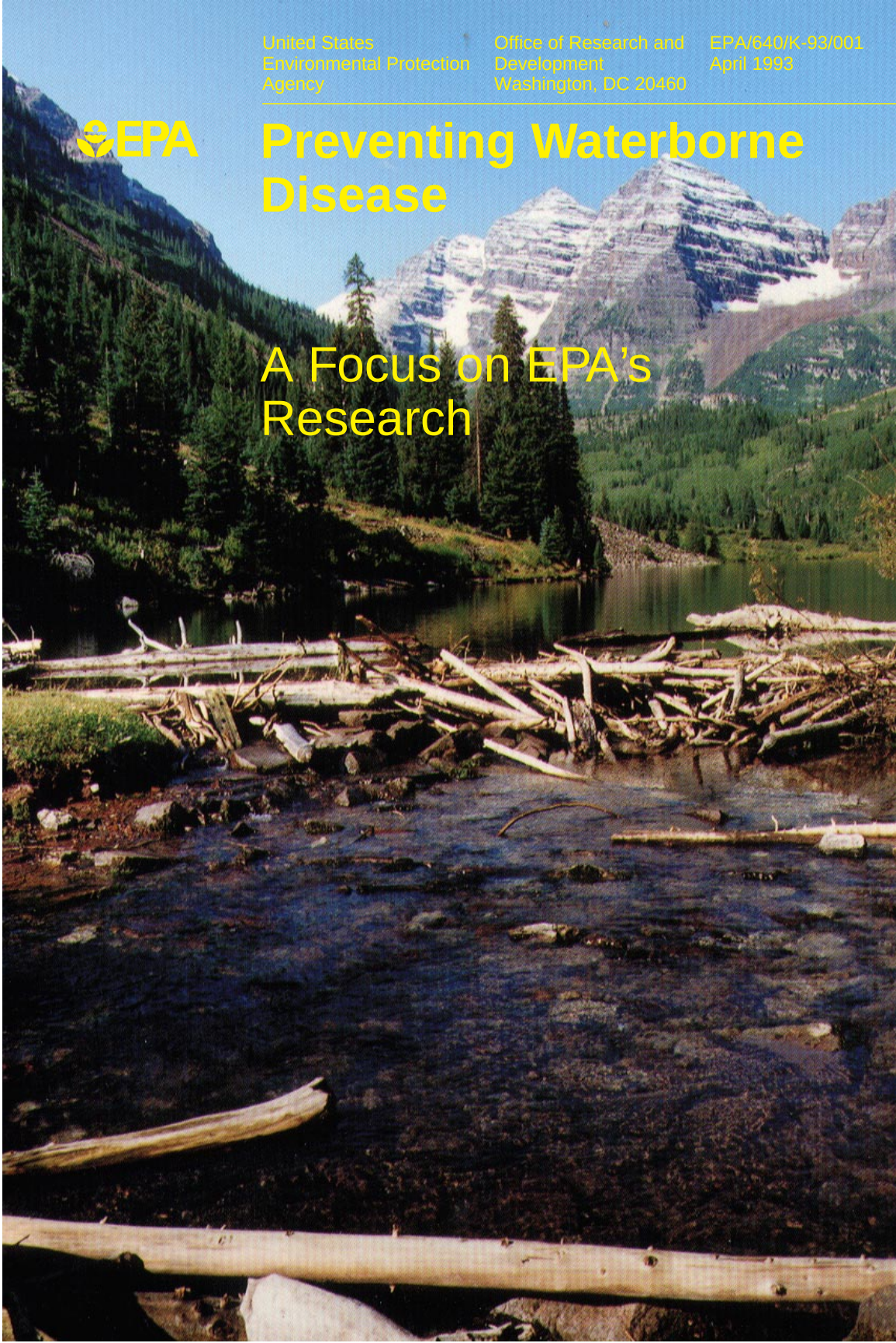
United States Environmental Protection Agency
Office of Research and Development
Washington, DC 20460
EPA/640/K-93/001
April 1993

EPA

Preventing Waterborne Disease

A Focus on EPA's Research



EPA's Office of Research and Development

The Office of Research and Development (ORD) conducts an integrated program of scientific research and development on the sources, transport and fate processes, monitoring, control, and assessment of risk and effects of environmental pollutants. These activities are implemented through its headquarters offices and National Research Laboratories and Centers. The research focuses on key scientific and technical issues to generate knowledge supporting sound decisions today, and to anticipate the complex challenges of tomorrow. With a strong, forward-looking research program, less expensive more effective solutions can be pursued and irreversible damage to the environment can be prevented.

Front cover photo by Lang Photography.

"The reported case total for the epidemic nears three-quarters of a million. Since the beginning of the epidemic in January 1991, the total number of reported cases is 746,968 with 6,448 deaths."

(*Cholera Epidemic in the Americas*, CDC Update, February 11, 1993)

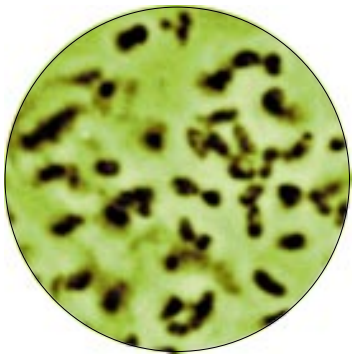
Although the above-listed statistics are alarming, the risk that extensive outbreaks of waterborne cholera will occur in the United States is minimal. Effective treatment of drinking water and sewage, coupled with adequate personal hygiene habits, has contributed to a successful line of defense against the spread of cholera in the U.S. Still, the ease of international travel has guaranteed the import of a wide variety of diseases not generally considered to be native to North America. Additionally, although fatalities caused by waterborne diseases have declined dramatically in the U.S. during this century, annual reports of water-related, microorganism-induced disease continue to number in the thousands. Just one waterborne outbreak of cryptosporidiosis in western Georgia (1987), for example, affected an estimated 13,000 people. In the "colonias" (poor settlements along the Texas-Mexico border), high levels of disease have been associated with the lack of public water supplies and inadequate waste treatment. While the words "typhoid fever" fade from our vocabulary, such terms as "*Giardia*," "*Legionella*," and "Norwalk virus" are becoming more familiar.

The United States Environmental Protection Agency (U.S. EPA), through its Office of Research and Development (ORD), is conducting research to better understand and prevent water contamination by harmful microorganisms. From monitoring our nation's ground water systems for viral pathogens...to developing more effective technology for large and small systems...to providing other nations with critical technical assistance, ORD scientists and engineers continue their mission to ensure safe waters. As the focus of our efforts adjusts to deal with emerging challenges, past and current successes add to our scientific arsenal against disease.

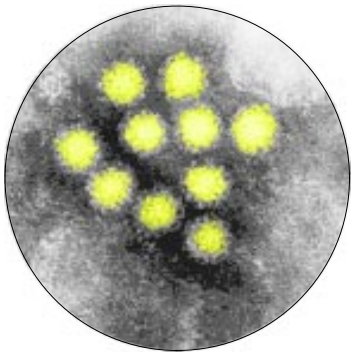
Researcher isolating infectious bacteria in one of ORD's pathogen containment suites.

Microorganisms Associated with Waterborne Disease

The following groups of microorganisms have been linked with the occurrence of waterborne disease. As each pathogen is isolated and identified as a threat to water quality, ORD researchers try to discover the most effective combination of barriers and disinfection methods to minimize risk of human exposure.



Bacteria. Bacteria are the most widely distributed life forms. Pathogenic bacteria range in length from approximately 0.4 to 14 µm (a µm or "micrometer" equals one one-thousandth of a millimeter) and 0.2 to 1.2 µm in width. Key bacterial pathogens responsible for waterborne disease include *Legionella*, *Salmonella typhi*, *Shigella*, and *Vibrio cholerae*.



