

In cooperation with the  
U.S. Environmental Protection Agency  
Office of Ground Water and Drinking Water

# Microbiological Monitoring for the U.S. Geological Survey National Water- Quality Assessment Program

Water-Resources Investigations Report 00-4018



U.S. Department of the Interior  
U.S. Geological Survey

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By Donna S. Francy, Donna N. Myers, and Dennis R. Helsel

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## CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
millimeter (mm)	0.03937	inch (in.)
micrometer (μm)	0.00003739	inch (in.)
milliliter (mL)	0.06102	cubic inch
microliter (μL)	0.00006102	cubic inch
liter (L)	2.113	pint, U.S.
liter	1.057	quart, U.S.
liter	0.2642	gallon, U.S.
square kilometer (km <sup>2</sup> )	0.3861	square mile
pound (lb)	0.4536	kilogram

**Temperature:** Temperature is given in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) by use of the following equation:

$$^{\circ}\text{F} = 1.8 (^{\circ}\text{C}) + 32$$

**Concentrations of bacteria** are given in colonies per 100 milliliters (col/100 mL) which is the same as colony forming units per 100 milliliters (CFU/100 mL)

**Chemical concentrations** in water are expressed in milligrams per liter (mg/L).

Other abbreviations used in this report:

APHA	American Public Health Association
CPE	Cytopathic effects, cell-culture analysis for enteric viruses
<i>C. perfringens</i>	<i>Clostridium perfringens</i> , a fecal-indicator bacterium
EDI	Equal discharge increment (streamwater sampling technique)
EWI	Equal width increment (streamwater sampling technique)
GWR	Ground Water Rule, USEPA
HIP	High Intensity Phase of the NAWQA Program
ICR	Information Collection Rule, USEPA
<i>E. coli</i>	<i>Escherichia coli</i> , a fecal-indicator bacterium
MCL	Maximum contaminant level
MF	Membrane-filtration method

MPN	Most-probable number method
mTEC	Membrane filter media for <i>Escherichia coli</i>
NASQAN	National Stream Quality Accounting Network, USGS
NAWQA	National Water-Quality Assessment Program, USGS
NWIS	National Water Information System, USGS
QA/QC	Quality assurance/quality control
RT-PCR	Reverse transcriptase, polymerase-chain reaction, method for enteric virus analysis
SDWA	Safe Drinking Water Act, USEPA
SWTR	Surface Water Treatment Rule, USEPA
TCR	Total coliform Rule, USEPA
USGS	U.S. Geological Survey
UCMR	Unregulated Contaminant Monitoring Rule, USEPA
USEPA	U.S. Environmental Protection Agency

# Microbiological monitoring for the U.S. Geological Survey National Water-Quality Assessment Program

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## Abstract

Data to characterize the microbiological quality of the Nation's fresh, marine, and estuarine waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. Methods and procedures vary with the objectives and practices of the parties collecting data and are continuously being developed or modified. Therefore, it is difficult to provide a nationally consistent picture of the microbial quality of the Nation's waters.

Study objectives and guidelines for a national microbiological monitoring program are outlined in this report, using the framework of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program. A national program is designed to provide long-term data on the presence of microbiological pathogens and indicators in ground water and surface water to support effective water policy and management. Three major groups of waterborne pathogens affect the public health acceptability of waters in the United States—bacteria, protozoa, and viruses. Microbiological monitoring in NAWQA would be designed to assess the occurrence, distribution, and trends of pathogenic organisms and indicators in surface waters and ground waters; relate the patterns discerned to factors that help explain them; and improve our understanding of the processes that control microbiological water quality.

## Introduction

On average, 26 waterborne-disease outbreaks have been documented each year in the United States over the past 25 years (Kramer and others, 1996). The persistence of outbreaks over time indicates that more progress is needed to meet the “drinkable and swimmable” goals of Federal water-quality legislation. Although significant improvements in drinking water and wastewater treatment have been achieved, waterborne disease outbreaks indicate that certain types and sources of waterborne pathogens (disease-causing organisms) are still a threat to human health in the United States (Craun, 1992). In particular, waterborne disease outbreaks caused by *Escherichia coli* O157:H7 were reported more frequently in 1995-96 than in previous years, and during that same period, *Cryptosporidium* and *Giardia* caused large outbreaks associated with recreational water quality (Levy and others, 1998).

Microbiological examination of water is used to determine the sanitary quality of water and the public health risk from waterborne disease. Although microbiological monitoring of finished waters is well established, microbiological monitoring of source waters and recreational waters is considered by some to be fragmented, incomplete, or virtually nonexistent in many parts of the Nation (Rose and others, 1999). Data to characterize the microbiological quality of source waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. For example, monitoring programs vary widely at the local level for recreational waters, and the result is the inconsistent use of indicator organisms

across the United States (U.S. Environmental Protection Agency, 1999a).

There is a need to identify human and animal factors associated with contamination of different source and recreational waters and to understand the processes that affect microbiological water quality. Concepts about the relation between the occurrence and distribution of microbiological contaminants and a range of environmental factors such as climate, hydrology, land use, and human and animal population densities need to be tested in areas that represent the national water-use patterns for public and domestic supply and for recreational uses.

The framework of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program is ideally suited to assess microbiological water quality on a national scale. Microbiological contamination of the Nation's water resources was among the top 10 priority water-quality issues identified by the National Advisory Committee to the NAWQA Program (R.J. Gilliom, oral commun., 1994). Although the issue has not been addressed nationally in the NAWQA program to date, microbiological contamination remains relevant and possibly of greater interest than in 1990 at the beginning of the Program's activities. National monitoring could provide long-term data on the presence of microbiological pathogens and indicators in ground waters and streams to support effective water policy and management. By nesting a microbiological assessment within the design of NAWQA, the wealth of additional water-quality and ancillary data currently being collected by NAWQA studies across the Nation can be used.

Information discussed in this report was developed, in part, from a pilot microbiological study in NAWQA completed in 1997 in cooperation with the U.S. Environmental Protection Agency (USEPA)—Office of Ground Water and Drinking Water. This report was written at the request of the USGS NAWQA Program and USEPA's Office of Ground Water and Drinking Water, who recognize the need for a nationally consistent microbiological assessment program.

### **NAWQA activities and their relation to microbiological issues**

Microbiological sampling has played a role in the assessment of water quality within the USGS on a local level and as a minor component of the NASQAN (National Stream Quality Accounting Network) (U.S.

Geological Survey, 1997) and the Benchmark (Mast and Turk, 1999) national streamwater-quality networks. Microbiological monitoring is, however, a key component of many state and local monitoring programs. The 305b Biennial Report to Congress on the quality of the Nation's water includes a microbiological assessment of the waters in each state that meet or do not meet the "swimmable" goal of the Clean Water Act (U.S. Environmental Protection Agency, 1996a). Microbiological monitoring is included in many local drinking-water programs to assess the suitability of the resource for human consumption, yet many of these data are unpublished and not easily accessible. Assessment of the microbiological quality of the Nation's waters merits consideration by the NAWQA Program because it continues to be a high priority water-quality issue among a broad range of public and private interests.

Indeed, the design and objectives of the NAWQA program are suited for the addition of microbiological sampling as a routine component of water-quality sampling. The NAWQA program defines the status and trends in water quality over a large and representative part of the United States. Program objectives in NAWQA Cycle I (1991-2001) are to relate the occurrence and distribution of water-quality constituents to natural and human factors and to provide interpreted information to those involved in policy planning and resource management. The emphasis in Program objectives in NAWQA Cycle II (beginning in 2001) will shift from occurrence and distribution studies to trend assessment and the understanding the processes controlling water quality.

The NAWQA program focus is on study units that are major hydrologic systems of the United States (Hirsch and others, 1988). Study units are geographically defined by a combination of ground- and surface-water features and usually encompass more than 10,000 km<sup>2</sup> of land area. Collectively they cover a diverse set of hydrologic systems, differing widely in natural and human factors that affect water quality. For Cycle 1, the NAWQA program collects data within 59 study units covering approximately 50 percent of the land surface and 60 percent of the water use and population of the United States. Sampling is ongoing in 15 to 20 of these study units at any one time.

Study-unit investigations begin with a review of available information (Gilliom and others, 1995) and planning for intensive sampling. Three years of sampling follows, called the High Intensity Phase (HIP).



During this phase, new data are gathered on streamwater quality, ground-water quality, stream habitat, fish, aquatic macroinvertebrates, and algae within the study unit.

Streams are sampled at indicator sites, at integrator sites, and in synoptic surveys, all of which could easily accommodate microbiological sampling. Indicator sites are stream-sampling sites located at outlets of drainage basins with homogeneous land use and physiography (Gilliom and others, 1995). Basins are chosen to be as large and representative as possible (typically 50-500 km<sup>2</sup>) while still encompassing primarily one type of land use and physiography. Integrator sites are stream-sampling sites located downstream from drainage basins that are large and complex, integrating runoff from multiple land uses. Most integrator sites are located on major streams with drainage basins that include a substantial proportion of the study unit area (typically 10-100 percent). In addition, sites are categorized in terms of frequency of sample collection. As part of the regular NAWQA sampling program, basic fixed sites are sampled 15 to 18 times a year for 2 years (monthly and during high and low flow). Intensive fixed sites are sampled more frequently over short periods. In addition, synoptic surveys over large parts of the study unit provide a spatial picture of study-unit conditions over a period of days or weeks or during a specific hydrologic condition.

NAWQA has three different study components for sampling ground-water quality. In the first component, land-use studies, the quality of recently recharged ground water (generally less than 10 years old) is compared and contrasted among the most important land use and hydrogeologic conditions in each study unit. Two to four land-use studies are usually conducted in each study unit, with approximately 30 shallow monitoring wells sampled in each study during the high-intensity phase. The second study component is subunit surveys, which are broad assessments of water-quality conditions of major aquifer systems within each study unit. Existing wells, including small public and private supply wells, are randomly selected and sampled to characterize the used resource. About 20-30 wells are sampled in each subunit. The third type of NAWQA ground-water study, the flowpath study, examines the fate and transport of contaminants along a specific ground-water flowpath.

## **Purpose and scope**

The purpose of this report is to outline a strategy for microbiological assessment of streamwater and ground water in NAWQA study units. These studies would be designed to collect, analyze, and interpret data on occurrence, distribution, and temporal trends in microbiological pathogens and their indicators in relation to processes and factors that affect their presence and transport. As part of this strategy, the design of field studies for testing new and (or) improved microbiological methods will be described. This report also contains information on microbiological sampling as it relates to water-quality standards and human health.

