Environmental Factors and Chemical and Microbiological Water-Quality Constituents Related to the Presence of Enteric Viruses in Ground Water From Small Public Water Supplies in Southeastern Michigan

By Donna S. Francy, Rebecca N. Bushon, Julie Stopar, Emma J. Luzano, and G. Shay Fout

In cooperation with the U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory

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Contents

Figures

Tables

Conversion Factors and Abbreviated Water-Quality Units

Multiply	By	To obtain
micrometer (µm)	0.00003937	inch (in.)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
square centimeter (cm^2)	0.1550	square inch (in^2)
square mile (mi^2)	2.59	square kilometer (km^2)
liter (L)	0.03531	cubic foot (ft^3)
milliliter (mL)	0.06102	cubic inch (in^3)
million gallons per day (Mgal/d)	3,786	cubic meters per day (m^3/d)

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

$$°F=1.8(°C) + 32$$

Abbreviated water-quality units used in this report: Chemical concentrations in water are reported in milligrams per liter (mg/L) and micrograms per liter (µg/L). Both units express the concentration of chemical constituents as weight (milligrams or micrograms) of chemical unit per unit volume (liter) of water. Turbidity is reported in Nephelometric Turbidity Units (NTU). Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of bacteria in water are reported in colonies per 100 milliliters (col/100 mL).

Concentrations of coliphage in water are reported in plaques per 100 milliliters (plaques/100 mL) or presence/absence per 100 milliliters or liter (P/A/100 mL or L), depending on the analytical method used.

Concentrations of viruses in water are reported in presence/absence per 50 liters or 500 liters, depending on the analytical method used (P/A per 50 L or 500 L).

Concentrations that are less than a specified value are reported as <, greater than are reported as >, less than or equal to are reported as ≤, and greater than or equal to are reported as ≥.

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Abstract

A study of small public ground-water-supply wells that produce water from discontinuous sand and gravel aquifers was done from July 1999 through July 2001 in southeastern Michigan. Samples were collected to determine the occurrence of viral pathogens and microbiological indicators of fecal contamination ("indicators"), determine whether indicators are adequate predictors of the presence of enteric viruses, and determine the factors that affect the presence of enteric viruses. Small systems are those that serve less than 3,300 people. Samples were analyzed for specific enteric viruses by reverse transcriptase-polymerase chain reaction (RT-PCR), for culturable viruses by cell culture, and for the indicators total coliforms, *Escherichia coli (E. coli)*, enterococci, and F-specific and somatic coliphage. Ancillary environmental and water-quality data were collected or compiled.

A total of 169 regular samples and 32 replicate pairs were collected from 38 wells. Replicate pairs were samples collected at the same well on the same date. One well was sampled 6 times, 30 wells were sampled five times, 6 wells were sampled twice, and 1 well was sampled once. By use of RT-PCR, enterovirus was found in four wells (10.5 percent) and hepatitis A virus (HAV) in five wells (13.2 percent). In two of these wells, investigators found both enterovirus and HAV, but on different sampling dates. Culturable viruses were found one time in two wells (5.9 percent), and neither of these wells was positive for viruses by use of RT-PCR on any sampling date. If results for all viruses are combined, 9 of the 38 small public-supply wells were positive for enteric viruses (23.7 percent) by either cell culture or RT-PCR.

One or more indicators were found in 18 of 38 wells. Total coliforms, *E. coli*, enterococci, and F-specific and somatic coliphage were found in 34.2, 10.5, 15.8, 5.9, and 5.9 percent, respectively, of the wells tested. In only 3 out of 18 wells were samples positive for an indicator on more than one date at the same well. The co-occurrence of enteric viruses and any indicator was 55.6 percent; five out of the nine virus-positive wells were also found to be positive for an indicator. Two wells with detections of viruses had a detection of total coliforms, one well had a detection of *E. coli,* one of enterococci, and one of F-specific coliphage. On a per sample basis, of 11 samples that were positive for enteric viruses, indicator bacteria co-occurred in only 2 samples, and coliphage were not present in any.

More virus-positive samples were found at sites served by septic systems than those served by sewerlines. Sampling condition (ground water or a mixture of tank and ground water), distance to septic system, type of and distance to nearest surface-water body, well characteristics, or land use were not related to the presence of viruses or indicators. Among continuous water-quality variables, statistically significant relations were found between total coliforms and dissolved organic carbon and between total coliforms and iron. There was a statistically significant relation between chloride concentrations >20 mg/L and detections of total coliforms. Presence of nitrate and nitrite was related to the presence of other indicators (*E. coli*, enterococci, and F-specific and somatic coliphage) or enteric viruses, but not to total coliforms. The data indicated that chloride-to-bromide (Cl:Br) ratios may be useful as a screening tool for total coliforms and enteric viruses but not for *E. coli*, enterococci, and F-specific and somatic coliphage.

This study provides evidence for fecal contamination of ground water from small public-supply wells, at least on an intermittent basis. Collecting data on multiple lines of evidence would be needed to reliably predict the presence of enteric viruses and protect public health. Future data collection toward this end could include repeat sampling several times a year for different indicators, measuring dissolved-organic carbon, nitrate plus nitrite, and (or) chloride concentrations, or determining Cl:Br ratios. The presence of a site served by a septic system is an indication that the well may be more vulnerable to contamination than a site served by a sewerline.

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Introduction

Fecal contamination of ground-water supplies originates from various sources including septic systems; sewerline breaks; overland flow and subsequent seepage from urban, agricultural, and natural areas; leachates from sanitary landfills; and recharge from surface waters or reclaimed wastewater. Septic-system effluent is a major source of microbiological contamination in domestic and public-supply wells; when a waterborne disease outbreak was recognized and a cause identified, dye studies often showed that septic tanks were the contamination source (Beller and others, 1997; Bergeisen and others, 1985; Ground Water Education in Michigan, 1992). In media with limited preferential flow paths, such as sand and gravel aquifers, viral contaminants from fecal sources may be more of a threat to ground water than bacterial or protozoan contaminants associated with fecal contamination. Because of their small size (0.023-0.080 μm) and net surficial charge, viruses have different transport properties in the subsurface than bacteria or protozoa do (Sinton and others, 1997; Schijven and Hassanizadeh, 2000). Moreover, microcosm studies have shown that viruses survive longer than bacteria in ground water (Gerba and Bitton, 1984).

Insufficient monitoring information is available on the occurrence of pathogenic viruses in ground water and the factors that affect the vulnerability of ground water to contamination. The paucity of information is especially apparent for those ground-water supplies classified as small systems—those that serve fewer than 3,300 people. Customers served by undisinfected small public ground-water supplies may be at higher risk for exposure to bacterial and viral contamination than customers served by undisinfected large public supplies because total-coliform monitoring is required less frequently for small systems than for larger systems. For example, small community systems are required to monitor for total coliforms one to three times each month, a frequency that may not be sufficient to detect problems with fecal contamination. Systems serving greater than 3,330 people are required to monitor for total coliforms from 4 to 480 times each month, depending on the size of the population served (U.S. Environmental Protection Agency, 2001).

As defined by U.S. Environmental Protection Agency (USEPA), a public water system is one that serves 25 or more people or has 15 or more service connections and operates at least 60 days per year (U.S. Environmental Protection Agency, 2000a). Public water systems include the following types: community, transient noncommunity, or nontransient noncommunity sources. A community water system serves at least 15 service connections used by year-round residents or regularly services at least 25 year-round residents. Noncommunity water systems do not have year-round residents. Transient noncommunity systems provide water in places such as a gas stations or campgrounds where people do not remain for long periods of time and do not serve at least 25 of the same persons over 6 months per year. Nontransient noncommunity wells supply 25 or more of the same people at least 6 months per year in places other than their residence (for example, schools or hospitals).

Public ground-water supplies (large and small) are located in all 50 States, many tribal lands, and most U.S. territories. The largest numbers of public ground-water supplies are in Wisconsin, Michigan, Pennsylvania, New York, and Minnesota. These five states account for more than 50,698 public ground-water supplies—one-third of the total number in the United States (U.S. Environmental Protection Agency, 2000a). The most important source of ground water in many parts of the upper Midwest is the surficial aquifer system, composed of sand and gravel largely of glacial origin. In the four-state area of Michigan, Wisconsin, Minnesota, and Iowa, the proportion of ground-water supply from the surficial aquifer system is 2.5 times that of the next largest water-producing zone (an aquifer composed of Cambrian-Ordovician bedrock) and 1.5 times that of all other water-producing zones combined (Olcott, 1992).

Because of the importance of ground-water supplies nationwide, and as the result of the growing concern over the contamination of ground water by microorganisms—in particular, viral and bacterial pathogens—the USEPA proposed the Ground Water Rule (GWR) (U.S. Environmental Protection Agency, 2000a). The proposed strategy of the GWR addresses risks through a multiple-barrier approach that relies on five major components:

- periodic sanitary surveys of ground-water systems,
- hydrogeologic assessments to identify wells sensitive to fecal contamination,
- source-water monitoring for systems drawing from contamination-sensitive wells without treatment or with other indications of risk,
- a requirement for correction of appreciable deficiencies and fecal contamination, and
- compliance monitoring to ensure that disinfection treatment is reliably operated where it is used.

Two of the key components of the proposed GWR were investigated during this study—hydrogeologic assessments to identify sensitive wells and source-water monitoring. The use of a hydrogeologic sensitivity assessment is important for identifying which ground waters may be sensitive to fecal contamination. The source-water monitoring component targets the microbiological indicators of fecal contamination—*E. coli*, somatic and F-specific coliphage, and enterococci—which the USEPA proposes to include in this GWR component on the basis of various ground-water and septic-system-effluent studies.

Building on the two components of the proposed GWR described above, the U.S. Geological Survey (USGS), in cooperation with the U.S. Environmental Protection Agency-Office of Research and Development, National Exposure Research Laboratory (USEPA-NERL), in Cincinnati, Ohio, studied the environmental factors and chemical and microbiological

water-quality constituents related to the presence of enteric viruses in ground water in aquifers serving small public water supplies.

Purpose and Scope

This report describes the relations among enteric viruses, microbiological indicators of fecal contamination, and selected chemical-quality constituents in ground-water samples from small public water systems in southeastern Michigan. The systems were sampled from one to six times each and analyzed for specific enteric viruses by reverse transcriptase-polymerase chain reaction (RT-PCR), for culturable viruses by cell culture, and for microbiological indicators of fecal contamination ("indicators")—total coliforms, *Escherichia coli* (*E. coli*), enterococci, and F-specific and somatic coliphage. Sampling was done from July 1999 through July 2001. Chemical-quality characteristics were determined for 31 wells during a single sampling round. Environmental, geologic, and land-use ancillary data were used to examine the hydrogeologic barriers and environmental factors that were related to the presence of viruses in ground water.

Sampling and analysis for indicators and viruses and compilation of ancillary information were designed to aid those developing the proposed and final GWR. Specific objectives were to (1) determine whether enteric viruses are found in waters from small public ground-water supplies, (2) determine whether enteric viruses are found in small public ground-water supplies at a higher frequency than has been found in other ground-water supplies, (3) determine whether indicators of fecal contamination are adequate predictors of the presence of enteric viruses, and (4) determine the factors that affect the presence of enteric viruses in small public ground-water supplies.

Enteric Viruses and Microbiological Indicators of Fecal Contamination

In this report, enteric viruses are defined as human pathogenic viruses invading the gastrointestinal tract of humans. The enteric viruses examined in this study are all RNA viruses and are readily transmitted through water. They include enterovirus, reovirus, rotavirus, hepatitis A virus (HAV), and Norwalk virus. Enteroviruses, which include poliovirus, coxsackievirus types A and B, and echovirus, can infect both the intestinal and upper respiratory tracts. These viruses can cause a variety of illnesses ranging from gastroenteritis to myocarditis and aseptic meningitis (Melnick, 1990). Enteroviruses account for an estimated 10-15 million symptomatic infections in the United States each year (Strikas and others, 1986). There are no data, however, that determine how many of these illnesses are directly or indirectly traceable to a well-water source.

Reovirus can also infect the human intestinal and upper respiratory tracts. "Reo" is short for respiratory enteric orphan because the first viruses discovered in this family were not shown to be associated with disease. This changed with the discovery of rotavirus in 1973 and more recently with Reovirus Type 3, which is now recognized as a human pathogen (Tyler and others, 2004). Rotavirus, a member of the reovirus family, is the most common cause of diarrhea in children, and one genotype of rotavirus has been shown to affect adults. Rotavirus affects approximately 2.7 million children younger than 5 years old each year in the United States, and it causes approximately one-third of diarrhea-associated hospitalizations and 800,000 deaths per year worldwide (Parashar and others, 1998).

HAV is characterized by a sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed by several days of jaundice. In susceptible individuals, the severity of the disease increases, leading to possible inflammation and necrosis of the liver, as well as permanent liver damage. HAV is distributed worldwide, occurring in both epidemic and sporadic fashions. An estimated worldwide incidence of HAV exceeds 1.4 million cases each year, at a health cost of $1.5-3 billion annually (Hollinger and Ticehurst, 1996). In the United States, 17,047 cases of HAV were reported to the Centers for Disease Control and Prevention in 1999 (Centers for Disease Control and Prevention, 1999).

The Norwalk virus is widely distributed worldwide, and it can cause acute epidemic and sporadic gastroenteritis. Symptoms of Norwalk virus infections include nausea, vomiting, diarrhea, and abdominal cramps. Recovery from this infection usually occurs within 2-3 days without serious or long-term health effects. In the United States, approximately 181,000 cases occur annually (Centers for Disease Control and Prevention, 1997).

Various methods for examining viral contamination in water samples are available, all with limitations. Immunological techniques offer a major reduction in time necessary for virus detection, but they are not sensitive enough to detect low numbers of viruses that are expected to be present in water samples (Gerba and others, 1986). RT-PCR techniques can be used to identify specific pathogens and can detect viruses that cannot grow in cell culture; for example, Norwalk virus. RT-PCR reduces the time required for the assay, and the initial and recurring costs are much less than cell-culture techniques; however, the method is unable to determine the infectivity of the viruses. It can detect only the presence or absence of pathogen-specific DNA or RNA.

Cell-culture techniques can determine the infectivity of the viruses, which is a major public-health issue. The cell-culture method determines the presence of culturable virus—mainly enterovirus and reovirus. The disadvantages to this method are increased cost and time for the assay, lack of methods for some enteric viruses of public-health concern, and inability to determine the particular strain of virus present in the sample. In this study, standard operating procedures (SOP) were used for processing and analyzing water samples for enteric viruses by the RT-PCR and cell-culture techniques.

Indicator bacteria, such as total coliforms, *E. coli*, and enterococci, indicate the possible presence of fecal contamination and are not generally disease-causing organisms. Total coliforms are a group of closely related gram-negative, rod-shaped bacteria that are found in the intestinal tract of humans and animals and grow at 35ºC. Total coliforms are also ubiquitous in the environment. They are used as a screening tool for fecal contamination and as presumptive evidence of biofilm formation or surface-water infiltration if a total-coliform-positive sample is collected at the tap. Although total coliforms are used to establish maximum contaminant levels for drinking-water regulations (U.S. Environmental Protection Agency, 2001), they may or not be of fecal origin. *E. coli*, a member of the total coliform group, grows at elevated temperatures (44.5ºC), and is a specific indicator of fecal contamination from warmblooded animals. Although most strains of *E. coli* are harmless and are present in the intestines of healthy individuals, some strains of *E. coli* are pathogenic. One pathogenic strain, *E. coli* 0157:H7, may cause kidney failure and death in the young and the aged. Enterococci are gram-positive, cocci-shaped bacteria that are not as ubiquitous as the total coliform group (Toranzos and others, 2002). They are always present in the feces of warmblooded animals, and they are more persistent in water than total coliforms are. Because of their different shape and survival rate, enterococci may provide a different assessment of the transport of fecal contamination in ground water than total coliforms (Francy and others, 2000). Total coliforms, *E. coli*, and enterococci are routinely measured by means of membrane filtration or most-probable-number techniques (American Public Health Association and others, 1998).

Coliphage are viruses that infect coliform bacteria. Two types of coliphage are used in water investigations—somatic coliphage attach to the cell wall of the bacterial host, and F-specific coliphage attach to the F-pili (produced at temperatures above 25ºC) of a suitable host. Both somatic and F-specific coliphages 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, however, may not always be a reliable indicator of septic-system-effluent contamination of wells. In a one-time sampling of 100 septic tanks and a quarterly sampling of 10 septic tanks for somatic and F-specific coliphage, investigators found coliphage in less than one-half of septic tanks at any one time (D.C. DeBorde, Montana HeadWaters, Inc., written commun., 1998). Two methods that are routinely used to analyze samples for coliphage are the single-agar layer (SAL) method and the two-step enrichment method. The SAL method is a quantitative, plaque assay method that is limited to sample volumes of 100 mL or less (Ijzerman and Hagedorn, 1992). The two-step enrichment method is a presence/absence method that can be used to analyze sample volumes of either 100 mL or 1 L (U.S. Environmental Protection Agency, 2000b).