Viruses. Viruses are inactive when outside of a living host cell. Viruses linked to waterborne disease have protein coats that provide protection from environmental hazards and range in size from 0.02 to 0.09 µm. Unlike bacteria and protozoa, they contain only one type of nucleic acid (RNA or DNA). Key pathogens include hepatitis A and Norwalk virus.

Protozoa. Protozoa, common in bodies of water, are much larger than bacteria and viruses. To survive harsh environmental conditions, some species can secrete a protective covering and form a resting stage called a "cyst." Encystment can protect protozoa from drinking water disinfection efforts and facilitate the spread of disease. Key protozoa being studied as agents of waterborne disease include *Giardia* and *Cryptosporidium*.

Why Can't Waterborne Pathogens Be Eliminated?

Microorganisms are present everywhere in our environment. Invisible to the naked eye, vast numbers of these microbes can be found in soil, air, food and water. Although humans are essentially free of microorganisms before birth, constant circumstances of exposure (e.g., breathing, eating, and drinking) quickly allow the establishment of harmless microbial flora in our bodies.

Microbial pathogens (microorganisms capable of causing disease), however, can and often do harm those who become infected. Moreover, diseases that healthy individuals "weather" well may prove fatal to individuals with compromised immune systems. In some cases, an infection can persist to create a "carrier state" where a disease-causing agent is harbored by the body (and spread) without any apparent symptoms.

Waterborne diseases are typically considered to be those diseases resulting from ingestion of contaminated water. Additional pathways of infection being studied by EPA include inhalation of water vapors as well as body contact during bathing (opportunistic pathogens) in the hospital environment.

Since voluntary water ingestion (drinking water) and bathing are universal practices and accidental ingestion during recreational activities (e.g., swimming, water skiing, wading) is common, inadequate protection of water integrity could lead to widespread outbreaks (the Centers for Disease Control defines an outbreak to be two or more cases of illness that can be traced to a common source). Because symptoms can be mild and short-lived, it is estimated that only a fraction of waterborne outbreaks is recognized, reported and investigated. Of these, the pathogenic agent is identified only half of the time. Additionally, experts believe that some food-related disease outbreaks may originate with an initial infection (e.g., of a restaurant worker) caused by contaminated drinking water.

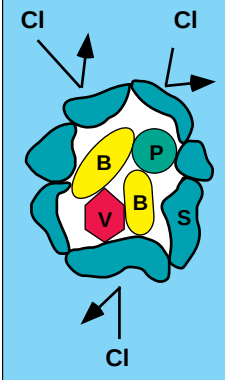
Bacteria, viruses and protozoa are the microorganism groups containing pathogens of primary concern in the study of waterborne diseases. To eliminate these pathogens from our water, especially from our drinking water, seems theoretically straightforward. Simply mix in a disinfectant, allow adequate contact time to assure inactivation (rendering the microbes unable to produce disease), and pump the water into the distribution lines.

In reality, many conditions render the above scenario unworkable. The physical characteristics of the water, primarily represented by dissolved and suspended solids content, can affect the disinfection process. The chemical content, both naturally occurring and anthropogenic (i.e., generated by humans), can also interfere with the chemical reactions desired during treatment and disinfection. Finally, pathogens associated (i.e., imbedded in or clumped) with higher organisms (e.g., algae, rotifers, worms) may be protected from the action of disinfectants.

To overcome these obstacles to disinfection, successful treatment of drinking and waste water generally includes a series of steps. The flowcharts in Figures 1 and 2 depict the steps involved in typical drinking and waste water treatment processes.

In the case of drinking water disinfection, once the impurities have been removed, enough disinfectant is added to inactivate pathogens. Addi-

It is estimated that swimmers and waders may ingest from 0.3 to 1.7 ounces of water per outing.



To kill or inactivate drinking water contaminants such as bacteria (B), protozoa (P), and viruses (V), adequate contact time with the disinfectant (chlorine or Cl in this representation) must be allowed. Adsorption to and clumping of solid particles (S) can inhibit the disinfection process.

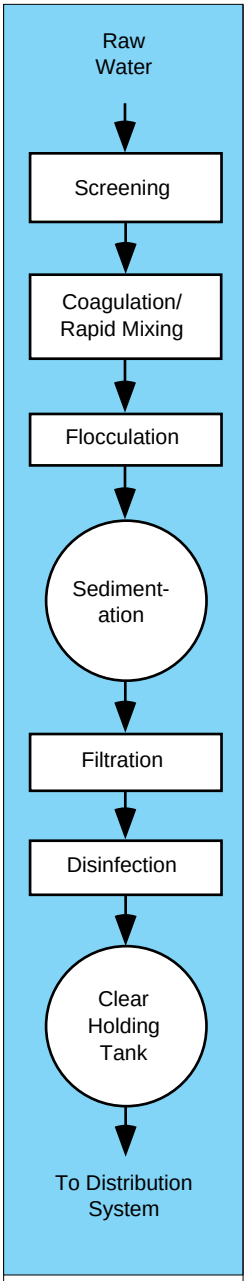


Figure 1. *Simplified flowchart of drinking water treatment processes.*

tionally, a residual level of disinfectant must be maintained throughout the distribution system to guard against potential problems (e.g., microorganisms entering through breaks in distribution lines or re-growth).

Proper distribution system operation and maintenance practices are essential deterrents of pathogen entry, recovery and survival. These practices (according to Geldreich *et al., 1992*) include:

- Systematic flushing of the entire distribution system "to get more movement of the chlorine residual into all parts of the pipe network...to remove static water from slow-flow sections, deadends and stratified water in storage tanks on a periodic basis;"
- Effecting repairs and replacement of distribution line components (e.g., broken mains and service meters) in a sanitary manner (i.e., soil-free replacement parts, disinfection and flushing of repaired lines, valves and fittings);
- Preventing pathogens from being drawn into the distribution system by maintaining continuous positive pressure and preserving barriers between public water supplies and sewage or storm water drainage;
- Varying the sample sites during routine monitoring to produce data more representative of the entire system.

While the importance of source water treatment to ensure safe drinking water seems obvious, the need to devote equal effort to pathogen reduction in wastewater is not always recognized. The release of untreated or inadequately treated wastewater into source waters drawn upon by other communities presents a significant health risk. Source waters heavily loaded with disease-causing microorganisms can reduce the effectiveness of "downstream" drinking water treatment processes. Such advances as ultraviolet light disinfection systems, initially investigated as a wastewater disinfection option several years ago, are presently becoming more widely accepted and reliable with recent design enhancements. This technology has been demonstrated to be capable of meeting existing disinfection criteria without the release of dangerous disinfection by-products.

What Progress Has Been Made?

Early in this century, the waterborne diseases of chief concern in the U.S. were typhoid fever and amebiasis. Of the 1,087 deaths associated with waterborne outbreaks between 1920 and 1991, 943 were attributed to typhoid fever while 102 were caused by amebiasis. Overall, 83% of the deaths occurred prior to 1936 and less than 1% occurred after 1970. Additionally, the number of outbreaks in community water systems since 1945 is about half as great as the number documented during the first half of this century. The reduction in fatalities and number of outbreaks indicates that progress has been made in the prevention of certain waterborne diseases. Much of the progress has been the result of increased implementation of important treatment practices (e.g., filtration, disinfection, sewage treatment). Although progress has been significant, the nation's water continues to be a source of disease. It must be rigorously monitored for indicators of fecal contamination.

