above areas may be substituted for two years of college.

Ancestral trait: Trait shared by a group of organisms as a result of descent from a common ancestor.

Aneuploidy: A chromosomal aberration in which certain chromosomes are present in extra copies or are deficient in number.

Anion: A negatively charged ion.

Anisogamous: Reproducing by the fusion of gametes that differ only in size, as opposed to gametes that are produced by oogamous species. Gametes of oogamous species, such as egg cells and sperm, are highly differentiated.

Annual: A plant that completes its entire life cycle in a single year or growing season.

Anterior: Referring to the head end of a bilaterally symmetical animal.

Anthropomorphism: Attributing a human characteristic to an inanimate object or a species other than a human.

Antibiotic: A chemical that kills or inhibits the growth of bacteria, often via transcriptional or translational regulation.

Antibody: A protein, produced by the B lymphocytes of the immune system that binds to a particular antigen.

Anticodon: The specialized base triplet on one end of a tRNA molecule that associates with a particular complementary codon on an mRNA molecule during protein synthesis.

Antidiuretic hormone: A hormone important in osmoregulation (it acts to reduce the elimination of water from the body.

Antigen: A foreign macromolecule that does not belong to the host organism and that elicits an immune response.

Apomorphic character: A derived phenotypic character, or homology, that evolved after a branch diverged from a phylogenetic tree.

Aposematic coloration: Serving as a warning, with reference particularly to colors and structures that signal possession of defensive device.

Aqueous solution: A solution in which water is the solvent.

Archaebacteria: A lineage of prokaryotes, represented today by a few groups of bacteria inhabiting extreme environments. Some taxonomists place archaebacteria in their own kingdom, separate from the other bacteria.

Archenteron: The endoderm-lined cavity formed during the gastrulation process that develops into the digestive tract of the animal.

Aristotle: A Greek philosopher often credited as the first to use empirical and deductive methods in logic.

Artificial selection: The selective breeding of domesticated plants and animals to encourage the occurrence of desirable traits.

Ascus: The elongate spore sac of a fungus of the Ascomycota group.

Asexual: A type of reproduction involving only one parent that produces genetically identical offspring by budding or division of a single cell or the entire organism into two or more parts.

Assortative mating: A type of nonrandom mating in which mating partners resemble each other in certain phenotypic characters.

Asymmetric carbon: A carbon atom covalently bonded to four different atoms or groups of atoms.

Atom: The smallest particle within a chemical element which does not come apart in ordinary chemical reactions; comprised of protons, neutrons and electrons.

Atomic number: The number of protons in the nucleus of an atom, unique for each element.

Atomic Theory: The physical theory of the structure, properties and behavior of the atom.

Atomic weight: The total atomic mass, which is the mass in grams of one mole of the atom (relative to that of 12C, which is designated as 12).

ATP (Adenosine triphosphate): A triply phosphorylated organic compound that functions as "energy currency" for organisms, thus allowing life forms to do work; it can be hydrolyzed in two steps (first to ADP and then to AMP) to liberate 7.3 Kcal of energy per mole during each hydrolysis.

ATPase: An enzyme that functions in producing or using ATP.

Autogenous model: A hypothesis which suggests that the first eukaryotic cells evolved by the specialization of internal membranes originally derived from prokaryotic plasma membranes.

Autoimmune disease: An immunological disorder in which the immune system goes awry and turns against itself.

Autonomic nervous system: A subdivision of the motor nervous system of vertebrates that regulates the internal environment; consists of the sympathetic and parasympathetic subdivisions.

Autopolyploid: A type of polyploid species resulting from one species doubling its chromosome number to become tetraploids, which may self-fertilize or mate with other tetraploids.

Autosome: Chromosomes that are not directly involved in determining sex.

Autotroph: An organism which is able to make organic molecules from inorganic ones either by using energy from the sun or by oxidizing inorganic substances.

Auxin: One of several hormone compounds in plants that have a variety of effects, such as phototropic response through stimulation of cell elongation, stimulation of secondary growth, and development of leaf traces and fruit.

Auxotroph: A nutritional mutant that is unable to synthesize and that cannot grow on media lacking certain essential molecules normally synthesized by wild-type strains of the same species.

Axon: A typically long outgrowth, or process, from a neuron that carries nerve impulses away from the cell body toward target cells.

Axoneme: An internal flagellar structure that occurs in some protozoa, such as *Giardia*, *Spironucleous*, and *Trichonmonas*.

B-cell lymphocyte: A type of lymphocyte that develops in the bone marrow and later produces antibodies, which mediate humoral immunity.

Bacteriophage: A virus that infects bacteria; also called a phage.

Bacterium: A unicellular microorganism of the Kingdom Monera. Bacteria are prokaryotes; their cells have no true nucleus. Bacteria are classified into two groups based on a difference in cell walls, as determined by Gram staining.

Balanced polymorphism: A type of polymorphism in which the frequencies of the coexisting forms do not change noticeably over many generations.

Barr body: The dense object that lies along the inside of the nuclear envelope in cells of female mammals, representing the one inactivated X chromosome.

Basal body: A cell structure identical to a centriole that organizes and anchors the microtubule assembly of a cilium or flagellum.

Base pairing: Complementary base pairing refers to the chemical affinities between specific base pairs in a nucleic acid: adenine always pairs with thymine, and guanine always pairs with cytosine. In pairing between DNA and RNA, the uracil of RNA always pairs with adenine. Complementary base pairing is not only responsible for the DNA double helix, but it is also essential for various in vitro techniques such as PCR (polymerase chain reaction). Complementary base pairing is also known as Watson-Crick pairing.

Base: A substance that reduces the hydrogen ion concentration in a solution.

Basement membrane: The floor of an epithelial membrane on which the basal cells rest.

Basidium: The spore-bearing structure of Basidiomycota.

Batesian mimicry: A type of mimicry in which a harmless species looks like a different species that is poisonous or otherwise harmful to predators.

Behavioral ecology: A heuristic approach based on the expectation that Darwinian fitness (reproductive success) is improved by optimal behavior.

Benign tumor: A noncancerous abnormal growth composed of cells that multiply excessively but remain at their place of origin in the body.

Benthic: Pertaining to the bottom region of an aquatic environment.

Beta pleated sheet: A zigzag shape, constituting one form of the secondary structure of proteins formed of hydrogen bonds between polypeptide segments running in opposite directions.

Bilateral symmetry: The property of having two similar sides, with definite upper and lower surfaces and anterior and posterior ends. The Bilateria are members of the branch of Eumetazoa (Kingdom Animalia) which possess bilateral symmetry.

Bile: A mixture of substances containing bile salts, which emulsify fats and aid in their digestion and absorption.

Binary fission: The kind of cell division found in prokaryotes, in which dividing daughter cells each receive a copy of the single parental chromosome.

Binomial nomenclature: Consisting of two names. In biology, each organism is given a *genus* name and a species name (i.e., the human is Homo sapiens.

Biogenesis: A central concept of biology, that living organisms are derived from other living organisms (contrasts to the concept of abiogenesis, or spontaneous generation, which held that life could be derived from inanimate material).

Biogeochemical cycle: A circuit whereby a nutrient moves between both biotic and abiotic components of ecosystems.

Biogeography: The study of the past and present distribution of species.

Biological magnification: Increasing concentration of relatively stable chemicals as they are passed up a food chain from initial consumers to top predators.

Biological species: A population or group of populations whose members have the potential to interbreed. This concept was introduced by Ernst Mayr.

Biomass: The total weight of all the organisms, or of a designated group of organisms, in a given area

Biome: A large climatic region with characteristic sorts of plants and animals.

Biosphere: The region on and surrounding the earth which is capable of supporting life. Theoretically, the concept may be ultimately expanded to include other regions of the universe.

BMR: The basal metabolic rate is the minimal energy (in kcal) required by a homeotherm to fuel itself for a given time. Measured within the thermoneutral zone for a postabsorptive animal at rest.

Carolus Linnaeus: Swedish botanist and originator of the binomial nomenclature system of taxonomic classification.

Cyst: A phase or a form of an organism produced either in response to environmental conditions or as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant cell wall.

Ecdysone: A steroid hormone that triggers molting in arthropods.

Ecological efficiency: The ratio of net productivity at one trophic level to net productivity at the next lower level.

Ecological niche: The sum total of an organism's utilization of the biotic and abiotic resources of its environment. The fundamental niche represents the theoretical capabilities and the realized niche represents the actual role.

Ecology: The study of how organisms interact with their environments.

Ecosystem: The sum of physical features and organisms occurring in a given area.

Ectoderm: The outermost tissue layer of an animal embryo. Also, tissue derived from an embryonic ectoderm.

Ectotherm: An organism that uses environmental heat and behavior to regulate its body temperature.

Edward Jenner: A pioneer of vaccination; used vaccination with material from cowpox lesions to protect people against smallpox.

Effector: The part of an organism that produces a response to a stimulus.

Electrical synapse: A junction between two neurons separated only by a gap junction, in which the local currents sparking the action potential pass directly between the cells.

Electrocardiogram: A plot of electrical activity of the heart over the cardiac cycle; measured via multiple skin electrodes.

Electrochemical gradient: Combined electrostatic and osmotic-concentration gradient, such as the chemiosmotic gradient of mitochondria and chloroplasts.