Previous Studies

Enteric Viruses in Ground-Water Supplies

The occurrence of pathogens and indicators in ground-water supplies has been the topic of recent studies. Data from other studies relevant to this investigation are summarized in table 1. These studies involved a variety of laboratories with different quality-assurance and quality-control requirements and with different analytical methods. The studies also varied with regard to sampling volumes, well selection procedures, and sampling procedures. However, a comparison of data from these studies is still warranted because it provides information on the occurrence of indicators and pathogens in large and small systems. No other published datasets with consistent methods and quality-assurance and quality-control requirements are available to fulfill this purpose. The most exhaustive review of occurrence studies is contained in the proposed GWR (U.S. Environmental Protection Agency, 2000a, p. 30207-39211). Few of the studies discussed in the GWR, however, were targeted to small public-water supplies, and not all of the investigations resulted in published works.

Of the 13 studies cited in the GWR, the USEPA describes an American Water Works Association Research Foundation (AWWARF) study as the broadest in scope (Abbaszadegan, Stewart, and others, 1999). A total of 539 ground-water samples from 448 large public-supply wells were analyzed for microorganisms; some wells were sampled more than once. Generally, higher percentages of detections of enteric viruses were found by use of the RT-PCR method than by use of the cell-culture method (table 1). Percent occurrence of indicator bacteria and coliphage ranged from 4.1 to 11 percent and of enteric viruses from 0.9 to 15 percent. A sequence analysis of RT-PCR results from 14 cell-culture samples positive for enterovirus revealed that the predominant virus was poliovirus type I in 13 samples and poliovirus type II in 1 sample (Abbaszadegan, Denhart, and others, 1999).

In a smaller scale AWWARF study than described above, investigators studied the occurrence of enteric viruses and indicators in ground water from large and small public-water supplies expected to be vulnerable to contamination (Lieberman and others, 2002). In phase 1 of this study, 94 wells were each sampled one time for indicator bacteria and somatic coliphage, and the percentage of detections ranged from 5 percent for somatic coliphage to 33 percent for total coliforms. In phase 2, a subset of 30 wells was sampled monthly for 1 year for fecal indicators and enteric viruses. Detections of indicators were considerably higher than those found in other studies and ranged from 20 percent for F-specific coliphage to 80 percent for total coliforms. Culturable enteroviruses and reoviruses were detected in 6 percent of monthly samples and 23 percent of the wells analyzed during the study (Lieberman and others, 2002; Dahling, 2002). Enteric viruses were detected by RT-PCR in 16 percent of the samples and 72 percent of the wells; reovirus was detected most frequently, in 62 percent of

Table 1. Summary of literature on the occurrence of enteric virus, bacterial indicators, and coliphage in ground-water-supply wells.

[ND, not determined]

Study	Number of wells	System type and aquifer information	Sampling frequency	Percentage of wells testing positive for indicated analysis											
				Enteric viruses[a]						Bacterial indicators				Coliphage	
				Cell culture	Enterovirus	Hepatitis A	Reovirus	Rotavirus	Norwalk	Total coliforms	*Escherichia coli*	Enterococci	Fecal coliform	Somatic	F-specific
Abbaszadegan and others, 1999	448	Large public, mixed geology	1 to 2	4.8	15	6.9	ND	14.0	0.9	9.9	ND	8.7	ND	4.1	11
Lieberman and others, 2002 (phase 1)	94	Large and small public, mixed geology, vulnerable to contamination	1	ND	ND	ND	ND	ND	ND	33	20	18	ND	5	ND
Lieberman and others, 2002 (phase 2); Dahling, 2002; Fout and others, 2003	30	Large and small public, mixed geology, vulnerable to contamination	12	23	38	14	62	0	21	80	50	70	ND	53	20
Davis and Witt, 2000	109	Large and small public, carbonate aquifer	2	0.9	12	ND	ND	ND	ND	ND	0	ND	1.8	1.8	12
Femmer, 2000	109	Large and small public, carbonate aquifer	1	0	ND	ND	ND	ND	ND	ND	8.3	ND	7.3	2.7	2.7
Banks and others, 2001	27	Small public, sand and gravel aquifer	1	3.7	ND	ND	ND	ND	ND	15	0	7.4	ND	3.7	7.4
Lindsey and others, 2002	59	Small noncommunity, fractured bedrock	1	8	ND	ND	ND	ND	ND	46	12	14	ND	8	4
Borschardt and others, 2003	50	Household, mixed geology, vulnerable to septic-system contamination	4	0	2	6	ND	2	2	28	2	10	ND	ND	4
Present study	38	Small public, sand and gravel aquifer	1 to 5	5.9	10	13.2	0	0	0	34	10	16	ND	2.9	5.9

[a] Specific viruses were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR).

the wells (Fout and others, 2003). Six percent of the samples were undetermined because of false negative results and 14 percent because of false positive results.

The USGS and the Missouri Department of Natural Resources did a two-phase study of large and small public ground-water supplies in a largely rural area characterized by carbonate aquifers, some with karstic features. Most of the 109 wells sampled during phase I of the study were constructed within the preceding 15 years (Davis and Witt, 2000). In phase I, as in the large AWWARF study, enteric viruses were found more often by use of RT-PCR than by use of cell culture, and F-specific coliphage were found more often than somatic coliphage. In phase II of the Missouri study, 109 wells that were drilled during or before 1970 were targeted for sampling (Femmer, 2000) and results were different than in phase I. In phase II, enteric viruses by cell culture were not found in any samples, RT-PCR analysis for enteric viruses was not done, *E. coli* and fecal coliforms were found more frequently than in phase I, somatic coliphage were found at about the same frequency, and F-specific coliphage were found less frequently.

A few other studies have been done since publication of the proposed GWR. In a study of 27 small public ground-water supplies in Maryland (Banks and others, 2001), culturable viruses were found in only one sample (3.7 percent). A slightly higher occurrence of culturable virus (8 percent) was found in a study of noncommunity water supplies in Pennsylvania—5 out of 59 wells sampled were positive (Lindsey and others, 2002). Borchardt and others (2003) sampled household wells in Wisconsin for enteric viruses and indicators, targeting wells near sites with high volumes of land-applied septic effluent or in subdivisions served by septic systems. In the Wisconsin study, HAV was the virus most frequently detected by RT-PCR, and culturable viruses were not found in any of the wells. In the study by Borchardt and others (2003), household supplies were sampled, so a smaller contributing population may have been targeted. In these three studies (Banks and others, 2001; Lindsey and others, 2002, Borchardt and others, 2003), percentage of occurrence of indicators ranged from 0 to 46 percent, and total coliforms were found most frequently.

Other researchers showed the importance of including a strong quality-assurance and quality-control program in the analysis of samples for enteric viruses. In one study (Lieberman and others, 2002), potential false positive and negative percentages were 6 and 14 percent, respectively. Borchardt and others (2003) found a potential false negative percentage of 8 percent. They hypothesized that natural waters contain dissolved compounds, such as organic acids, that may inhibit the PCR. Borchardt and others (2003) found that the 16 inhibited samples were from 14 different wells and that the majority of inhibited samples were collected during the winter, when ground-water recharge in Wisconsin was at the lowest rate. Although a strong quality-assurance and quality-control program is also important in the analysis of enteric virus samples by cell culture, these data are seldom included in published results.

Factors That Affect the Presence of Enteric Viruses in Ground-Water Supplies

Considerable work has been done to determine the factors that affect the presence of enteric viruses in ground water, focusing on the transport and survival of viruses in the subsurface (Gerba and Bitton, 1984; Yates and Yates, 1988; Dowd and Pillai, 1997; Sinton and others, 1997). The following review is limited to a sampling of relevant occurrence studies and recent investigations in the study area in Michigan.

In the large AWWARF study, ancillary environmental, geologic, and water-quality data were compiled to assess factors that affected a well's vulnerability to fecal contamination (Abbaszadegan, Stewart, and others, 1999). The majority of wells testing positive for enteric viruses by cell culture or RT-PCR were less than 150 ft away from a sewage source. No relation could be found between geologic formation and detection of indicators or pathogens; however, geological categories were simplified, and confounding variables were not considered. Statistically significant relations were found between the presence of indicators or pathogens and other variables; these included minimum screen distance, amount of unsaturated soil, type of well screen, type of nearby surface-water source, and concentrations of nitrate and various metals.

In phase I of the Missouri study (Davis and Witt, 2000), contrary to what might be expected, the enteric-virus-positive sample and 10 of the 13 coliphage-positive samples were from wells in a confined aquifer or in an unconfined aquifer where karst was not substantial. In contrast, the highest median nitrate plus nitrite concentrations were found in samples from wells in an unconfined aquifer with karst features. In the phase II study, bacteria were detected in the unconfined and confined Ozark aquifers and alluvial aquifers and in areas of agricultural, forested, urban, and mixed land use. A vulnerability assessment based on previous microbiological contamination, geohydrologic barriers, and well construction was done for 41 wells for which data were available. In 12 of these 41 wells, microorganisms were detected; of the 12 wells, 6 were rated as "high vulnerability" and 5 as "unknown vulnerability." No microorganisms were detected in any of the wells rated as "low vulnerability."

Other studies examined the use of chemical constituents to assess fecal contamination of ground water. In a study of shallow homeowner wells in Texas (<350 ft deep), all wells with elevated nitrate concentrations (>4 mg/L) also had detectable concentrations of fecal coliforms or fecal streptococci (Brooks and Cech, 1979). In this same study, the relations between depth of wells or proximity to septic systems and elevated nitrate concentrations were found to be statistically significant. A study of well-water quality in Washington indicated that concentrations of nitrate, chloride, and calcium increased over time in wells in unsewered areas but not in sewered areas. A sodium-calcium exchange mechanism was suggested as being responsible for calcium increases in wells in unsewered areas (DeWalle and others, 1980). Borchardt and others (2003) found that chloride concentration, but not nitrate

concentration, was a fair predictor of the presence of viruses in water from household wells.

In 1996–98, the USGS completed two ground-water-quality studies in the surficial aquifers of southeastern Michigan investigated during this study (Thomas, 2000). Although analyses for microorganisms were not done, this earlier study provided relevant information on how recent residential land use (1975–90) affected the chemical quality of ground water. The studies involved sampling 30 shallow monitoring wells, 24 domestic wells, and 4 small public-supply wells within the study area. Results showed that young, shallow waters (less than 50 ft deep and post-1953) had significantly higher median concentrations of nitrate, chloride, sodium, potassium, calcium, and dissolved solids than older, deeper waters (Thomas, 2000). Chloride/bromide ratios were shown to be useful in assessing the effects of human activities on ground-water quality (Thomas, 2000). Elevated chloride/bromide ratios (those greater than 400) were associated with constituents linked with human activities—nitrate, volatile organic compounds, and pesticides. For example, in all 13 samples with elevated nitrate concentrations (>2 mg/L), chloride/bromide ratios were greater than or equal to 400.

Indicators of Fecal Contamination in the Subsurface

Characterizing the occurrence of enteric viruses in ground-water supplies is known to be difficult and time-consuming because human viruses are present in fecal waste only when the source population is infected, many different types of enteric viruses exist, and assay techniques are complex and costly. Therefore, it is desirable to identify a fecal indicator organism that is easy to detect and may act as a surrogate for enteric viruses. Bacteria are considered inadequate indicators of viruses in the subsurface because of the greater transport distance and survival time for viruses as compared with bacteria. This result is especially true in porous media; in nonporous media (such as karst and fractured bedrock), the difference in transport efficiency for viruses as compared to bacteria is probably not substantial. Instead, coliphage are considered better indicators of the transport and survival of viruses in the subsurface.

Studies that examined the relations between the presence of enteric viruses and indicator bacteria or coliphage in ground-water supplies have reported mixed results. In the large AWWARF study (Abbaszadegan, Stewart, and others, 1999), statistically significant correlations were not found between the presence of virus and indicator bacteria or coliphage. Similarly, in phase I of the Missouri Ozark study (Davis and Witt, 2000), coliphage and indicator bacteria were not present in any sample that was positive for enteric viruses. In phase 2 of the AWWARF study, where wells vulnerable to contamination were sampled (Richard J. Lieberman, U.S. Environmental Protection Agency, written commun., 2001), a statistically significant positive correlation was found between total infectious virus and total coliforms, *E. coli*, enterococci, or somatic or F-specific coliphage; the relation between viruses and F-specific coliphage had the highest Spearman's correlation coefficient (0.388). Out of these indicators, total coliforms showed the lowest false negative rate; that is, out of 20 samples that were positive for enteric viruses, only 2 were negative for total coliforms. In a study of household wells, total coliforms were found in one of five samples that were positive for enteric viruses; *E. coli*, enterococci, and F-specific coliphage were not found in any virus-positive samples (Borchardt and others, 2003). Out of five samples from noncommunity supply wells that were positive for total culturable virus (Lindsey and others, 2002), two were positive for *E. coli,* enterococci, or *Clostridium perfringens*; three were positive for total coliforms, somatic coliphage, or F-specific coliphage; and four were positive for at least one indicator.

Environmental Setting

The public-supply wells chosen for sampling were in four counties on the edge of the Detroit/Ann Arbor Metropolitan Area in southeastern Michigan (fig. 1)—Oakland, Macomb, Livingston, and Washtenaw. This area was selected for study because it was part of a previously studied "subunit survey," a study design used by the USGS National Water-Quality Assessment Program to evaluate the quality of ground water used for domestic and public supply (Gilliom and others, 1995, p. 28). Subunits are defined as distinct parts of major aquifer systems that are relatively similar to one another with regard to water-quality characteristics.

Whereas the cities of Detroit and Ann Arbor rely on surface water for their public supplies, many of the surrounding townships and counties depend on ground water. In 1995, ground water in Oakland, Macomb, Livingston, and Washtenaw Counties supplied more than 30 percent of the total 270 Mgal/d water used. Public-supply systems serve more than 1.8 million people in this area, and of this, 13.5 percent are served from ground-water sources (C. Luukkonen, U.S. Geological Survey, written commun., 2001).

Most public and domestic wells in this area (including those for this study) produce water from discontinuous sand and gravel aquifers screened at depths from 50 to 150 ft. In some places, the aquifer is unconfined, but more often the aquifer is semiconfined or confined by poorly permeable glacial till. Although southeastern Michigan is underlain by bedrock aquifers, these aquifers are not as productive as the surficial deposits overlying them (Grannemann and others, 2000). In some areas, the bedrock is of low permeability and the water may have concentrations of brine or methane contributing to poor water quality (Thomas, 2000). In Macomb and Oakland Counties, only 2 to 3 percent of wells were finished in a bedrock aquifer; in Washtenaw County, only 13 percent; and in Livingston County, only 15 percent (Michigan Department of Natural Resources, Wellkey Database, written commun., 1999).