Some Waterborne Diseases of Concern in the United States

Disease	Microbial Agent	General Symptoms
Amebiasis	Protozoan (*Entamoeba histolytica*)	Abdominal discomfort, fatigue, diarrhea, flatulence, weight loss
Campylo-bacteriosis	Bacterium (*Campylobacter jejuni*)	Fever, abdominal pain, diarrhea
Cholera	Bacterium (*Vibrio cholerae*)	Watery diarrhea, vomiting, occasional muscle cramps
Cryptospor-idiosis	Protozoan (*Cryptosporidium parvum*)	Diarrhea, abdominal discomfort
Giardiasis	Protozoan (*Giardia lamblia*)	Diarrhea, abdominal discomfort
Hepatitis	Virus (hepatitis A)	Fever, chills, abdominal discomfort, jaundice, dark urine
Shigellosis	Bacterium (*Shigella* species)	Fever, diarrhea, bloody stool
Typhoid fever	Bacterium (*Salmonella typhi*)	Fever, headache, constipation, appetite loss, nausea, diarrhea, vomiting, appearance of an abdominal rash
Viral Gastroenteritis	Viruses (Norwalk, rotavirus and other types)	Fever, headache, gastrointestinal discomfort, vomiting, diarrhea

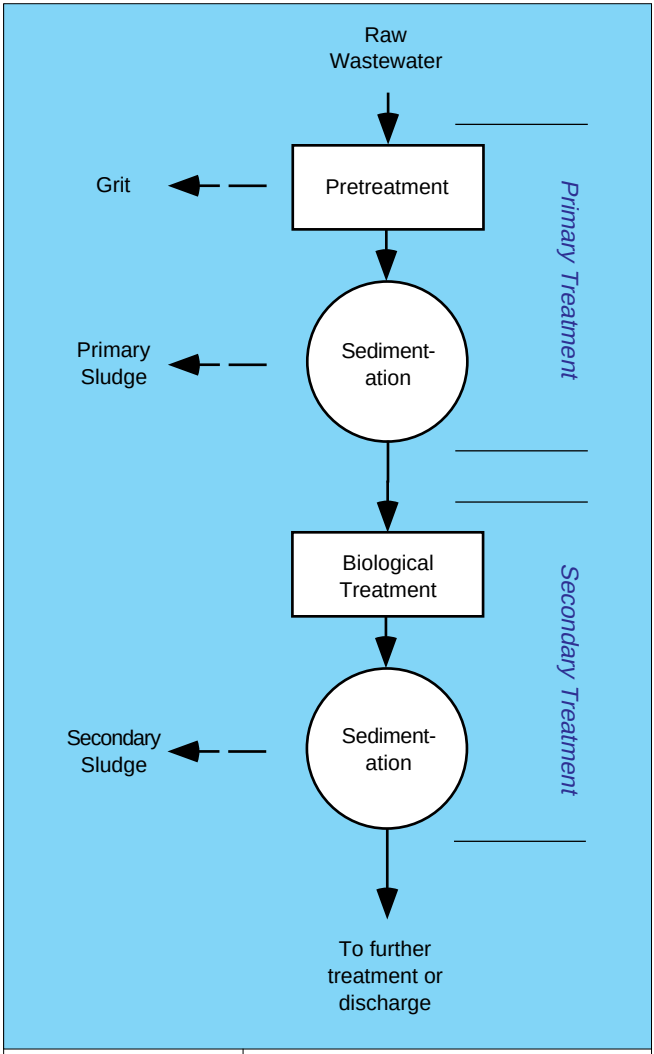


Figure 2. *Simplified flowchart of typical wastewater treatment processes.*

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

Relating to prevention of waterborne disease, the SDWA required EPA to:

1) set numerical standards, referred to as Maximum Contaminant Levels (MCLs — the highest allowable contaminant concentrations in drinking water) or treatment technique requirements for contaminants in public water supplies;

2) issue regulations requiring monitoring of all regulated and certain unregulated contaminants, depending on the number of people served by the system, the source of the water supply, and the contaminants likely to be found;

3) set criteria under which systems are obligated to filter water from surface water sources; it must also develop procedures for states to determine which systems have to filter;

4) develop disinfection rules for all public water supplies; and

5) require all states to develop Wellhead Protection Programs designed to protect from sources of contamination areas around wells that supply public drinking water systems.

Through the Surface Water Treatment Rule (SWTR), EPA has set treatment requirements to control microbiological contaminants in public water systems using surface water sources (and ground-water sources under the direct influence of

surface water). These requirements include the following:

1) treatment must remove or inactivate at least 99.9% of *Giardia lamblia* cysts and 99.99% of viruses;

2) all systems must disinfect, and are required to filter if certain source water quality criteria and site-specific criteria are not met;

3) the regulations set criteria for determining if treatment, including turbidity (suspended particulate matter) removal and disinfection requirements, is adequate for filtered systems; and

4) all systems must be operated by qualified operators as determined by the states.

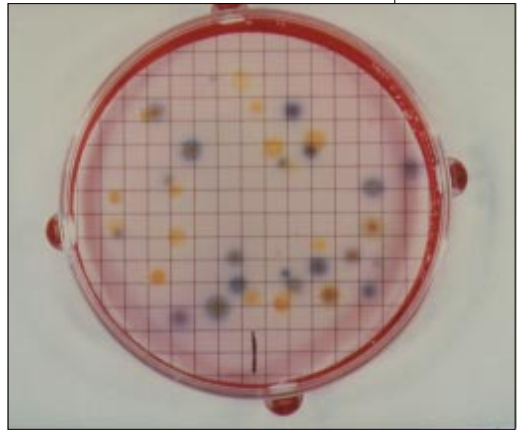
Current EPA Research – Barriers to Contamination

Although water treatment and disinfection techniques are quite effective at microbe reduction, finished drinking water is not sterile. Survival and regrowth of microorganisms in drinking water distribution systems can lead to the deterioration of water quality and even noncompliance of a supply. Regrowth has largely been associated with heterotrophic bacteria (i.e., those bacteria – including pathogens – that require preformed organic compounds as carbon and energy sources). Bacterial growth occurs on the walls of the distribution system (referred to as "biofilms") and in the water either as free living cells or cells attached to suspended solids. A multi-faceted phenomenon, bacterial regrowth is influenced primarily by temperature, residence time in mains and storage units, the efficacy of disinfection, and nutrients.

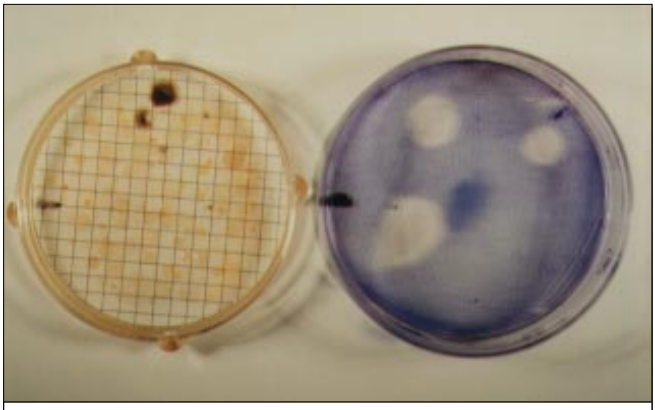
Assimilable organic carbon (AOC) is the portion of the total organic carbon (TOC) dissolved in water that is easily used by microorganisms as a carbon source (i.e., nutrients). ORD researchers are currently investigating treatment processes to control AOC. One promising process is biologically active filtration wherein bacterial communities are intentionally established in the filters to use up, or biodegrade, the AOC as it passes through. This treatment process must be employed before final disinfection so that bacteria escaping from the filter can be properly controlled. As described in Figure 1, most water utilities do not disinfect with chlorine until late in the treatment train. This limits the formation of disinfection by-products (i.e., those compounds like chloroform produced when chlorine reacts with naturally occurring organic carbon).