The information in this report can be used by the NAWQA Program and by NAWQA study units to identify issues and objectives, and to design and implement sampling programs. Sampling objectives and guidelines outlined herein can be adapted for use in other programs of the USGS or in other Federal, state, and local programs.

## **Overview of microbiological pathogens and indicators**

Microbiological data collected in USGS programs are used by water-resource managers to determine compliance with recreational and drinking-water regulations established by the USEPA and the States. The USGS has been involved in studies that address recreational water quality (Francy and others, 1996; Francy and Darner, 1998; Myers and others, 1998; Whitman and others, 1999) and quality of water from domestic wells (Bickford and others, 1996) or public-supply wells (Davis and Witt, 1998). Other USGS microbiological studies were done to investigate the distribution and variability of fecal-indicator bacteria in streams (Embrey, 1992; Myers, 1992) and to evaluate fecal-indicator monitoring methods (Francy and others, 1993). These types of studies are all within the mission of the USGS and can serve as examples, along with this report, as to the types of microbiological sampling that can be accomplished through NAWQA and other USGS programs.

Recreational waters are monitored to assess the health risk to people engaged in water-contact recreation. Swimmers are at risk of contracting gastrointestinal illness, skin rashes, and ear and eye infections from contact with fecal-contaminated water. In 1986, the USEPA recommended that criteria based on con-

centrations of *Escherichia coli* (*E. coli*) and enterococci be included in state recreational water-quality standards (U.S. Environmental Protection Agency, 1986a). This recommendation was based on the results of USEPA studies in which a statistically significant relation was found between the rate of swimming-associated gastrointestinal illness and the concentration of *E. coli* or enterococci at freshwater beaches (Dufour, 1984) and the concentration of enterococci at marine beaches (Cabelli, 1981). These same studies found no statistical relation between fecal-coliform concentrations—the fecal indicator in widespread use at that time—and swimming-associated gastrointestinal illness. Some states, however, continue to rely on total or fecal coliforms in regulatory standards even though *E. coli* and enterococci are the preferred and most useful indicators of the quality of recreational waters for body contact.

In recognizing the inconsistent use of indicator organisms for monitoring recreational waters, the USEPA recently developed the Beaches Environmental Assessment, Closure and Health (BEACH) Program (U.S. Environmental Protection Agency, 1999a). In this program, the USEPA intends to move toward adopting *E. coli* and enterococci into all state standards and to strengthen beach-monitoring programs in the United States.

Removal and disinfection of pathogens from drinking-water supplies has long been a means to decrease the public health risk. In the Safe Drinking Water Act (SDWA) amendments of 1986, Congress directed USEPA to develop national requirements for filtration as a treatment technique for surface water and for disinfection of all water supplies (Macler, 1995). As a result, the USEPA enacted the Surface Water Treatment Rule, the Total Coliform Rule, the Information Collection Rule, and the Interim Enhanced Surface Water Treatment Rule. USEPA is developing the Long Term 1 Surface Water Treatment Rule, the Long Term 2 Surface Water Treatment Rule, the Ground Water Rule, and the Unregulated Contaminant Monitoring Rule (UCMR). Microbiological monitoring is included in most of these regulations to assess the public health risk of potable water supplies. No microbial contaminants listed on the Candidate Contaminant List of the UCMR are proposed for monitoring at this time because standard methods were not available when the UCMR was promulgated.

Under the Surface Water Treatment Rule (SWTR) (U.S. Environmental Protection Agency,

1989a), the USEPA requires water treatment in place of water testing for microbial contaminants that are difficult to detect, such as *Giardia* and viruses. The rule requires the disinfection and filtration of all surface-water systems and ground-water systems under the direct influence of surface water, unless the system meets certain source-water-quality and system-operation criteria. To avoid filtration, the system must maintain a watershed control program, limit total coliform violations, and have no history of waterborne-disease outbreaks. The SWTR allows systems to use alternative treatment if they can demonstrate two orders of magnitude removal of *Giardia* using such treatment; riverbank filtration is one potential alternative treatment to avoid conventional filtration. The Interim Enhanced Surface Water Treatment Rule added *Cryptosporidium* to these requirements.

The Total Coliform Rule (TCR) was promulgated at the same time as the SWTR and applies to all systems, including ground-water systems (Macler, 1995). Under the TCR, the USEPA established standards for community and noncommunity water systems based on total coliforms (U.S. Environmental Protection Agency, 1989b). The USEPA established a maximum contaminant level (MCL) based on the presence or absence of total coliforms in a percentage of samples collected each month. All samples testing positive for total coliforms must also be tested for fecal coliforms or *E. coli*.

In 1996, the USEPA established the Information Collection Rule (ICR) (U.S. Environmental Protection Agency, 1996b). Under the ICR, large public water systems that use surface water, or ground water under the direct influence of surface water, were required to monitor source waters over an 18-month period for *Giardia*, *Cryptosporidium*, and total culturable viruses. In addition, bacterial indicators of fecal contamination (total coliforms and fecal coliforms or *E. coli*) were being monitored to assess how well they predict the presence and levels of microbial contamination. USEPA will use information generated by the ICR, along with other concurrent sampling efforts, to develop national occurrence estimates of the presence and levels of microbial contamination in source waters. This will determine the requirements of the Long Term 2 Surface Water Treatment Rule.

The Interim Enhanced Surface Water Treatment Rule applies to public water systems that use surface water or ground water under the direct influence of surface water and serve at least 10,000 people (U.S.

Environmental Protection Agency, 1998a). The rule is being developed to strengthen protection against microorganisms and includes treatment requirements for waterborne pathogens, *Cryptosporidium* in particular. The primary focus of the Interim Enhanced Surface Water Treatment Rule is to minimize *Cryptosporidium* levels in finished water.

The 1996 Amendments to the SDWA requires USEPA to establish criteria for a monitoring program for unregulated contaminants. The Unregulated Contaminant Monitoring Regulation (UCMR) will affect the assessment of source waters and potentially the treatment and distribution of finished waters, both from ground and surface sources. The UCMR includes a list of 57 drinking-water contaminant candidates, 10 of which are microbiological contaminants (bacteria, viruses, protozoa, and algae). All but 1 of the 10 microbiological contaminants are proposed for occurrence assessment over the next 5-10 years (U.S. Environmental Protection Agency, 1999b). At this time, very little information is available on the source waters where monitoring is needed—those most at risk for contamination.

Currently, the USEPA is gathering information to promulgate requirements for disinfection of ground-water systems (Macler, 1995) under the Ground Water Rule (GWR). This is the result of the growing concern over the contamination of ground water by microorganisms, and in particular, viral pathogens. Identifying which ground waters are or may be vulnerable to fecal contamination is an important issue in the development of ground-water disinfection criteria. One approach to vulnerability assessment is to examine the occurrence of viruses in certain hydrogeologic and land-use settings.

By adding microbiological analyses to existing NAWQA activities, the USGS can provide information to regulators and others on the occurrence and distribution of microbiological pathogens and indicators in streamwaters and ground waters of the Nation. The public health acceptability of waters in the United States is affected by four major groups of waterborne organisms—bacteria, protozoa, viruses, and algae (including cyanobacteria). Bacterial indicators of sewage and animal wastes have typically been used to determine the sanitary quality of water and the public-health risk from waterborne disease. Viruses and protozoa are transmitted to humans through fecal contamination of water and food. Certain species of algae pose a threat to human health by the toxins they pro-

duce, but in themselves are not human pathogens nor of fecal origin. This report addresses water-quality monitoring for the three groups of microorganisms that are pathogenic to humans and are transmitted through fecal contamination of water, and excludes the fourth group of nonfecal origin, the algae.

## Bacteria

Waterborne bacterial pathogens in the United States include species in the genera *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, *Yersinia*, and pathogenic strains of *E. coli*. Because bacterial pathogens generally appear intermittently in low concentrations in the environment and because methods of culturing are difficult, fecal-indicator bacteria are used to indicate the possible presence of pathogens. The most widely used bacterial indicators include total coliforms, fecal coliforms, *E. coli*, fecal streptococci, enterococci, and *Clostridium perfringens* (*C. perfringens*). A good indicator organism should be applicable in all types of water; unable to reproduce in ambient waters; be harmless to man and other animals; lend itself to easy, quantitative testing procedures; be of warmblooded animal origin; correlate with fecal contamination; and be present in waters in greater numbers than and survive as long as or longer than pathogens.

The historical definition of the total-coliform group has been based on the method used for detection (lactose fermentation) rather than on systematic bacteriology (American Public Health Association and others, 1998). Total coliforms are defined as aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation at 35°C within 48 hours (Britton and Greeson, 1989). Elevated temperature tests identify those genera of total coliform bacteria that belong in the more specific fecal-coliform group. Fecal coliforms are total coliforms capable of producing gas from lactose at 44.5°C. *Escherichia coli* is a species of the fecal-coliform group.

Total coliforms include several genera that are found in the human intestine; however, some genera are also found in soils, on vegetation, and in industrial wastes. This multiplicity of sources makes the sanitary significance of total coliforms difficult to establish (Palmer and others, 1984). They are used as a rough measure of source-water quality and as a screen for fecal contamination. In addition, speciation of the total-coliform group may provide information on treatment effectiveness and the source of colonization

of a distribution system or well (American Public Health Association, 1998, p. 9-1).

The fecal-coliform indicator used to assess fecal contamination of water has been faulted because of nonfecal sources of at least one member of the fecal-coliform group. For example, thermotolerant *Klebsiella* species have been observed in pulp- and paper-mill effluents, textile-processing-plant effluent, cotton-mill wastewaters, and sugar-beet wastes, in the absence of fecal contamination (U.S. Environmental Protection Agency, 1986a). Alternatively, *E. coli* is a natural inhabitant of the gastrointestinal tract of warm-blooded animals and is direct evidence of fecal contamination from warmblooded animals.

The fecal streptococci are a group of fecal-indicator bacteria that include a variety of species and strains that are all gram positive cocci. Although the normal habitat of fecal streptococci is the gastrointestinal tract of warmblooded animals, some species are not exclusive to animals (American Public Health Association, 1998, p. 9-74). In fact, studies on the distribution of fecal streptococci in water indicate that at least one strain commonly found in environmental samples is ubiquitous and can exist for extended periods in soil and water (Geldreich, 1976). Fecal streptococci, therefore, have limited value as an indicator of fecal contamination in environmental samples. The enterococci group is a subgroup of the fecal streptococci, and it is considered a more specific indicator of fecal contamination. The enterococci are differentiated from other streptococci by their ability to grow in 6.5 percent chloride, at pH 9.6, and at elevated temperatures. The enterococci method is valuable for determining the extent of fecal contamination of recreational surface waters, especially marine waters (American Public Health Association, 1998, p. 9-75). In addition, because enterococci cells are a different shape and have different survival rates than members of the coliform group, enterococci may be useful in assessing transport of fecal contamination in ground water.