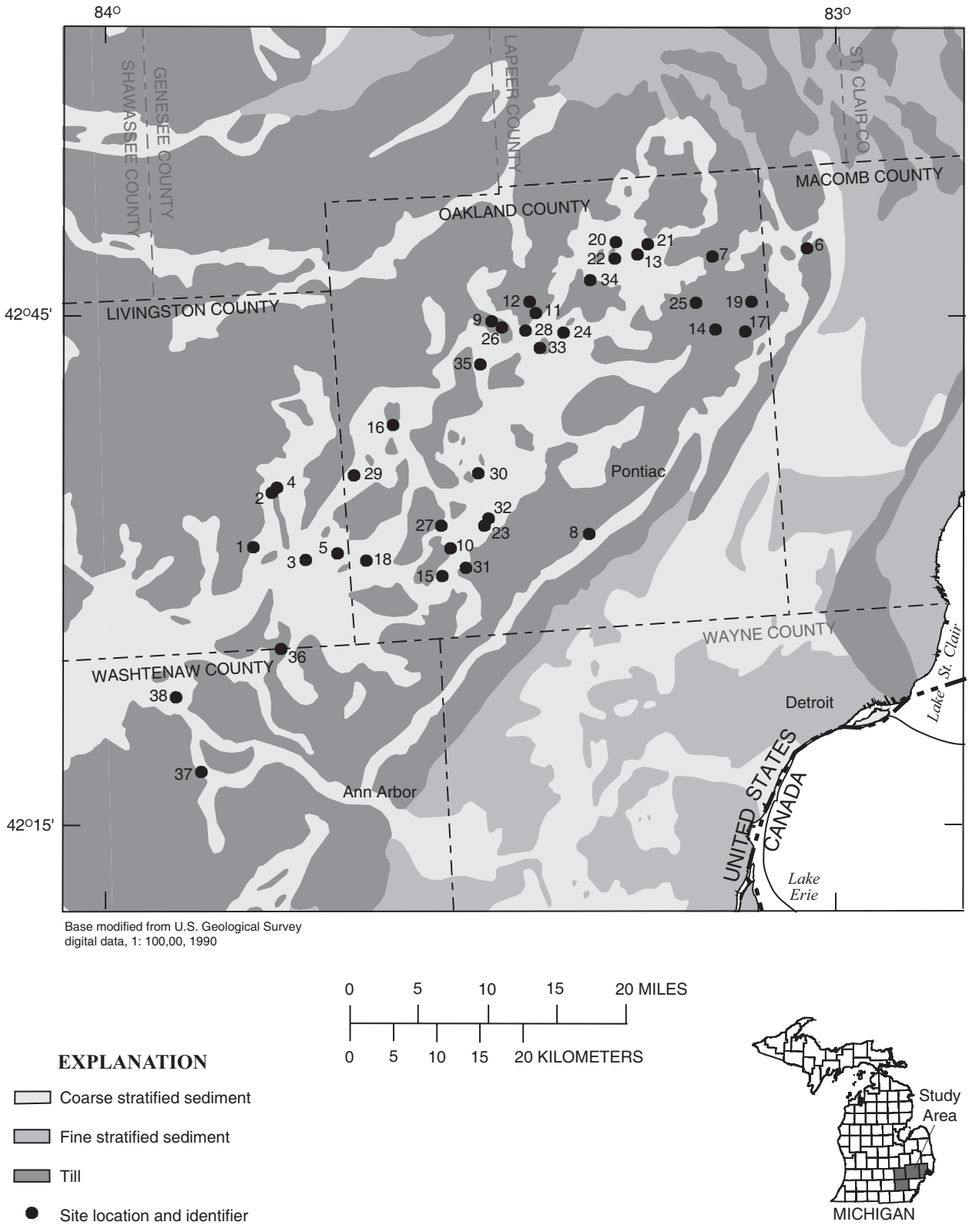


Figure 1. Well location and surficial geology of the study area, southeastern Michigan.

Within Oakland, Macomb, Livingston, and Washtenaw Counties, deposits of surficial sediments are as thick as 400 ft and consist of glacial outwash and till (fig. 1). The hydrogeology of this region has been described by Weisler and others (1952), Mozola (1953), Twenter and Knutilla (1972), Rogers (1996), Aichele (2000), and Thomas (2000). The area stretching from Ann Arbor to the northwestern corner of Macomb County consists of moraines deposited northeast to southwest. Between the moraines, thick layers of outwash were deposited by meltwaters during the last glacial retreat. In addition, meltwater lakes formed as the glaciers retreated, depositing pockets of lacustrine sediments (Aichele, 2000). A typical well log shows interbedded sand, gravel, and clay layers. Glacial sediments form a two-layer aquifer system. The upper layer is typically unconfined, and the lower layer is typically semiconfined by discontinuous clay or till layers (Thomas, 2000).

The average annual precipitation of the area is approximately 32 in. (Midwestern Climate Center, 1995). Ground water is recharged primarily by precipitation, which percolates through outwash deposits and discharges through river systems and wells. The climate is temperate, with average temperature ranges from -10°C in winter to 30°C in summer. The landscape is peppered with numerous small lakes and wetlands, creating complicated flow patterns for surficial drainage. However, on a regional scale, surface water and ground water flow southeast toward Lake Erie and Lake Saint Clair.

The outskirts of the Detroit Metropolitan Area are rapidly developing as a result of population growth; therefore, land use is a complicated intermingling of types. Based on 1995 land use surveys (Southeastern Michigan Council of Governments, 2001), urban area accounted for 42 percent of the total area in Oakland County, 37.5 percent in Macomb County, 13.8 percent in Washtenaw County, and 14.4 percent in Livingston County. Agriculture accounted for 12 percent of the land in Oakland County, more than 30 percent in Macomb and Livingston Counties, and 46.4 percent in Washtenaw County. Since 1995, many new suburbs have been built, and urban development continues to replace agricultural and forested land.

Methods of Study

The 38 wells included in this study with ancillary well and location information are listed in table 8 (at the back of the report). Wells selected for sampling were small public-supply wells that serve from 25 to 3,300 people and tap ground water not under the direct influence of surface water. Several of the ground-water sources were composed of two- or three-well systems; therefore, some samples were composites from more than one well.

Well selection was done in six steps: (1) identify primary and alternate candidate wells, (2) write and phone well owners, (3) visit sites to confirm well-selection criteria, (4) obtain permission from well owners, (5) select a final set of 34 wells, and (6) add substitute wells as needed.

Initially, 160 wells that met the following criteria were identified through contacts with local experts at the state and county levels in Michigan: (1) well produces water from sand-and-gravel deposits, (2) casing material is PVC or galvanized steel, (3) well annulus is grouted, (4) well is intact without cracks in the casing, (5) well is used as a drinking-water source, and (6) well water is not disinfected before distribution. A random-selection program (Scott, 1990) was used to identify 40 primary and alternate wells. After identification of primary and alternate wells, letters describing the study and containing a "permission to sample" signature block were mailed to candidate well owners. If the owner responded positively to the sampling request, a reconnaissance visit was made to determine the suitability of the well for this study. If samples could not be obtained from a primary well because of inaccessibility or for any other reason, the alternate wells were considered for inclusion in the study. However, because of the paucity of available suitable wells, other wells not selected as primary or alternate wells were examined for inclusion in the study.

In the manner described above, a candidate list of 34 wells was compiled. Seven wells were omitted at the request of the well owner or because they were found to be unsuitable. One or two samples were collected from these seven wells before the wells were removed from the study, and these data are reported herein. Following the same steps as outlined above, the investigators identified and sampled four substitute wells later in the study.

Sampling Procedures

Thirty-eight wells were sampled for indicators and pathogens from July 1999 through July 2001. One well was sampled 6 times (to facilitate collecting a replicate spike sample), 30 wells were sampled 5 times, 6 wells were sampled twice, and one well was sampled once—a total of 169 samples. Samples were collected and analyzed for chemical-quality constituents once from the 31 wells where at least five samples for indicators and pathogens were available.

USGS technicians designed a portable self-contained enteric virus sampling apparatus with easy-to-operate control valves. The sampler design was based on the requirements and procedures described in the USEPA Information Collection Rule (U.S. Environmental Protection Agency, 1996a). The sampling apparatus contains a regulator module, cartridge housing module, and a discharge module. A regulator module consisting of a backflow regulator connected to a pressure regulator (Watts Regulator Product Series 8, North Andover, Mass.) and gage is joined to the cartridge housing containing an autoclaved, positively-charged ZetaPor Virosorb 1 MDS filter cartridge (Cuno Inc., Meriden, Conn.). The discharge module is connected by way of a quick-connect coupling to a hose leading to the inlet of a water meter (Neptune Systems,

San Jose, Calif.). Before sampling and after each use, the samplers and cartridge housing were cleaned with a nonphosphorus soap solution for 30 minutes and rinsed with deionized water. They were then sterilized with a 10 percent bleach solution, dechlorinated with a 0.2 percent thiosulfate solution, and finally, rinsed with sterile deionized water to remove residue.

The wells were sampled from the tap nearest to the pump and ahead of chlorination or softening. Although an effort was made to collect untreated water from the aquifer whenever possible, sampling limitations at some wells made it necessary to include some ground water from a storage tank.

Upon arrival at the field site, the sample tap was flame sterilized and connected to the samplers by plastic attachments, which had been sterilized with bleach, and then rinsed with thiosulfate solution and sterile deionized water. In order to collect a second concurrent sample to be used as a replicate spike, a Y-splitter and second sampler was used. A four-parameter water-quality meter was connected to the virus sampler discharge hose to monitor temperature, specific conductance, pH, and dissolved oxygen before and during collection of the virus sample, according to procedures described in Wilde and Radtke (1998). Before filtering the sample for viruses, samplers were rinsed with 80 L of well water to remove any residue that may have been left from the sterilization procedure. The well was then pumped for 5–15 minutes until specific conductance and pH stabilized; for some wells, dissolved oxygen and temperature remained variable from intermittent pumping. After purging was completed, a sterile 1 MDS filter was inserted into the cartridge housing. After 1,000 L was pumped through the sampler and filter, the influent line was disconnected and samples for bacteria and coliphage analysis were collected in two sterile 3-L bottles directly from the tap. If required, a chemical-quality sample was collected in appropriately designated sample containers at this time. Sampling continued until 2,000 L was filtered through the 1 MDS filter. Upon sample completion, the cartridge housing containing the 1 MDS filter was removed from the sampler(s), drained, placed on ice, and mailed overnight to the USGS Ohio District Microbiology Laboratory (ODML) along with a 3-L sample and replicate spike (if collected). Samples were processed at the USGS ODML within 24 hours of sample collection. Chemical-quality samples, when collected, were packed on ice and shipped overnight to the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado. The second 3-L sample was analyzed for indicator bacteria (described below) and turbidity (Hach Company, 1989) at the USGS Michigan District within 6 hours of sample collection.

Microbiological Analyses

Ground-water samples were analyzed for enteric viruses by RT-PCR and cell-culture methods; for total coliforms, *E. coli*, and enterococci by membrane-filtration methods; and for somatic and F-specific coliphage by SAL and two-step enrichment methods.

Enteric Virus Analysis by RT-PCR

The SOP for RT-PCR was developed and written by USEPA (Fout and others, 2003). Oligonucleotide primers and probes used in this study were designed as described in Fout and others (2003) except for HAV, and are listed in table 9 (at back of report). HAV primers and probes were redesigned from those previously used (Denis-Mize and others, 2004; Fout and others, 2003) on the basis of new sequence data obtained with standard sequence-analysis software.

Viruses attached to the 1 MDS filters with charge interactions were eluted by use of a beef extract solution at a high pH and then concentrated with celite (Ohio Valley Specialty Chemical, Marietta, Ohio). Two elution procedures were performed for each filter, one upon receipt of sample and one after an overnight storage of the filter at room temperature in a beef extract solution (Francy and others, 2004). An aliquot of the concentrated eluate from each elution was kept at the ODML for analysis by RT-PCR. A second aliquot of the eluate was transferred to a laboratory at the Ohio State University for analysis by cell culture.

Viruses in the eluate for RT-PCR analysis were further concentrated by ultracentrifugation through a sucrose gradient and then treated with a solvent mixture designed to remove inhibitors (Fout and others, 2003; Francy and others 2004). Organic compounds, such as humic substances, can act as inhibitors by interfering with the activity of the enzymes used in the RT-PCR step. Because all of these viruses are RNA viruses, the RNA was reverse-transcribed to DNA by the enzyme reverse transcriptase (RT). The DNA was then processed using PCR, which enzymatically amplifies specific DNA sequences by means of oligonucleotide primers that flank the region of interest in the target DNA. From June 2000 to November 2001, each sample was subjected to two multiplex RT-PCR reactions. Each multiplex reaction included multiple primers, which targeted specific viruses. Reaction A detected RNA from enterovirus, reovirus, and rotavirus; and reaction B detected RNA from HAV and Norwalk virus. This method was prone to false-negative and false-positive results as indicated by the quality-control samples. Therefore, the method was modified and used to analyze samples from March 2003 to February 2004 by use of single reaction RT-PCR for enterovirus and HAV only. The same primers, probes, and reaction conditions were used in the modified method.

PCR products were analyzed by nucleic acid hybridization (Fout and others, 2003) to confirm the identity of viral RNA and eliminate the potential for erroneous interpretation of nontarget nucleic acids potentially present in the sample. Oligonucleotide probes, labeled with a nonradioactive compound 3′-digoxigenin and specific to each virus group, were used to confirm identity. Some of the samples were subjected to agarose gel electrophoresis to identify presumptive viral-positive samples; however, only the samples confirmed during hybridization were considered positive. Results were recorded

as the presence or absence of each virus per 50 L, based on the effective sample volume analyzed by RT-PCR.

Enteric Virus Analysis by Cell Culture

The assay for culturable virus involved a modified version of the USEPA Information Collection Rule method (U.S. Environmental Protection Agency, 1996a; G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1999). A roller bottle containing a monolayer of Buffalo Green monkey kidney (BGM) cells was inoculated with 40 mL of eluate and incubated at 36.5ºC on the roller apparatus. Each culture was examined microscopically for the appearance of cytopathic effects (cell disintegration or changes in cell morphology) (CPE) for a total of 14 days. If more than 75 percent of the cell monolayer showed signs of CPE, the culture was frozen at –70ºC; all negative cultures were placed in the freezer after 14 days. A second passage on all cultures was done with 75-cm^2 flasks. Cultures negative after 14 days of the second passage were confirmed as CPE negative. The cell-culture method using BGM cells targets enterovirus (poliovirus, echovirus, and coxsackie virus) and reovirus. Results were recorded as the presence or absence of culturable viruses per 500 L, based on the effective sample volume analyzed by the cell-culture method.

Indicator Bacteria

Analyses of samples for total coliforms, *E. coli,* and enterococci were by use of the MI and mEI membrane-filtration methods. The MI method allows the simultaneous detection of total coliforms and *E. coli* on one medium (U.S. Environmental Protection Agency, 2000c). Two enzyme substrates are included in MI agar—a fluorogen reacts with an enzyme found in total coliforms (ß-galactosidase), and a chromogen reacts with an enzyme found in *E. coli* (ß-glucuronidase). After 24 hours of incubation at 35°C, total coliform colonies fluoresce under a long-wave ultraviolet light, and *E. coli* colonies appear blue under natural light. The mEI method (USEPA Method 1600) allows the detection of enterococci in 24 hours with an incubation at 41°C (U.S. Environmental Protection Agency, 1997b). The mEI medium contains a substrate, indoxyl ß-D-glucoside, that turns blue when cleaved by an enzyme present in enterococci (ß-glucosidase). All colonies with any blue halo are recorded as enterococci, regardless of colony color. Membrane-filtration results are recorded as colonies per 100 mL.

Coliphage

Two methods were used for coliphage: (1) the SAL, direct plating method with induction of ß-galactosidase and (2) the two-step enrichment presence-absence method (P/A). Intitially, 100 mL of sample was used for the SAL method because large volumes are impractical for the method. When a draft of USEPA Method 1601 (U.S. Environmental Protection Agency, 2000b) was available for the P/A method, 1-L samples by the enrichment P/A method were added to the analytical scheme in January 2000, and then 100-mL samples were added in March 2000 for comparison purposes. For both methods, the host cultures were *E. coli* C for somatic coliphage and *E. coli* F-amp (resistant to streptomycin and ampicillin) for F-specific coliphage.

The SAL method is a modification of a method developed by Ijzerman and Hagedorn (1992). Briefly, 100-mL sample volumes were mixed with agar medium, *E. coli* host culture, chemicals that induce the ß -galactosidase enzyme and enhance the visibility of plaques (a clearing in the bacterial lawn), and appropriate antibiotics. The mixture was poured into four 150- x 15-mm plates and incubated at 35ºC for 24 hours. If a phage particle is present in the sample, it attaches to an *E. coli* cell and replicates, causing death of the cell and cell lysis. This process continues until a plaque is visible. Results are recorded as number of plaques per 100 mL.

The enrichment P/A method (U.S. Environmental Protection Agency, 2000b) was developed in response to the need to analyze large sample volumes for detection of viruses in ground water. For the enrichment step, 100-mL and 1-L sample volumes are supplemented with magnesium chloride, log-phase host *E. coli*, and tryptic soy broth. Divalent cations are included in the enrichment solution to enhance phage infectivity. After 24 hours of incubation at 36°C, samples are spotted onto a lawn of suitable host bacteria and incubated overnight. Positive results for coliphage appear as a clear halo around the spot. Bacteria from the sample grow on the spot while phage radiate from the spot to lyse the surrounding *E. coli* lawn. Results are recorded as presence or absence per unit volume of sample.

Ancillary Environmental and Water-Quality Information

Ancillary geological data and well and site information were collected by the USGS or compiled from various sources. Geological data and data on well construction and depth were obtained from drillers' logs from the Michigan Department of Natural Resources. These data included well-construction date, casing depth, lithology, and amount of clay above the screened interval. Percent clay was calculated by dividing the amount of clay above the screened interval by the casing depth. USGS field workers filled out project-specific well and site inventory forms with ancillary information obtained through observations or consultation with well owners or operators. These data included sampling condition (whether the water sampled was directly from the aquifer or was stored in a tank), type of sewage treatment and collection system (septic system or sewerline), distance from well to septic system (if applicable), and type of and estimation of distance to nearby surface-water bodies (by visual inspection). Data on the population served for community water systems

were obtained from discussions with well owners or from water-system-review information sheets obtained from well owners.