To accomplish disinfection earlier in treatment, some water utilities employ ozonation. While ozone is a very strong disinfectant, it also converts a portion of the TOC into AOC. ORD researchers are examining the advantages (e.g., disinfection of bacteria, viruses and protozoan cysts, control of color, control of taste and odor, enhance-

Currently, it is estimated that there will be over 100,000 violations of the SDWA annually. Nearly half of these will be for MCL violations. Of these, the majority will be microbiological violations by small systems.



Single step membrane filter procedure for enumerating E. coli *in recreational waters. The yellow colonies are* E. coli *while the blue, red and purple colonies are other coliforms.*



In situ *cytotoxicity test for heterotrophic bacteria found in drinking water. Heterotrophs recovered from drinking water form individual yellow colonies (left) that can be transferred to a tissue cell culture (right). Formation of plaques (i.e., clear areas caused by destruction of infected cells) in the tissue culture can indicate virulence and signal the need for further action.*

In the lesser developed countries, waterborne disease is still a major problem. The World Health Organization has estimated that more people die annually of water-related diarrheal illnesses than of cancer or AIDs.

ment of coagulation, and partial oxidation of the naturally occurring organic carbon that reacts with chlorine) and disadvantages of ozone (e.g., enhancement of AOC, conversion of bromide to bromate, and formation of its own disinfection by-products like formaldehyde).

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

EPANET can be useful for analyzing the loss of disinfectant residual, designing water quality sampling programs, performing drinking water exposure risk assessments, and calibrating network hydraulic models. It can provide insight into how changes in water source utilization, pumping water storage levels, use of satellite treatment, and targeted pipe cleaning and replacement would affect drinking water quality.

In support of small community and non-community (less than 3300 people) drinking water treatment systems, ORD researchers are designing, modifying and testing "Hybrid Drinking Water Treatment Package Plants." These package plants are factory-built, skid-mounted, and ready to be operated in the field with minimal site preparation. They exhibit lower capital cost than custom designed facilities built onsite and can incorporate any drinking water treatment process. Promising technologies being considered for incorporation include membranes, advanced oxidation, bag filters, and photocatalytic oxidation. By merging, modifying, and adapting conventional treatment trains with innovative treatment technologies, a broader variety of contaminants (including pathogens) can be removed and SDWA compliance can be facilitated.

Concern has recently mounted over the ability of certain pathogenic protozoan (*Cryptosporidium)* cysts to survive treatment processes and enter the distribution system. ORD, in its project entitled "Evaluation of Particulate Removal Processes," is designing and testing the most effective filtration techniques to physically remove the cysts. Being studied are slow sand (see Figure 3), diatomaceous earth, and coagulation/rapid sand filtration processes. Results of this research will build upon earlier work with filtration of *Giardia lamblia*.

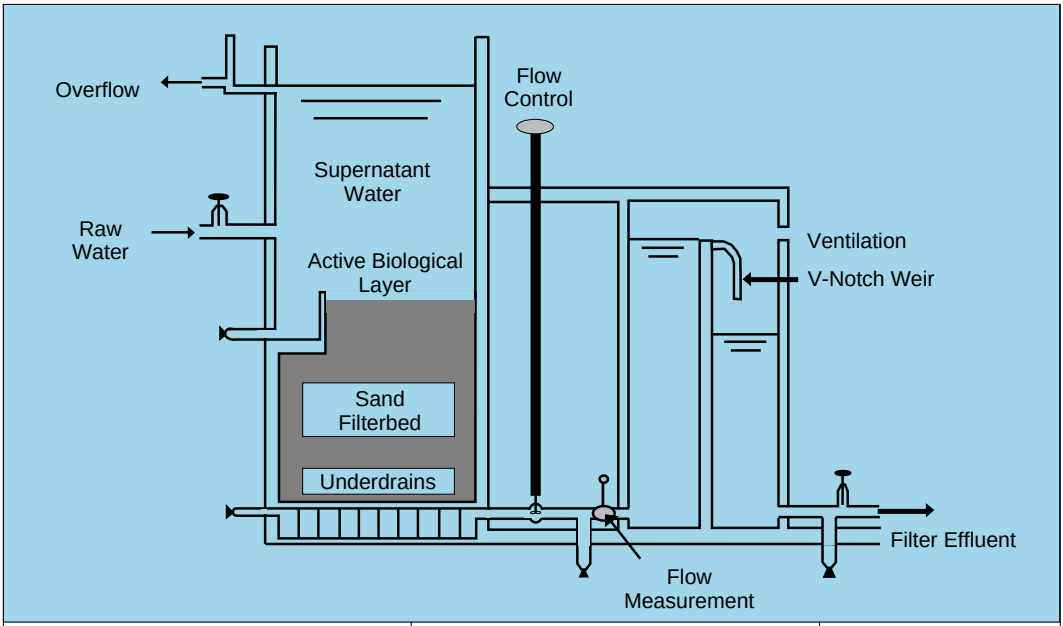


Figure 3. Basic elements of a slow sand filter.

Current EPA Research — Pathogenic Intestinal Protozoa

During the last 20 years, significant improvements have been made in the quantitative methods for detecting pathogenic intestinal protozoa (particularly *Giardia* and *Cryptosporidium*) in water. Additionally, there has been progress in standardizing these methods. Current methods, however, are still time-consuming and skill-intensive (requiring highly trained analysts), and lack the ability to indicate viability (whether the cyst is dead or alive) or infectivity. The latter item has clouded the development of quantitative risk assessments.

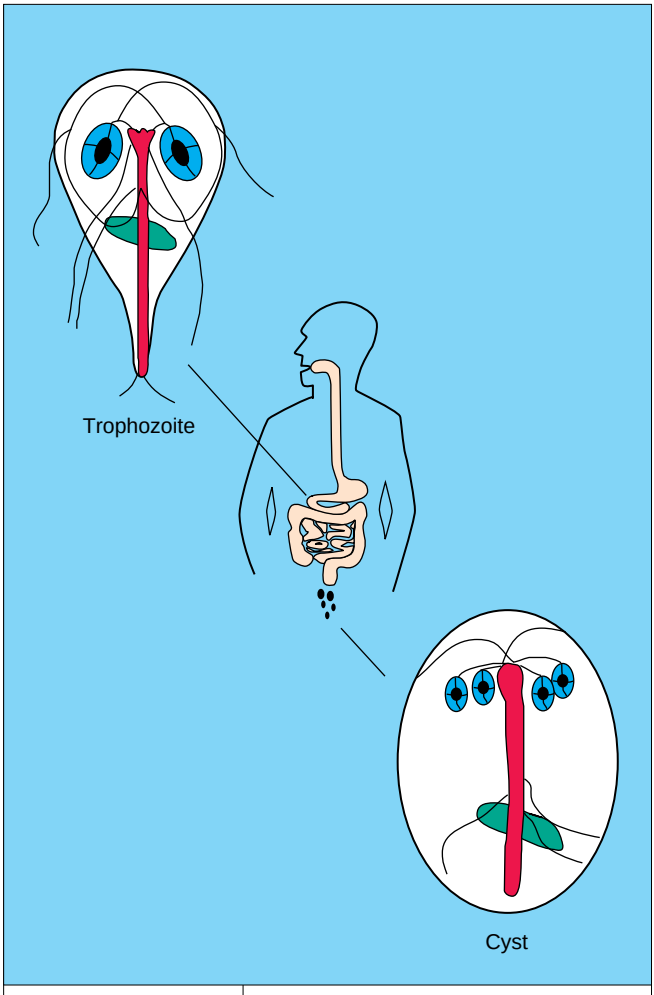
The cosmopolitan nature of intestinal protozoa, and the certainty that all surface water supplies must be contaminated with these organisms, has been established through studies in animals (beavers, muskrats, and birds) and by occurrence studies in sewage throughout all parts of the country. Seasonal and geographic differences have been recognized and data on concentrations in various waters have been collected.