*Clostridium perfringens* is present in large numbers in human and animals wastes, and its spores are more resistant to disinfection and environmental stresses than is *E. coli*. *Clostridium perfringens* has been suggested as a conservative tracer of past fecal contamination and as an indicator for chlorinated water in distribution systems (Bisson and Cabelli, 1980). *Clostridium perfringens*, however, is probably not an appropriate indicator for most recreational waters because spores in the sediment are resuspended

into the water column from swimmer or wave disturbances (Bisson and Cabelli, 1980). One exception is that *C. perfringens* may be a reliable indicator of streamwater quality in tropical climates, where warm water temperatures support the growth and reproduction of *E. coli* and aerobic conditions preclude the growth and sporulation of *C. perfringens* (Fujioka and Shizumura, 1985). *Clostridium perfringens* has also been found to be a sensitive indicator of microorganisms entering streams from point sources but not a reliable indicator of nonpoint sources (Sorenson and others, 1989). Detection of *C. perfringens* in water has been proposed as an indicator of the presence and density of pathogenic viruses and possibly other stress-resistant microorganisms (U.S. Environmental Protection Agency, 1996c).

### Protozoa

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

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

The USEPA-required method for detection of *Giardia* and *Cryptosporidium* in source and drinking water under the ICR involves nominal porosity filtration and indirect fluorescent antibody procedures (U.S. Environmental Protection Agency, 1996c). The ICR method has been criticized for being difficult to implement, being characterized by poor recovery of target organisms, and yielding highly variable results (U.S. Environmental Protection Agency, 1996b). As a result, the USEPA supported the development of Method 1622 for *Cryptosporidium* (U.S. Environmental Protection Agency, 1998b), and Method 1623 for

*Giardia* and *Cryptosporidium* (U.S. Environmental Protection Agency, 1999c). Method 1622 was validated through an interlaboratory study and revised as a final, valid method in January 1999. The interlaboratory validation of Method 1623 is currently being done.

## Viruses

More than 100 types of human pathogenic viruses may be present in fecal-contaminated waters (Havelaar and others, 1993). Treatment processes and watershed-management strategies designed on the basis of bacteriological criteria do not necessarily protect against viral infection because viruses are generally more persistent in the environment and are not removed as completely by treatment. In addition, because of their smaller size, viruses (0.023 to 0.080  $\mu\text{m}$ ) are transported further in ground water than bacteria (0.5 to 3  $\mu\text{m}$ ) or protozoan pathogens (4 to 15  $\mu\text{m}$ ) (Abbaszadegan and others, 1998). Because of the importance of viruses as a major public health concern, new methods for detection of enteric viruses and the search for indicators of viral contamination continue.

The current method for culturing enteric viruses under the ICR (U.S. Environmental Protection Agency, 1996c) is recognized as being difficult to implement; therefore, the ICR does not preclude the use of additional methods for research purposes. In addition, cell-culture methods are not available or suitable for all viruses of public health concern. One method, reverse-transcriptase-polymerase chain reaction (RT-PCR), a gene-probe method that amplifies and recognizes the nucleic acids of target viruses, has been adequately validated by the USEPA (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and is becoming widely used for environmental monitoring of enteric viruses. The RT-PCR method, however, does not determine the infectivity of the virus, and it is technically demanding, time consuming, and costly for routine use.

Because monitoring of enteric viruses is recognized as being difficult and time consuming, some researchers advocate the use of coliphage as indicator viruses for fecal contamination (Sobsey and others, 1995). Coliphages are bacteriophages that infect and replicate in coliform bacteria. The two main groups of coliphages that are considered as candidates for viral indicators are somatic and F-specific coliphages. Somatic coliphages infect coliform bacteria by attachment to the outer cell membrane or cell wall. They are

widely distributed in both fecal-contaminated and uncontaminated waters; therefore, they may not be reliable indicators of fecal contamination (Sobsey and others, 1995). F-specific coliphages attach only to the F-pilus of coliforms that carry the F+ plasmid; F-pili are made only by bacteria grown at higher temperatures. Hence, F-specific coliphages found in environmental samples presumably come from warmblooded animals or sewage (Handzel and others 1993).

Although somatic and F-specific coliphages are not consistently found in feces, they are found in high numbers in sewage and are thought to be reliable indicators of the sewage contamination of waters (International Association of Water Pollution Research and Control, 1991). Coliphage is also recognized to be representative of the survival and transport of viruses in the environment. To date, however, coliphage has not been found to correlate with the presence of pathogenic viruses.

## Occurrence and distribution of microbiological indicators in ground water and streamwater, 1997

The USGS collected data on the occurrence and distribution of microbiological indicators in 1997 to test concepts for monitoring in a nationally consistent program. Sampling and testing for bacterial and viral indicators in streamwater and ground water were done at sites already established as part of the NAWQA program. The data collected in 1997 are presented elsewhere (Francy and others, 2000) and are summarized in this section. The objectives of this study were to

- determine which microbiological sampling and analysis procedures are technically feasible for incorporation into a long-term, national monitoring effort,
- begin to describe the occurrence and distribution of selected microbiological pathogens and their indicators in streams and aquifers,
- relate the presence of microorganisms to human and natural factors that may affect the sanitary quality of water, and
- provide baseline microbiological data for future long-term monitoring of water resources.

Samples were collected for microbiological analysis from three to four stream sites in each of five NAWQA study units. Each sample was collected to provide data from a range of flow conditions, with 6 to

10 samples collected from each stream site from March through September 1997. Sites were selected to assess concentrations of microorganisms at locations indicative of nonpoint sources, at reference sites (NAWQA indicator sites), or at locations near source waters for public utilities (NAWQA integrator sites).

Ground-water samples were collected for microbiological analysis at existing single-family, community, or noncommunity wells in three study units as part of NAWQA subunit surveys and at USGS-installed monitoring wells in one study unit as part of a land-use study. The goal of sampling at ground-water sites was to provide a regional summary of concentrations of microorganisms in settings similar to those used for sources of drinking water.

Streamwater and ground-water samples were analyzed for total coliforms, *E. coli*, *C. perfringens*, and coliphage. Total coliforms were found in 99 percent, *E. coli* in 97 percent, and *C. perfringens* in 73 percent of streamwater samples analyzed for each bacterium. Total coliforms were found in 20 percent, *E. coli* in less than 1 percent, and *C. perfringens* in none of the ground-water samples analyzed for each bacterium. Although coliphage analyses were done on many of the samples, contamination in the laboratory and problems in discerning discrete plaques precluded quantification.

In assessing the bacteriological quality of streamwater, monitoring for *E. coli* and *C. perfringens* provided useful information on the distributions of microorganisms in natural waters. Because concentration distributions of total coliforms were similar to those of *E. coli*, the two indicators were moderately correlated; and because total coliforms are ubiquitous in the environment, only monitoring for *E. coli* is needed to assess fecal contamination in streamwater. Monitoring for *C. perfringens* may provide information on the presence and survival of stress-resistant organisms, such as *Cryptosporidium* or *Giardia*; however, this correlation needs to be further investigated.

In assessing the bacteriological quality of ground water, monitoring for total coliforms provided useful information. All total-coliform colonies should be tested to determine whether they are *E. coli*, which is an indicator of fecal contamination. In this study, because higher percentages of detections of total coliforms were found by use of two alternative methods than were found for the most widely used method, further work is needed to determine the best method for recovery of these fecal-indicator bacteria from

ground water. *Clostridium perfringens* was not detected in any of the ground-water samples and may be present in numbers too low to routinely detect in ground-water samples.

Problems with contamination of samples in the laboratory precluded quantification of coliphage in ground water and streamwater. Quality-control samples for coliphage analysis are used in many laboratories to estimate negative bias (positive-control samples) because the procedures to detect coliphage are not well established and most investigators are concerned with low coliphage recoveries. This study, however, showed that contamination of samples in the analytical process is highly probable and that both positive and negative controls need to be included whenever samples are analyzed.

Adequate assessment of the human and natural factors that may affect the sanitary quality of water requires a greater diversity of sites and more detailed information than that obtained during this study. For example, streams should be selected to include a wide range of climatic conditions to evaluate the effect of mean annual temperature on concentrations of microbiological indicators. Land use, however, was diverse enough to have a significant and discernible effect on concentrations of bacterial indicators in streamwater. Other factors related to land use that could be further investigated include information on soil-drainage properties, number and types of point sources, detailed information on livestock densities and waste amounts, and water-management strategies in the basin.

For ground-water samples, a diversity of lithologies, aquifer types, and land-use categories among study units are needed to adequately assess the relation between detections of microbiological indicators and other factors. Other factors not investigated in this study and worth investigating in future studies include population densities in unsewered areas, soil-drainage properties, and solid-phase chemistry of sediment cores. Increasing potential for nitrate to enter ground water was found by NAWQA land-use studies in areas with well-drained and permeable soils that are underlain by sand and gravel or karst, conditions that enable rapid downward movement of water (U.S. Geological Survey, 1999) and perhaps microorganisms. In another study, a relation was found between the degree of iron oxide coating on sand grains under saturated flow conditions and sorption of microorganisms (Ryan and others 1999); perhaps other solid-phase chemical properties of sediment cores are equally important.

In this study, the presence of a septic system on the property and well depth were found to be related to detections of total coliforms in ground water, although these relations were not statistically significant. In other studies, the presence of a septic field near a well or seepage of sewage was implicated in more incidences of well contamination (DiNovo and Jaffe, 1984; Yates and others, 1985; Yates and Yates, 1988) and incidences of outbreaks of waterborne disease (Craun and Calderon, 1996) than any other known source. Information on the proximity of wells to septic tanks and feedlots and on the direction of ground-water flow from septic tanks and feedlots may help identify which wells are more vulnerable than others to contamination.

Because of the high number of nondetections of total coliforms in ground-water samples (80 percent), a larger data set may provide greater insight into factors that affect detections of microbiological indicators in ground water. Future studies may focus on a variety of well categories including single-family domestic wells, small community or noncommunity wells, and public-supply wells. This focus on water supplies would help determine the extent to which current regulations are effective in the assessment of ground-water quality and whether modifications to current regulations are needed to adequately protect public health.