Electrogenic pump: An ion transport protein generating voltage across a membrane.

Electromagnetic spectrum: The entire spectrum of radiation; ranges in wavelength from less than a nanometer to more than a kilometer.

Electron microscope: A microscope that focuses an electron beam through a specimen, resulting in resolving power a thousandfold greater that that of a light microscope. A transmission EM is used to study the internal structure of thin sections of cells; a scanning EM is used to study the ultrastructure of surfaces.

Electron transport chain: A series of enzymes found in the inner membranes of mitochondria and chloroplasts. These are involved in transport of protons and electrons across the membrane during ATP synthesis.

Electron: A negatively charged subatomic particle of an atom or ion. In atoms, the number of electrons present is equal to the number of positively charged protons present. Hence, atoms are electrically neutral.

Electronegativity: A property exhibited by some atoms whereby the nucleus has a tendency to pull electrons toward itself.

Electronic charge unit: The charge of one electron (1.6021 x 10e: 19 coulomb).

Electrostatic force: The attraction between particles with opposite charges.

Electrostatic gradient: The free-energy gradient created by a difference in charge between two points, generally the two sides of a membrane.

Element: Any substance that cannot be broken down into another substance by ordinary chemical means.

Elimination: The release of unabsorbed wastes from the digestive tract.

Emergent property: A property exhibited at one level of biological organization but not exhibited at a lower level. For example, a population exhibits a birth rate, an organism does not.

Emulsion: A suspension, usually as fine droplets of one liquid in another.

Endergonic: A phenomenon which involves uptake of energy.

Endocrine: A phenomenon which relates to the presence of ductless glands of the type typically found in vertebrates. The endocrine system involves hormones, the glands which secrete them, the molecular hormone receptors of target cells, and interactions between hormones and the nervous system.

Endocytosis: A process by which liquids or solid particles are taken up by a cell through invagination of the plasma membrane.

Endoderm: The innermost germ layer of an animal embryo.

Endodermis: A plant tissue, especially prominent in roots, that surrounds the vascular cylinder; all endodermal cells have Casparian strips.

Endomembrane system: The system of membranes inside a eukaryotic cell, including the membranous vesicles which associate with membrane sheets and/or tubes.

Endometrium: The inner lining of the uterus, which is richly supplied with blood vessels that provide the maternal part of the placenta and nourish the developing embryo.

Endonuclease: An enzyme that breaks bonds within nucleic acids. A restriction endonuclease is an enzyme that breaks bonds only within a specific sequence of bases.

Endoplasmic reticulum: A system of membrane-bounded tubes and flattened sacs, often continuous with the nuclear envelope, found in the cytoplasm of eukaryotes. Exists as rough ER, studded with ribosomes, and smooth ER, lacking ribosomes.

Endorphin: A hormone produced in the brain and anterior pituitary that inhibits pain perception.

Endoskeleton: An internal skeleton.

Endosperm: A nutritive material in plant seeds which is triploid (3n) and results from the fusion of three nuclei during double fertilization.

Endosymbiotic: 1) An association in which the symbiont lives within the host 2) A widely accepted hypothesis concerning the evolution of the eukaryotic cell: the idea that eukaryotes evolved as a result of symbiotic associations between prokaryote cells. Aerobic symbionts ultimately evolved into mitochondria; photosynthetic symbionts became chloroplasts.

Endothelium: The innermost, simple squamous layer of cells lining the blood vessels; the only constituent structure of capillaries.

Endothermic: In chemistry, a phenomenon in which energy is absorbed by the reactants. In physiology, this term concerns organisms whose thermal relationship with the environment is dependent substantially on internal production of heat.

Endotoxin: A component of the outer membranes of certain gram-negative bacteria responsible for generalized symptoms of fever and ache.

Energy: The capacity to do work by moving matter against an opposing force.

Enhancer: A DNA sequence that recognizes certain transcription factors that can stimulate transcription of nearby genes.

Entropy: A type of energy which is not biologically useful to do work (in contrast to free energy).

Envelope: 1) (nuclear) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (virus) A structure which is present on the outside of some viruses (exterior to the capsid).

Enzyme: A protein, on the surface of which are chemical groups so arranged as to make the enzyme a catalyst for a chemical reaction.

Epicotyl: A portion of the axis of a plant embryo above the point of attachment of the cotyledons; forms most of the shoot.

Epidermis: The outermost portion of the skin or body wall of an animal.

Epinephrine: A hormone produced as a response to stress; also called adrenaline.

Epiphyte: A plant that nourishes itself but grows on the surface of another plant for support, usually on the branches or trunks of tropical trees.

Episome: Genetic element at times free in the cytoplasm, at other times integrated into a chromosome.

Epistasis: A phenomenon in which one gene alters the expression of another gene that is independently inherited.

Epithelium: An animal tissue that forms the covering or lining of all free body surfaces, both external and internal.

Epitope: A localized region on the surface of an antigen that is chemically recognized by antibodies; also called antigenic determinant.

Equation: A precise representation of the outcome of a chemical reaction, showing the reactants and products, as well as the proportions of each.

Equilibrium: In a reversible reaction, the point at which the rate of the forward reaction equals that of the reverse reaction. (constant) At equilibrium, the ratio of products to reactants. (potential) The membrane potential for a given ion at which the voltage exactly balances the chemical diffusion gradient for that ion.

Ernst Mayr: Formulated the biological species concept.

Erythrocyte: A red blood corpuscle.

Esophagus: An anterior part of the digestive tract; in mammals it leads from the pharynx to the stomach.

Essential: 1) An amino or fatty acid which is required in the diet of an animal because it cannot be synthesized. 2) A chemical element required for a plant to grow from a seed and complete the life cycle.

Estivation: A physiological state characterized by slow metabolism and inactivity, which permits survival during long periods of elevated temperature and diminished water supplies.

Estradiol: 1,3,5(10)-estratriene- 3,17 beta-diol C18H24O2. This is the natural hormone - present in pure form in the urine of pregnant mares and in the ovaries of pigs.

Estrogen: Any of a group of vertebrate female sex hormones.

Estrous cycle: In female mammals, the higher primates excepted, a recurrent series of physiological and behavioral changes connected with reproduction.

Estrus: The limited period of heat or sexual receptivity that occurs around ovulation in female mammals having estrous cycles.

Estuary: That portion of a river that is close enough to the sea to be influenced by marine tides.

Ethylene: The only gaseous plant hormone, responsible for fruit ripening, growth inhibition, leaf abscission, and aging.

Eubacteria: The lineage of prokaryotes that includes the cyanobacteria and all other contemporary bacteria except archaebacteria.

Euchromatin: The more open, unraveled form of eukaryotic chromatin, which is available for transcription.

Eucoelomate: An animal whose body cavity is completely lined by mesodern, the layers of which connect dorsally and ventrally to form mesenteries.

Eukaryote: A lifeform comprised of one or more cells containing a nucleus and membrane - bound organelles. Included are members of the Kingdoms Protista, Fungi, Plantae and Animalia.

Eumetazoa: Members of the subkingdom that includes all animals except sponges.

Eutrophic: A highly productive condition in aquatic environments which owes to excessive concentrations of nutrients which support the growth of primary producers.

Evaginated: Folded or protruding outward.

Evaporative cooling: The property of a liquid whereby the surface becomes cooler during evaporation, owing to the loss of highly kinetic molecules to the gaseous state.

Eversible: Capable of being turned inside out.

Evolution: All of the changes that have transformed life on earth from its earliest beginnings to the diversity that characterizes it today. As used in biology, the term evolution means descent with change.

Excitable cells: A cell, such as a neuron or a muscle cell that can use changes in its membrane potential to conduct signals.

Excitatory postsynaptic potential: An electrical change (depolarization) in the membrane of a postsynaptic neuron caused by the binding of an excitatory neurotransmitter from a presynaptic cell to a postsynaptic receptor. This phenomenon facilitates generation of an action potential in the PSP.

Excretion: Release of materials which arise in the body due to metabolism (e.g., CO_2, NH3, H20).

Exergonic: A phenomenon which involves the release of energy.

Exocytosis: A process by which a vesicle within a cell fuses with the plasma membrane and releases its contents to the outside.

Exon: A part of a primary transcript (and the corresponding part of a gene) that is ultimately either translated (in the case of mRNA) or utilized in a final product, such as tRNA.

Exoskeleton: An external skeleton, characteristic of members of the phylum, Arthropoda.

Exotoxin: A toxic protein secreted by a bacterial cell that produces specific symptoms even in the absence of the bacterium.

Exponential: (population growth) The geometric increase of a population as it grows in an ideal, unlimited environment.

Extraembryonic membranes: Four membranes (yolk sac, amnion, chorion, allantois) that support the developing embryo in reptiles, birds, and mammals.

Extrinsic: External to, not a basic part of; as in extrinsic isolating mechanism.

F plasmid: The fertility factor in bacteria, a plasmid that confers the ability to form pili for conjugation and associated functions required for transfer of DNA from donor to recipient.

F1 generation: The first filial or hybrid offspring in a genetic cross-fertilization.

F2 generation: Offspring resulting from interbreeding of the hybrid F1 generation.

Facilitated diffusion: Passive movement through a membrane involving a specific carrier protein; does not proceed against a concentration gradient.