Work on cross-species infectivity of animal and human cysts has established that beavers and muskrats may at least be secondary reservoirs for giardiasis. Also, while it appears that avian cysts are not infective for mammals, we cannot now distinguish avian and mammalian cysts in a water sample. The goal of an ORD project entitled "Development of Gene Probes for Speciation of *Giardia*" is to develop and test the application of genetic and molecular probe methods to allow classification of detected *Giardia* species.

Gene sequences have been mapped in species of *Giardia* shed by animals (e.g., herons and mice) and compared with corresponding gene sequences in human-hosted *Giardia*. Preliminary results indicate that through these mapped se-

In Minnesota, 100% of the muskrats and 7% of the beavers examined were positive for Giardia. *In four Northeastern states (Maine, New Hampshire, New York, and Vermont), the corresponding figures were 94% for muskrats and 17% for beavers.*

Erlandsen *et al.*, 1990.



Two-stage life cycle of Giardia lamblia*: the active trophozoite stage and the environmentally-resistant, resting cyst stage. Cellular components shown above include nuclei (blue), axonemes (red), and median bodies (green).*

quences, once labelled with a detectable probe, human type *Giardia* can be differentiated from bird and mouse *Giardia*. Probes have been synthesized and experiments show that one reacts with 10 different *G. lamblia* human isolates but not with *G. psittaci* (associated with birds) or *G. muris* (associated with mice). It is hoped that progress in speciation of *Giardia* can be applied to the study of *Cryptosporidium*.

Current *Giardia* detection methods are unable to distinguish viable from nonviable cysts. A practical detection method for viable cysts would allow assessing the significance of positive reports and may allow establishment of numerical standards under the SDWA. The objectives of the projects "Molecular Probes for the Detection of Protozoan Parasites" and "Induction of Stress Proteins as a Measure of *Giardia* Cyst Viability" are to discover, separate and amplify specific genetic sequences (DNA or RNA) associated with viable *Giardia* cysts. If these specific sequences can be identified, probes can be developed to allow testing for viable cysts only.

Practical methods for isolation, identification and quantification of waterborne pathogens such as *Giardia* are not yet available. Isolation and identification methods are needed before control methods can be evaluated and regulatory decisions can be made regarding required treatment processes and MCLs. The goal of ORD's project entitled "Immunological Methods for Detection of Etiological Agents of Waterborne Disease" is to develop innovative immunologic methods for the detection, identification and enumeration of pathogenic microorganisms. Immunologic methods may provide the sensitivity and specificity needed for detection since low numbers of target organisms may be present in large volumes of water along with high numbers of the normal flora and fauna.

To accomplish this, the pathogenic agents will be isolated and their antigens (proteins that stimulate the body to produce antibodies) will be used to produce specific antisera for immunologic tests (e.g., immunofluorescent assay, enzyme immunoassay, radioimmunoassay).

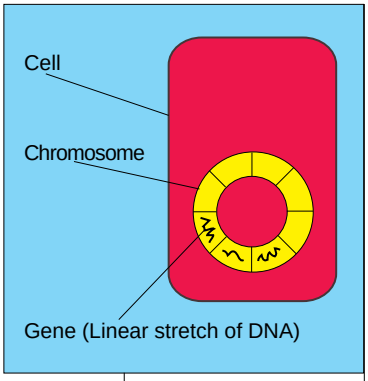
Because standardized procedures for detecting pathogenic protozoa do not now exist, confusion in the interpretation of results obtained by

different laboratories occurs. The goal of the project entitled "Standardized Methods for Detecting Pathogenic Protozoa in Water" is to develop standardized methods for detecting *Giardia* and *Cryptosporidium* in water. These methods will also assist EPA in assessing research findings relevant to regulatory activities under the SDWA.

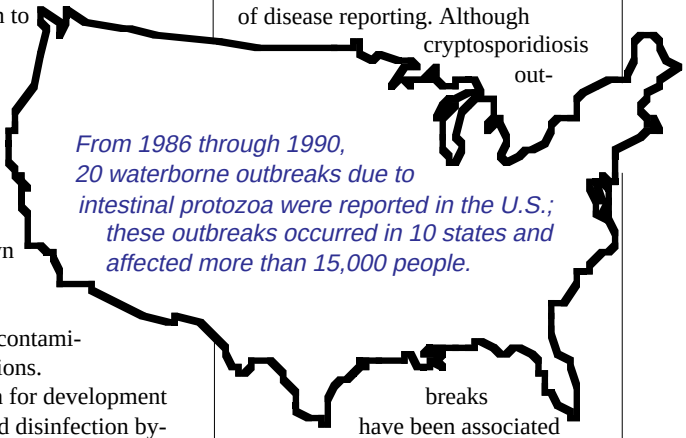
Cryptosporidium is the only microorganism on the Office of Ground Water and Drinking Water's list of contaminants to be addressed in the next round of regulations. Quantitative risk assessment for this organism is hampered by the unavailability of any human infectious dose data and by the scarcity of animal dose data. Additionally, very little is known of the longevity and protective ability of the body's immune response to *Cryptosporidium* infection.

The objective of the project entitled "*Cryptosporidium* Infectious Dose and Immune Response" is to determine *Cryptosporidium* infectious dose and the associated immune response in human volunteers. Organisms known to be infectious for humans will be obtained from infected calves and administered in drinking water to the volunteers. Conclusions drawn from this project could help shape future maximum contaminant level regulations.

In preparation for development of disinfection and disinfection by-product rules, information on the occurrence of *Giardia* cysts and *Cryptosporidium* oöcysts in source waters and throughout the drinking water treatment process must be collected. Through the project entitled "Cyst and Oöcyst Levels in the Ohio River," ORD is monitoring monthly one raw water sample (collected from the river) plus samples from five different points in the drinking water treatment process. Although current methods are based on microscopic examination of concentrated samples obtained from large volumes of water, immunofluorescence membrane assays and gene probe techniques are being used for this project. Findings from this project will also be used in a nationwide survey for occurrence of these organisms in water supplies.



In the early 1980s, a waterborne disease study in Washington State suggested that certain elements were required for a good waterborne disease surveillance and investigation program. Since that time, computer hardware and software have been introduced which may increase the potential for improved efficiency of disease reporting. Although cryptosporidiosis outbreaks have been associated with drinking water, the relative significance of drinking water in the transmission of this disease is unknown. The project entitled "Surveillance of Waterborne Disease/



From 1986 through 1990, 20 waterborne outbreaks due to intestinal protozoa were reported in the U.S.; these outbreaks occurred in 10 states and affected more than 15,000 people.

An epidemiological investigation involves the study and occurrence of disease within a population. In the study of waterborne disease, epidemiological data can indicate a need for additional drinking water treatment (e.g., filtration).

Cryptosporidiosis Epidemiological Feasibility Study" is underway to: 1) systematically evaluate waterborne disease strategies, computer software and educational programs in local and state health departments, and 2) design an epidemiological study to address the significance of drinking water in the transmission of cryptosporidiosis. Products from these efforts could shed light on the understanding and tracking of waterborne disease outbreaks throughout the world.