## **Goals and objectives of microbiological monitoring in a National Water-Quality Assessment Program**

The goal of microbiological monitoring within the NAWQA Program would be to continue the focus of NAWQA Cycle I objectives; that is, to assess the occurrence, distribution, and trends of pathogenic organisms and indicators in surface and ground waters and to relate the patterns discerned to factors that help explain them. In addition, microbiological sampling and objectives will fit the anticipated goal of Cycle II and include enhanced efforts towards understanding the processes that control microbiological water quality. Six objectives are identified to achieve these goals.

### **Objective 1—Identify the human and natural factors associated with occurrence of pathogens and indicators from fecal sources**

A variety of point and nonpoint sources including sewage-treatment plants; septic tanks; overflows from sanitary, combined, and storm sewers; feedlots; animal-production facilities; pasture lands; and rangelands continuously or intermittently contribute fecal waste to streams and ground water. Three additional potential sources of fecal contamination to ground water include induced infiltration of surface water to ground water by pumping wells; land disposal of biosolids; and injection or infiltration of reclaimed water through artificial recharge. Contamination from fecal sources is driven by hydrologic and hydrometeorological factors such as rainfall, which results in runoff to streams or infiltration to ground water (DeWalle and others, 1980; Gerba and Bitton, 1984; Snowdon and Cliver, 1989).

Past studies provide some insight into the factors that affect microbiological water quality of streams. Contamination is more likely where animal or human populations are highest (Brooks and Cech, 1979; Howell and others, 1995). Using results from the 1997 pilot NAWQA study at 17 stream sites in various hydroclimatic regions of the Nation, Francy and others (2000) showed that elevated concentrations of fecal-indicator organisms were more likely to be found in streams draining urban and agricultural land uses than in streams draining forested and mixed land uses. In another study, higher concentrations of *Giardia* and *Cryptosporidium* were found in waters receiving industrial and sewage effluents than in waters not receiving these wastes and (or) having more extensive watershed protection practices (LeChevallier and others, 1991). The presence of livestock (Sherer and others, 1988) and recreational activities (Varness and others, 1978) was also shown to be positively associated with elevated concentrations of fecal-indicator bacteria.

In the 1997 pilot NAWQA study (Francy and others, 2000), well depth and the presence of a septic system on the residential lot appeared to be the major factors associated with detection of total coliforms in ground water sampled from domestic and small public-supply wells. Total coliforms may indicate fecal contamination but can also be associated with non-fecal contamination of the well casing or distribution system. Similar results are reported by Sandhu and others (1979) for wells near septic systems in South

Carolina, by DeWalle and others (1980) for wells near septic systems in Washington, by Vaughn and others (1983) for wells near septic tank leach fields in New York, and by Brooks and Cech (1979) for wells near septic tanks in Texas.

Examining the relations of water-quality constituents to the presence of fecal indicators and pathogens may provide some insight into the sources of contamination. In the 1997 pilot study, statistically significant correlations were found between concentrations of bacterial indicators and chemical or physical water-quality characteristics that may be associated with sewage in streamwater samples: dissolved organic carbon, ammonia and organic nitrogen, total phosphorus, nitrate and nitrite, chloride, suspended sediment, and specific conductance (Francy and others, 2000). In a study of ground-water quality (Bickford and others, 1996), statistically significant relations were found between concentrations of some bacterial indicators and dissolved organic carbon, dissolved solids, ammonia plus organic nitrogen, and chloride.

NAWQA indicator and integrator stream sites are chosen to address the human and natural factors that affect water quality. These same factors are likely to be related to the microbiological water quality; in particular, land use, population density, animal density, water and wastewater management strategies, physiography and climate, geology, and soil-drainage properties.

Indicator sites provide the best opportunity for relating microbiological contamination of streamwater to specific land use and natural factors. Sampling at indicator sites will provide an understanding of how concentration changes as streamflow changes. Sampling at fixed sites will allow comparison of sites with different magnitudes of sources. Comparisons for a specific hydrologic condition, such as low streamflows, can be accomplished through spatial synoptic studies of contamination across a gradient of source magnitudes. For questions dealing directly with animal waste, indicator sites draining watersheds with high densities of domestic animals or animal-production facilities would also be appropriate.

For ground water, all three types of studies will address Objective 1. Comparisons of microbiological contamination between land-use wells or subunit survey wells differing in amounts of nearby sources will best indicate the effect of source magnitude on contamination. For example, a gradient in magnitude of sources is expected in urban areas compared to agri-

cultural areas and will be detected in results of land-use studies. Flowpath investigations will provide the understanding of why these broader patterns occur, based on detailed study of the transport mechanisms through a variety of flow systems with different fracturing, soil, or geologic characteristics. Flowpath studies could be designed to specifically address microbial sources, transport in the subsurface, and effects on receptors (in this case, drinking-water wells).

Questions to be answered include the following: Which factors, such as human and animal population density and presence of sewage-treatment plants or septic systems, affect concentrations of fecal indicators and pathogens in streamwater and ground water? At what levels of upstream human activity and land use do these become important for downstream water supplies? How do natural factors like geology, soil texture and drainage, slope, streamflow, and infiltration relate to the presence or absence of microbiological contaminants in streamwater and ground water? Can water-management strategies take advantage of timing of contamination to avoid microbiological contamination of streams? Which chemical or physical water-quality characteristics best correlate to the presence of microbial pathogens and indicators? Which chemical, physical, or biological surrogates (diatoms, algae, turbidity, temperature) occur coevally with pathogen breakthrough along a flow path? How does solid-phase chemistry of sediments affect transport of microorganisms along a flow path?

## **Objective 2—Characterize the quality of streamwater and ground water in relation to beneficial uses for human consumption and recreation**

Microbial contamination of fresh and estuarine surface water can directly affect public health because of the use of these resources for drinking water, recreation, and shellfish propagation. The microbiological quality of all fresh waters is a concern because of the Nation's current and future dependence on these sources for domestic and public supply. On a national scale, data are insufficient to characterize the microbiological quality of our Nation's waters that serve these beneficial uses. In addition, there is a national need for information on the microbiological quality of source waters and factors related to source-water vulnerability to microbiological contamination. This information is needed to develop water- and wastewater-treatment



regulations and develop proactive policies for the protection of watersheds.

For surface waters, USEPA will continue to need information from occurrence studies of various fecal-indicator organisms, viruses, and protozoans to develop treatment requirements for water entering water-treatment plants. For example, the ICR (U.S. Environmental Protection Agency, 1996b) investigates the balance between the production of disinfection byproducts and the degree of chlorination and filtration needed to eliminate pathogens such as *Cryptosporidium* and *Giardia*. The occurrence and distribution of fecal organisms in source waters can help USEPA and water suppliers assess the need for increased filtration and disinfection requirements for surface-source waters. Local and state governments and private water suppliers, whose water-treatment plants are subject to monitoring and treatment regulations under the SWTR (U.S. Environmental Protection Agency 1989a), need to know more about environmental factors such as land use and hydrologic factors that affect the microbiological quality of source waters.

The USEPA needs more information on the occurrence of viruses and fecal indicators in ground water in support of the GWR, which is under development and which may mandate disinfection for certain types of ground-water supplies (Berger, 1993; Macler, 1996; Macler and Pontius, 1997). Diagnostic measures (tools) that assess the vulnerability of ground water to viral contamination are also a need of the USEPA (Macler, 1996; Macler and Pontius, 1997). Dutka and others (1990) found coliphage in ground waters even in the absence of indicator bacteria, suggesting that bacteria may be insufficient indicators for the presence of viruses. Factors such as aquifer lithology or texture may affect viral survival and transport (Robertson and Edberg, 1997). Because viruses are negatively charged at neutral pH and less negatively charged at lower pH values (Yates and Yates, 1988), they travel farther in sandy or fractured materials with a neutral or alkaline pH than in silty, clayey, or unfractured and relatively more acidic materials (Gannon and others, 1991; Malard and others, 1994). Bacteria and protozoans are larger and in most environments are more readily attenuated (subject to natural disinfection) by soils and aquifer materials than are viruses (Abbaszadegan and others, 1998; Berger, 1993; Yates, 1995; Robertson and Edberg, 1997). In fact, the presence of protozoan pathogens in ground water is thought to indicate the influence of surface water (Macler, 1996).

Another important water use is recreation. There are more outbreaks of waterborne disease from fecal-contaminated recreational water than from fecal-contaminated drinking water, although fewer individuals per outbreak are affected by the former (Herwaldt and others, 1991). Yet, some states continue to rely on less useful indicators of recreational quality, total or fecal coliform bacteria, rather than the preferred indicators, *E. coli* and enterococci (Natural Resources Defense Council, 1998). Although more monitoring programs are being developed for public bathing beaches (U.S. Environmental Protection Agency, 1999d), few programs are being developed for recreational waters, like scenic and recreational rivers, where there are no beaches. More monitoring and the use of recommended indicators are needed for most types of recreational waters (U.S. Environmental Protection Agency, 1999a).

Some NAWQA integrator and indicator sites are drinking-water sources or recreational streams; these sites provide the best locations in the NAWQA program for addressing Objective 2. Relating contamination at these sites to gradients of upstream characteristics will provide information valuable for filling many of the above-mentioned knowledge gaps. Currently, the frequency of sampling at streamwater sites is not high enough at locations used for body-contact recreation—specially designed studies would be needed if this objective is to be met. For ground waters, sampling of subunit survey wells, most of which are domestic or public supply wells, provide the best means for evaluating factors affecting waters used for human consumption.

Questions to answer at a national scale include the following: What microbiological pathogens and indicators are present at levels of concern in source waters and recreational waters? What is the health risk of swimming in our Nation's recreational streams and drinking water from untreated or partially treated public and domestic ground-water supplies? How do land use and hydrologic factors affect the potability of surface-water supplies? What factors affect the vulnerability of ground-water supplies to viral contamination?

### **Objective 3—Determine within-year distribution of microbiological water quality and how it relates to water management, climate, and season**

Survival times of pathogens and their indicators have been shown to be seasonally and climatically related to temperature (Flint, 1987), survival being greater at colder water temperatures or at water temperatures near that of the human or animal hosts. Temperature is described as probably the most important factor influencing virus inactivation in the environment (Yates and Yates, 1988). Studies in the tropics have suggested that organisms like *E. coli*, which is the preferred indicator for fresh temperate waters, may persist for a much longer time or possibly replicate in fresh tropical waters, reducing its usefulness as an indicator of fecal contamination (Hernandez-Delgado and others, 1991).

The seasonality of most water-based recreation in temperate climates has resulted in regulations in many states that require disinfection of wastewater discharges to streams only from late spring to early fall but not outside those times. Although this water-management strategy protects swimmers, boaters, and other recreationists during times when exposure is greatest, it may conflict with the need to protect source-water quality all year long in rivers and lakes that serve multiple uses of recreation, water supply, and waste assimilation.