Facultative: An organism which exhibits the capability of changing from one habit or metabolic pathway to another, when conditions warrant. (anaerobe) An organism that makes ATP by aerobic respiration if oxygen is present but that switches to fermentation under anaerobic conditions.

Fat: A biological compound consisting of three fatty acids linked to one glycerol molecule.

Fate map: A means of tracing the fates of cells during embryonic development.

Fatty acid: A long carbon chain carboxylic acid. Fatty acids vary in length and in the number and location of double bonds; three fatty acids linked to a glycerol molecule form fat.

Fauna: The animals of a given area or period.

Feature detector: A circuit in the nervous system that responds to a specific type of feature, such as a vertically moving spot or a particular auditory time delay.

Feces: Indigestible wastes discharged from the digestive tract.

Feedback: The process by which a control mechanism is regulated through the very effects it brings about. Positive feedback is when the effect is amplified; negative feedback is when the effect tends toward restoration of the original condition. Feedback inhibition is a method of metabolic control in which the end-product of a metabolic pathway acts as an inhibitor of an enzyme within that pathway.

Fermentation: Anaerobic production of alcohol, lactic acid or similar compounds from carbohydrate resulting from glycolysis.

Fibrin: The activated form of the blood: clotting protein fibrinogen, which aggregates into threads that form the fabric of the clot.

Fibroblast: A type of cell in loose connective tissue that secretes the protein ingredients of the extracellular fibers.

Fibronectins: A family of extracellular glycoproteins that helps embryonic cells adhere to their substrate as they migrate.

Fitness: The extent to which an individual passes on its genes to the next generation. Relative fitness is the number of offspring of an individual compared to the mean.

Fixation: 1) Conversion of a substance into a biologically more usable form, for example, CO2 fixation during photosynthesis and N2 fixation. 2) Process of treating living tissue for microscopic examination.

Fixed: action pattern (FAP): A highly: stereotyped behavior that is innate and must be carried to completion once initiated.

Flaccid: Limp; walled cells are flaccid in isotonic surroundings, where there is no tendency for water to enter.

Flagellin: The protein from which prokaryotic flagella are constructed.

Flagellum: A long whiplike appendage that propels cells during locomotion in liquid solutions. The prokaryote flagellum is comprised of a protein, flagellin. The eukaryote flagellum is longer than a cilium, but as a similar internal structure of microtubules in a"9 + 2" arrangement.

Flame cell: A flagellated cell associated with the simplest tubular excretory system, present in flatworms: it acts to directly regulate the contents of the extracellular fluid.

Flora: The plants of a given area or period.

Flow cytometer: A particle: sorting instrument capable of counting protozoa.

Fluid feeder: An animal that lives by sucking nutrient-rich fluids from another living organism.

Fluid mosaic model: The currently accepted model of cell membrane structure, which envisions the membrane as a mosaic of individually inserted protein molecules drifting laterally in a fluid bilayer of phospholipids.

Follicle stimulating hormone (FSH): A gonadotropic hormone of the anterior pituitary that stimulates growth of follicles in the ovaries of females and function of the seminiferous tubules in males.

Follicle: A jacket of cells around an egg cell in an ovary.

Food chain: Sequence of organisms, including producers, consumers, and decomposers, through which energy and materials may move in a community.

Food web: The elaborate, interconnected feeding relationships in an ecosystem.

Foot candle: Unit of illumination; the illumination of a surface produced by one standard candle at a distance of one foot.

Formula: A precise representation of the structure of a molecule or ion, showing the proportion of atoms which comprise the material.

Founder effect: The difference between the gene pool of a population as a whole and that of a newly isolated population of the same species.

Fractionation: An experimental technique which involves separation of parts of living tissue from one another using centrifugation.

Fragmentation: A mechanism of asexual reproduction in which the parent plant or animal separates into parts that reform whole organisms.

Frameshift mutation: A mutation occurring when the number of nucleotides inserted or deleted is not a multiple of 3, thus resulting in improper grouping into codons.

Free energy of activation: See Activation energy.

Free energy: Usable energy in a chemical system; energy available for producing change.

Frequency: dependent selection: A decline in the reproductive success of a morph resulting from the morph's phenotype becoming too common in a population; a cause of balanced polymorphism in populations.

Functional group: One of several groups of atoms commonly found in organic molecules. A functional group contributes somewhat predictable properties to the molecules which possess them.

Fundamental niche: The total resources an organism is theoretically capable of utilizing.

G: (protein) A membrane protein that serves as an intermediary between hormone receptors and the enzyme adenylate cyclase, which converts ATP to cAMP in the second messenger system in nonsteroid hormone action. Depending on the system, G proteins either increase or decrease cAMP production.

G1 phase: The first growth phase of the cell cycle, consisting of the portion of interphase before DNA synthesis is initiated.

G2 phase: The second growth phase of the cell cycle, consisting of the portion of interphase after DNA synthesis but before mitosis.

Gaia hypothesis: An idea, first formulated by James E. Lovelock in 1979, which suggests that the biosphere of the earth exists as a "superorganism" which exhibits homeostatic self- regulation of the environment-biota global system.

Gametangium: The reproductive organ of bryophytes, consisting of the male antheridium and female archegonium; a multichambered jacket of sterile cells in which gametes are formed.

Gamete: A sexual reproductive cell that must usually fuse with another such cell before development begins; an egg or sperm.

Gametophyte: A haploid plant that can produce gametes.

Ganglion: A structure containing a group of cell bodies of neurons.

Gap junction: A narrow gap between plasma membranes of two animal cells, spanned by protein channels. They allow chemical substances or electrical signals to pass from cell to cell.

Gastrula: A two-layered, later three-layered, animal embryonic stage.

Gastrulation: The process by which a blastula develops into a gastrula, usually by an involution of cells.

Gated ion channel: A membrane channel that can open or close in response to a signal, generally a change in the electrostatic gradient or the binding of a hormone, transmitter, or other molecular signal.

Gel electrophoresis: In general, electrophoresis is a laboratory technique used to separate macromolecules on the basis of electric charge and size; the technique involves application of an electric field to a population of macromolecules which disperse according to their electric mobilities. In gel electrophoresis, the porous medium through which the macromolecules move is a gel.

Gel: Colloid in which the suspended particles form a relatively orderly arrangement.

Gene amplification: Any of the strategies that give rise to multiple copies of certain genes, thus facilitating the rapid synthesis of a product (such as rRna for ribosomes) for which the demand is great.

Gene cloning: Formation by a bacterium, carrying foreign genes in a recombinant plasmid, of a clone of identical cells containing the replicated foreign genes.

Gene delivery: This is a general term for the introduction of new genetic elements into the genomes of living cells. The delivery problem is essentially conditioned by the fact that the new genetic elements are usually large, and by the presence of the outer cell membrane and the nuclear membrane acting as barriers to incorporation of the new DNA into the genome already present in the nucleus. Viruses possess various natural biochemical methods for achieving gene delivery; artificial gene delivery is one of the essential problems of "genetic engineering". The most important barrier is apparently the outer cell membrane, which is essentially a lipid barrier, and introduction of any large complex into the cell requires a fusion of one kind or another with this membrane. Liposomes, which consist of lipid membranes themselves, and which can fuse with outer cell membranes, are thus potential vehicles for delivery of many substances, including DNA.

Gene flow: The movement of genes from one part of a population to another, or from one population to another, via gametes.

Gene pool: The sum total of all the genes of all the individuals in a population.

Gene regulation: Any of the strategies by which the rate of expression of a gene can be regulated, as by controlling the rate of transcription.

Gene: The hereditary determinant of a specified characteristic of an individual; specific sequences of nucleotides in DNA.

Genetic drift: Change in the gene pool as a result of chance and not as a result of selection, mutation, or migration.

Genetic recombination: The general term for the production of offspring that combine traits of the two parents.

Genetics: The science of heredity; the study of heritable information.

Genome: The cell's total complement of DNA.

Genomic equivalence: The presence of all of an organism's genes in all of its cells.

Genomic imprinting: The parental effect on gene expression. Identical alleles may have different effects on offspring depending on whether they arrive in the zygote via the ovum or via the sperm.

Genomic library: A set of thousands of DNA segments from a genome, each carried by a plasmid or phage.

Genotype: The particular combination of genes present in the cells of an individual.

Genus: A taxonomic category above the species level, designated by the first word of a species' binomial Latin name.

Glial cell: A nonconducting cell of the nervous system that provides support, insulation, and protection for the neurons.

Glomerulus: A capillary bed within Bowman's capsule of the nephron; the site of ultrafiltration.

Glucose: A six carbon sugar which plays a central role in cellular metabolism.

Glycocalyx: The layer of protein and carbohydrates just outside the plasma membrane of an animal cell; in general, the proteins are anchored in the membrane, and the carbohydrates are bound to the proteins.

Glycogen: A long, branched polymer of glucose subunits that is stored in the muscles and liver of animals and is metabolized as a source of energy.

Glycolysis: A metabolic pathway which occurs in the cytoplasm of cells and during which glucose is oxidized anaerobically to form pyruvic acid.

Glycoprotein: A protein with covalently linked sugar residues. The sugars may be bound to OH side chains of the polypeptide (O: linked) or the amide nitrogen of asparagine side chains (N: linked).

Glycosidic: A type of bond which links monosaccharide subunits together in di- or polysaccharides.