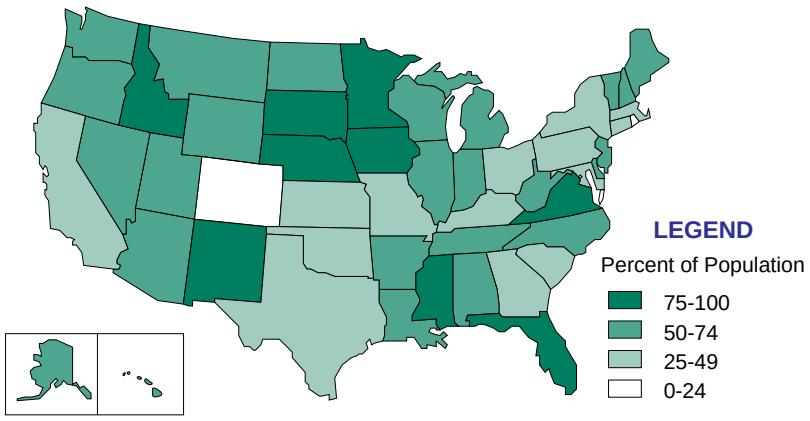
Current EPA Research — Viruses

Traditionally, methods for detecting and identifying viruses have relied on slow cell culture methods. Existing methods may underestimate the quantity of viruses present or alternatively produce false negatives when viruses are actually present in sampled water. Some viruses (e.g., hepatitis A and Norwalk) simply cannot be detected by the commonly used cell culture/plaque assay methods. Given the health risks presented by viruses, it is essential to develop more information on the nature and extent of viral contamination in our nation's waters. The objective of ORD's project entitled "Practical Methods for Monitoring Viral Pathogens in Surface and Groundwater Source Waters to Define Level of Treatment" is to develop improved methods for detection of waterborne viruses. In addition to supporting EPA's risk assessment efforts relating to water quality, these methods will provide the means to support the establishment of new virological standards and to permit the formation of effective options for regulatory decisions.

This project will focus on the development of biotechnology methods based on recognition of viral-specific nucleic acids within infected cell systems. The use of a biotechnology approach that employs DNA probes to detect the presence of viruses is faster, less expensive and easier to perform than traditional plaque assay methods.

The Science Advisory Board's (SAB) *Reducing Risk* report to EPA describes pollutants in drinking water as one of the four highest risks to

Percentage of State Populations Served by Ground Water for Domestic Supply

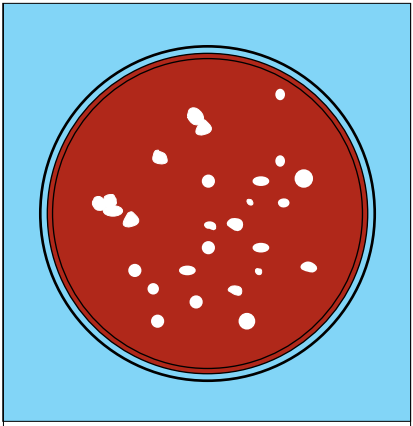


Source: 1990 State Section 305(b) Water Quality Reports

human health. With over 50% of the U.S. population relying on ground water as their primary source of drinking water, the need for groundwater resource protection, including protection from pathogens, is clear.

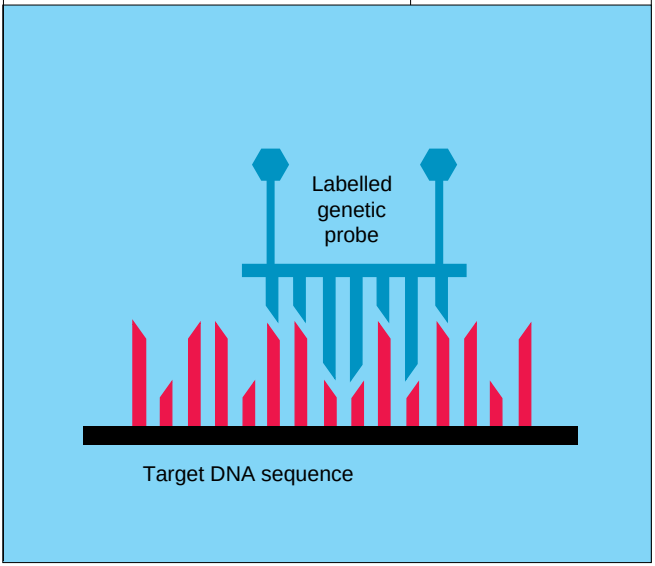
"Monitoring of Ground Water for Human Enteric Viruses" is a current project to address the mandate of the SDWA that EPA establish treatment requirements and criteria for ground water systems. A virus occurrence survey of vulnerable ground water systems is being performed to support requirements for minimum levels of virus inactivation and ultimately a ground water disinfection rule. A number of public ground water systems will be identified and ranked according to vulnerability to fecal contamination. Of these, 25 systems will be monitored for the presence of viruses through tissue culture methods and gene probe techniques.

Norwalk and Norwalk-like viruses cause viral gastroenteritis (the second leading cause of illness in the U.S.) in consumers of contaminated water and food. Since these viruses cannot be grown and identified in tissue cultures, they cannot be detected in water samples by current monitoring techniques. A small amount of Norwalk virus is available for studies. This virus preparation has been isolated from stool samples of infected individuals and used in an enzyme immunoassay for the detection of Norwalk virus. Because immunologic methods require a high virus concentration, the etiologic agent responsible for most waterborne outbreaks of gastroenteritis usually is not determined. Since the viral density in environmental samples is normally too low for direct immunologic detection and since there is no known cell culture method for this virus, the genetic material of the Norwalk virus particle must currently be amplified using a biotechnology approach called polymerase chain reaction (PCR).



Once virus particles infect cells in a single layer tissue culture, cellular damage (clear areas or "plaque-forming units" in the brown agar depicted at left) becomes apparent. The plaque assay is used for identification, counting, and purification of viruses.

Although known to be highly infectious, the infectious dose for Norwalk virus is unknown. The only safety-tested virus inoculum (a microorganism-containing specimen that has been shown to be free of other pathogens) available cannot be used for infectious dose studies because it is not possible to determine the virus concentration. The project entitled "Develop a Dose-



Hybridized probe (in blue) binds with target genetic sequence (in red) to make it detectable by radiation or color.

Preparing stock tissue cell cultures for isolation and identification of viruses in water samples.

Although the rate of water transport through their gills varies greatly, oysters have been found to filter as many as 154 gallons per day. In waters exposed to human sewage, these shellfish can filter out and concentrate pathogens as well as food.

Response Curve for Norwalk Virus" is approaching this problem in four phases: 1) cell cultures capable of growing the Norwalk virus will be developed; 2) Norwalk viruses will be grown in cell culture, purified and safety-tested for use in volunteer studies; 3) a measure of the number of total and infectious virus particles in the purified sample will be established; and 4) a human volunteer study will be initiated to determine the amount of virus particles required to cause disease.

The Clean Water Act stipulates that the nation's rivers, lakes, and coastal waters be swimmable and fishable. Water quality standards based on established criteria to achieve this goal must be developed. The project entitled "Shellfish Methods and Exposure Response Assessment/Viruses" is being conducted to develop methods for detecting and enumerating contamination of shellfish and shellfish growing waters by human enteric viruses. Shellfish growing in polluted waters are known to concentrate these viruses during feeding. Since shellfish are often eaten raw or insufficiently cooked, subjecting shellfish waters to human wastes constitutes a public health risk.