Seasonal patterns of rainfall and snowmelt not only can affect surface-water quality through transport of microbial contaminants to streams and lakes by way of runoff, but also can affect ground-water quality through infiltration during wet seasons or floods. Urbanized streams have been shown to contain elevated concentrations of fecal bacteria during periods of rainfall and runoff (Novotony and others, 1985) from street refuse, animal waste, combined-sewer overflows, and sewage-treatment bypasses. Rainfall events in less populated environments may also cause increased fecal contamination in streams from accumulated fecal debris in watersheds, even in arid environments (Tunnick and Brickler, 1984). Schulmeyer (1995) showed that above-normal precipitation and runoff to a river increased the possibility of microbial contamination of its alluvial aquifer from induced infiltration. Heavy rains were found to influence the intermittent contamination of domestic wells with fecal indicators through rapid infiltration (DeWalle and others, 1980).

Seasonal and climatic patterns of pathogens and indicators can be determined at any of the surface or ground-water sites established in the NAWQA program. To best relate these to causative factors, however, surface water should be sampled at indicator sites and ground water at land-use sites, the results of which can be compared across the United States. Effects of infiltration or other seasonal factors can also be measured in wells that are influenced by surface waters or surface processes and at wells along a flowpath.

Questions to be answered include the following: Do climatic factors such as mean annual temperature and mean annual precipitation differentially affect the concentrations of pathogens and indicators in stream-water and ground water in similar land-use settings? Are concentrations of pathogens and indicators higher in warm seasons compared to cool seasons? Does the lack of year-round disinfection of sewage discharges in some areas negatively affect the microbiological quality for downstream water users? Do setback distances for wells (the distance to the nearest potential source of fecal contamination) need to be greater in cold climates than in warm climates in order to reduce viral contamination to acceptable levels?

### **Objective 4—Describe the occurrence and distribution of *E. coli*, enterococci, and other indicators of fecal contamination in relation to the occurrence and distribution of bacterial, viral, and protozoan pathogens**

Because routine monitoring for pathogens is often impractical, fecal-indicator bacteria are typically used as a measure of microbiological water quality. Fecal-indicator bacteria indicate the presence of fecal contamination and the possible presence of pathogenic microorganisms. The presence of *E. coli* in water is direct evidence of the presence of human or other warm-blooded-animal waste (Dufour, 1984); however, *E. coli* and other bacterial indicators are not considered reliable indicators for all pathogens, especially protozoan and viral pathogens. For example, in some outbreaks of waterborne viral disease when enteric viruses were detected in water, the levels of bacterial indicators were within water-quality standards (Sobsey and others, 1995). In a study of viral transport through a shallow, sandy aquifer, occurrence of viruses was not statistically related with either total or fecal coliforms (Vaughn and others, 1983). In a survey of surface waters used for potable water supplies, no correlation between concentrations of bacterial indica-

tors and either *Cryptosporidium* or *Giardia* was found (Rose and others, 1991).

Other microorganisms have been suggested as indicators of the presence of pathogens. Coliphage, a virus that infects coliform bacteria, has been suggested as a possible indicator for human enteric viruses (Sobsey, 1995; International Association of Water Pollution Research and Control, 1991) because coliphage is more morphologically similar to enteric viruses than bacterial indicators of fecal contamination. *Clostridium perfringens*, a spore-forming bacterium, has been suggested as an indicator for protozoan and viral pathogens because of its resistance to environmental extremes and disinfection. *Clostridium perfringens* was found to correlate with human enteric viruses, *Giardia*, and *Cryptosporidium* in streamwater samples (Payment and Franco, 1993).

A national monitoring strategy could incorporate these newly suggested indicators as part of assessment activities and evaluate their usefulness as indicators, as called for by Rose and others (1999). For example, one criterion for a good indicator is that the indicator be absent from microbiologically safe water and always be present with a source of fecal contamination. This criterion can be tested on a national scale by determining the occurrence and distribution of these viral and protozoan indicators in relation to the presence of human and animal waste in streamwater and ground water in a variety of environmental and hydroclimatic settings.

NAWQA indicator and integrator surface-waters sites are suitable for relating the occurrence of fecal indicators to the presence of pathogenic microorganisms. Similarly, land-use study, subunit survey, and flowpath study wells are appropriate for addressing this objective in ground-water studies. Particular attention can be directed towards waters that are source waters for public supplies. Source waters would include a subset of the integrator sites for surface waters and the subunit survey wells for ground waters.

Questions to be answered include the following: How well does coliphage correlate with the presence of human and other animal waste and enteric viruses? How well does *C. perfringens* correlate with human and other animal waste and protozoan or viral pathogens? Are there threshold concentrations for bacterial and viral indicators in streamwater and ground water below which pathogens are not present and above which pathogens are detected? If so, can these threshold concentrations be used to develop risk-based

human-health standards for viral and protozoan indicators in source waters and recreational waters? Can some of the more easily determined microbial indicators be used as surrogates for pathogens that are more difficult, time consuming, and expensive to identify? Are *E. coli*, enterococci, or coliphage suitable indicators for the transport of enteric viruses in subsurface materials with different geochemical properties?

### **Objective 5—Establish a long-term monitoring effort to identify trends and changes in microbiological water quality**

Over the past two decades, repeated evaluations of available water-quality information showed that the United States needed long-term national water-quality assessment to support effective water policy and management (Gilliom and others, 1995). The NAWQA program was designed to fill this need and has addressed the chemical quality of ground water and the chemical and aquatic biological or ecological quality of surface water. There still remains a great need to describe the status of and trends in microbiological water quality and associated human-health issues. With increasing populations and urbanization, this is a critical time to begin a long-term microbiological monitoring program.

There is a lack of monitoring data collected in a consistent manner on a national scale to identify long-term trends in microbiological water quality. Currently, microbiological monitoring is accomplished through a myriad of state and local monitoring programs. These distributed state and local programs often use a variety of collection methods, analysis methods, and analytical laboratories that make interpretation of nationally aggregated data sets difficult at best.

Trends can and should be determined at all of the types of sites sampled by NAWQA. Trends at integrator and subunit survey wells will provide information on changes in source waters, and therefore directly answer questions about comparisons to treated waters and public health. Trends at indicator sites, and land-use and flowpath wells, can be directly related to watershed and contributing-area characteristics. These trends allow answers to the “Why is quality changing?” questions.

Questions to answer include the following: What long-term trends and changes in microbiological quality are found in our Nation’s waters? How do changes in land use, point sources, and other factors

affect microbiological water quality? Do changes in watershed-management practices affect microbiological water quality over the long term? What data could currently contribute to a national microbial assessment, and are there locations for which little or no data exist?

### **Objective 6—Field test new and (or) improved microbiological methods to determine their applicability for incorporation into national, state, and local monitoring efforts**

Total and fecal-coliform bacteria, *E. coli*, and enterococci are being used routinely for monitoring purposes because methods of analysis are straightforward, quantitative, and inexpensive. Conversely, pathogens are rarely used on a routine basis for monitoring because analytical methods are complex, qualitative to semiquantitative, expensive, and very time consuming.

New methods for detecting and quantifying microbiological pathogens and indicators in water are developed and laboratory tested by the USEPA and others in the research community. Currently, new methods are available for fecal-indicator bacteria, somatic and F-specific coliphage, *C. perfringens*, *Cryptosporidium*, *Giardia*, and enteric viruses. The data collected by use of these methods can be used to characterize, in a nationally consistent manner, the performance of new methods in relation to contaminant sources and background conditions.

Each method must be field validated before acceptance as a USEPA- or USGS-approved method for monitoring the sanitary quality of water. The framework of the NAWQA program can be used to test these methods and to ensure that the method is acceptable for use in a large-scale field program. To be acceptable, methods should be widely accepted, have known levels of bias and variability, and be relatively easy to apply in field operations or have holding times long enough to allow shipping to a central laboratory for analysis.

Locations to address this objective would include a subset of sites across the range of types of NAWQA sampling sites. Testing at integrator sites and subunit survey wells would ensure that methods work at levels typical of source waters and treated supplies. Testing at indicator sites and land-use wells would be appropriate for locations strongly influenced by single factors, such as human or other animal wastes.

Questions to answer include the following: Is there a technically feasible method for coliphage analysis that includes adequate quality-assurance and quality-control procedures? Can the reverse-transcriptase polymerase-chain reaction (RT-PCR) method for enteric viruses be used to detect the presence of enteric viruses in all types of natural waters? Does the method for detection of *Cryptosporidium* in water have consistent levels of bias and variability in a variety of natural waters? Are new methods for fecal-indicator bacteria superior in recovery of target organisms to the currently used methods?

### **Recommendations for data collection and analysis**

A national microbiological monitoring program has been designed to meet the objectives listed above. This program, outlined in the following sections of this report, addresses retrospective analysis, sampling strategies, collection and analysis methods, documentation and data management, and quality assurance and quality control. Microbiological monitoring should be considered for inclusion in surface- and ground-water quality studies in all NAWQA study units.

#### **Retrospective analysis**

A retrospective analysis of available state and local microbiological monitoring data should be included in the study-unit investigation. This review and analysis of existing data provides a historical perspective on microbiological water quality in the study unit and evaluates priorities for study design (Gilliom and others, 1995). These data may be used to differentiate between microbiological problems in ground water from those associated with surface water.

#### **Sampling strategies**

Sampling activities for stream sites and ground-water sites are summarized in table 1 and described below. Sampling strategies involve the selection of station locations and of microbiological indicators and pathogens to target for monitoring.