Data on population densities were obtained from the Center for International Earth Science Information Network of Columbia University (1997), wherein U.S. Census Bureau Data were converted to GIS format. Information on land use was obtained from geographic information system (GIS) coverages established by the Southeastern Michigan Council of Governments (2001) and generated from 1:24,000 aerial photographs taken in 1995. From these coverages, detailed land use within a 500-m radius around the well was identified.

Chemical-quality sampling included determinations of major constituents (including bromide and chloride), nutrients, boron, dissolved organic carbon, and alkalinity. All chemical analyses were done according to procedures published in Fishman and Friedman (1989), Patton and Truitt (1992), Fishman (1993), Struzeski and others (1996), Brenton and Arnett (1993), and U.S. Environmental Protection Agency (1996b). Constituents associated with anthropogenic sources—concentrations of nitrate plus nitrite, ammonia plus organic nitrogen, chloride, chloride/bromide ratios, boron, and dissolved organic carbon—were determined for use as multiple lines of evidence to corroborate detections of microorganisms. Analyses of samples for major ions were included to characterize ground-water quality.

Quality Assurance and Quality Control

Standard field and data management quality-assurance practices for USGS water-quality activities in Ohio are described in Francy and others (1998). USGS Ohio personnel did quality-assurance checks at the Lansing, Mich., office to ensure that these same quality-assurance procedures were followed. Standard laboratory quality-assurance and quality-control practices are described for the ODML in Francy and others (2004). Laboratory quality-assurance practices for the OSU laboratory doing cell-culture analysis are described in U.S. Environmental Protection Agency (1996a). The USEPA and USGS did two quality-assurance checks of the OSU laboratory to ensure that these practices were followed.

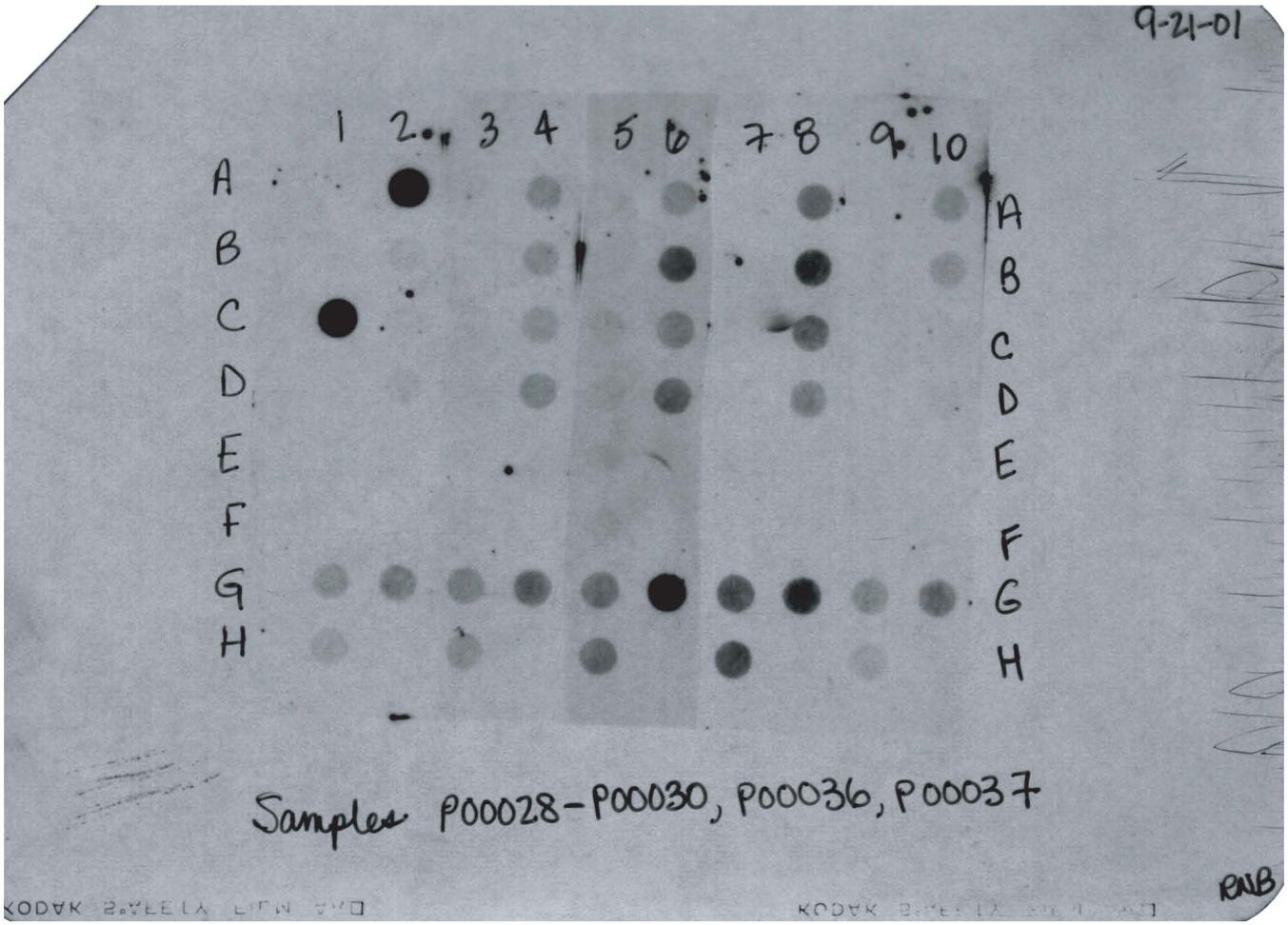
At the ODML, quality-control samples were included for analyses of samples for coliphage and enteric viruses. The procedures for coliphage are detailed in Francy and others (2004). Quality-control samples for enteric virus analysis by RT-PCR are listed and described in table 10 (at back of report). Figure 2 shows an example of hybridization results and associated quality-control samples. Because of the failure of RT-PCR quality-control samples to produce desired results, some samples had to be analyzed more than once to obtain conclusive results.

At the Lansing, Mich., office, quality-control samples were collected during field activities. For bacterial analysis, filter blanks for every sample for total coliforms, *E. coli*, and enterococci were analyzed—a 50- to 100-mL aliquot of sterile buffered water, plated before the sample. The filter blank is used to assess contamination of reagents and equipment used in the membrane-filtration procedure. All filter blanks were negative. Four equipment blanks for enteric viruses were collected throughout the sampling period. An equipment blank is a blank solution (sterile reagent water) used to determine potential contamination from the equipment cleaning and sterilization processes and during preservation, transportation, and handling of the sample (Francy and others, 1998). To collect the equipment blank for enteric viruses, after the apparatus had been cleaned and sterilized, 20 L of sterile reagent water was pumped through the apparatus and collected on a 1 MDS filter. Analysis for enteric virus contamination was then done on the filter eluate by means of the RT-PCR and cell-culture methods. Two equipment blanks were negative for the viruses tested and two were negative for two viruses (enterovirus and HAV) and inconclusive for three other viruses tested.

A replicate spike for enteric viruses was collected once at each of the 31 wells that were sampled 5 times and was collected at 1 well that was sampled twice. Replicate spikes are done to determine whether the water matrix inhibits or prevents detection of the target virus and to assess the reproducibility of the collection and analytical procedures. To collect a replicate spike, the field crew filtered all but 10 L of ground water and collected the remaining 10 L in a sterile container. The filled container and 1 MDS filter were sent by overnight mail to the ODML. In the laboratory, 1 mL of attenuated live poliovirus vaccine, Sabin strains types 1, 2, and 3 (Lederle Laboratories, Pearl River, NY), at a concentration of approximately 4,000 plaques per mL, was added to the 10 L of ground water. (RT-PCR results later showed that the concentration of poliovirus spike was much greater than it needed to be to produce a positive response.) The spiked 10-L sample was then passed through the associated 1 MDS filter. Analysis was done on 32 replicate spikes with the RT-PCR method and on 6 replicate spikes with the cell-culture method.

Enteric Viruses and Microbiological Indicators of Fecal Contamination in Small Public Ground-Water Supplies

Field personnel made onsite water-quality measurements and collected 169 regular samples for indicators and pathogens from July 1999 through July 2001; 32 replicate spike samples for enteric viruses by RT-PCR also were collected during this period (table 11, at back of report). Water temperature, specific conductance, dissolved oxygen, and pH were measured at the time of sampling except when there were equipment problems; turbidity was not routinely measured because the required equipment was not always available. Except for equipment problems with three samples, all regular samples were analyzed for indicator bacteria. All regular samples were analyzed for coliphage by the SAL method, and samples



	1	2	3	4	5	6	7	8	9	10
A	Negative process control	Matrix spike 29	Negative process control	Matrix spike 29	Negative process control	Matrix spike 29	Negative process control	Matrix spike 29	Negative process control	Matrix spike 29
B	Sample 28	Matrix spike 30	Sample 28	Matrix spike 30	Sample 28	Matrix spike 30	Sample 28	Matrix spike 30	Sample 28	Matrix spike 30
C	Sample 29	Matrix spike 36	Sample 29	Matrix spike 36	Sample 29	Matrix spike 36	Sample 29	Matrix spike 36	Sample 29	Matrix spike 36
D	Sample 30	Matrix spike 37	Sample 30	Matrix spike 37	Sample 30	Matrix spike 37	Sample 30	Matrix spike 37	Sample 30	Matrix spike 37
E	Sample 36	PCR negative control	Sample 36	PCR negative control	Sample 36	PCR negative control	Sample 36	PCR negative control	Sample 36	PCR negative control
F	Sample 37	Hybridization negative control	Sample 37	Hybridization negative control	Sample 37	Hybridization negative control	Sample 37	Hybridization negative control	Sample 37	Hybridization negative control
G	Seeded process control	PCR positive control	Seeded process control	PCR positive control	Seeded process control	PCR positive control	Seeded process control	PCR positive control	Seeded process control	PCR positive control
H	Matrix spike 28	---	Matrix spike 28	—	Matrix spike 28	---	Matrix spike 28	—	Matrix spike 28	---

Figure 2. Example of hybridization results from reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for enteric viruses.Columns 1 and 2 show results for enterovirus; 3 and 4 for reovirus; 5 and 6 for rotavirus; 7 and 8 for hepatitis A virus; 9 and 10 for Norwalk virus.)

collected later in the sampling period were also analyzed by use of the newly published P/A method. Because of the high cost of analysis and time constraints towards the end of the sampling period, only 93 of the 169 samples were analyzed for enteric viruses by the cell-culture method. Cell-culture analyses were done from 1 to 5 times at 34 wells and were not done at 4 wells (wells 5, 33, 34, and 35).

Quality-Control Considerations for Enteric Viruses by RT-PCR

All 169 regular and 32 replicate spike samples were analyzed for enterovirus and HAV by use of RT-PCR. Eighty-two regular samples and six replicate spike samples analyzed early in the analysis period (when the multiplex RT-PCR procedure was in use) also were analyzed for reovirus, rotavirus, and Norwalk virus. The presence of indigenous HAV, reovirus, rotavirus, and Norwalk virus could be determined in replicate spike samples. However, because an enterovirus (poliovirus) was spiked into the 32 replicate spike samples, it was not possible to determine the presence of indigenous enterovirus in these samples. All 32 replicate spike samples were correctly identified as positive for enterovirus by RT-PCR (indicated by a "PS" in table 11 to distinguish these results from an indigenous detection of "P").

The inclusion of 32 replicate pairs (the regular and replicate spike sample collected on the same date at the same well) for HAV and six replicate pairs for reovirus, rotavirus, and Norwalk virus provides information on the reproducibility of the sampling and analysis methods. (The replicate spikes are indicated using a "c" footnote on the sampling date and replicate pairs are highlighted in table 11.) The replicate pairs for reovirus, rotavirus, and Norwalk virus provided matching results in all cases; however, none were positive for any virus. Of the 32 replicate pairs for HAV, however, the results matched in all but four samples. In these four samples for HAV, three replicate pairs had inconclusive data in one sample and viruses absent in the other (wells 10, 25, and 35), and one replicate pair had virus present in one sample and absent in the other (well 28). The data on well 28 showed that results are not always reproducible, even in samples collected at the same well on the same date. This difficulty with reproducibility may be due to low concentrations of viruses in ground water and (or) intermittent occurrence of viruses in ground water.

Because of different results in four replicate pairs for HAV, the data from replicate pairs were treated as two separate samples for summarizing analytical results for quality-control purposes. Analyzing data in this manner, enterovirus and HAV were detected in less than 3 percent of samples (table 2). The highest potential false positive percentage (22.7 percent) was found for rotavirus. Potential false positives result from cross contamination during inhibitor removal or RT-PCR (negative process control or PCR negative control is positive; see table 10). Because all hybridization negative controls were negative, cross contamination did not occur during hybridization (data not shown). The highest potential false negative percentage (38.6 percent) was found for Norwalk virus. Potential false negatives result when inhibi-

Table 2. Results of enteric virus analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) of regular and replicate spike samples collected from small-public-supply wells in southeastern Michigan, July 1999 through July 2001.

Virus	Total samples analyzed	Number of samples (percent)			
		Positive	Negative	Potential false positive[a]	Potential false negative[b]
Enterovirus	169	4 (2.4)	154 (91.1)	1 (0.6)	10 (5.9)
Hepatitis A virus	201	5 (2.5)	176 (87.6)	5 (2.5)	15 (7.5)
Reovirus	88	0	69 (78.4)	5 (5.7)	14 (15.9)
Rotavirus	88	0	54 (61.4)	20 (22.7)	14 (15.9)
Norwalk virus	88	0	40 (45.5)	14 (15.9)	34 (38.6)

[a]Potential false positive results occurred when the negative process control was positive and (or) the PCR negative control was positive.

[b]Potential false negative results occurred when the matrix spike was negative, the seeded process control was negative, and (or) the PCR positive control was negative.

tors of RT-PCR are present in the ground water (matrix spike is negative), the RT-PCR reaction failed to reach completion (seeded process control is negative), and (or) the RT-PCR and hybridization reactions failed to reach completion (PCR positive control is negative). Potential false positive and negative percentages for enterovirus and HAV were less than 10 percent, the highest being the potential false negative percentage for HAV (7.5 percent). All of the potential false negatives for HAV were caused by failure of the matrix spike to provide a positive result; all of the potential false positives for HAV were caused by a positive result in the negative process control (data not shown).

Inconclusive results are caused by potential false positives and (or) false negatives and can be examined by well and by date to see if patterns emerge. Inconclusive results were found for both enterovirus and HAV in the same sample on only two occasions (table 11, wells 4 and 27). All of the 11 wells with inconclusive results for enteroviruses had one inconclusive result each. Nine of the wells with inconclusive results for enterovirus were in samples collected in June and July 1999 when the multiplex reaction method was in use and not enough sample was left to rerun the analysis by use of the single-reaction RT-PCR method. Of the 14 wells with inconclusive results for HAV, nine wells had one inconclusive result, four had two, and one had three. Inconclusive results for HAV were found in January, February, April, May, June, July, August, October, and November. Inconclusive results, therefore, were not seasonal; rather, they were the result of the RT-PCR method working poorly with a particular batch of samples.

Occurrence of Enteric Viruses and Microbiological Indicators of Fecal Contamination

The percentages of detection of enteric viruses by cell culture and of five viruses by RT-PCR are summarized in table 3. For this summary, results from replicate pairs were combined, and detection in either sample from the pair was considered positive for the virus. Of the 93 samples from 34 wells analyzed for enteric viruses by cell culture, only 2 were positive (wells 10 and 25). The two samples positive for culturable viruses were not positive for any other virus by RT-PCR, and the wells from which they came from were negative for all viruses on all other sampling dates (table 11). Enterovi-

Table 3. Detections of enteric virus by reverse transcriptase-polymerase chain reaction (RT-PCR), culturable viruses, and microbiological indicators of fecal contamination from small-public-supply wells in southeastern Michigan, July 1999 through July 2001.

[L, liter; mL, milliliter; P/A, presence/absence; SAL, single agar layer]

Indicator or pathogen	By well			By sample		
	Number of wells analyzed	Positive results	Percent positive	Number of samples analyzed	Positive results	Percent positive
Culturable virus	34	2	5.9	93	2	2.2
Enteric virus by RT-PCR						
Enterovirus	38	4	10.5	169	4	2.4
Hepatitis A virus	38	5	13.2	169	5	2.5
Reovirus	34	0	0.0	82	0	0.0
Rotavirus	34	0	0.0	82	0	0.0
Norwalk virus	34	0	0.0	82	0	0.0
Total coliforms	38	13	34.2	167	14	8.4
Escherichia coli	38	4	10.5	167	4	2.4
Enterococci	38	6	15.8	167	7	4.2
Coliphage - F specific						
100 mL SAL	38	0	0.0	169	0	0.0
100 mL P/A	32	0	0.0	106	0	0.0
1 L P/A	34	2	5.9	121	2	1.7
Coliphage - Somatic						
100 mL SAL	38	0	0.0	169	0	0.0
100 mL P/A	32	0	0.0	106	0	0.0
1 L P/A	34	1	2.9	121	1	0.8

rus and HAV were found in 10.5 and 13.2 percent of the wells, respectively, by use of RT-PCR. Although only one virus was found on each sampling date at a particular well, enterovirus and HAV were both found in two wells (wells 11 and 28), but on different sampling dates. If results for all viruses are combined, 9 of the 38 wells were positive for enteric viruses (23.7 percent) by either cell culture or RT-PCR. On a per-sample basis, 11 of the samples collected were positive for any virus.