Glyoxysome: A type of microbody found in plants, in which stored lipids are converted to carbohydrates.

Golgi apparatus: A system of concentrically folded membranes found in the cytoplasm of eukaryotic cells. Plays a role in the production and release of secretory materials such as the digestive enzymes manufactured in the pancreas.

Gonadotropin: Refers to a member of a group of hormones capable of promoting growth and function of the gonads. Includes hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are stimulatory to the gonads.

Graded potential: A local voltage change in a neuron membrane induced by stimulation of a neuron, with strength proportional to the strength of the stimulus and lasting about a millisecond.

Granum: A stacklike grouping of photosynthetic membranes in a chloroplast.

Gravitropism: A response of a plant or animal in response to gravity.

Greenhouse effect: The warming of the Earth due to atmospheric accumulation of carbon dioxide which absorbs infrared radiation and slows its escape from the irradiated Earth.

Gregor Mendel: The first to make quantitative observations of the patterns of inheritance and proposing plausible explanations for them.

Growth factor: A protein that must be present in a cell's environment for its normal growth and development.

Guard cell: A specialized epidermal cell that regulates the size of stoma of a leaf.

Gymnosperm: A vascular plant that bears naked seeds not enclosed in any specialized chambers.

Habit: In biology, the characteristic form or mode of growth of an organism.

Habitat: The kind of place where a given organism normally lives.

Habituation: The process that results in a long-lasting decline in the receptiveness of interneurons to the input from sensory neurons or other interneurons (sensitization, adaptation).

Half: The average amount of time it takes for one-half of a specified quantity of a substance to decay or disappear.

Haploid: The condition of having only one kind of a given type of chromosome.

Hardy-Weinberg theorem: An axiom maintaining that the sexual shuffling of genes alone cannot alter the overall genetic makeup of a population.

Haustorium: In parasitic fungi, a nutrient-absorbing hyphal tip that penetrates the tissues of the host but remains outside the host cell membranes.

Haversian system: One of many structural units of vertebrate bone, consisting of concentric layers of mineralize bone matrix surrounding lacunae, which contain osteocytes, and a central canal, which contains blood vessels and nerves.

Heat of vaporization: The amount of energy absorbed by a substance when it changes state to a gas. Water absorbs approximately 580 calories per gram when it changes from liquid water to water vapor.

Heat: The total amount of kinetic energy due to molecular motion in a body of matter. Heat is energy in its most random form.

Helper T cell: A type of T cell that is required by some B cells to help them make antibodies or that helps other T cells respond to antigens or secrete lymphokines or interleukins.

Hemagglutinin: A surface antigen on influenza viruses which controls infectivity by associating with receptors on host erythrocytes or other cells.

Hematopoiesis: The formation of blood.

Hematopoietic stem cells: Cells found in the bone marrow of adult mammals which give rise to erythroid stem cells, lymphoid stem cells, and myeloid stem cells. Such cells give rise to erythrocytes and a variety of types of lymphocytes and leucocytes.

Hemoglobin: An iron-containing respiratory pigment found in many organisms.

Hemolymph: In invertebrates with open circulatory systems, the body fluid that bathes tissues.

Hemophilia: A genetic disease resulting from an abnormal sex-linked recessive gene, characterized by excessive bleeding following injury.

Hepatic: Pertaining to the liver.

Heredity: A biological phenomenon whereby characteristics are transmitted from one generation to another by virtue of chemicals (i.e. DNA) transferred during sexual or asexual reproduction.

Herpesvirus: A double stranded DNA virus with an enveloped, icosahedral capsid.

Hertz: A unit of frequency equal to one cycle per second.

Heterochromatin: Nontranscribed eukaryotic chromatin that is so highly compacted that it is visible with a light microscope during interphase.

Heterochrony: Evolutionary changes in the timing or rate of development.

Heterocyst: A specialized cell that engages in nitrogen fixation on some filamentous cyanobacteria.

Heterogamy: The condition of producing gametes of two different types (contrast with isogamy).

Heteromorphic: A condition in the life cycle of all modern plants in which the sporophyte and gametophyte generations differ in morphology.

Heterosporous: Referring to plants in which the sporophyte produces two kinds of spores that develop into unisexual gametophytes, either male or female.

Heterotroph: An organism dependent on external sources of organic compounds as a means of obtaining energy and/or materials. Such an organism requires carbon ("food") from its environment in an organic form. (synonym-organotroph).

Heterozygote advantage: A mechanism that preserves variation in eukaryotic gene pools by conferring greater reproductive success on heterozygotes over individuals homozygous for any one of the associated alleles.

Heterozygous: The condition whereby two different alleles of the gene are present within the same cell.

Histamine: A substance released by injured cells that causes blood vessels to dilate during an inflammatory response.

Histology: The study of tissues.

Histone: A type of protein characteristically associated with the chromosomes of eukaryotes.

HIV-1: Acute human immunodeficiency virus type 1 is the subtype of HIV (human immune deficiency virus) that causes most cases of AIDS in the Western Hemisphere, Europe, and Central, South, and East Africa. HIV is a retrovirus (subclass lentivirus), and retroviruses are single: stranded RNA viruses that have an enzyme called reverse transcriptase. With this enzyme the viral RNA is used as a template to produce viral DNA from cellular material. This DNA is then incorporated into the host cell's genome, where it codes for the synthesis of viral components. An HIV-1 infection should be distinguished from AIDS. Acquired immunodeficiency syndrome (AIDS) is a secondary immunodeficiency syndrome resulting from HIV infection and characterized by opportunistic infections, malignancies, neurologic dysfunction, and a variety of other syndromes.

Holoblastic: A type of cleavage in which there is complete division of the egg, as in eggs having little yolk (sea urchin) or a moderate amount of yolk (frog).

Home range: An area within which an animal tends to confine all or nearly all its activities for a long period of time.

Homeobox: Specific sequences of DNA that regulate patterns of differentiation during development of an organism.

Homeostasis: A phenomenon whereby a state or process (for example, within an organism) is regulated automatically despite the tendency for fluctuations to occur.

Homeothermic: Capable of regulation of constancy with respect to temperature.

Homeotic genes: Genes that control the overall body plan of animals by controlling the developmental fate of groups of cells.

Homeotic: (mutation) A mutation in genes regulated by positional information that results in the abnormal substitution of one type of body part in place of another.

Homologous chromosomes: Chromosomes bearing genes for the same characters.

Homologous structures: Characters in different species which were inherited from a common ancestor and thus share a similar ontogenetic pattern.

Homology: Similarity in characteristics resulting from a shared ancestry.

Homoplasy: The presence in several species of a trait not present in their most common ancestor. Can result from convergent evolution, reverse evolution, or parallel evolution.

Homosporous: Referring to plants in which a single type of spore develops into a bisexual gametophyte having both male and female sex organs.

Homozygous: Having two copies of the same allele of a given gene.

Hormone: A control chemical secreted in one part of the body that affects other parts of the body.

Host range: The limited number of host species, tissues, or cells that a parasite (including viruses and bacteria) can infect.

Humoral immunity: The type of immunity that fights bacteria and viruses in body fluids with antibodies that circulate in blood plasma and lymph, fluids formerly called humors.

Hybrid vigor: Increased vitality (compared to that of either parent stock) in the hybrid offspring of two different, inbred parents.

Hybrid: In evolutionary biology, a cross between two species. In genetics, a cross between two genetic types.

Hybridization: The process whereby a hybrid results from interbreeding two species; 2) DNA hybridization is the comparison of whole genomes of two species by estimating the extent of hydrogen bonding that occurs between single-stranded DNA obtained from the two species.

Hybridoma: A hybrid cell that produces monoclonal antibodies in culture, formed by the fusion of a myeloma cell with a normal antibody-producing lymphocyte.

Hydration shell: A "covering" of water molecules which surrounds polar or charged substances in aqueous solutions. The association is due to the charged regions of the polar water molecules themselves.

Hydrocarbon: Any compound made of only carbon and hydrogen.

Hydrogen bond: A type of bond formed when the partially positive hydrogen atom of a polar covalent bond in one molecule is attracted to the partially negative atom of a polar covalent bond in another.

Hydrogen ion: A single proton with a charge of +1. The dissociation of a water molecule (H2O) leads to the generation of a hydroxide ion (OH-) and a hydrogen ion (H+).

Hydrolysis: The chemical reaction that breaks a covalent bond through the addition of hydrogen (from a water molecule) to the atom forming one side of the original bond, and a hydroxyl group to the atom on the other side.

Hydrophilic: Having an affinity for water.

Hydrophobic interaction: A type of weak chemical bond formed when molecules that do not mix with water coalesce to exclude the water.

Hydrophobic: The physicochemical property whereby a substance or region of a molecule resists association with water molecules.

Hydrostatic: Pertaining to the pressure and equilibrium of fluids. A hydrostatic skeleton is a skeletal system composed of fluid held under pressure in a closed body

compartment; the main skeleton of most cnidarians, flatworms, nematodes, and annelids.

Hydroxyl group: A functional group consisting of a hydrogen atom joined to an oxygen atom by a polar covalent bond. Molecules possessing this group are soluble in water and are called alcohols.

Hydroxyl ion: The OH- ion.