The network of fixed stream sites established by each NAWQA study unit in Cycle I and described

**Table 1.** Sampling activities for characterizing microbiological water quality

Design components	Sampling strategies	Sampling frequency (annual)	Targeted characteristics
<b>Streamwater for the occurrence and distribution assessment</b>			
Water-column studies	Basic Fixed Sites: 3-5 Integrator Sites; 4-8 Indicator Sites	16-18	<i>Escherichia coli</i> or enterococci <i>Clostridium perfringens</i>
	Intensive Fixed Sites: 1-2 Integrator Sites and 1-4 Indicator Sites		
	Basic fixed-interval and extreme-flow sampling for 2 years		
Water-column studies	Select 2 Fixed Sites	9	Somatic and F-specific coliphage <i>Cryptosporidium</i> <i>Giardia</i> Enteric viruses New methods for bacterial indicators
	Semimonthly and extreme-flow sampling for 2 years		
Water-column synoptic	Variable and issue specific	Variable	Variable, but usually a few microorganisms in each study
<b>Ground-water studies</b>			
Subunit survey Land-use survey	All wells in each survey (typically 20-30 wells)	Once	Total coliforms <i>Escherichia coli</i> Enterococci Somatic and F-specific coliphage Testing of new methods
Repeat sampling	Five wells in each survey	4	Total coliforms <i>Escherichia coli</i> Enterococci Somatic and F-specific coliphage Enteric viruses
Flowpath study	Special studies that examine microbial transport	Variable	Variable, but usually a few microorganisms in each study

in Gilliom and others (1995) can be used to form a core of fixed sites to characterize microbiological water quality and address most objectives described in the previous section. A subunit of sites, however, must be established to focus on different priority items related to public health microbiology. For streamwater, these may include sites that are sources of drinking water for public utilities, sites that are used by swimmers and boaters, and sites that are impacted by non-point sources of fecal contamination. For ground water, emphasis should be placed in selecting study-unit survey wells that are drinking-water supplies serving a range of populations. More emphasis should also be placed on ground-water flowpath studies that allow investigators to trace a microbial contaminant from its source to a receptor well.

The following sampling strategy is recommended at streamwater sites:

- Sample all basic and intensive fixed sites for bacterial indicators—*E. coli* or enterococci (with enterococci being preferred in estuarine waters) and *C. perfringens*—approximately 16-18 times per year during the 2-year sampling period of the HIP. If state guidelines dictate the use of other bacterial indicators for monitoring recreational waters (such as fecal coliforms), include them in the streamwater-monitoring strategy.
- Sample for viral and protozoan indicators and pathogens at a lower frequency and at fewer streamwater sites than for bacterial indicators. These include somatic and F-specific coliphage, *Cryptosporidium*, *Giardia*, and enteric viruses.

Stream sampling for viral and protozoan indicators and pathogens is recommended to be done semimonthly and during three high streamflows for a total of nine samples per year at each site. This sampling frequency may also be used for testing new analytical methods for bacterial indicators.

- Select two fixed sites for sampling for viral and protozoan indicators and pathogens and testing new analytical methods (for example, one integrator and one indicator site). Select those sites that are source waters for public water supplies and (or) represent a gradient of potential sources of fecal contamination.
- Do a synoptic streamwater study to address a local issue or problem that has transfer value to regional or national issues.

For ground-water sampling, subunit surveys are suited for addressing most objectives of microbiological sampling. Subunit surveys include examination of the types of ground waters that are source waters for domestic and public-supply wells. Subunit surveys typically contain 20 to 30 wells. Land-use studies would also be used when direct relations with associative factors are to be determined, especially when samples are taken from wells that represent the types of ground waters found in domestic wells.

We recommend the following activities for ground-water sampling at subunit survey and land-use study wells:

- Add microbiological monitoring to all wells in land-use studies and subunit surveys.
- Although well locations in land-use studies and subunit surveys are randomly selected, some consideration should be given to stratifying the sampling design. For example, select ground-water wells for a subunit survey to assess different well depths and distances to sources of fecal contamination, such as feedlots or septic systems.
- To aid in the evaluation of the effectiveness of current regulations, select wells for a subunit survey that include community wells and transient and nontransient noncommunity wells, as well as domestic wells. Although information on domestic wells provides little benefit to USEPA, this information will be of value to state and local agencies and water providers.
- Sample each subunit or land-use study well once for bacterial and viral indicators—total coliforms, *E. coli* (for total-coliform positive plates), entero-

cocci, and somatic and F-specific coliphage.

Target these studies to the temporal period during which detection is most likely. Testing of new methods for bacterial and viral indicators may also be completed on these wells.

- Save any positive *E. coli* isolates for serotyping to identify any pathogenic *E. coli* strains.
- Select five wells that were positive for one or more of the bacterial and viral indicators for additional sampling and analyses (repeat sampling). Because so little is known about the recurrence of pathogens and indicators in ground-water samples, include repeat sampling at these five wells on a quarterly/seasonal basis for 1 year. This repetition is especially important for wells that are completed in unconfined, highly permeable aquifers wherein seasonal factors or the influence from surface waters needs to be considered.
- In addition to the bacterial and viral indicators listed above, include enteric virus analysis as part of repeat sampling protocol.

Flowpath studies are not appropriate for routine microbial monitoring but should be included as special studies that examine microbial transport in the subsurface. Flowpath studies that emphasize microbial transport are different than the transect design and sampling strategy described in Gilliom and others (1995) for NAWQA Cycle I. Instead of a well transect along a flowpath beginning or terminating at a stream (Cycle I), flowpath studies should be designed to specifically address microbial sources, transport in the subsurface, and effects on receptors. Flowpath studies should be designed to address local issues and geological conditions.

- The receptor may be a public-supply well, a well field infiltrated with reclaimed wastewater, or a community well receiving streamwater by induced infiltration. The source may be a septic system, recharge water, contaminated streamwater, or nonpoint sources from a feedlot.
- Monitor for microbiological indicators and pathogens that address local concerns and issues.
- Collect ancillary data on solid-phase chemistry and particle size of sediment cores and ground-water chemistry that may be used to transfer results to studies in other areas.

### **Sample collection and analysis methods**

Methods for collection and analysis of microbiological pathogens and indicators are continuously being

developed or modified to reflect recent technological advances. The methods described in this report are the current methods approved or recommended by USEPA. As new methods are developed, tested, and approved, they can be incorporated into the NAWQA program, as appropriate.

Sterile conditions must be maintained during collection, preservation, storage, and analysis of microbiological samples. Complete discussions of equipment cleaning and sterilizing procedures are given in Myers and Sylvester (1997) and in U.S. Environmental Protection Agency (1978). Samples for analysis of total coliforms, *E. coli*, and enterococci are processed on site or at a nearby laboratory by field personnel. Samples for analysis of *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and enteric viruses are collected by field personnel and shipped to a central laboratory for analysis.

**Sampling procedures.** The reader is referred to Myers and Sylvester (1997) and U.S. Environmental Protection Agency (1996c) for complete discussions of sampling procedures, parts of which are included in this plan.

**Streamwater sample collection.** When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The sample volumes needed for each analytical method are listed in table 2.

The standard samplers used in the USGS can be used to collect streamwater samples for bacterial and viral indicators, *Cryptosporidium*, and *Giardia* providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

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

samples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

- Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).
- Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for *E. coli* and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for *C. perfringens* and coliphage analysis.

For *Cryptosporidium* and *Giardia* analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.

- Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not dechlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
- Composite the sample in a 10-L cubitainer that is presterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for *Cryptosporidium* analysis. The sample does not have to be kept on ice during transport.

At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RT-PCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the

**Table 2.** Analysis methods and sample volumes for streamwater and ground-water studies

[Abbreviations: L, liter; mL, milliliter; APHA, American Public Health Association and others; USEPA, U.S. Environmental Protection Agency]

Microbiological pathogen or indicator	Sample volume	Analytical method	Reference
<b>Streamwater sites</b>			
Bacterial indicators	0.01 to 100 mL	Membrane filtration	
<i>Escherichia coli</i>		mTEC method	USEPA, 1986b
Enterococci		mEI method	USEPA, 1997
<i>Clostridium perfringens</i>	10 to 100 mL	mCP method	USEPA, 1996c
Somatic and F-specific coliphage	100 mL	Single-agar layer method with $\beta$ -galactosidase induction	Ijzerman and Hagedorn, 1992
<i>Cryptosporidium</i> and <i>Giardia</i>	10 L	Method 1623—Filtration, immunomagnetic separation, fluorescent antibody staining	USEPA, 1999c
Enteric viruses	100 L	Reverse transcriptase, polymerase chain reaction (RT-PCR) Cell culture	Shay Fout, written commun., USEPA, 1997; USEPA, 1996c
New analytical methods	0.01 to 100 mL	Modified mTEC	Bennett Smith, written commun., USEPA, 1997
<b>Ground-water sites</b>			
Total coliforms and <i>Escherichia coli</i>	200 mL	MI method	Brenner and others, 1993
Pathogenic <i>Escherichia coli</i>	Isolates from MI plates	Serotyping	APHA, 1998, Section 9260 F.
Enterococci	200 mL	mEI method	USEPA, 1997
Coliphage	1 L	Enrichment	USEPA, 1999e
Enteric viruses	2,000 L	Reverse transcriptase, polymerase chain reaction (PCR); cell culture	Shay Fout, written commun., USEPA, 1997; USEPA, 1996c
New analytical methods	200 mL	Colilert method	Idexx Laboratories, Westbrook, Maine

cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb<sup>1</sup> 1MDS filter (Cuno, Meriden, Conn.). The sampling equipment is obtained from the analyzing laboratory; for example, the USGS Ohio District Laboratory has modified the sampling apparatus (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) into a self-contained box with easy-to-use control valves. The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent

<sup>1</sup>Use of trade, brand, or firm names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

to a central laboratory for virus elution, concentration, and detection.

**Ground-water sample collection.** Collecting ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.

- Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
- In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
- Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps.

Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible. Because this is operationally prohibitive for private domestic wells, a tap that yields water directly from the well and



before entering the holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.

- Document the stage of the distribution system from which water was collected and details about the distribution system, including the type of tank and condition of the tank and pipes. In addition, if the well can easily be opened for inspection, document the condition of the well, including the sanitary seal (if any) and the amount of debris in the well. Any information on the location of the well, including proximity to septic systems or feedlots, should also be documented in the field at the time of sampling.

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

Because ground water is less prone to microbiological contamination than surface water, larger volumes of ground water are needed than of surface water.

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

**Sample preservation and storage.** Holding times for samples before processing are 6 hours for total coliforms, *E. coli*, and enterococci and 24 hours

for *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and the 1MDS filters for enteric viruses by RT-PCR and cell culture.

- After collection, immediately store the sample on ice.
- Be sure to keep the sample out of direct sunlight, because ultraviolet rays kill microorganisms.
- Add sodium thiosulfate to sample bottles for bacterial and viral indicators if the water collected contains residual chlorine. (Samples may have residual chlorine if the sampling site is downstream from a wastewater-treatment plant that chlorinates its effluents). Add ethylene diamine-tetracetic acid to sample bottles when water is suspected to contain trace elements such as copper, nickel, and zinc at concentrations greater than 1 mg/L (Britton and Greeson, 1989, p. 5-6; U.S. Environmental Protection Agency, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). (Sodium thiosulfate or ethylene diaminetetracetic acid are not added to containers for *Cryptosporidium* and *Giardia*).

**Analytical methods.** Details of the analytical methods for microbiological monitoring are beyond the scope of this report, but they are summarized briefly below and in table 2.