The percentages of detections of indicators are summarized in table 3. For indicator bacteria, total coliforms were found most often, in 34.2 percent of the 38 wells tested. Of the 13 wells that were positive for total coliforms, only 4 were positive for *E. coli*. Enterococci were found in 6 of the 38 wells tested (15.8 percent). On a per-sample basis, the percentages of detections for indicator bacteria were lower, ranging from 2.4 to 8.4 percent. F-specific and somatic coliphage were detected in less than 6 percent of the wells, or 1.7 percent of the samples collected during this study. Somatic or F-specific coliphage were detected by use of 1-L P/A method in three samples and were not detected when 100-mL sample volumes were analyzed by either method (table 11).

For all samples with detections of indicator bacteria, plate counts were outside the ideal range of 20–80 colonies (table 11). For total coliforms, 2 out of 18 samples were above the ideal range, and the rest were below. All *E. coli* concentrations were low, ranging from 1 to 6 col/100 mL. For all but one of the seven detections of enterococci, counts were below the ideal range.

The importance of repeat sampling for multiple indicators is shown by the absence of recurrence and co-occurrence of indicator bacteria and coliphage. Out of the 18 wells with at least one detection of indicator bacteria or coliphage, in only 3 wells (wells 7, 9, and 23) were indicators detected on more than one sampling day. (These sample dates are in bold and italics in table 11.) For well 7, total coliforms and *E. coli* were detected in one sample, and only enterococci were detected in a second sample. Indicator bacteria were detected in three samples from well 9. In well 23, somatic coliphage were detected in one sample, and F-specific coliphage were detected in a second sample; no indicator bacteria were found in any samples from this well. As for the co-occurrence of indicator bacteria in the same sample (these data are in bold and italics in table 11), one sample contained total coliforms, *E. coli*, and enterococci (well 9, 11/16/99), and another sample contained both total coliforms and enterococci (well 26, 1/3/00). The presence of total coliforms and *E. coli* in the same sample is not considered a co-occurrence because *E. coli* is one species in the total coliform group.

Microbiological Indicators of Fecal Contamination as Predictors of the Presence of Enteric Viruses

Comparisons were made to determine whether indicators were adequate predictors of the presence of enteric viruses. Of 11 samples that were positive for enteric viruses, indicator bacteria co-occurred in only 2 samples, and coliphage were not present in any (table 11). In the sample collected from well 10 on July 28, 1999, culturable viruses and enterococci were present. In the sample collected from well 37 on June 26, 2000, enterovirus by RT-PCR and total coliforms were present. Because of the low co-occurrence of indicators and enteric viruses in the same sample, subsequent data analyses were done on a per well basis. In four out of nine wells where viruses were detected, no indicator was found on any sampling date (table 4). Two wells with detections of viruses had a detection of total coliforms, one well had a detection of

Table 4. Small-public-supply wells in southeastern Michigan with detections of enteric viruses, July 1999 through July 2001, and detections of microbiological indicators from the same well.

[RT-PCR, reverse transcriptase-polymerase chain reaction; + is positive and - is negative for microbiological indicator]

Well number	Viruses detected	Total coliforms only	Total coliforms and *Escherichia coli*	Enterococci	Somatic coliphage	F-specific coliphage
5	Hepatitis A by RT-PCR	-	-	-	-	+
6	Hepatitis A by RT-PCR	-	-	-	-	-
10	Culturable viruses	-	-	+	-	-
11	Enterovirus and hepatitis A by RT-PCR	-	-	-	-	-
19	Hepatitis A by RT-PCR	+	-	-	-	-
25	Culturable viruses	-	-	-	-	-
28	Enterovirus and hepatitis A by RT-PCR	-	-	-	-	-
35	Enterovirus by RT-PCR	-	+	-	-	-
37	Enterovirus by RT-PCR	+	-	-	-	-

E. coli, one of enterococci, and one of F-specific coliphage. Only one type of indicator was found in any of the virus-positive wells.

The accuracy of the indicators to predict the presence of the enteric viruses tested during this study was generally poor (table 5). A data-analysis method reported by Borchardt and others (2003) yielded true-positive rates of 11.1 percent for three indicators and 33.3 percent for total coliforms. If the detection of any indicator was taken as a predictor for enteric viruses, the true-positive rate rose to 55.6 percent. That means that slightly more than half of the time a virus was detected, an indicator also was detected. True-negative rates and negative predictive values were high because of large numbers of negative values for indicators and pathogens. Positive predictive values ranged from 16.7 to 50 percent. The 50-percent result for coliphage is biased high because only two samples were positive for coliphage, one of which had a virus detection.

Environmental Factors and Chemical-Quality Constituents Related to the Presence of Indicators and Enteric Viruses

Chemical-quality constituents were sampled for and analyzed at least once during the period of study for 31 wells (table 12, at back of report). For seven wells, the well was removed from the study before chemical-quality samples could be collected. Five wells were sampled twice for chemical quality—to confirm a high nitrate plus nitrite (well 9) or boron (well 38) concentration or to assess the variability in chemical-quality results (wells 28, 29, and 34). The high nitrate plus nitrite and boron concentrations were confirmed. A visual inspection was made to determine the variability between sample pairs. For sample pairs collected at the same well on the same day (well 34) or at the same well on different days (wells 28 and 29), the variability was small compared to the analytical range for all constituents. Therefore, average concentrations for each constituent were used in all subsequent data analysis.

The relations between chemical-quality and environmental variables and detections of three different groups of microorganisms were examined: (a) total coliforms, (b) indicators excluding total coliforms ("other indicators"), and (c) enteric viruses by cell culture and RT-PCR. Total coliforms were grouped separately because of the effect their detection had on the relations when included with other indicators. In addition, total coliform may or may not indicate fecal contamination. For statistical analyses, environmental and chemical-quality variables were considered to be either continuous or categorical. Well-information data on ground-water sources that were composed of two or three-well systems were averaged (for example, table 8, well 2).

Categorical variables were ranked in as many as four hypothesized risk categories, with 1 being the highest risk and 4 being the lowest risk (table 6). Presence or absence of nitrate plus nitrite was considered a categorical variable because this constituent was detected in less than one-half of the samples. For other constituents, natural breaks in the continuous data were used to develop categories. Fisher's exact test was used to assess the relations between detections of microorganisms and categorical variables (Sokal and Rohlf, 1981, p. 731). A one-sided test was used to test most variables except for those with more than two risk categories. Fisher's exact test measures the relations between two variables by determining their independence. For the one-sided test, the null hypothesis is rejected (the two variables are dependent) if the indicator is not detected when the variable is at low risk and the indicator is present when the variable is at high risk.

Setting the significance level at 0.1, statistically significant dependencies were found between detections of microorganisms and sewage-system type, nitrate plus nitrite, chloride,

Table 5. Predictive accuracy of microbiological indicators for virus occurrence in the same small-public-supply well in southeastern Michigan, July 1999 through July 2001.

Indicator	True-positive rate (percent)[a]	True-negative rate (percent)[b]	Positive predictive value (percent)[c]	Negative predictive value (percent)[d]
Total coliforms	33.3	65.5	23.1	76.0
Escherichia coli	11.1	89.7	25.0	76.5
Enterococci	11.1	82.8	16.7	75.0
Coliphage	11.1	96.6	50.0	77.8
Any indicator	55.6	55.2	27.8	80.0

[a] Percentage of virus-positive wells that were also found to be positive by the indicator.

[b] Percentage of virus-negative wells that were also found to be negative by the indicator.

[c] Percentage of indicator-positive wells that were positive for virus.

[d] Percentage of indicator-negative wells that were negative for virus.

Table 6. Rankings of environmental and chemical-quality categorical variables by risk of fecal contamination and relations to detections of microorganisms in small-public-supply wells in southeastern Michigan, July 1999 through July 2001.

[p-values were determined by use of the one-sided Fisher's exact test, except where indicated otherwise; values in bold are significant at α=0.1; GW, ground water; mg/L, milligrams per liter; <, value is less than concentration indicated; ≥, value is greater than or equal to concentration indicated]

Parameter	Rank				P-value		
	High Risk			Low Risk	Total coliforms	Other indicators[a]	Enteric viruses
	1	2	3	4			
Sampling condition	GW and storage tank	GW	--	--	0.423	0.451	0.826
Sewerage-system type	Septic system	Sewerline	--	--	0.327	0.713	**0.088**
Distance to septic system (feet)	20-299 feet	≥ 300-1,000 feet	--	--	0.570	0.556	0.870
Type of nearest surface water[b]	Flowing water (creek/stream/canal)	Other (wetland, ditch, pond, lake, spring)	None within 3/4 mile	--	0.231	0.642	0.734
Distance to nearest surface water[b]	< 100 feet	100-500 feet	1/4 to 3/4 mile	None within 3/4 mile	0.559	0.761	0.531
Nitrate plus nitrite	Presence	Absence	--	--	0.680	**0.03**	**0.086**
Ammonia plus organic nitrogen	≥ 0.2 mg/L	< 0.2 mg/L	--	--	0.127	0.713	0.894
Chloride	≥ 20 mg/L	< 20 mg/L	--	--	**0.021**	0.682	0.632
Chloride to bromide ratios	≥ 300	< 300	--	--	**0.087**	0.973	**0.087**

[a] Other indicators include *Escherichia coli* , enterococci, and somatic and F-specific coliphage.

[b] p-values were determined by use of the two-sided Fisher's exact test.

and chloride-to-bromide ratios (Cl:Br). For example, the relation between sewage-system type and presence of enteric viruses was significant (p=0.088) because a greater number of virus detections were found at sites with septic systems than with sewerlines than would be expected by chance alone, as follows:

Virus detection	Sewerage-system type	
	Septic system	Sewerline
Not detected	13	16
Detected	7	2

Similarly, the presence of enteric viruses was significantly related to nitrate plus nitrite (p=0.086) and the presence of total coliforms to Cl:Br because of the following sample results:

Virus detection	Nitrate plus nitrite	
	Detected	Not detected
Not detected	3	19
Detected	4	5

Total coliform detection	Chloride-to-bromide ratio	
	≥ 300	< 300
Not detected	10	13
Detected	7	2

The relations between detections of microorganisms and continuous variables were examined by use of the Wilcoxon rank-sum test (Helsel and Hirsch, 1992, p. 118). None of the well-characteristic variables (casing depth, clay above screen, or age of well) or land-use variables (population density and percent of land use) were related to detections of any groups of microorganisms (table 7) at α=0.1. The only statistically significant relations were between total coliforms and dissolved organic carbon and between total coliforms and iron. Although the relations between total coliforms and ammonia plus organic nitrogen and chloride were not statistically significant, low p-values indicate some association that may be significant with a larger dataset. Similarly, the relation between other indicators and iron concentrations showed some association with a low p-value (p=0.1211). None of the continuous variables showed any association with detections of enteric viruses.

Cl:Br ratios were used by other investigators to describe the effects of anthropogenic sources on water quality (Whittemore, 1984, 1988; Eberts and others, 1990; Thomas, 2000; Jagucki and Darner, 2001). In areas where halite is not naturally occurring, natural waters have lower Cl:Br ratios than those waters affected by anthropogenic sources (such as road salt, domestic sewage, and water-softener backwash). From the results from a recent investigation, Thomas (2000) concluded that there are no natural sources of halite in the glacial sediments or in the near-surface bedrock in the study area. Brine is defined as water having a dissolved-solids concentration greater than 100,000 mg/L (Eberts and others, 1990) and is enriched in bromide relative to chloride. Brine is found in deep aquifers as fossil water and is often brought to the land surface as a byproduct of oil and gas production (Jagucki and Darner, 2001).

Binary mixing curves, based on the relations between chloride concentrations and Cl:Br ratios, were constructed to show how Cl:Br ratios change with the addition of increasing amounts of halite, brine, and sewage (figs. 3*A*-3*D*). In the mixing curve (fig. 3*A*), the background ground-water end-member was established by using the sample with the lowest chloride concentration (well 23). Other end-members were established as follows: halite from Knuth and others (1990), domestic sewage from Davis and others (1998) and Peavy (1978), and brine from Eberts and others (1990). The lines drawn between end-members show different mixtures of the two end-members; for example, the water from well 25 consists of a mixture of background ground water and halite, whereas well 9 consists of less background ground water and more halite. In a study of the glacial aquifer in southeast Michigan and the same study area reported in this report, Thomas (2000) established a theoretical maximum Cl:Br ratio of 400 for natural waters; those waters with Cl:Br ratios greater than 400 were likely affected by anthropogenic sources.

The placement on a binary mixing curve of the 32 wells for which water-quality data were available is shown on figure 3*A*. At least half of the wells were characterized as producing background ground water, and most of the other wells were characterized as producing mixtures of ground water and halite or ground water and sewage. Only one well had water with a chloride concentration and a Cl:Br ratio consistent with a mixture of ground water and brine (well 7). Thirteen wells had Cl:Br ratios greater than 400, indicating possible anthropogenic influences, as defined by Thomas (2000).

Detections of the three groups of microorganisms, described above, are shown on binary mixing curves (figs. 3*B*, 3*C*, and 3*D*). Of the nine wells having at least one positive sample for total coliforms, seven plotted near or above a Cl:Br ratio of 400 and were considered a mixture of ground water and halite or ground water and sewage (fig. 3*B*). The other two total-coliform-positive wells plotted in an area characterized as background ground water or a mixture of ground water and brine. Of the four wells positive for enterococci, two were characterized as having anthropogenic influence and two were not (fig. 3*C*). Three wells were positive for *E. coli* and two were positive for coliphage; of these five, only one (*E. coli* positive well) was characterized by anthropogenic influence based on a theoretical maximum Cl:Br ratio of 400 for natural waters (fig. 3*C*). Of the nine wells where viruses were detected, seven plotted near or above a Cl:Br ratio of 400, indicating anthropogenic influence (fig. 3*D*).

The results from two wells described above merit further discussion. Well 9 had multiple lines of evidence to confirm

Table 7. Relations between detections of microorganisms and continuous environmental and chemical-quality variables of small-public-supply wells in southeastern Michigan, July 1999 through July 2001.

[p-values were determined by use of the Wilcoxon rank-sum test; values in bold are significant at α=0.1; mi^2, square mile; µg/L, micrograms per liter; mg/L, milligrams per liter; <, value is less than concentration indicated]

Variable	Range	Total coliforms			*Escherichia coli*, enterococci, and (or) coliphage			Enteric viruses by cell culture or RT-PCR		
		Median			Median			Median		
		Detections	Non-detections	p-value	Detections	Non-detections	p-value	Detections	Non-detections	p-value
Casing depth (feet)	45 to 282	84	111	0.303	136	104	0.5506	102	111	0.706
Clay above screen (percent)	0 to 96	53	47	0.348	49	51	0.9868	49	51	0.643
Age of well as of Jan. 1999 (years)	0.8 to 44.6	20.5	15.3	0.606	12.7	20.0	0.3857	13.1	16.2	0.891
Population density (people per mi^2)[a]	6.6 to 4,400	470	740	0.261	340	675	0.6189	470	610	0.706
Percent of urban land use[a]	0 to 98.9	29.8	45.4	0.394	38.6	43.2	0.8285	45.5	46.5	0.959
Percent of agricultural land use[a]	0 to 71.4	5.8	1.3	0.472	3.6	0.6	0.4588	7.2	1.3	0.164
Percent for forest, open land, and water[a]	0 to 81.2	46.8	43.4	0.569	51.5	42.7	0.1689	41.8	45.8	0.631
Boron (µg/L)	19 to 500	29	37	0.390	29	37	0.218	30	33	0.586
Nitrogen, ammonia plus organic (mg/L)	<0.01 to 5.2	0.24	0.16	0.116	0.17	0.16	0.9433	0.16	0.18	0.471
Dissolved organic carbon (mg/L)	<0.33 to 3.9	1.6	0.86	**0.004**	1.4	0.94	0.3263	0.82	0.98	0.629
Chloride (mg/L)	2.2 to 109	33.9	11.3	0.103	4.9	17.6	0.2809	17.9	13.55	0.769
Iron (µg/L)	<10 to 4,120	1620	940	**0.062**	1550	940	0.1211	1260	970	0.722

[a] Value given is within a 500 meter radius of the well.