Hyperosmotic: A solution with a greater solute concentration than another, a hypoosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

Hyperpolarization: An electrical state whereby the inside of the cell is made more negative relative to the outside than was the case at resting potential. A neuron membrane is hyperpolarized if the voltage is increased from the resting potential of about -70 mV, reducing the chance that a nerve impulse will be transmitted.

Hypertrophy: Abnormal enlargement, excessive growth.

Hypha: A fungal filament.

Hypocotyl: The portion of the axis of a plant embryo below the point of attachment of the cotyledons; forms the base of the shoot and the root.

Hypoosmotic solution: A solution with a lesser solute concentration than another, a hyperosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

Hypothesis: A formal statement of supposition offered to explain observations. Note that a hypothesis is only useful if it can be tested. Even if correct, it is not scientifically useful if untestable.

Hypothetico-deductive: A method used to test hypotheses. If deductions formulated from the hypothesis are tested and proven false, the hypothesis is rejected.

Imaginal disk: An island of undifferentiated cells in an insect larva, which are committed (determined) to form a particular organ during metamorphosis to the adult.

Imbibition: The soaking of water into a porous material that is hydrophilic.

Immune response: 1) A primary immune response is the initial response to an antigen, which appears after a lag of a few days. 2) A secondary immune response is the response elicited when the animal encounters the same antigen at a later time. The secondary response is normally more rapid, of greater magnitude and of longer duration than the primary response.

Immunoglobulins: The class of proteins comprising the antibodies.

Immunological: 1) Immunological distance is the amount of difference between two proteins as measured by the strength of the antigen-antibody reaction between them. 2) Immunological tolerance is a mechanism by which an animal does not mount an immune response to the antigenic determinants of its own macromolecules.

Immunomagnetic separation (IMS): A purification procedure that uses microscopic, magnetically responsive particles coated with an antibodies targeted to react with a specific pathogen in a fluid stream. Pathogens are selectively removed from other debris using a magnetic field.

Imprinting: A type of learned behavior with a significant innate component, acquired during a limited critical period.

In vitro fertilization: Fertilization of ova in laboratory containers followed by artificial implantation of the early embryo in the mother's uterus.

Incomplete dominance: A type of inheritance in which F1 hybrids have an appearance that is intermediate between the phenotypes of the parental varieties.

Indeterminate: 1) A type of cleavage exhibited during the embryonic development in deuterostomes, in which each cell produced by early cleavage divisions retains the capacity to develop into a complete embryo; 2) A type of growth exhibited by plants: they continue to grow as long as they live, because they always retain meristematic cells capable of undergoing mitosis.

Induced fit: The change in shape of the active site of an enzyme so that it binds more snugly to the substrate, induced by entry of the substrate.

Induction: 1) The ability of one group of embryonic cells to influence the development of another. 2) A method in logic which proceeds from the specific to general and develops a general statement which explains all of the observations. Commonly used to formulate scientific hypotheses.

Industrial melanism: Melanism which has resulted from blackening of environmental surfaces (tree bark, etc.) by industrial pollution. This favors survival of melanic forms such as moths which rest on tree bark and are less likely to be seen by predators.

Infectious: 1) An infectious disease is a disease caused by an infectious microbial or parasitic agent. 2) Infectious hepatitis is the former name for hepatitis A. 3) Infectious mononucleosis is an acute disease that affects many systems, caused by the Epstein: Barr virus.

Inflammatory response: A line of defense triggered by penetration of the skin or mucous membranes, in which small blood vessels in the vicinity of an injury dilate and become leakier, enhancing infiltration of leukocytes; may also be widespread in the body.

Ingestion: A heterotrophic mode of nutrition in which other organisms or detritus are eaten whole or in pieces.

Inhibitory postsynaptic potential: An electrical charge (hyperpolarization) in the membrane of a postsynaptic neuron caused by the binding of an inhibitory neurotransmitter from a presynaptic cell to a postsynaptic receptor.

Initial precision and recovery (IPR): Four aliquots of spiking suspension analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Inner cell mass: A cluster of cells in a mammalian blastocyst that protrudes into one end of the cavity and subsequently develops into the embryo proper and some of the extraembryonic membranes.

Inositol triphosphate: The second messenger, which functions as an intermediate between certain nonsteroid hormones and the third messenger, a rise in cytoplasmic Ca++ concentration.

Insertion: A mutation involving the addition of one or more nucleotide pairs to a gene.

Insight learning: The ability of an animal to perform a correct or appropriate behavior on the first attempt in a situation with which it has had no prior experience.

Insulin: The vertebrate hormone that lowers blood sugar levels by promoting the uptake of glucose by most body cells and promoting the synthesis and storage of glycogen in the liver; also stimulates protein and fat synthesis; secreted by endocrine cells of the pancreas called islets of Langerhans.

Integral protein: A protein of biological membranes that penetrates into or spans the membrane.

Interbreed: To breed with another kind or species; hybridize.

Interferon: A chemical messenger of the immune system, produced by virus: infected cells and capable of helping other cells resist the virus.

Interleukin: 1: A chemical regulator (cytokine) secreted by macrophages that have ingested a pathogen or foreign molecule and have bound with a helper T cell; stimulates T cells to grow and divide and elevates body temperature. Interleukin: 2, secreted by activated T cells, stimulates helper T cells to proliferate more rapidly.

Intermediate filament: A component of the cytoskeleton that includes all filaments intermediate in size between microtubules and microfilaments.

Interneuron: An association neuron; a nerve cell within the central nervous system that forms synapses with sensory and motor neurons and integrates sensory input and motor output.

Internode: The segment of a plant stem between the points where leaves are attached.

Interstitial cells: Cells scattered among the seminiferous tubules of the vertebrate testis that secrete testosterone and other androgens, the male sex hormones.

Interstitial fluid: The internal environment of vertebrates consisting of the fluid filling the spaces between cells.

Intertidal zone: The shallow zone of the ocean where land meets water.

Intrinsic rate of increase: The difference between number of births and number of deaths, symbolized as rmax; maximum population growth rate.

Introgression: Transplantation of genes between species resulting from fertile hybrids mating successfully with one of the parent species.

Intron: The noncoding, intervening sequence of coding region (exon) in eukaryotic genes.

Invagination: The buckling inward of a cell layer, caused by rearrangements of microfilaments and microtubules; an important phenomenon in embryonic development.

Inversion: 1) An aberration in chromosome structure resulting from an error in meiosis or from mutagens; reattachment in a reverse orientation of a chromosomal fragment to the chromosome from which the fragment originated. 2) A phenomenon which occurs during early development of sponges at which time the external ciliated cells become inward-directed.

Invertebrate: An animal without a backbone; invertebrates make up about 95% of animal species.

Ion: A charged chemical formed when an atom or group of atoms has more or less electrons than protons (rather than an equal number).

Ionic bond: A chemical bond due to attraction between oppositely charged ions.

Irruption: A rapid increase in population density often followed by a mass emigration.

Isogamy: A condition in which male and female gametes are morphologically indistinguishable.

Isomer: Molecules consisting of the same numbers and kinds of atoms, but differing in the way in which the atoms are combined.

Isosmotic: Solutions of equal concentration with respect to osmotic pressure.

Isotope: An atomic form of an element, containing a different number of neutrons than another isotope. Isotopes vary from one another with respect to atomic mass.

Juxtaglomerular apparatus (JGA): Specialized tissue located near the afferent arteriole that supplies blood to the kidney glomerulus; JGA raises blood pressure by producing renin, which activates angiotensin.

K- selection: The concept that life history of the population is centered upon producing relatively few offspring that have a good chance of survival.

Karyogamy: The fusion of nuclei of two cells, as part of syngamy.

Karyotype: A method of classifying the chromosomes of a cell in relation to number, size and type.

Keystone predator: A species that maintains species richness in a community through predation of the best competitors in the community, thereby maintaining populations of less competitive species.

Kilocalorie: A thousand calories; the amount of heat energy required to raise the temperature of 1 kilogram of water by primary C.

Kin selection: A phenomenon of inclusive fitness, used to explain altruistic behavior between related individuals.

Kinesis: A change in activity rate in response to a stimulus.

Kinetic energy: The energy of motion. Moving matter does work by transferring some of its kinetic energy to other matter.

Kinetochore: A specialized region on the centromere that links each sister chromatid to the mitotic spindle.

Kingdom: A taxonomic category, the second broadest after domain.

Krebs cycle: A chemical cycle involving eight steps that completes the metabolic breakdown of glucose molecules to carbon dioxide; occurs wihin the mitochondrion; the second major stage in cellular respiration. Also called citric acid cycle or tricarboxylic acid (TCA) cycle.

Laboratory blank: See Method blank

Laboratory control sample (LCS): See Ongoing precision and recovery (OPR) standard

Lacteal: A tiny lymph vessel extending into the core of the intestinal villus and serving as the destination for absorbed chylomicrons.

Lagging strand: A discontinuously synthesized DNA strand that elongates in a direction away from the replication fork.

Lamarck: Proposed, in the early 1800s, that evolutionary change may occur via the inheritance of acquired characteristics. This idea, which has since been discredited, holds that the changes in characteristics which occur during an individual's life can be passed on to its offspring.

Larva (pl. larvae): A free-living, sexually immature form in some animal life cycles that may differ from the adult in morphology, nutrition, and habitat.