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

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

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

Testing of new microbiological monitoring methods and comparing the recoveries of new methods to the USEPA-approved method can be done by use of the NAWQA network. For ground-water samples, for example, one may include a commercially available MPN kit, Colilert (Idexx Laboratories, Westbrook, Maine), for simultaneous detection of total coliforms and *Escherichia coli*. For streamwater sampling, one may include a single-step modified mTEC medium with 5-bromo-6-chloro-3-indolyl $\beta$ -d-glucuronide (Bennett Smith, USEPA, Cincinnati, Ohio, oral commun., 1997); this method was developed to replace the mTEC method. Other new methods can be added to the monitoring program for field testing as they are developed.

**Laboratory analysis.** Samples need to be kept on ice and shipped to a central laboratory for analysis of coliphage, *C. perfringens*, *Cryptosporidium*, *Giardia*, and enteric viruses by the current analytical methods.

The single-agar layer (SAL), direct plating method with induction of  $\beta$ -galactosidase (Ijzerman and Hagedorn, 1992) is recommended for detection of somatic and F-specific coliphage in streamwater samples. In this method, 100-mL sample volumes are mixed with an agar medium, *E. coli* host culture, chemicals that induce the  $\beta$ -galactosidase enzyme, and appropriate antibiotics. The mixtures are poured into four 150- x 15-mm plates and incubated at 35°C. Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is dark blue is visible within each plaque. Viral plaques are easily identified and enumerated by the distinct blue circle. Because of contamination by naturally occurring bacteria in streamwater samples, antibiotic-resistant host-culture strains, *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for somatic and F-specific coliphage, respectively.

Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water. Because the SAL method is impractical for sample volumes above 100 mL, an alternative method should be used for ground-water sample analysis. One example, currently being tested by USEPA, is a two-step enrichment presence-absence method (U.S. Environmental Protection Agency, 1999e).

Samples for enumeration of *C. perfringens* are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c). Standard MF

techniques are used, and the plates are incubated anaerobically for 24 hours at 44.5°C. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as *C. perfringens*. In the laboratory, *C. perfringens* analyses are done on 100-, 30-, and 10-mL volumes of streamwater. In the case of a high-flow or high-turbidity streamwater sample, lower sample volumes may be plated.

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

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

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

For cell-culture analysis, the sample eluate is added to a monolayer of a continuous cell line derived from African green monkey kidney cells (U.S. Environmental Protection Agency, 1996c). Each cell cul-

ture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water.

**Ancillary data.** To adequately assess the human and natural factors that may affect the sanitary quality of water, detailed, accurate ancillary data are needed.

Water-quality data collected as part of the regular NAWQA program may be used to relate concentrations or detections of microorganisms to other water-quality constituents or measurements. These include streamflow, specific conductance, temperature, pH, turbidity, and concentrations of suspended sediment, major ions and metals, organic and inorganic nitrogen and phosphorus, and dissolved and suspended organic carbon.

As part of the regular NAWQA program, environmental, geologic, and land-use data are collected and can be used as factors related to the distribution of microorganisms. For ground-water and streamwater sites, these data should include the following:

- land-use settings (urban, residential, row crop, mining, and so on)
- land cover (urban, agricultural, rangeland, forestland, wetland, barren land)
- population density (number of people per square kilometer)
- geologic setting (bedrock and surficial geology)
- climatic data (average annual temperature and precipitation)
- surficial geology (soil types and soil drainage properties)
- nearby domestic animal types and densities

For ground-water sites, specific information on the well and aquifer, already collected as part of the regular NAWQA program, may aid in determining which factors make a well more vulnerable to microbial contamination and transport:

- lithology
- aquifer type (confined, unconfined, or semi-confined/unknown)
- fracturing (fractured, unfractured, or karstic bedrock)
- age of well
- grouting (grouted or not grouted,
- well-casing material
- depth to top of open interval
- solid-phase chemistry of sediment cores

More detailed information on some of these factors is needed to thoroughly assess the effect on microbiological water quality. For ground water and streamwater, population and farm-animal densities need to be examined together. The number of animal units (1,000 lb of animal) is a more useful measurement of farm-animal density than the number of animals. This is because, for example, one chicken contributes far less waste than one cow does. In addition, countywide animal densities may not be accurate enough. A large-animal facility in one corner of the county a considerable distance from a stream has much less effect on water quality than free-range cattle with access to a stream or confined cattle near a stream. Similarly, human population densities should be obtained from block-group or more detailed census coverages and not from countywide densities. This is especially important in assessing population density associated with a well, because population of the county may be concentrated in a few areas and may or may not affect ground-water quality at the sampling location.

In a microbiological sampling program, some data not already collected as part of the regular NAWQA program may need to be collected to thoroughly assess human and other animal factors that affect microbiological water quality.

- For streamwater, identify watershed-protection strategies in the basin and place sites into categories based on decreasing watershed protection: (1) protected watersheds, (2) watershed with limited access, (3) recreational use, (4) agricultural row-crop use, (5) agricultural livestock use, (6) sewage discharge, (7) industrial-urban discharges. A watershed receiving pollution from multiple sources should be given the highest applicable rating (LeChevallier and others, 1991).
- For ground-water sites, identify and collect additional information that may aid in determining the vulnerability of the well to microbial contamination (Abbaszadegan and others, 1998): (1) type of nearest surface-water body (flowing, flowing and standing, or standing), (2) distance to nearest surface-water body, (3) type of nearest sewage source (sewage and agricultural, industrial and natural, none) (4) distance to nearest sewage source, (5) type of nearest agricultural source

(animals, animals and crops, crops, none), and (6) distance to nearest agricultural source.

In addition, because the factors that affect microbiological contamination of a well are often localized, observations obtained by the field or reconnaissance crew are critical to evaluating the well.

- Document the condition of the distribution system and the condition of the well, including the sanitary seal (if any) and the amount of debris in the well.
- Document the location of the well, including proximity to septic systems and feedlots and numbers of animals on the lot. Include information on the presumed direction of ground-water flow from a potential sewage source.

### Documentation, data management, and data analysis

Site conditions and field activities are documented on standard USGS surface- or ground-water field-note forms. These include site information, types of samples collected, field measurements, sampling conditions, calibration procedures, and bacterial indicator information and results. Because the analyses for coliphage, *C. perfringens*, *Cryptosporidium*, *Giardia*, and enteric viruses are done by a central laboratory, proper sample chain-of-custody procedures must be followed. Documentation forms and procedures can be obtained by the analytical laboratory.

Microbiological data are stored in the National Water Information System (NWIS) of the USGS. Field personnel are responsible for entering and validating total coliform, *E. coli*, and enterococci data. The analyzing laboratories will provide data on the presence or concentration of coliphage, *C. perfringens*, *Cryptosporidium*, and enteric virus to project personnel for input into their local NWIS data base. A list of the NWIS parameter codes for the microbiological analyses described in this report can be found in table 3.

NAWQA personnel in the study units will gather detailed ancillary data and provide these data to a national synthesis team responsible for data analysis, reports, and journal-article preparation. Data will be aggregated at the national level in a consistent fashion in a data base for use in data analysis and reports. Reports on local conditions will be written by study-unit personnel.

Data analysis for microbiology is beyond the scope of this report. Nevertheless, some generaliza-

tions can be made to aid study units in the preparation of sampling plans and interpretation of data.

Units of measurement for microorganisms are diverse and dependent on the target organism, type of method, and known limitations (table 3). For example, because recoveries of *Cryptosporidium* and *Giardia* are highly variable, percent recovery for each water matrix are determined as well as number of oocysts and cysts. In interpreting data on enteric viruses, one must be aware that the RT-PCR method detects the presence or absence of genetic material from specific target viruses that are infectious or noninfectious; the cell-culture method, conversely, quantifies the number of infectious units but does not identify the specific virus.

Distributions of bacterial concentrations commonly are asymmetrical because of the occurrence of many low concentrations and a few extremely high concentrations in the sample population. The frequency curves of these distributions usually have a long right tail and thus are said to be positively skewed. An arithmetic mean computed from data with a right-skewed distribution is considerably higher than the median. For this reason, the logarithmic transformation is usually necessary to convert data with a right skewed distribution to a symmetric distribution resembling the normal distribution.

Assuming that the microbiological data have been normalized through a base 10 logarithmic transformation (have a lognormal distribution), the geometric mean is the best estimate of central tendency. The geometric mean is the antilog of the mean of the  $\log_{10}$  values. The geometric mean and interval are more efficient measures of the median and its confidence interval when the data are truly lognormal. The sample median and its interval are more appropriate and more efficient if the logarithms of data still exhibit skewness and (or) outliers (Helsel and Hirsch, 1992, p. 73).

Boxplots are particularly valuable graphical techniques for display of microbiological data (Helsel and Hirsch, 1992, p. 24-26). These data are best presented by calculating the median, quartiles, and (or) quantiles and extremes of the distribution of sample concentrations.

If the microbiological data follow a lognormal distribution, standard parametric statistical tests (t-tests and analysis of variance) may be used to compare two or more groups of data. Often, however, microbiological data fail to follow a normal distribution, even after a log transformation; in this case, a nonparamet-

ric test must be used. These tests generally involve ranking the data before performing a hypothesis test. A variety of nonparametric methods can be used to compare the medians between two or more groups of microbiological data (Helsel and Hirsch, 1992, p. 118, 142, and 159).

Microbiological data sets are often represented as categorical variables in which the response variable is nominal (no ordering to the categories). An obvious example of categorical data is the presence or absence of enteric viruses in water. Although total coliforms in ground water are quantified by membrane filtration methods, these data are often treated as categorical because of the low number of total coliform detections and the fact that the presence of even one total coliform in a well is an indication of a water-quality

problem. When one variable is continuous and one is categorical, a Kruskal-Wallis test may be used; when both variables are categorical, however, contingency tables can be used to assess association (Helsel and Hirsch, 1992, p. 377).