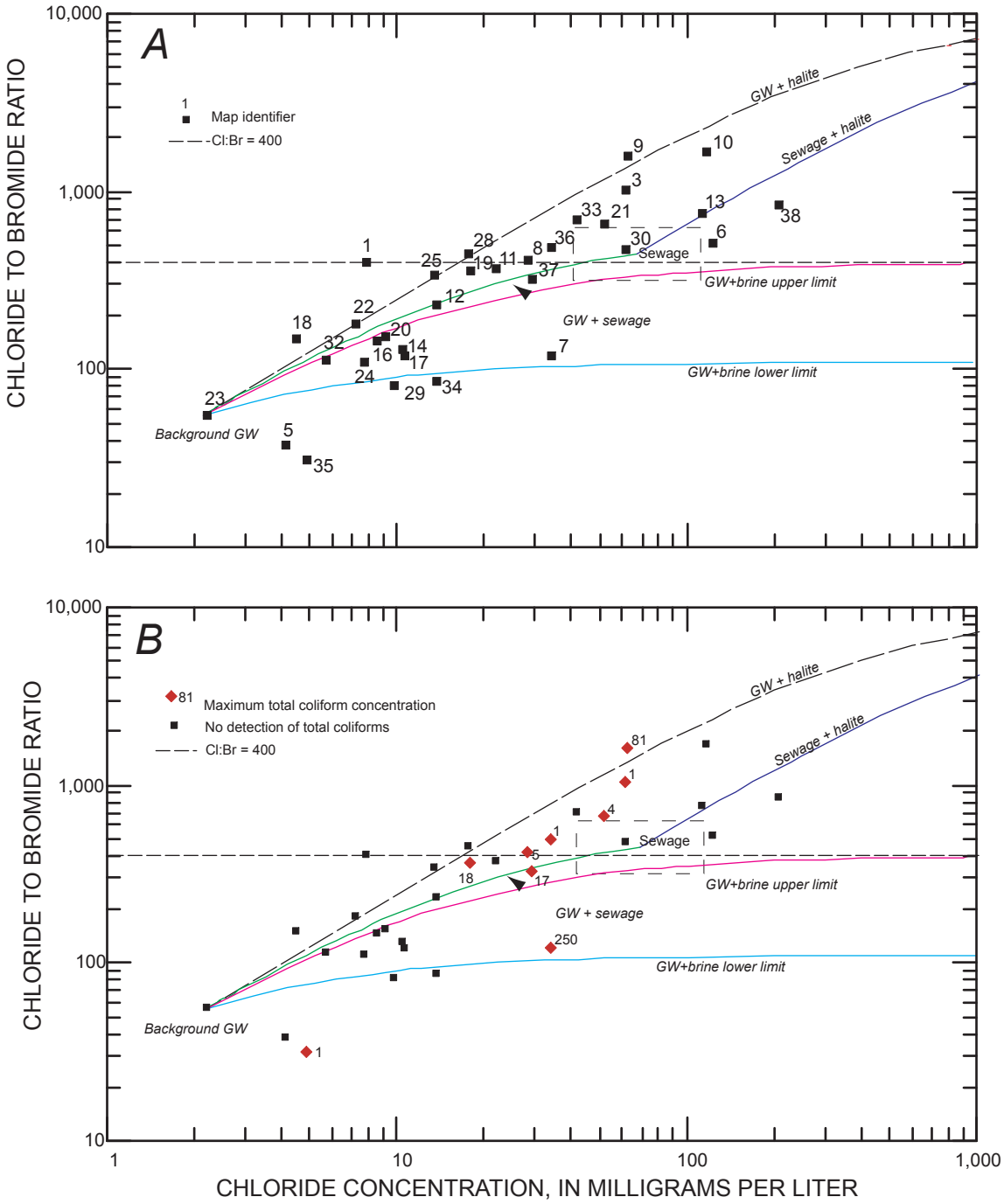
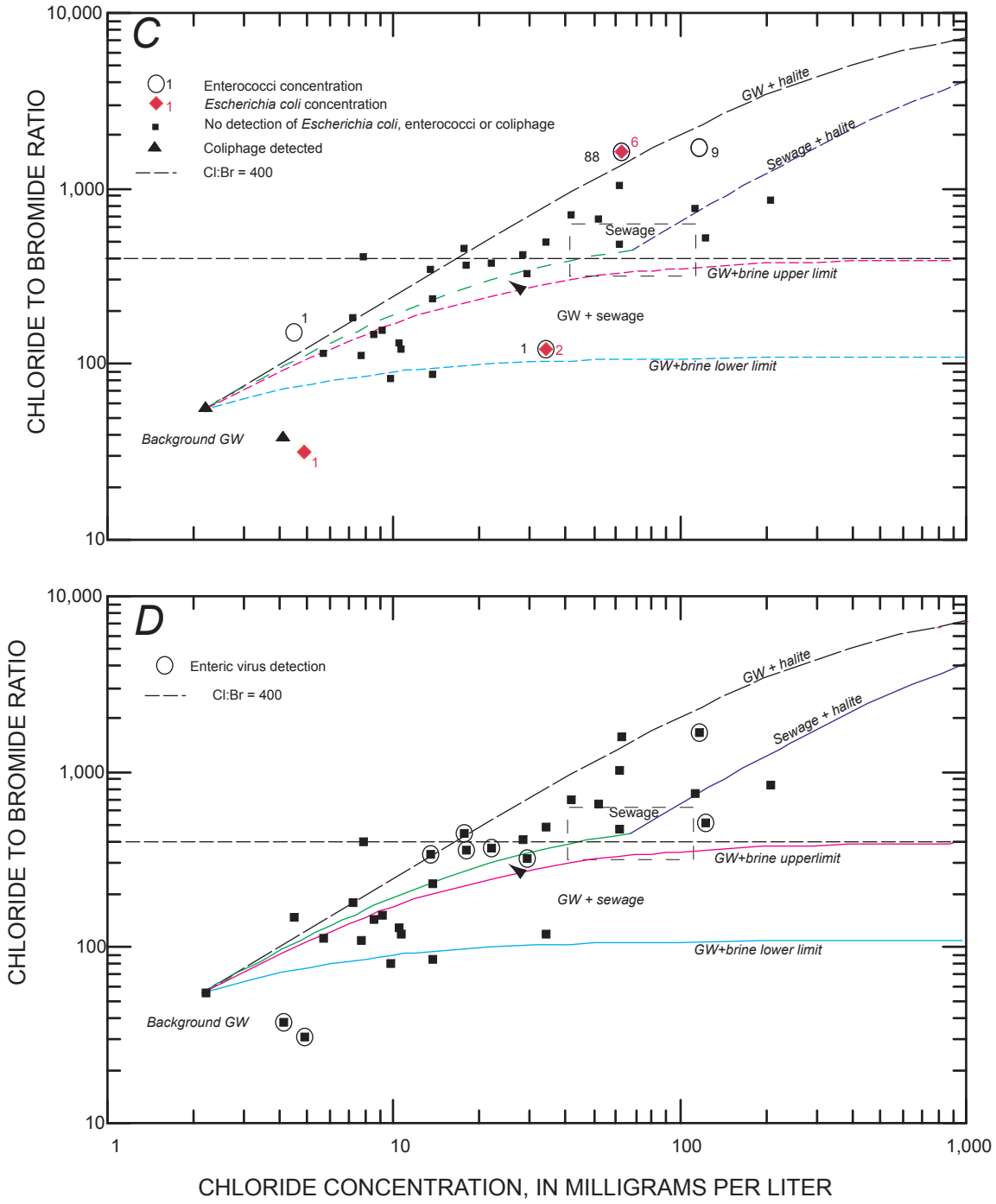


Figure 3. Chloride concentrations in relation to chloride to bromide (Cl:Br) ratios for small-public- supply wells sampled July 1999 through July 2001: *A*, Distribution of wells by map number, and at least one detection of *B*, total coliforms, *C*, enterococci, *Escherichia coli,* and (or) coliphage, and *D*, enteric viruses. (Halite end-member from Knuth and others, 1990; domestic sewage end-member from Davis and others, 1998, and Peavy, 1978; sewage has a Cl:Br ratio of 30–600 and chloride concentration of 37–101 mg/L (Peavy, 1978, as cited in Thomas, 2000); brine end-members from Eberts and others, 1990; a Cl:Br ratio of 400 is the theoretical maximum for natural water (Thomas, 2000).)



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the influence of anthropogenic sources—the presence of indicators (table 11), classification as a mixture of ground water and halite (fig. 3*A*), and high ammonia, ammonia plus organic nitrogen, and dissolved organic carbon concentrations (table 12). Although water from well 9 also had a high iron concentration, high iron is not considered as evidence of anthropogenic sources. In well 9, however, no viruses were found. Well 38 had the highest concentration of boron (506 µg/L) and was classified as a mixture of sewage and halite, yet no indicators or viruses were found. Boron is present in detergents, and elevated concentrations (above background levels) may be the result of anthropogenic sources.

Summary, Conclusions, and Suggestions for Future Study

Viruses have been identified as a threat to ground-water supplies in the United States. Insufficient monitoring information is available, however, on the occurrence of pathogenic viruses in ground water and the factors that affect the vulnerability of ground water to contamination. This result is especially apparent for small ground-water systems—those that serve less than 3,300 people—where monitoring requirements are less stringent than for larger systems. In the proposed Ground Water Rule (GWR), USEPA included the monitoring of indicators of fecal contamination (*E. coli*, coliphage, and enterococci), and identified viruses and bacteria as threats to ground-water supplies of all sizes. Hydrogeologic assessments of the vulnerability of a ground-water source to contamination are also a component of the GWR. Building on these two components of the proposed GWR, the U.S. Geological Survey (USGS), in cooperation with the U.S. Environmental Protection Agency-Office of Research and Development, National Exposure Research Laboratory (USEPA-NERL), in Cincinnati, Ohio, studied the occurrence of microbiological indicators of fecal contamination (“indicators”)—total coliforms, *Escherichia coli* (*E. coli*), enterococci, and F-specific and somatic coliphage—and enteric viruses in small public ground-water supplies and assessed the factors that affect their vulnerability to contamination. Although total coliforms are used to establish maximum contaminant levels for drinking-water regulations, they may or may not be of fecal origin. The information in this report can be used by those developing the proposed and final GWR.

Thirty-eight wells in southeastern Michigan were sampled from one to six times each for indicators and enteric viruses from July 1999 through July 2001. Samples were collected from a four-county area in small community and non-community wells that produce water from discontinuous sand and gravel aquifers. In some places, the aquifer is unconfined, but more often the aquifer is semiconfined or confined by poorly permeable glacial till. Analyses of samples for enteric viruses were done by use of two methods—reverse transcriptase-polymerase chain reaction (RT-PCR) and cell culture. The RT-PCR method detects the presence of viral RNA from specific target viruses. In this study, all samples were analyzed for enterovirus and hepatitis A virus (HAV) by use of either the multiplex or the single-reaction RT-PCR method. Some samples were analyzed early in the study for three additional viruses (reovirus, rotavirus, and Norwalk virus) by use of a multiplex RT-PCR method that was later dropped because it frequently failed quality-control tests. The cell-culture method determines the presence of culturable virus—mainly enterovirus and reovirus. Somatic and F-specific coliphage were analyzed by use of the single-agar layer (SAL) and presence and absence (P/A) methods. Analysis of samples for indicator bacteria included total coliforms and *E. coli* by use of the MI method and enterococci by use of the mEI method. Ancillary water-quality, environmental, geologic, and land-use data were collected and compiled for each well.

The inclusion of quality-control samples is important for interpreting results in any water-quality sampling program. In this study, it was shown that this inclusion is especially important when analyzing samples for enteric viruses by RT-PCR. Replicate pairs are samples collected at the same well on the same date. However, in the only replicate pair that contained a virus detection, the results did not match—one sample was positive and one was negative for HAV. This finding showed that results on the presence of enteric viruses in ground water are not always reproducible, even in samples collected at the same well on the same date. The inconsistency of results may be because viruses are usually present in low concentrations in ground water, and so the probability of detecting a virus in an aliquot of 2,000 L is also low.

Other quality-control samples are used to assess the percentage of potential false positive and false negative results. The highest potential false positive and false negative percentages were found for rotavirus (22.7 percent) and Norwalk virus (38.6 percent), respectively. These samples were analyzed by use of the multiplex RT-PCR method, which frequently resulted in quality-control problems. In another study where the multiplex RT-PCR method was used (Lieberman and others, 2002), overall potential false positive and false negative percentages were 6 and 14 percent, respectively. Potential false negative percentages for enterovirus and HAV were only 5.9 and 7.5 percent in this study; most of these samples were analyzed by use of the single-reaction RT-PCR method, and potential false negative results were caused by inhibition of the matrix spike. Using the single-reaction RT-PCR method, Borchardt and others (2003) found a similar potential false negative percentage caused by inhibition of the matrix spike.

After including the results from quality-control samples, enterovirus was found in four wells (10.5 percent) and hepatitis A virus (HAV) in five wells (13.2) by use of RT-PCR. In two wells, investigators found both enterovirus and HAV, but on different sampling dates. Enteric viruses by cell culture (culturable viruses) were found two wells (5.9 percent); viruses by use of RT-PCR were not found in either of these wells on any sampling date. Total coliforms, *E. coli*, entero-

cocci, and F-specific and somatic coliphage were found in 34.2, 10.5, 15.8, 5.9, and 5.9 percent, respectively, of the wells tested.

The percentages of enteric viruses found in this study by well can be compared to those found in other studies (table 1). Although other studies were done with a variety of sampling and analytical procedures and well-selection criteria, the comparisons are still warranted because they provide valuable information on the occurrence of indicators and pathogens in large and small systems. The percentage of detections of culturable viruses were in the same range as those in other studies except for phase 2 of one study where investigators targeted wells vulnerable to contamination (Lieberman and others, 2002; Dahling, 2002). For enterovirus by RT-PCR, detections in our study were in the same range as those found by Abbaszadegan, Stewart, and others (1999) and Davis and Witt (2000), greater than the percentage found by Borchardt and others (2003), and considerably less than that found by Lieberman and others (2002) and Fout and others (2003). In Borchardt and others (2003), household supplies were sampled, so a smaller contributing population may have been targeted. The second highest percentage of detections of HAV was found in this study among the studies reported. This percentage (13 percent) was higher than those found in studies of large public (Abbaszadegan, Stewart, and others, 1999) and household supplies (Borchardt and others, 2003), but not higher than that found in a study that targeted wells vulnerable to contamination (Lieberman and others, 2002; Fout and others, 2003).

The percentages of indicators found in this study by well can be compared to those found in other studies (table 1). The percentages were similar to the range of percentages found in two other studies where small noncommunity (Lindsey and others, 2002) and household wells (Borchardt and others, 2003) were sampled. In contrast, the percentage found in this study for total coliforms (34 percent) was greater than that found in a study of large public-supply wells (Abbaszadegan, Stewart, and others, 1999) (9.9 percent) and of small public-supply wells (Banks and others, 2001) (15 percent). In this study, F-specific coliphage were found in only 5.9 percent of the wells sampled. These results are similar to those found in other studies (Femmer, 2000; Banks and others, 2001; Lindsey and others, 2002; and Borchardt and other, 2003) where percentages were in the single digits, but less than those found in Abbaszadegan, Stewart, and others (1999) or Davis and Witt (2000). For somatic coliphage, the low percentage of detections found in this study (2.9 percent) was similar to those found in other studies, except for phase 2 of the Lieberman study (53 percent). *E. coli* and enterococci detections in this study were 10 and 16 percent, and in the mid-range among the studies reported.

This study reinforced the importance of repeat sampling from the same well because of the intermittent occurrence of indicators and viruses. In only 3 out of 18 wells were samples positive for an indicator on more than one date. In only two out of nine wells were samples positive for a virus on more than one date. In the large American Water Works Association Research Foundation study (Abbaszadegan, Stewart, and others, 1999), repeat sampling was recommended because when a well tested positive for one of the assays, it was likely to test positive in future sampling for another assay. In phase I of the Missouri study (Davis and Witt, 2000), an indicator or pathogen found during the first round of sampling was not found at the same well during the second round of sampling. Also in the Missouri study, when a culturable virus was found in a sample or in a well, no enteric virus was found by use of RT-PCR. In the present study, indicators were poor predictors of the presence of enteric viruses in individual samples. Out of 11 samples positive for enteric viruses, one had the co-occurrence of enterococci and another of total coliforms. On a per well basis, indicators were better predictors; more than half of the time, when a virus was detected an indicator also was detected. By using the detection of any indicator as a predictor for enteric viruses, the true-positive rate rose to 55.6 percent. This percentage was higher than using total coliforms alone (33.3 percent), or using *E. coli*, enterococci, or coliphage (all were 11.1 percent) as predictors of the presence of enteric viruses.