Lateral line system: A mechanoreceptor system consisting of a series of pores and receptor units (neuromasts) along the sides of the body of fishes and aquatic

amphibians; detects water movements made by an animal itself and by other moving objects.

Lateral meristems: The vascular and cork cambia, cylinders of dividing cells that run most of the length of stems and roots and are responsible for secondary growth.

Law of independent assortment: Mendel's second law, stating that each allele pair segregates independently during gamete formation; applies when genes for two traits are located on different pairs of homologous chromosomes.

Law of segregation: Mendel's first law, stating that allele pairs separate during gamete formation, and then randomly re-form pairs during the fusion of gametes at fertilization.

Leading strand: The new continuously complementary DNA strand synthesized along the template strand in the 5' --- > 3' direction.

Leukocyte: A white blood cell; typically functions in immunity, such as phagocytosis or antibody production.

Levels of organization: A basic concept in biology is that organization is based on a hierarchy of structural levels, with each level building on the levels below it.

Lichen: An organism formed by the symbiotic association between a fungus and a photosynthetic alga.

Life: (table) A table of data summarizing mortality in a population.

Ligament: A type of fibrous connective tissue that joins bones together at joints.

Ligand: A ligand is a molecule that binds specifically to a receptor site of another molecule. A ligase is an enzyme which catalyzes such a reaction. For example, a DNA ligase is an enzyme which catalyzes the covalent bonding of the 3' end of a new DNA fragment to the 5' end of a growing chain.

Ligase: Ligases are enzymes that catalyze the "stitching together" of polymer fragments. DNA ligase, for example, catalyzes phosphodiester bond formation between two DNA fragments, and this enzyme is involved in normal DNA replication, repair of damaged chromosomes, and various in vitro techniques in genetic engineering that involve linking DNA fragments.

Lignin: A hard material embedded in the cellulose matrix of vascular plant cell walls that functions as an important adaptation for support in terrestrial species.

Limbic system: A group of nuclei (clusters of nerve cell bodies) in the lower part of the mammalian forebrain that interact with the cerebral cortex in determining emotions; includes the hippocampus and the amygdala.

Linked genes: Genes that are located on the same chromosomes.

Lipid: One of a family of compounds, including fats, phospholipids, and steroids, that are insoluble in water.

Lipoprotein: A protein bonded to a lipid; includes the low-density lipoproteins (LDLS) and high-density lipoproteins (HDLS) that transport fats and cholesterol in the blood.

Liposome: Liposomes are vesicles (spherules) in which the lipid molecules are spontaneously arranged into bilayers with hydrophilic groups exposed to water molecules both outside the vesicle and in the core.

Locus: A particular place along the length of a certain chromosome where a specified allele is located.

Logistic population growth: A model describing population growth that levels off as population size approaches carrying capacity.

Lymphocyte: Lymphocytes (lymph cells, lympho- leukocytes) are a type of leukocyte (white blood cell) responsible for the immune response. There are two classes of lymphocytes: 1) the B- cells, when presented with a foreign chemical entity (antigen), change into antibody producing plasma cells; and, 2) the T- cells interact directly with foreign invaders such as bacteria and viruses. The T- cells express various surface marker macromolecules. For example, CD4+ is the notation for a specific expressed T-cell surface marker that can be identified by assay.

Lysis: The destruction of a cell by rupture of the plasma membrane.

Lysogenic cycle: A type of viral replication cycle in which the viral genome becomes incorporated into the bacterial host chromosome as a prophage.

Lysosome: A membrane-bounded organelle found in eukaryotic cells (other than plants). Lysosomes contain a mixture of enzymes that can digest most of the macromolecules found in the rest of the cell.

Lysozyme: An enzyme in perspiration, tears, and saliva that attacks bacterial cell walls.

Lytic cycle: A type of viral replication cycle resulting in the release of new phages by death or lysis of the host cell.

M phase: The mitotic phase of the cell cycle, which includes mitosis and cytokinesis.

Macroevolution: Evolutionary change on a grand scale, encompassing the origin of novel designs, evolutionary trends, adaptive radiation, and mass extinction.

Macromolecule: A giant molecule of living matter formed by the joining of smaller molecules, usually by condensation synthesis. Polysaccharides, proteins, and nucleic acids are macromolecules.

Macrophage: An amoeboid cell that moves through tissue fibers, engulfing bacteria and dead cells by phagocytosis.

Major histocompatibility complex: A large set of cell surface antigens encoded by a family of genes. Foreign MHC markers trigger T-cell responses that may lead to rejection of transplanted tissues and organs.

Malignant tumor: A cancerous growth; an abnormal growth whose cells multiply excessively, have altered surfaces, and may have unusual numbers of chromosomes and/or aberrant metabolic processes.

Malphighian tubule: A unique excretory organ of insects that empties into the digestive tract, removes nitrogenous wastes from the blood, and functions in osmoregulation.

Mantle: A heavy fold of tissue in mollusks that drapes over the visceral mass and may secrete a shell.

Marsupial: A mammal, such as a koala, kangaroo, or opossum, whose young complete their embryonic development inside a maternal pouch called the marsupium.

Mass number: The sum of the number of protons plus the number of neutrons in the nucleus of an atom; unique for each element and designated by a superscript to the left of the elemental symbol.

Matrix spike (MS): A sample prepared by adding a known quantity of organisms to a specified amount of sample matrix for which an independent estimate of target analyte concentration is available. A matrix spike is used to determine the effect of the matrix on a method's recovery efficiency.

Matrix: The nonliving component of connective tissue, consisting of a web of fibers embedded in homogeneous ground substance that may be liquid, jellylike, or solid.

Matter: Anything that takes up space and has mass.

Mechanoreceptor: A sensory receptor that detects physical deformations in the body environment associated with pressure, touch, stretch, motion, and sound.

Median bodies: Prominent, dark-staining, paired organelles consisting of microtubules and found in the posterior half of *Giardia*. In *G. intestinalis* (from humans), these structures often have a claw-hammer shape, while in *G. muris* (from mice), the median bodies are round.

Medulla oblongata: The lowest part of the vertebrate brain; a swelling of the hindbrain dorsal to the anterior spinal cord that controls autonomic, homeostatic functions, including breathing, heart and blood vessel activity, swallowing, digestion, and vomiting.

Medusa: The floating, flattened, mouth-down version of the cnidarian body plan. The alternate form is the polyp.

Megapascal: A unit of pressure equivalent to 10 atmospheres of pressure.

Meiosis: A two-stage type of cell division in sexually reproducing organisms that results in gametes with half the chromosome number of the original cell.

Membrane potential: The charge difference between the cytoplasm and extracellular fluid in all cells, due to the differential distribution of ions. Membrane potential affects the activity of excitable cells and the transmembrane movement of all charged substances.

Mesenteries: Membranes that suspend many of the organs of vertebrates inside fluid-filled body cavities.

Mesoderm: The middle primary germ layer of an early embryo that develops into the notochord, the lining of the coelom, muscles, skeleton, gonads, kidneys and most of the circulatory system.

Mesosome: A localized infolding of the plasma membrane of a bacterium.

Messenger: (RNA) A type of RNA synthesized from DNA in the genetic material that attaches to ribosomes in the cytoplasm and specifies the primary structure of a protein.

Metabolism: The sum total of the chemical and physical changes constantly taking place in living substances.

Metamorphosis: The resurgence of development in an animal larva that transforms it into a sexually mature adult.

Metanephridium: A type of excretory tubule in annelid worms that has internal openings called nephrostomes that collect body fluids and external openings called nephridiopores.

Metastasis: The spread of cancer cells beyond their original site.

Metazoan: A multicellular animal. Among important distinguishing characteristics of metazoa are cell differentiation and intercellular communication. For certain multicellular colonial entities such as sponges, some biologists prefer the term "parazoa".

Method blank: An aliquot of reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, and procedures that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Microbody: A small organelle, bounded by a single membrane and possessing a granular interior. Peroxisomes and glyoxysomes are types of microbodies.

Microevolution: A change in the gene pool of a population over a succession of generations.

Microfilament: Minute fibrous structure generally composed of actin found in the cytoplasm of eukaryotic cells. They play a role in motion within cells.

Microscope: An instrument which magnifies images either by using lenses in an optical system to bend light (light microscope) or electromagnets to direct the movement of electrons (electron microscope).

Microtubule: A minute tubular structure found in centrioles, spindle apparati, cilia, flagella, and other places in the cytoplasm of eukaryotic cells. Microtubules play a role in movement and maintenance of shape.

Microvillus: Collectively, fine, fingerlike projections of the epithelial cells in the lumen of the small intestine that increase its surface area.

Mimicry: A phenomenon in which one species benefits by a superficial resemblance to an unrelated species. A predator or species of prey may gain a significant advantage through mimicry.

Missense: (mutation) The most common type of mutation involving a base- pair substitution within a gene that changes a codon, but the new codon makes sense, in that it still codes for an amino acid.

Mitochondrial matrix: The compartment of the mitochondrion enclosed by the inner membrane and containing enzymes and substrates for the Krebs cycle.

Mitochondrion: An organelle that occurs in eukaryotic cells and contains the enzymes of the citric acid cycle, the respiratory chain, and oxidative phosphorylation. A mitochondrion is bounded by a double membrane.