### Quality assurance and quality control

Quality-assurance/quality-control (QA/QC) practices are established for all phases of data collection and analysis in the field and laboratory, as well as during data entry and validation. An important part of the quality-assurance process is adequate training of field personnel to ensure that procedures are followed correctly and in a consistent manner. The USGS plans to offer special training courses in microbiological

**Table 3.** National Water Information System Parameter Codes

Microorganism	Method	Parameter Code	Unit of measurement
Total coliforms	MI agar	90900	
Total coliforms	Colilert	90908	
<i>Escherichia coli</i>	mTEC agar	31633	
<i>Escherichia coli</i>	Modified mTEC agar	90902	Colonies per 100 milliliters
<i>Escherichia coli</i>	MI agar	90901	
<i>Escherichia coli</i>	Colilert	50468	
Enterococci	mEI agar	90909	
<i>Clostridium perfringens</i>	mCP agar	90915	
Coliphage, <i>E. coli</i> CN-13 host	Single-agar layer	90903	Plaques per 100 milliliter
Coliphage, <i>E. coli</i> F-amp host	Single-agar layer	90904	
Coliphage	Enrichment	Not available	Presence/absence per 3 liters
<i>Cryptosporidium</i> and <i>Giardia</i>	Filtration, immunomagnetic separation, fluorescent antibody	99761	Oocysts or cysts per 10 liters
		99762	Percent recovery
Enterovirus	RT-PCR	99766	
Reovirus	RT-PCR	99767	
Rotavirus	RT-PCR	99768	Presence/absence per 50 liters
Hepatitis-A virus	RT-PCR	99769	
Calicivirus	RT-PCR	99771	
Enteric virus	Cell culture	90910	Most probable number of infectious units per 100 liters

concepts and sampling and analytical techniques, specifically designed for those who will be conducting microbiological studies. Quality control involves the collection of data to estimate the measurable components of quality in the laboratory and field processes (Francy and others, 1998); these are summarized in table 4.

**Quality assurance and quality control in the field.** Quality-assurance and quality-control procedures will be strictly followed during all stages in the field; special attention must be get paid to the possibility of contamination during all collection and processing steps. Myers and Sylvester (1997) and Francy and others (1998) provide complete discussions of

**Table 4.** Field and laboratory quality-control samples for microbiology

Type of sample	Analysis	Frequency of collection	Purpose
<b>Field</b>			
Membrane-filtration (MF) equipment blank	Total coliform, enterococci, <i>Escherichia coli</i>	Every sample	Analytical bias—determine sterility of equipment and supplies
MF procedure blank	Total coliform, enterococci, <i>Escherichia coli</i>	Every fourth sample	Analytical bias—measure the effectiveness of the rinsing technique
Equipment blank	Enteric virus	One sample at beginning of project	Sampling and analytical bias—ensure equipment cleaning and sterilization techniques are adequate
Field blank	Coliphage, <i>Cryptosporidium</i> and <i>Giardia</i> , enteric virus	Periodically, 5 percent of the samples collected	Sampling and analytical bias—ensure equipment cleaning and sterilization techniques are adequate, assess field contamination of samples
Replicate	Total coliform, <i>Escherichia coli</i> , enterococci, <i>Clostridium perfringens</i> , coliphage	Five percent of the samples collected	Sampling and analytical variability
	<i>Cryptosporidium</i> and <i>Giardia</i> , enteric virus	One per year	
Matrix spike	<i>Cryptosporidium</i> and <i>Giardia</i>	Every sample	Recovery efficiency
Field spike (poliovirus)	Enteric viruses using RT-PCR and cell culture methods	From 2 to 5 percent of the samples collected	Sampling and analytical bias
<b>Laboratory</b>			
Reference cultures	Total coliform, enterococci, <i>Escherichia coli</i> , <i>Clostridium perfringens</i>	Each lot of media and reagents	Ensure MF culture media and buffered water are performing adequately
Negative control	Coliphage	Each batch of samples	Determine laboratory contamination
Positive control	Coliphage	Each batch of samples	Ensure method is executed properly
Method specific	<i>Cryptosporidium</i> and <i>Giardia</i> , enteric virus	As described in method protocols	Determine analytical bias

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

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

For bacteria samples, membrane-filtration (MF) equipment and MF procedure blanks are used to estimate analytical bias. Field personnel should do the following:

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

If contamination from a MF equipment or procedure blank is found, results are suspect and are qualified or not reported.

Proper and consistent procedures for counting and identifying target colonies will be followed, as described in Myers and Sylvester (1997).

- After counting, turn the plate 180° and ensure the second count is within 5 percent of the first count. Have a second analyst check calculations of bacterial concentrations in water for errors.

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

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

- For enteric virus analysis, collect one equipment blank after collection of the first sample to ensure that equipment cleaning and sterilization techniques are adequate.
- For coliphage, *Cryptosporidium*, *Giardia*, and enteric virus analyses, collect field blanks periodically. At a minimum, the number of field blanks should equal 5 percent of the total number of samples collected.

Five percent of samples collected for bacterial and viral indicators (total coliforms, *E. coli*, enterococci, *C. perfringens*, and coliphage) should be nested replicate samples to estimate sampling and analytical variability. For streamwater samples, concurrent replicates to estimate sampling variability are collected by alternating subsamples in each vertical between two collection bottles. For ground-water samples, sequential replicates are collected one after another into separate sterile bottles. Concurrent and sequential replicates are then analyzed in duplicate (split replicates) to estimate analytical variability.

- Because of the expense associated with collection and analysis of samples for pathogens (*Cryptosporidium* and enteric viruses), collect only one replicate sample per year at a site wherein detection of pathogens was found in an earlier sample.

To assess analytical bias of the sampling and analytical method, 2 to 5 percent of the samples collected for enteric virus should be field matrix spikes.

- Run all but 10 L of ground water through the 1 MDS filter and collect the remaining 10 L in a carboy. In the laboratory, the poliovirus vaccine will be added to the 10 L and then passed through the same 1MDS filter. Analysis will be done by use of the cell-culture and RT-PCR methods.
- All cell-culture positive samples are serotyped to identify or discount laboratory contamination.

Because of the variability in the performance of Method 1623 for recovery of *Cryptosporidium* and *Giardia*, each sample will be collected in duplicate—one will be a regular sample and the other a matrix spike. The laboratory will add a known quantity of cysts and oocysts to the matrix spike to determine recovery efficiency, as described in USEPA (1999c).

**Quality assurance and quality control in the laboratory.** All production analytical laboratories that provide chemical, radiochemical, and biologi-

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

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

Reference cultures are used by the central laboratory to evaluate the performance of the test procedures, including media and reagents. Pure cultures of *E. coli*, *Enterobacter aerogenes*, and *Streptococcus faecalis* (American Type Culture Collection, Rockville, Md.) are used to ensure that MF culture media and buffered water are performing adequately. A pure culture of *C. perfringens*, isolated from a sewage sample and verified by standard procedures, is used to evaluate the test procedure and each lot of media and reagents.

Because contamination of samples from coliphage during the analytical procedure is highly probable (Francy and others, 2000), a negative control of host and sterile buffered water is run concurrently with each batch of samples. In addition, to ensure that the method is being executed properly, a positive-control sewage sample is run with each batch of samples. A laminar flow safety hood is recommended for processing the samples for coliphage analysis. Alternatively, a separate coliphage room may be established to discourage laboratory contamination during the analytical process. An ultraviolet light is installed and operated for 8 hours every night in the safety hood or coliphage room to reduce contamination.

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

## Summary of objectives and recommendations

Although waterborne disease outbreaks continue to be threat to human health in the United States, current local monitoring program for microbiological pathogens and their indicators are inadequate to identify the human and animal factors associated with microbiological contamination on a national scale. This report was written to help fill this gap by providing a strategy for microbiological assessment of our Nation's streamwater and ground water.

The objectives and the design of the USGS NAWQA program are suited to assess microbiological water quality on a national scale. Monitoring for three groups of microorganisms that affect the public health acceptability of waters in the United States and are transmitted through fecal contamination can be added to the existing NAWQA program: the bacteria, protozoa, and viruses. A pilot microbiological sampling program, which included sampling for bacterial and viral indicators, was conducted in six NAWQA study units in 1997. Information gathered during the pilot provided information on the microorganisms to include and the factors to evaluate in a long-term, national monitoring effort.

On the basis of the NAWQA pilot, information gathered in other USGS studies, and on other published studies, six objectives were identified to include in a national monitoring program.

- Objective 1—Identify the human and natural factors associated with occurrence of pathogens and indicators from fecal sources.
- Objective 2—Characterize the quality of streamwater and ground water in relation to beneficial uses for human consumption and recreation.
- Objective 3—Determine within-year distribution of microbiological water quality and how it relates to water management, climate, and season.
- Objective 4—Describe the occurrence and distribution of *E. coli*, enterococci, and other indicators of fecal contamination in relation to the occurrence and distribution of bacterial, viral, and protozoan pathogens.
- Objective 5—Establish a long-term monitoring effort to identify trends and changes in microbiological water quality.
- Objective 6—Field test new and (or) improved microbiological methods to determine their applicability.



cability for incorporation into national, state, and local monitoring efforts.

The network of fixed stream sites established by each NAWQA study unit in Cycle I can be used to form a core of fixed sites; however, a subunit of sites must be established to focus on different priority items related to public health microbiology.

We propose to sample streamwater sites for the bacterial indicators *E. coli* or enterococci and *C. perfringens*. At lower frequencies, samples would be collected for somatic and F-specific coliphage, *Cryptosporidium*, *Giardia*, enteric viruses, and for testing new analytical techniques for bacterial indicators. NAWQA synoptic streamwater sites may be used to address a local issue or problem, and the microorganisms included would depend on local concerns.

For ground-water studies, NAWQA subunit surveys and land-use studies are suitable for addressing the above objectives. We recommend that each well be sampled for bacterial and viral indicators—total coliforms, *E. coli*, enterococci, and somatic and F-specific coliphage. Identify up to five wells that were positive for one or more of these microorganisms for repeat sampling (four times per year). Repeat sampling includes enteric viruses in addition to bacterial and viral indicators. In addition, each study unit should conduct ground-water flowpath studies that trace a microbial contaminant from its source to a receptor well.

Sampling strategies have been designed and the methods of sampling and analysis were selected to meet the objectives above. For ground-water and streamwater sampling, one can generally follow standard sampling protocols under sterile sampling conditions. Sometimes, however, one must use a specialized protocol required for particular target organisms. The current analytical methods approved or recommended by USEPA will be used for microbiological sample analyses. As new methods are developed, tested, and approved they can be incorporated into the NAWQA microbiological program.

To adequately assess the human and natural factors that may affect the sanitary quality of water, collection of detailed, accurate ancillary data is needed. This includes water-quality, environmental, geologic, and land-use data collected as part of the regular NAWQA program, and any additional data needed to thoroughly assess how human and animal factors affect microbiological water quality.

Established documentation, data management, data analysis and QA/QC procedures are essential to ensure the accuracy and consistency of any monitoring program. Standard field-note forms and chain-of-custody procedures and forms will be used and data will be stored consistently in the USGS data base. Field and laboratory personnel will receive adequate and consistent training. Quality-control samples will be collected to define any bias and variability in the collection or analytical procedures.

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