The relations between environmental or water-quality variables and the presence of indicators and enteric viruses were determined. Sewage-system type was found to be related to the presence of enteric viruses; more virus-positive samples were found at sites served by septic systems than those served by sewerlines. Similarly, Borchardt and others (2003) found that all of the virus-positive wells were in subdivisions served by septic systems. Sampling condition (whether the water sampled was directly from the aquifer or was stored in a tank), distance to septic, type of and distance to nearest surface-water body, well characteristics, or land use were not related to the presence of viruses or indicators. Abbaszadegan, Stewart, and others (1999) found a relation between the presence of indicators or pathogens and well construction and environmental variables; however, this was a large study of 448 wells. Among continuous water-quality variables, the only statistically significant relations were between total coliforms and dissolved organic carbon and between total coliforms and iron. Chloride or ammonia plus organic nitrogen concentrations as continuous variables were not statistically significant, but they showed some association with the presence of total coliforms that may be significant with a larger dataset. None of the continuous water-quality variables showed any association with detections of enteric viruses. Examining chloride as a categorical variable, however, a statistically significant relation between chloride concentrations $\geq$ 20 mgl/L and detections of total coliforms was found. Also, relations were statistically significant between chloride to bromide ratios (Cl:Br) $\geq$ 300 and detections of total coliforms or enteric viruses. Presence of nitrate and nitrite was related to the presence of some microorganisms. Although higher dissolved-organic carbon, nitrogen, or chloride concentrations are often associated with anthropogenic contamination, the reason for the association between iron and total coliforms is not known.

If Cl:Br ratios were used as a screening technique to identify waters influenced by anthropogenic sources and thus susceptible to fecal contamination, most of the total-coliform positive wells and virus-positive wells (88 percent each) would be properly identified. However, two out of four wells positive for enterococci (50 percent), two out of three positive for *E. coli* (67 percent), and both wells positive for coliphage were plotted as consisting of mostly background ground water, not strongly influenced by anthropogenic sources (figs. 2*B* and 2*C*). Although more data are needed, this preliminary study shows that Cl:Br ratios may be useful as a screening tool for total coliforms and enteric viruses, but not for all indicators.

Overall, if results for all viruses are combined, 9 of the 38 small public-supply wells were positive for enteric viruses (23.7 percent) by either cell culture or RT-PCR. These results confirm the contamination of small public-supply wells, at least on an intermittent basis. Results of this study show multiple lines of evidence are needed to predict the presence of viruses and protect public health. A data-collection program that would yield multiple lines of evidence might include repeat sampling several times a year for several different microbiological indicators of fecal contamination, measuring dissolved-organic carbon, nitrate plus nitrite, and (or) chloride concentrations, or determining Cl:Br ratios. The presence of a site served by a septic system is an indication that the well may be more vulnerable to contamination. In any sampling program for enteric viruses, however, a rigorous quality-assurance program with numerous quality-control samples would be needed for proper interpretation of enteric-virus results.

Future studies may focus on ground-water supplies vulnerable to contamination—those with previous detections of fecal microorganisms; high concentrations of water-quality constituents associated with fecal contamination; and (or) sources of contamination evident, such as a nearby septic system. This study could include repeat sampling at a well positive for an enteric virus by cell culture or RT-PCR to determine how frequently virus-positive samples are indicator negative. In addition, future studies could be designed to investigate the occurrence of indicators and pathogens in both large and small systems using consistent methods, well-selection criteria, and quality-assurance and quality-control requirements.

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Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001.

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	USGS site identification number	County	Population served[a]	Construction date	Casing depth (ft)	Clay above screen (ft)	Percent clay[b]	Sampling condition[c]	Sewerage type
1	**VIR093-1**	Livingston	Transient	Dec-90	55	20	36	Tank	Septic
2	**VIR093-2**	Livingston	200	Dec-72	114	90	79	Tank	Septic
				May-73	110	90	82		
3	**VIR093-3**	Livingston	95	Jan-67	74	38	51	Tank	Septic
4	**VIR093-4**	Livingston	150	Jun-78	80	52	65	Tank	Septic
				Aug-78	77	71	92		
5	**VIR093-5**	Livingston	Transient	Mar-86	150	120	80	Tank	Septic
6	**VIR099-1**	Macomb	Transient	Jul-90	72	66	92	GW	Septic
7	**VIR125-1**	Oakland	Transient	Sep-97	73	49	67	GW	Sewer, was septic
8	**VIR125-2**	Oakland	670	Feb-54	94	85	90	Tank	Sewer
				Sep-54	163	17	10		
9	**VIR125-3**	Oakland	112	Jun-93	64	34	53	Tank	Septic
10	**VIR125-4**	Oakland	80	Dec-88	60	50	83	GW	Sewer
11	**VIR125-5**	Oakland	935	Mar-74	211	43	20	GW	Septic
12	**VIR125-6**	Oakland	935	Aug-88	252	168	67	GW	Septic
13	**VIR125-7**	Oakland	100	Apr-67	45	1	2	Tank	Sewer
14	**VIR125-8**	Oakland	250	Apr-92	157	69	44	Tank	Septic
				Feb-93	154	64	42		
15	**VIR125-9**	Oakland	365	Jun-87	90	74	82	Tank	Sewer
				Aug-87	85	64	75		
16	**VIR125-10**	Oakland	30	Feb-98	198	0	0	GW	Sewer
				Jun-98	194	0	0		
17	**VIR125-11**	Oakland	500	Oct-87	105	70	67	Tank	Septic
18	**VIR125-12**	Oakland	290	May-69	208	78	38	Tank	Sewer
				1998	216	--	--		
19	**VIR125-13**	Oakland	236	Aug-73	216	88	41	Tank	Septic
				Jun-74	206	104	50		

Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued*.

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	USGS site identification number	County	Population served[a]	Construction date	Casing depth (ft)	Clay above screen (ft)	Percent clay[b]	Sampling condition[c]	Sewerage type
20	**VIR125-14**	Oakland	260	Oct-73	126	--	--	GW	Sewer
				Oct-73	125	59	47		
21	**VIR125-15**	Oakland	2929	May-77	84	30	36	GW	Sewer
22	**VIR125-16**	Oakland	750	Mar-73	117	30	26	GW	Sewer
				1978	84	84	100		
				Apr-97	138	75	54		
23	**VIR125-17**	Oakland	365	Jun-64	283	151	53	Tank	Septic
				Jan-65	281	107	38		
24	**VIR125-18**	Oakland	160	Sep-74	246	79	32	Tank	Sewer
				1978	--	--	--		
25	**VIR125-19**	Oakland	250	Jul-90	79	16	20	Tank	Septic
				Nov-90	75	16	21		
26	**VIR125-20**	Oakland	50	Jun-90	134	42	31	GW	Septic
27	**VIR125-21**	Oakland	935	Apr-74	83	46	55	Tank	Sewer
				Apr-80	84	0	0		
28	**VIR125-22**	Oakland	80	Sep-71	102	50	49	Tank	Sewer
				Sep-71	102	50	49		
29	**VIR125-23**	Oakland	101	Oct-91	81	25	31	Tank	Septic
30	**VIR125-24**	Oakland	118	Sep-73	113	87	77	Tank	Sewer
				Aug-74	106	65	62		
				Sep-74	114	79	69		
31	**VIR125-25**	Oakland	75	Jun-86	159	117	74	Tank	Sewer
				Jul-86	157	141	90		
32	**VIR125-26**	Oakland	290	Mar-56	239	159	67	Tank	Septic
				--	247	159	64		
33	**VIR125-27**	Oakland	300	Jul-79	95	70	74	GW	Sewer
34	**VIR125-28**	Oakland	282	May-70	80	60	75	GW	Sewer
35	**VIR125-30**	Oakland	Transient	Sep-60	137	18	13	Tank	Septic

Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	USGS site identification number	County	Population served[a]	Construction date	Casing depth (ft)	Clay above screen (ft)	Percent clay[b]	Sampling condition[c]	Sewerage type
36	**VIR161-1**	Livingston	Transient	May-86	63	38	60	Tank	Sewer, was septic
37	**VIR161-2**	Washtenaw	25	Dec-85	72	69	96	GW	Septic
38	**VIR161-3**	Washtenaw	Transient	Apr-84	122	39	32	GW	Septic

[a]Population served is given for community wells and nontransient noncommunity wells except where indicated as transient.

[b]Percent clay was calculated by dividing the clay above the screen (feet) by the total casing depth (feet).

[c]"Ground water" is water sampled directly from the aquifer, and "tank" is ground water stored in a tank before sampling.

Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	Distance to septic (ft)	Nearest surface-water body	Distance to nearest surface-water body	Population density (people per square mile within 500-meter radius of well)	Percentage of land use within 500-meter radius of the well		
					Urban	Agricultural	Forest, open land, and water
1	500	None	NA	557	45	11	43
2	100	Lake	50 ft	815	39	15	46
3	150	Lake	1/8 mi	315	26	0	74
4	--	Lake	3/4 mi	537	26	36	38
5	200	Lake	1/2 mi	46	5	0	63
6	75	Spring	300 ft	467	20	20	50
7	NA	Lake	300 ft	7	8	9	66
8	NA	Drainage ditch	300 ft	3,800	99	0	0
9	75	Drainage ditch	150 ft	471	38	15	47
10	NA	Pond	300 ft	4,365	49	5	42
11	300	Drainage ditch	100 ft	1,226	71	10	19
12	100	Drainage ditch	50 ft	272	51	8	40
13	NA	None	NA	434	19	0	81
14	1,000	Creek	1/2 mi	268	42	0	42
15	NA	Wetland	120 ft	471	46	27	25
16	NA	Lake	1/4-1/2 mi	742	24	0	75
17	50	None	NA	610	15	71	13
18	NA	None	NA	3,835	62	3	26
19	1,000	Pond	1/4 mi	945	57	8	36

Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	Distance to septic (ft)	Nearest surface-water body	Distance to nearest surface-water body	Population density (people per square mile within 500-meter radius of well)	Percentage of land use within 500-meter radius of the well		
					Urban	Agricultural	Forest, open land, and water
20	NA	Pond	50 ft	822	25	1	73
21	NA	Pond	30 ft	1,269	60	0	29
22	NA	None	NA	835	42	0	57
23	300	Lake	1/4 mi	2,430	48	2	50
24	NA	None	NA	106	27	0	44
25	300	Creek	50 ft	86	45	7	47
26	390	None	NA	209	25	0	53
27	NA	Wetland	200 ft	1,399	55	15	30
28	NA	Small drainage/wetland	100 ft	1,949	90	0	10
29	100	Wetland	100 ft	351	28	0	57
30	NA	Lake	1/4 mi	832	53	1	46
31	NA	Pond	15 ft	96	44	2	54
32	300	Canal	200 ft	2,569	77	0	23
33	NA	Pond	100 ft	1,694	71	0	16
34	NA	None	NA	878	44	4	52
35	110	Pond	300 ft	89	0	6	70

Table 8. Characteristics of small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; ft, feet; mi, miles; GW, ground water; NA, not applicable; --, no data]

Map number	Distance to septic (ft)	Nearest surface-water body	Distance to nearest surface-water body	Population density (people per square mile within 500-meter radius of well)	Percentage of land use within 500-meter radius of the well		
					Urban	Agricultural	Forest, open land, and water
36	NA	Lake	1/4 mi	832	27	0	48
37	300	None	NA	156	30	38	32
38	20	Stream	1/4 mi	30	10	0	38

Table 9. Oligonucleotide primers and probes used for virus detection in the reverse transcriptase-polymerase chain reaction (RT-PCR) method.

Name	Virus	Sequence (5'→3')
		PCR primers
MRD 13	Enterovirus	ACC GGA TGG CCA ATC CAA
MRD 14		CCT CCG GCC CCT GAA TG
MRD 154	Rotavirus	GCT GGC GTG TCT ATG GAT TCA
MRD 155		CAA AAC GGG AGT GGG GAG C
MRD 188	Reovirus	ACG TTG TCG CAA TGG AGG TGT
MRD 189		GTG CTG AGA TTG TTT TGT CCC AT
HPA2130	Hepatitis A	AGA GTG AAT GTT TAT CTT TCA GC
HPA2253		GGG ATC TGG AAC ATT CTG
MRD 211	Norwalk	CAA GCC CCC CAA GGT GAA T
MRD 212		GGC GCA TGG TTT GTT GAT TTC
		Hybridization probes
MRD 32	Enterovirus	ACT ACT TTG GGT GTC CGT GTT TC
ROTAP1	Rotavirus	TGC ACT TCG TTT AAG AAT GA
ROTAP2		TCG GTT AAG AAT GAA ACA AGT
ROTAP3		TGC ACT AAG AAT GAG GAT GA
ROTAP4		AAA GTA TTT CGC ACT CAG AAT
ROTAP5		TGC ATT AAG AAT GAG GAT GA
ROTAP6		TGT GTT AAG AAT GAG GAT GAA
REOP1	Reovirus	AAC GGT CAT CAG ATC G
REOP2		ACG GTC ATC AGG TCG
REOP3		AAT GGT CAT CAG GTC G
HPA2227P	Hepatitis A	GTT TTT CAA CAA CAG TTT C
HPAVP1382P		GTT TCT CAA CAA CAG TTT C
HPAP1431P		CTT CTC CAC CAC TGT TT
MRD 214	Norwalk	CCA GGG GGT ATG CAG GAA AC

Table 10. Quality-control samples for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Sample type	Description	Purpose
Replicate spikes (collected in the field, spiked in the lab)	Enterovirus (poliovirus vaccine) spiked into a second environmental sample during filtration	Recovery efficiency Inhibitor removal efficiency Cross-contamination of samples during processing and analysis
Equipment blank	Sterile water subjected to all phases of sample collection, handling, processing and analysis	Adequacy of equipment cleaning and disinfection and contamination potential during sample collection and processing
Negative-process control	40 milliliters of sterile water analyzed with each batch of five samples, beginning at inhibitor removal	Cross-contamination of samples during inhibitor removal and RT-PCR
Seeded process control	Virus mixes added to a duplicate negative process control with each batch of five samples before RT-PCR	Verify proper completion of RT-PCR reaction
Matrix spikes	Virus mixes added to duplicate environmental samples before RT-PCR	Indicate whether inhibitors of RT-PCR were present in environmental samples
PCR positive controls	RT-PCR products from positive control virus mixes added to sterile water (also used as hybridization positive control)	Verify the proper completion of the RT-PCR and hybridization reaction
PCR negative controls	Sterile water added at the RT-PCR step	Contamination during RT-PCR
Hybridization negative controls	Sterile water added to hybridization wells	Cross-contamination of sample during hybridization

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001.