Because there are more than 100 waterborne virus types that may cause clinical outbreaks in humans, monitoring efforts are essential to ensure the virological safety of waters and particularly the reliability of water and wastewater treatment practices. This information can only be provided through increased monitoring and assessment programs of each major pathway leading to the deposition of human enteric viruses into the nation's waters. These viruses are responsible for serious illnesses ranging from hepatitis to myocarditis to central nervous system disorders to acute gastroenteritis. The general recommendation has been that drinking water should be free of human enteric viruses and that recreational water viral limits be set. The goal of the project entitled "National Monitoring and Assessment Program: Status and Long-Term Trends in Human Enteric Virus Pollutants in the Aquatic Ecosystems" is to establish a national viral survey program focusing on the following five factors: 1) selection of monitoring sites based on those most likely to have broad public health importance; 2) field sampling that will result in the collection of adequate and representative sample volumes to safeguard against false negatives; 3) concentration procedures to increase the density of viruses so that they can be effectively assayed; 4) standard protocols for viral detection using both gene probe and classical plaque assay techniques; and 5) parallel biological and chemical analysis that will serve to determine the quality of the water source.

Current EPA Research — Bacteria

The new National Primary Drinking Water Regulations require that all drinking water samples testing positive for total coliforms be further tested for the presence of either fecal coliforms or *E. coli*. There is a method currently available that allows the simultaneous detection of total coliforms and *E. coli* in a broth medium in 24 hours; however, there is no equivalent method for use with membrane filters. Development of such a method will allow those who prefer to obtain counts of these organisms in their distribution systems to use a membrane filter method and to have results within the 24-hour time frame. Through the project "Development of a Membrane Filter Medium for the Simultaneous Detection of Total Coliforms and *E. coli*," a membrane filter medium on which both total coliforms and *E. coli* can be distinguished from noncoliforms will be developed and patented.

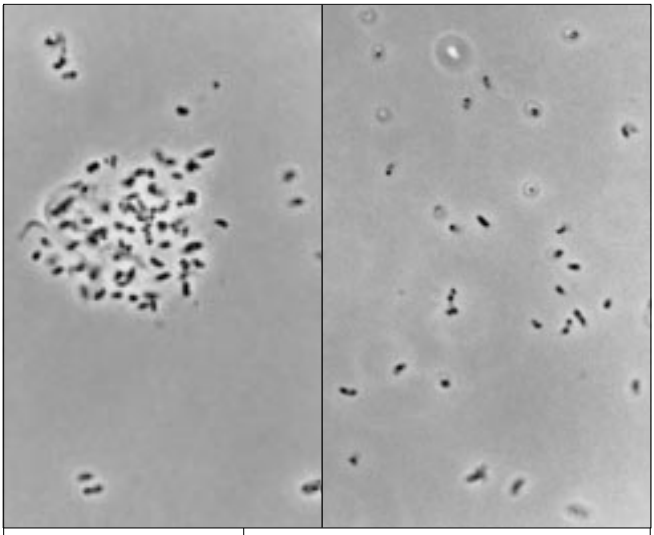
E. coli are fecal organisms that when present in drinking water are indicative of fecal pollution. Logistical concerns in sample handling and holding require evaluation of conditions for optimizing sample stability and longevity. No current regulations exist for handling samples for analysis of *E. coli*. Through the project entitled "Optimal Sample Holding Conditions for Analysis of Fecal *E. coli* in Drinking Water," sample temperature and holding time will be determined for *E. coli* or fecal coliform analysis methods (i.e., Colilert and M-FC agar). Relative recovery of methods and storage conditions will be assessed for optimal *E. coli* recovery.

The requirement (through the SDWA amendments) to test all coliform-positive drinking water samples for either fecal coliforms or *E. coli* is new. Data from available methods for detecting chlorine-damaged *E. coli* in drinking water are limited. The objective of the project entitled "Detection of Low Numbers of Chlorine-Stressed *E. coli* in Drinking Water" is to evaluate and compare the abilities of a commercial method (Colilert) and a standard coliform method (EC-MUG) to recover low numbers of chlorine-stressed *E. coli* from potable water. Pure cultures of *E. coli* will be washed, nutrient-stressed in finished drinking water, and treated with chlorine. The chlorine-stressed *E. coli* will then be enumerated, diluted to levels that would be found in marginally unsafe drinking water and assayed in multiple tubes by the three methods. These experiments will be repeated using naturally occurring *E. coli* from diluted human fecal specimens, contaminated source waters and effluents.

The golden metallic sheen of the colonies at left indicate the presence of total coliforms and the possibility that the sampled water supply is contaminated.

The infectious bacterial agent identified from the stools of cholera victims is *Vibrio cholerae*. The epidemic in Latin America has prompted a renewed interest in control measures for this disease. Through the project entitled "Inactivation of *Vibrio cholerae* Biotype El Tor and Biotype Classical by Chlorination," it has been determined that the strain responsible for the epidemic in Peru is capable of reverting to a variant which is more resistant

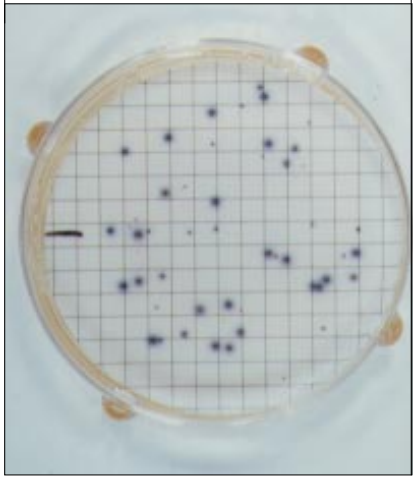
In the U.S., the presence of coliform bacteria in drinking water is used as an indicator of possible microbiological contamination. When total coliforms are detected, fecal coliform or E. coli *analysis must be performed.*



A rugose (rough-surfaced) variant (above left) of Vibrio cholerae *01 is able to form aggregates. ORD studies have indicated that this variant is more resistant to disinfection than the smooth strain (above right).*

to chlorination than the typical smooth variety of *Vibrio cholerae*. Cells of the variant appear to be imbedded in a gelatinous mucoid material, facilitating the formation of aggregates, which renders them more resistant to disinfection. Although the variant is more resistant, studies have indicated that all strains are readily inactivated through adequate chlorination.

The *Legionella pneumophila* bacterial strains that cause community- and hospital-acquired pneumonia are usually spread via finished drinking water. Certain free living amoebae (protozoa) support the multiplication of *L. pneumophila* in drinking water systems. These amoebae may also be responsible for enhancing the virulence (capacity of a microorganism to cause disease) of the *Legionellae* and for protecting them from adverse environmental factors such as high temperature and chlorine disinfection. The project entitled "Multiplication of *Legionellae* in Amoebae and Assessment of Virulence" will determine the effect of intracellular growth of *Legionella* in amoebae on virulence and as protection against chlorine and high temperature. To accomplish this, a method will be established to study the ability of various types of amoebae to provide a protective niche for the multiplication of *Legionellae* under adverse environmental conditions. Combinations of *Legionella* isolates and specific amoebae that result in high yields of *Legionella* after intracellular growth will be used to study the effects of intracellular growth on virulence. Preliminary studies on the ability of amoebae to supply iron to *Legionellae* growing intracellularly showed no obvious associations between growth and iron concentration.