Mitosis: A process of cell division in eukaryotic cells conventionally divided into the growth period (interphase) and four stages: prophase, metaphase, anaphase, and telophase. The stages conserve chromosome number by equally allocating replicated chromosomes to each of the daughter cells.

Modern synthesis: A comprehensive theory of evolution emphasizing natural selection, gradualism, and populations as the fundamental units of evolutionary change; also called Neo-Darwinism.

Molarity: A common measure of solute concentration, referring to the number of moles of solute in 1 L of solution.

Mold: A rapidly growing, asexually reproducing fungus.

Mole: The number of grams of a substance that equals its molecular weight in daltons and contains Avogadro's number of molecules.

Molecular formula: A type of molecular notation indicating only the quantity of the constituent atoms.

Molecule: Two or more atoms of one or more elements held together by ionic or covalent chemical bonds.

Molting: A process in arthropods in which the exoskeleton is shed at intervals to allow growth by secretion of a larger exoskeleton.

Monera: The kingdom of life forms that includes all of the bacteria.

Monoclonal antibody: A defensive protein produced by cells descended from a single cell; an antibody that is secreted by a clone of cells and, consequently, is specific for a single antigenic determinant.

Monoecious: Referring to an organism having the capacity of producing both sperm and eggs.

Monohybrid cross: A breeding experiment that employs parental varieties differing in a single character.

Monomer: A small molecule, two or more of which can be combined to form oligomers (consisting of a few monomers) or polymers (consisting of many monomers).

Monophyletic: A term used to describe any taxon derived from a single ancestral form that gave rise to no species in other taxa.

Monosaccharide: A simple sugar; a monomer.

Monozygotic twins: Monozygotic twins are genetically identical, derived from the division and autonomous development of a single zygote (fertilized egg).

Morphogenesis: The development of body shape and organization during ontogeny.

Morphospecies: Species defined by their anatomical features.

Mosaic evolution: The evolution of different features of an organism at different rates.

Mosaic: A pattern of development, such as that of a mollusk, in which the early blastomeres each give rise to a specific part of the embryo. In some animals, the fate of the blastomeres is established in the zygote.

Motor nervous system: In vertebrates, the component of the peripheral nervous system that transmits signals from the central nervous system to effector cells.

MPF: M: phase promoting factor: A protein complex required for a cell to progress from late interphase to mitosis; the active form consists of cyclin and cdc2, a protein kinase.

Mucosa: Refers to the mucous tissue lining various tubular structures in the body.

Mullerian mimicry: A mutual mimicry by two unpalatable species.

Multigene family: A collection of genes with similar or identical sequences, presumably of common origin.

Must: This action, activity, or procedural step is required.

Mutagen: A chemical or physical agent that interacts with DNA and causes a mutation.

Mutagenesis: The creation of mutations.

Mutation: A spontaneous or induced change in a gene's or chromosome's structure or number. The resulting individual is termed a mutant.

Mutualism: A symbiotic relationship in which both the host and the symbiont benefit.

Mycelium: The densely branched network of hyphae in a fungus.

Myelin sheath: An insulating coat of cell membrane from Schwann cells that is interrupted by nodes of Ranvier where saltatory conduction occurs.

Myofibrils: Fibrils arranged in longitudinal bundles in muscle cells (fibers); composed of thin filaments of actin and a regulatory protein and thick filaments of myosin.

Myoglobin: An oxygen-storing, pigmented protein in muscle cells.

Myosin: A type of protein filament that interacts with actin filaments to cause cell movement, such as contraction in muscle cells.

NAD+: Nicatinamide adenine dinucleotide (oxidized); a coenzyme present in all cells that assists enzymes in transferring electrons during the redox reactions of metabolism.

Nanometer: A unit of measure (length). 1 nm is equal to 1 x 10: 9 m, or 1/1,000,000 mm.

Negative control: See Method blank.

Negative feedback: A primary mechanism of homeostasis, whereby a change in a physiological variable that is being monitored triggers a response that counteracts the initial fluctuation.

Neuraminidase: A surface enzyme possessed by some influenza viruses which help the virus penetrate the mucus layer protecting the respiratory epithelium and also plays a role in budding of new virus particles from infected cells.

Neuron: A nerve cell; the fundamental unit of the nervous system, having structure and properties that allow it to conduct signals by taking advantage of the electrical charge across its cell membrane.

Neurosecretory cells: Cells that receive signals from other nerve cells, but instead of signaling to an adjacent nerve cell or muscle, release hormones into the blood stream.

Neurotransmitter: The chemical messenger released from the synaptic terminals of a neuron at a chemical synapse that diffuses across the synaptic cleft and binds to and stimulates the postsynaptic cell.

Neutral variation: Genetic diversity that confers no apparent selective advantage.

Neutralization reactions: Chemical reactions between acids and bases where water is an end product.

Neutron: An uncharged subatomic particle of about the same size and mass as a proton.

Nomenclature: The method of assigning names in the classification of organisms.

Noncompetitive inhibitor: A substance that reduces the activity of an enzyme by binding to a location remote from the active site, changing its conformation so that it no longer binds to the substrate.

Noncyclic electron flow: A route of electron flow during the light reactions of photosynthesis that involves both photosystems and produces ATP, NADPH, and oxygen; the net electron flow is from water to NADP+.

Noncyclic photophosphorylation: The production of ATP by noncyclic electron flow.

Nondisjunction: An accident of meiosis or mitosis, in which both members of a pair of homologous chromosomes or both sister chromatids fail to separate normally.

Nonpolar: Electrically symmetrical. For example, in many molecules with covalent bonds, the electrons are shared equally; the poles are electrically neutral.

Nonsense mutation: A mutation that changes an amino acid codon to one of the three stop codons, resulting in a shorter and usually nonfunctional protein.

Norm of reaction: The range of phenotypic possibilities for a single genotpe, as influenced by the environment.

Nuclear: 1) (envelope) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (pore) An opening of the nuclear envelope which allows for the movement of materials between the nucleus and surrounding cytoplasm.

Nuclease: This term refers to any enzyme that acts on nucleic acids, e.g., Dnase, Rnase, endonuclease, etc.

Nucleic: (acid) A polymer composed of nucleotides that are joined by covalent bonds (phosphodiester linkages) between the phosphate of one nucleotide and the sugar of the next nucleotide.

Nucleoid: The region that harbors the chromosome of a prokaryotic cell. Unlike the eukaryotic nucleus, it is not bounded by a membrane.

Nucleolus (pl. nucleoli): A specialized structure in the nucleus, formed from various chromosomes and active in the synthesis of ribosomes.

Nucleolus: A small, generally spherical body found within the nucleus of eukaryotic cells. The site of ribosomal RNA synthesis.

Nucleoside: An organic molecule consisting of a nitrogenous base joined to a five-carbon sugar.

Nucleosome: The basic, beadlike unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound around a protein core composed of two copies of each of four types of histone.

Nucleotide: The basic chemical unit (monomer) of a nucleic acid. A nucleotide in RNA consists of one of four nitrogenous bases linked to ribose, which in turn is linked to phosphate. In DNA, deoxyribose is present instead of ribose.

Nucleus: A membrane-bound organelle containing genetic material. Nuclei are a prominent internal structure seen both in *Cryptosporidium* oocysts and *Giardia* cysts. In *Cryptosporidium* oocysts, there is one nucleus per sporozoite. One to four nuclei can be seen in *Giardia* cysts.

Nucleus: The membrane bound organelle of eukaryotic cells that contains the cell's genetic material. Also the central region of an atom, composed of protons and neutrons.

Null: In the scientific method, the hypothesis which one attempts to falsify.

Ongoing precision and recovery (OPR) standard: A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Oocyst and cyst spiking suspension: See Spiking suspension.

Oocyst and cyst stock suspension: See Stock suspension.

Oocyst: The encysted zygote of some sporozoa; e.g., *Cryptosporidium*. The oocyst is a phase or form of the organism produced as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant outer wall.

Positive control: See Ongoing precision and recovery standard.

PTFE: Polytetrafluoroethylene.

Quantitative transfer: The process of transferring a solution from one container to another using a pipette in which as much solution as possible is transferred, followed by rinsing of the walls of the source container with a small volume of rinsing solution (e.g., reagent water, buffer, etc.), followed by transfer of the rinsing solution, followed by a second rinse and transfer.

Reagent water blank: see Method blank.

Reagent water: Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD): The standard deviation divided by the mean times 100.

Robert Hooke: Coined the term "cell" to describe the structures he saw while examining a piece of cork using a microscope.

RSD: See Relative standard deviation

Should: This action, activity, or procedural step is suggested but not required.

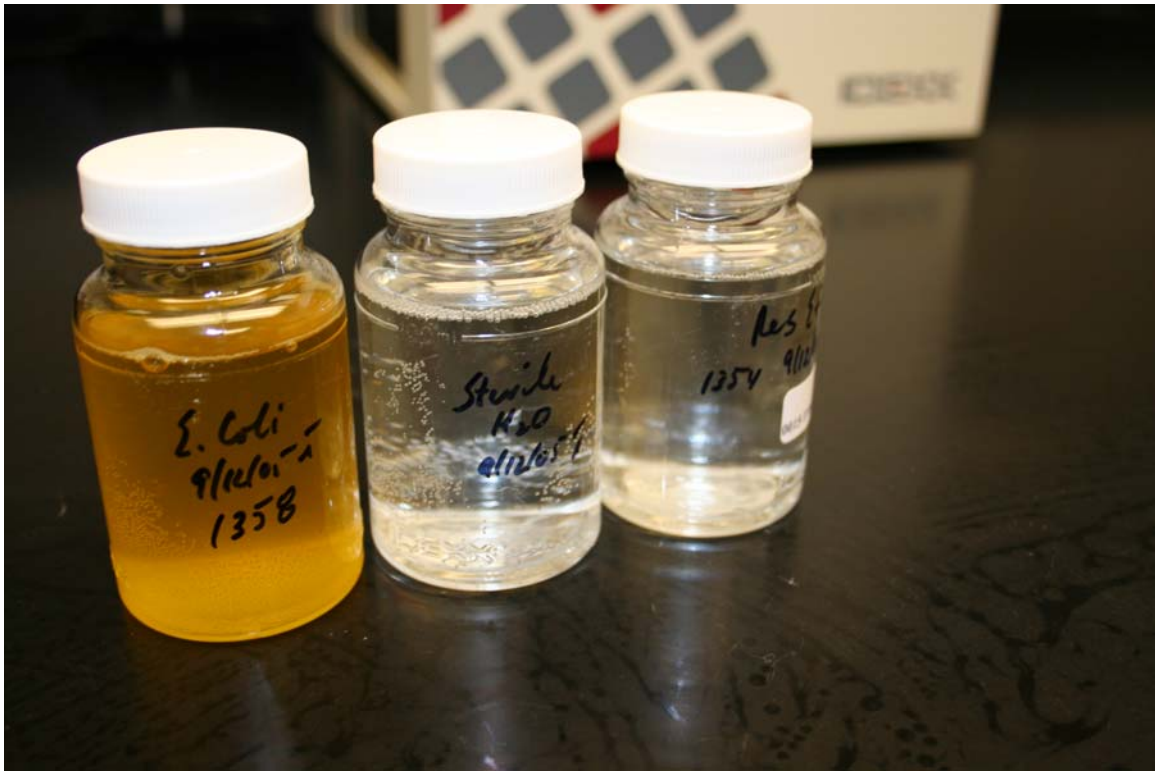
Spiking suspension: Diluted stock suspension containing the organism(s) of interest at a concentration appropriate for spiking samples.

Sporozoite: A motile, infective stage of certain protozoans; e.g., *Cryptosporidium*. There are four sporozoites in each *Cryptosporidium* oocyst, and they are generally banana-shaped.

Stock suspension: A concentrated suspension containing the organism(s) of interest that is obtained from a source that will attest to the host source, purity, authenticity, and viability of the organism(s).

Succession: Transition in the species composition of a biological community, often following ecological disturbance of the community; the establishment of a biological community in an area virtually barren of life.

Thomas Malthus: Formulated the concept that population growth proceeds at a geometric rate.



Simple Math Conversion Factors

1 PSI = 2.31 Feet of Water
1 Foot of Water = .433 PSI
1.13 Feet of Water = 1 Inch of Mercury
454 Grams = 1 Pound
1 Gallon of Water = 8.34 Pounds
1 mg/L = 1 PPM
17.1 mg/L = 1 Grain/Gallon
1% = 10,000 mg/L
694 Gallons per Minute = MGD
1.55 Cubic Feet per Second = 1 MGD
60 Seconds = 1 Minute
1440 Minutes = 1 Day
.746 kW = 1 Horsepower

LENGTH
12 Inches = 1 Foot
3 Feet = 1 Yard
5280 = 1 Mile

AREA
144 Square Inches = 1 Square Foot
43,560 Square Feet – 1 Acre

VOLUME
1000 Milliliters = 1 Liter
3.785 Liters = 1Gallon
231 Cubic Inches = 1 Gallon
7.48 Gallons = 1 Cubic Foot
62.38 Pounds = 1 Cubic Foot of Water

Dimensions

SQUARE: Area (sq.ft) = Length X Width
Volume (cu.ft) = Length (ft) X Width (ft) X Height (ft)

CIRCLE: Area (sq.ft) = 3.14 X Radius (ft) X Radius (ft)

CYLINDER: Volume (Cu. ft) = 3.14 X Radius (ft) X Radius (ft) X Depth (ft)

PIPE VOLUME: .785 X Diameter ^{2} X Length = Area X 7.48 for Gallons

SPHERE: $\frac{(3.14)\ (\text{Diameter})^3}{(6)}$

Circumference = 3.14 X Diameter

Flow Rate

Multiply	—>	to get
to get	<—	Divide
cc/min	1	mL/min
cfm (ft^3/min)	28.31	L/min
cfm (ft^3/min)	1.699	m^3/hr
cfh (ft^3/hr)	472	mL/min
cfh (ft^3/hr)	0.125	GPM
GPH	63.1	mL/min
GPH	0.134	cfh
GPM	0.227	m^3/hr
GPM	3.785	L/min
oz/min	29.57	mL/min

General Conversions

POUNDS PER DAY= Concentration (mg/L) X Flow (MG) X 8.34

PERCENT EFFICIENCY = $\frac{\text{In} - \text{Out}}{\text{In}}$ X 100

TEMPERATURE: $^0F = (^0C \times 9/5) + 32$ 9/5 =1.8
$^0C = (^0F - 32) \times 5/9$ 5/9 = .555

CONCENTRATION: Conc. (A) X Volume (A) = Conc. (B) X Volume (B)

FLOW RATE (Q): Q = A X V (Quantity = Area X Velocity)

FLOW RATE (gpm): Flow Rate (gpm) = $\frac{2.83 \text{ (Diameter, in)}^2 \text{ (Distance, in)}}{\text{Height, in}}$

% SLOPE = $\frac{\text{Rise (feet)}}{\text{Run (feet)}} \times 100$

ACTUAL LEAKAGE = $\frac{\text{Leak Rate (GPD)}}{\text{Length (mi.) X Diameter (in)}}$

VELOCITY = $\frac{\text{Distance (ft)}}{\text{Time (Sec)}}$

N = Manning's Coefficient of Roughness
R = Hydraulic Radius (ft.)
S = Slope of Sewer (ft/ft.)

HYDRAULIC RADIUS (ft) = $\frac{\text{Cross Sectional Area of Flow (ft)}}{\text{Wetted pipe Perimeter (ft)}}$

WATER HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960}$

BRAKE HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Efficiency}}$

MOTOR HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Eff. X Motor Eff.}}$

MEAN OR AVERAGE = $\frac{\text{Sum of the Values}}{\text{Number of Values}}$

TOTAL HEAD (ft) = Suction Lift (ft) X Discharge Head (ft)

SURFACE LOADING RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Surface Area (sq. ft)}}$
(gal/min/sq.ft)

MIXTURE STRENGTH (%) = $\frac{\text{(Volume 1, gal) (Strength 1, \%) + (Volume 2, gal) (Strength 2,\%)}}{\text{(Volume 1, gal) + (Volume 2, gal)}}$

INJURY FREQUENCY RATE = $\frac{\text{(Number of Injuries) 1,000,000}}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = $\frac{\text{Volume of Basin (gals) X 24 hrs}}{\text{Flow (GPD)}}$

FLUORIDE ION PURITY (%) = $\frac{\text{(Molecular weight of Fluoride) (100\%)}}{\text{Molecular weight of Chemical}}$

INJURY FREQUENCY RATE = $\frac{\text{(Number of Injuries) 1,000,000}}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = Volume of Basin (gals) X 24 hrs
Flow (GPD)

BY-PASS WATER (gpd) = Total Flow (GPD) X Plant Effluent Hardness (gpg)
Filtered Hardness (gpg)

HARDNESS
HARDNESS (mg/L as $CaCO_3$) = A (mls of titrant) X 1000
Mls of Sample

Ca HARDNESS as mg/L $CaCo_3$ = 2.5 X (Ca, mg/L)

Mg HARDNESS as mg/L $CaCo_3$ = 4.12 (Mg, mg/L)

ALKALINITY TOTAL = Mls of Titrant X Normality X 50,000
(mg/L) Mls of Sample

EXCHANGE CAPACITY (grains) = Resin Volume (cu. ft) X Removal Capacity

HARDNESS TO GRAIN/GALLON = Hardness (mg/L) X gr./gal
17.1 mg/L

LANGELIER INDEX = pH - pH_s

CHEMICAL ADDITION

CHEMICAL FEED RATE = Chemical Feed (ml/min)
(gpm) 3785 ml/gal

CHLORINE DOSE (mg/L) = Chlorine Demand (mg/L) + Chlorine Residual (mg/L)

POLYMER % = Dry Polymer (lbs.)
Dry Polymer (lbs.) + Water (lbs.)

DESIRED PAC = Volume (MG) X Dose (mg/L) X 8.34
(lbs./MG) 1 MG

PAC (lbs./gal) = PAC (mg/L) X 3.785 (1/gallon)
1000 (mg/g) X 454 (g/lb.)

FILTRATION
FILTRATION RATE = Flow Rate (gpm)
(gpm/sq. ft) Surface Area (sq. ft)

BACKWASH PUMPING RATE = Filter Area (sq. ft) X Backwash Rate (gpm/sq. ft)
(gpm)

FILTRATION RATE = Flow Rate (gpm)
(gpm/sq. ft) Filter Area (sq. ft)

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