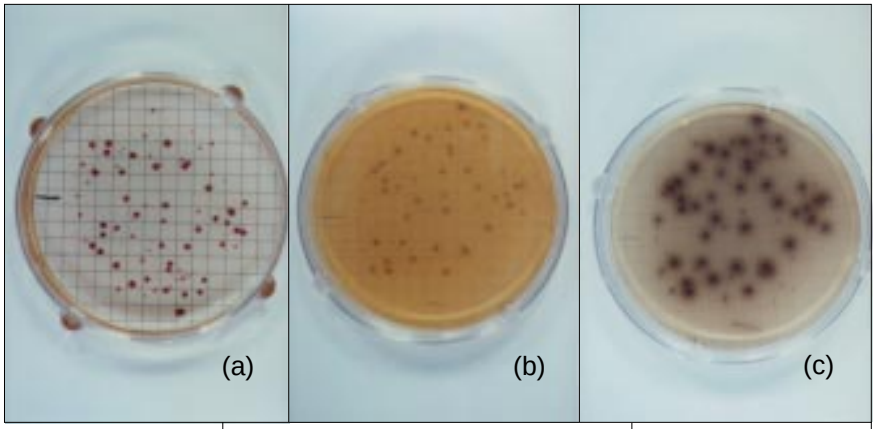


This one step method (developed by ORD) allows enterococci (blue colonies) enumeration in just 24 hours.

EPA is required by the SDWA to establish appropriate controls and regulations for potable water. ORD's project entitled "Develop Methods for Identifying Potential Bacterial Pathogens in Drinking Water" will develop a data base on potential health hazards (i.e., pathogenicity) associated with bacteria commonly found in water distribution systems. To accomplish this, three rodent species will be compromised using nitrous oxides or immunosuppressive agents, and the animals subsequently

will be challenged via the gastrointestinal route.

Although virulence is usually measured *in vivo* (animal research), the need for extensive animal testing can be significantly reduced by the development of a battery of *in vitro* (cell culture) tests for traits known to be virulence-related. This battery can be used to predict the potential an organism has for causing disease in exposed populations. Through the project entitled "Develop *In Vitro* Methods for Identifying Potential Bacterial Pathogens in Drinking Water," model systems will be developed that can be used to determine the potential pathogenicity of bacteria found in potable water distribution systems. Additionally, gene probe and other assays to identify known opportunistic pathogens will be developed and evaluated.



Two step, 48 hour membrane filter test for enumerating enterococci in recreational waters. (a) and (b) Two perspectives of colonies (red) present at 24 hours. (c) At 48 hours, colonies with black halos are identified as enterococci.

Bacteria common to drinking water distribution systems colonize point-of-entry, granular activated carbon (GAC) filters where they are able to grow to very high densities. Subsequent to reaching the high densities the bacteria begin sloughing off the GAC filters. The number of bacteria in the filter effluent (i.e., water flowing out of the filter) is significantly higher than in the influent water. This amplification of bacteria in drinking water is of concern to EPA because GAC filters are being considered as a substitute for central potable (i.e., fit for drinking) water treatment in small communities where the treatment system has been overwhelmed by organic substances that may be harmful to human health. EPA's Office of Ground Water and Drinking Water (OGWDW), however, does not want to recommend the use of these filters if the possibility exists that their use poses an acute disease risk due to bacteria that grow on the filters. The health significance of the bacteria known to adsorb and grow on GAC filters used in the home will be evaluated. The OGWDW will use this information to develop appropriate controls and regulations for this type of drinking water treatment as required by the SDWA.

The objective of ORD's project entitled "Health Effects Associated with Point-of-Entry GAC Filters" is to determine if a significant health hazard is associated with the use of granular activated carbon, point-of-entry, whole house filters. To accomplish this, a suitable study site will be selected based on the following criteria: 1) the water in the delivery system must meet EPA and local drinking water standards; and 2) the water distribution system should contain a bacterial population whose density is as high as possible and still acceptable under local regulations.

Analysis of potable water or cooling tower water for Legionella pneumophila *requires approximately five to seven days for growth of the organisms on the initial isolation medium and another five to seven days to confirm the identity of these organisms. Gene probe techniques could reduce analysis time to one day.*

After a distribution system meeting the above criteria has been found, a volunteer population of appropriate size will be selected from among the water system customers. The selected population will be randomly divided into GAC user and non-user groups. Point-of-entry, GAC filters will be installed in the homes of the randomly selected user group. The health status of both groups will be monitored over a predetermined period of time and during this time interval the bacterial population in the water system and the filter effluent will be monitored on a routine basis. In the event of an illness where a bacterial agent is diagnosed as the cause, the GAC filter will be removed and examined for the presence of the organism determined to be the agent of the disease in that household unit. If an association between illness or disease and the use of GAC filters is observed, health advisory guidelines will be established or processes that will eliminate the causative organisms will be developed.

Some believe that exposure to fecal pollution through recreational waters or ingestion of contaminated shellfish causes greater health risks if the pollution is of human rather than animal origin. Before the relative risks of human versus animal fecal pollution can be assessed, it is necessary to develop a microbiological method for distinguishing human from animal pollution. Current methods detect fecal pollution but do not reveal the source. The objective of the project entitled "Method to Distinguish Non-Human Fecal Pollution from Human Fecal Pollution" is to develop a gene probe specific for *E. coli* that inhabit the human intestine for use as an indicator of the presence of human fecal contamination in water. The probe will be field tested at several sites in which fecal pollution is exclusively from human sources, exclusively from animal sources and from mixed sources.

Shigella species are among the most common and significant pathogens associated with wastewater and

EPA researcher using the transmission electron microscope to detect pathogens unable to be detected by other methods.

sludge. Because of their low infective doses, these organisms may be hazardous even if present in low numbers in wastewaters that are recycled for potable use or sludges that are applied to agricultural land. *Shigellae* are very difficult to detect in environmental samples by conventional methods because of their biochemical similarities to *E. coli*. The use of current gene probe technology in the project entitled "Detection of Enteroinvasive *Shigella* in Wastewaters and Sludges" should enable us to detect *Shigellae* in sludges and wastewaters that would appear to be free of these pathogens if analyzed by conventional methods.

Conclusion

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

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

Human enteric bacteria being subcultured in an anaerobic hood.

EPA Publications

The EPA publications listed below may provide more detailed information on the subjects discussed in this document. These references and additional copies of this brochure can be requested at no charge (while supplies are available) from EPA's Center for Environmental Research Information (CERI). Once the CERI inventory is exhausted, clients will be directed to the National Technical Information Service (NTIS) where documents can be purchased.

Environmental Pollution Control Alternatives: Drinking Water Treatment for Small Communities, EPA/625/5-90/025.

Methods for the Investigation and Prevention of Waterborne Disease Outbreaks, EPA/600/1-90/005a.

Microbiological Methods for Monitoring the Environment — Water and Wastes, EPA/600/8-78/017.

Seminar Publication: Control of Biofilm Growth in Drinking Water Distribution Systems, EPA/625/R-92/001.

Test Methods for *Escherichia coli* and Enterococci in Water by the Membrane Filter Procedure, EPA/600/4-85/076.

USEPA Manual of Methods for Virology, EPA/600/4-84/013 and updates.

Waterborne Disease Outbreaks - Selected Reprints of Articles on Epidemiology, Surveillance, Investigation, and Laboratory Analysis, EPA/600/1-90/005b.

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Geldreich, E.E., K.R. Fox, J.A. Goodrich, E.W. Rice, R.M. Clark, and D.L. Swerdlow. 1992. Searching for a water supply connection in the Cabool, Missouri disease outbreak of *E. coli* 0157:H7. *Wat. Res.*, 26: 1127-1137.

This publication was prepared by Patrick Burke of ORD's National Risk Management Research Laboratory, Cincinnati, Ohio. Contributors and reviewers include Alfred Dufour, Walter Jakubowski, Robert Safferman, Shay Fout, Gerard Stelma and Terry Covert of the National Exposure Research Laboratory - Cincinnati, and Robert Clark, Kim Fox, Edwin Geldreich, Richard Miltner, Donald Reasoner, and Eugene Rice of the National Risk Management Research Laboratory. Thanks to Al Lang and Jim O'Dell for photographic support.