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
1	11/2/1999	12.9	471	0.4	7.3	--	<1	<1	<1	<1
	3/21/2000	9.5	459	0.1	7.6	--	<1	<1	<1	<1
	7/26/2000	20.3	473	0.3	7.4	--	<1	<1	<1	<1
	1/17/2001	9.5	457	0.4	7.4	13	<1	<1	<1	<1
	1/17/2001[b]	--	--	--	--	--	--	--	--	--
	6/26/2001	12	486	0.4	7.4	16	<1	<1	<1	<1
2	7/7/1999	11.1	378	1.8	7.7	--	<1	<1	<1	<1
	12/2/1999	10.6	581	2.0	7.3	--	K8	<1	<1	<1
3	11/3/1999	10.8	735	0.1	7.2	--	<1	<1	<1	<1
	3/1/2000	10.6	706	0.1	7.2	--	<1	<1	<1	<1
	8/2/2000	11.4	742	0.1	7.1	--	E1	<1	<1	<1
	2/28/2001	10.4	706	0.1	7.2	0.9	<1	<1	<1	<1
	6/20/2001	10.8	745	0.1	6.9	9.8	<1	<1	<1	<1
	6/20/2001[b]	--	--	--	--	--	--	--	--	--
4	7/6/1999	11.8	405	3.3	7.0	--	K2	<1	<1	<1
5	3/13/2001	7.8	574	0.1	7.4	15	<1	<1	<1	<1
	4/2/2001	8.7	558	0.4	7.5	18	<1	<1	<1	<1
	4/2/2001[b]	--	--	--	--	--	--	--	--	--
	5/9/2001	10.7	588	0.2	7.3	15	<1	<1	<1	<1
	6/13/2001	11.3	594	0.1	7.2	13	<1	<1	<1	<1
	7/10/2001	12.9	604	0.1	7.0	2.7	<1	<1	<1	<1
6	10/26/1999	10	1,040	0.6	7.0	--	<1	<1	<1	<1
	2/28/2000	9.1	1,140	0.2	7.2	--	<1	<1	<1	<1
	7/24/2000	10.6	1,100	2.8	7.0	--	<1	<1	<1	<1
	12/4/2000	9.4	1,090	0.2	6.8	1.8	<1	<1	<1	<1
	12/4/2000[b]	--	--	--	--	--	--	--	--	--
	3/7/2001	9.2	1,150	0.2	7.1	4.1	<1	<1	<1	<1
7	***8/3/1999***	11.3	505	0.7	--	3.0	K250	K2	<1	<1
	12/7/1999	10.7	721	0.1	7.2	--	<1	<1	<1	<1
	5/8/2000	10.5	768	0.1	7.2	1.0	--	--	--	<1
	10/25/2000	11.3	737	0.1	7.0	--	<1	<1	K1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	3/20/2001	9.7	707	0.1	7.2	1.2	<1	<1	<1	<1
	3/20/2001[b]	--	--	--	--	--	--	--	--	--
8	7/27/1999	13.8	--	1.2	--	--	<1	<1	<1	<1
	11/30/1999	10.9	679	1.5	7.2	--	<1	<1	<1	<1
	4/18/2000	11	--	--	7.4	--	<1	<1	<1	<1
	4/18/2000[b]	--	--	--	--	--	--	--	--	--
	8/29/2000	11.5	717	1.3	7.1	--	K5	<1	<1	<1
	2/13/2001	11	741	3.0	7.2	25	<1	<1	<1	<1
9	***9/1/1999***	11.9	707	0.1	7.1	--	K3	<1	<1	<1
	11/16/1999	10.8	635	0.2	7.3	--	*K81*[a]	*K6*	*K88*	<1
	3/27/2000	10.5	689	0.1	7.3	38	<1	<1	K1	<1
	8/14/2000	12.6	713	0.2	7.7	--	<1	<1	<1	<1
	2/26/2001	10.7	689	0.1	7.2	55	<1	<1	<1	<1
	2/26/2001[b]	--	--	--	--	--	--	--	--	--
10	7/28/1999	13.4	1,150	0.1	6.8	--	<1	<1	K9	<1
	12/16/1999	11.5	1,170	0.1	7.0	--	<1	<1	<1	<1
	5/15/2000	12.3	1,110	0.1	7.0	--	<1	<1	<1	<1
	10/31/2000	12.1	1,100	0.1	7.1	--	<1	<1	<1	<1
	10/31/2000[b]	--	--	--	--	--	--	--	--	--
	1/31/2001	10.7	1,100	0.1	7.1	30	<1	<1	<1	<1
11	6/30/1999	11	--	--	--	22	<1	<1	<1	<1
	11/15/1999	11	618	<.1	7.3	--	<1	<1	<1	<1
	3/28/2000	11.5	621	<.1	7.3	--	<1	<1	<1	<1
	3/28/2000[b]	--	--	--	--	--	--	--	--	--
	8/9/2000	11	638	0.1	7.3	--	<1	<1	<1	<1
	11/6/2000	11.1	604	1.3	7.2	0.9	<1	<1	<1	<1
12	6/29/1999	11.7	577	0.2	7.3	--	<1	<1	<1	<1
	11/17/1999	11.5	556	0.2	7.4	--	<1	<1	<1	<1
	3/29/2000	11.7	558	0.1	7.3	--	<1	<1	<1	<1
	9/5/2000	10.7	561	0.1	7.3	--	<1	<1	<1	<1
	1/22/2001	11.2	562	0.1	7.3	16	<1	<1	<1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

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Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	1/22/2001[b]	--	--	--	--	--	--	--	--	--
13	11/1/1999	10.8	979	0.8	7.2	--	<1	<1	<1	<1
	2/22/2000	10	998	1.7	7.3	--	<1	<1	<1	<1
	2/22/2000[b]	--	--	--	--	--	--	--	--	--
	7/12/2000	10.6	994	0.1	7.3	--	<1	<1	<1	<1
	2/20/2001	10.1	999	1.1	7.0	15	<1	<1	<1	<1
	4/24/2001	10.6	1,000	0.4	7.0	15	<1	<1	<1	<1
14	8/18/1999	11.4	400	3.2	--	--	<1	<1	<1	<1
	1/12/2000	10	472	7.3	7.3	--	<1	<1	<1	<1
	6/12/2000	10.2	587	3.6	7.4	--	<1	<1	<1	<1
	11/28/2000	11.7	500	1.1	7.2	2.1	<1	<1	<1	<1
	11/28/2000[b]	--	--	--	--	--	--	--	--	--
	3/26/2001	5.3	566	0.4	7.1	1.2	<1	<1	<1	<1
15	7/21/1999	11.9	500	4.1	7.3	--	<1	<1	<1	<1
	12/14/1999	10.3	676	2.7	7.6	--	<1	<1	<1	<1
16	8/16/1999	22.1	412	3.6		--	<1	<1	<1	<1
	1/10/2000	11.1	332	4.5	7.4	--	<1	<1	<1	<1
	5/16/2000	11.4	531	2.6	7.4	--	<1	<1	<1	<1
	10/23/2000	11.3	536	2.9	7.2	--	<1	<1	<1	<1
	6/11/2001	11.5	533	2.6	7.3	20	<1	<1	<1	<1
	6/11/2001[b]	--	--	--	--	--	--	--	--	--
17	8/2/1999	16.5	550	1.6	--	--	<1	<1	<1	<1
	2/9/2000	9.7	683	0.2	7.2	--	<1	<1	<1	<1
	8/21/2000	21.9	712	0.3	7.3	--	<1	<1	<1	<1
	11/15/2000	10.5	658	0.1	7.0	5.4	<1	<1	<1	<1
	11/15/2000[b]	--	--	--	--	--	--	--	--	--
	3/28/2001	7.8	667	0.6	7.2	20	<1	<1	<1	<1
18	8/9/1999	12	400	2.1	7.5	--	--	--	--	<1
	12/20/1999	11.9	583	1.5	7.3	--	<1	<1	<1	<1
	5/17/2000	11.4	852	1.3	7.3	14	<1	<1	<1	<1
	11/1/2000	11.4	573	2.1	7.1	--	<1	<1	K1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

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Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	11/1/2000[b]	--	--	--	--	--	--	--	--	--
	5/23/2001	11.6	588	2.2	7.2	13	<1	<1	<1	<1
19	10/5/1999	10.3	588	2.8	7.3	--	K18	<1	<1	<1
	2/2/2000	9.9	579	1.9	7.4	--	<1	<1	<1	<1
	2/2/2000[b]	--	--	--	--	--	--	--	--	--
	6/13/2000	10.1	586	0.1	7.4	--	<1	<1	<1	<1
	2/14/2001	10.4	572	2.2	7.4	14	<1	<1	<1	<1
	4/10/2001	10.2	579	2.3	7.3	12	<1	<1	<1	<1
20	8/17/1999	11.9	380	1.0	--	--	<1	<1	<1	<1
	1/11/2000	11.1	456	2.2	7.5	--	<1	<1	<1	<1
	5/30/2000	11.2	515	0.9	7.5	--	<1	<1	<1	<1
	11/29/2000	10.4	496	1.1	7.2	7.5	<1	<1	<1	<1
	11/29/2000[b]	--	--	--	--	--	--	--	--	--
	3/27/2001	9.9	495	3.6	7.4	7.8	<1	<1	<1	<1
21	11/9/1999	11.7	554	0.1	7.5	--	<1	<1	<1	<1
	4/24/2000	11.6	564	0.1	7.6	12	<1	<1	<1	<1
	8/1/2000	11.6	584	0.3	7.3	--	K4	<1	<1	<1
	12/6/2000	11.4	576	0.1	7.3	26	<1	<1	<1	<1
	3/12/2001	11.5	577	0.1	7.4	20	<1	<1	<1	<1
	3/12/2001[b]	--	--	--	--	--	--	--	--	--
22	10/6/1999	10.3	491	0.1	7.4	7.7	<1	<1	<1	<1
	1/18/2000	10.5	429	1.1	7.5	--	<1	<1	<1	<1
	5/31/2000	10.6	503	0.1	7.4	--	<1	<1	<1	<1
	8/15/2000	10.9	498	0.2	7.3	--	<1	<1	<1	<1
	3/21/2001	10.3	486	1.5	7.5	13	<1	<1	<1	<1
	3/21/2001[b]	--	--	--	--	--	--	--	--	--
23	10/25/1999	11.4	525	2.1	7.3	--	<1	<1	<1	<1
	2/15/2000	11.2	526	1.5	7.4	--	<1	<1	<1	<1
	2/15/2000[b]	--	--	--	--	--	--	--	--	--
	6/27/2000	11.4	528	0.1	7.4	--	<1	<1	<1	<1
	2/7/2001	11.3	512	2.6	7.2	15	<1	<1	<1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	6/5/2001	11.5	519	2.2	7.3	24	<1	<1	<1	<1
24	6/22/1999	10.7	470	0.4	7.4	--	<1	<1	<1	<1
	11/10/1999	11	463	3.2	7.5	--	<1	<1	<1	<1
	4/17/2000	10.6	468	0.7	7.5	--	<1	<1	<1	<1
	8/28/2000	10.7	462	4.8	7.3	--	<1	<1	<1	<1
	2/27/2001	12.3	459	0.7	7.6	9.5	<1	<1	<1	<1
	2/27/2001[b]	--	--	--	--	--	--	--	--	--
25	10/4/1999	11	521	2.3	7.4	--	<1	<1	<1	<1
	1/26/2000	10.2	514	1.3	7.2	14	<1	<1	<1	<1
	1/26/2000[b]	--	--	--	--	--	--	--	--	--
	6/14/2000	10.7	536	2.2	7.3	--	<1	<1	<1	<1
	2/21/2001	10.6	535	3.2	7.3	24	<1	<1	<1	<1
	4/11/2001	10.6	520	2.8	7.4	17	<1	<1	<1	<1
26	8/31/1999	15.6	798	0.3	7.1	--	<1	<1	<1	<1
	1/3/2000	10.5	679	0.5	7.2	--	*K1*[a]	<1	*K2*	<1
27	7/20/1999	13.2	320	2.2	7.1	--	<1	<1	K4	<1
	12/9/1999	11.3	960	0.5	7.0	--	<1	<1	<1	<1
28	11/22/1999	11.2	569	3.1	7.4	--	<1	<1	<1	<1
	2/29/2000	10.8	558	2.3	7.5	--	<1	<1	<1	<1
	2/29/2000[b]	--	--	--	--	--	--	--	--	--
	7/19/2000	11.7	571	2.9	7.4	--	<1	<1	<1	<1
	3/6/2001	10.6	560	7.5	7.4	2.0	<1	<1	<1	<1
	6/27/2001	11.7	589	8.6	7.1	0.2	<1	<1	<1	<1
29	6/23/1999	10.8	692	0.1	7.3	--	<1	<1	<1	<1
	12/1/1999	10.9	697	0.1	7.4	--	<1	<1	<1	<1
	4/19/2000	10.9	700	0.1	7.6	--	<1	<1	<1	<1
	9/20/2000	10.6	727	0.1	7.4	--	<1	<1	<1	<1
	11/20/2000	10.5	714	0.1	7.1	20	<1	<1	<1	<1
	4/4/2001	10.4	688	0.1	7.4	20	<1	<1	<1	<1
	4/4/2001[b]	--	--	--	--	--	--	--	--	--
30	8/11/1999	12	510	2.6	7.6	--	<1	<1	<1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	1/5/2000	11.2	633	1.7	7.6	--	<1	<1	<1	<1
	5/23/2000	12.6	652	2.2	7.6	11	<1	<1	<1	<1
	11/27/2000	11.2	708	3.9	7.3	3.9	<1	<1	<1	<1
	11/27/2000[b]	--	--	--	--	--	--	--	--	--
	5/22/2001	10.8	645	1.9	7.4	8.6	<1	<1	<1	<1
31	8/4/1999	12	--	2.3	--	15	K1	K1	<1	<1
	12/15/1999	10.8	588	3.7	7.3	--	<1	<1	<1	<1
32	10/27/1999	11.3	484	2.3	7.4	--	<1	<1	<1	<1
	2/16/2000	11	463	1.0	7.6	--	<1	<1	<1	<1
	6/28/2000	11.5	489	1.3	7.5	--	<1	<1	<1	<1
	1/16/2001	10.8	489	1.7	7.4	12	<1	<1	<1	<1
	6/4/2001	11.2	479	0.9	7.4	13	<1	<1	<1	<1
	6/4/2001[b]	--	--	--	--	--	--	--	--	--
33	1/23/2001	10.8	737	0.1	7.2	0.2	<1	<1	<1	<1
	3/5/2001	10.5	775	0.1	7.2	0.8	<1	<1	<1	<1
	3/5/2001[b]	--	--	--	--	--	--	--	--	--
	4/26/2001	10.7	849	0.1	7.0	0.2	<1	<1	<1	<1
	5/29/2001	10.6	805	0.1	7.2	--	<1	<1	<1	<1
	6/25/2001	10.7	755	0.1	7.1	0.1	<1	<1	<1	<1
34	2/6/2001	10.6	528	0.2	7.5	28	<1	<1	<1	<1
	3/19/2001	10.4	531	0.1	7.4	26	<1	<1	<1	<1
	3/19/2001[b]	--	--	--	--	--	--	--	--	--
35	3/14/2001	10.4	481	0.1	7.5	22	<1	<1	<1	<1
	4/3/2001	10.2	484	0.1	7.4	24	K1	K1	<1	<1
	4/3/2001[b]	--	--	--	--	--	--	--	--	--
	5/7/2001	10.7	503	0.1	7.3	26	<1	<1	<1	<1
	6/12/2001	10.3	509	0.1	7.3	22	<1	<1	<1	<1
	7/9/2001	10.4	513	0.3	7.2	17	<1	<1	<1	<1
36	11/8/1999	11.8	733	5.4	7.6	--	K1	<1	<1	<1
	3/8/2000	11.4	707	4.0	6.9	--	<1	<1	<1	<1
	7/17/2000	13.2	796	2.8	7.7	--	<1	<1	<1	<1

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Water temperature (°C)	Specific conductance (µS/cm)	Oxygen, dissolved (mg/L)	pH (standard units)	Turbidity (NTU)	Total coliforms (col/100mL)	*Escherichia coli* (col/100mL)	Enterococci (col/100mL)	Coliphage, F-specific, SAL (plaques/100mL)
	1/3/2001	10.4	647	3.5	6.9	--	<1	<1	<1	<1
	1/3/2001[b]	--	--	--	--	--	--	--	--	--
	7/23/2001	13.6	564	3.7	6.7	0.7	<1	<1	<1	<1
37	9/29/1999	12.2	753	<.1	7.1	17	<1	<1	<1	<1
	2/8/2000	11.9	767	0.1	7.2	--	<1	<1	<1	<1
	2/8/2000[b]	--	--	--	--	--	--	--	--	--
	6/26/2000	12	813	0.1	7.1	--	K17	<1	<1	<1
	5/21/2001	12.3	862	0.1	7.1	28	<1	<1	--	<1
	7/11/2001	12.1	891	0.1	6.7	37	<1	<1	<1	<1
38	9/28/1999	11.9	1,440	<.1	7.4	10	<1	<1	<1	<1
	1/4/2000	10.5	1,440	0.1	7.5	--	<1	<1	<1	<1
	5/22/2000	11.2	1,420	0.3	7.4	13	<1	<1	<1	<1
	8/22/2000	12.6	1,410	0.3	7.2	--	<1	<1	<1	<1
	6/18/2001	13	1,540	3.2	7.3	8.1	<1	<1	<1	<1
	6/18/2001[b]	--	--	--	--	--	--	--	--	--

[a] Italics indicate the detection of more than one bacterial and (or) viral indicator in the same sample.

[b] Replicate samples were spiked with enterovirus.

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
1	11/2/1999	<1	--	--	--	--	--	A	A	A	A	A
	3/21/2000	<1	--	--	--	--	--	A	A	A	A	A
	7/26/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	1/17/2001	<1	A	A	A	A	--	A	A	--	--	--
	1/17/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
	6/26/2001	<1	A	A	A	A	--	A	A	--	--	--
2	7/7/1999	<1	--	--	--	--	<1.0	I	A	I	I	I
	12/2/1999	<1	--	--	--	--	<1.0	A	A	A	I	I
3	11/3/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	3/1/2000	<1			A	A	--	A	A	A	A	A
	8/2/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	2/28/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/20/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/20/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
4	7/6/1999	<1	--	--	--	--	<1.0	I	I	I	I	I
5	3/13/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/2/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/2/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
	5/9/2001	<1	A	A	**P**	A	--	A	A	--	--	--
	6/13/2001	<1	A	A	A	A	--	A	**P**	--	--	--
	7/10/2001	<1	A	A	A	A	--	A	A	--	--	--
6	10/26/1999	<1	--	--	--	--	<1.0	A	**P**	A	A	A
	2/28/2000	<1	--	--	A	A	--	A	A	A	A	A
	7/24/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	12/4/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	12/4/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	3/7/2001	<1	A	A	A	A	--	A	A	--	--	--
7	***8/3/1999***	<1	--	--	--	--	<1.0	A	A	A	A	I
	12/7/1999	<1	--	--	--	--	<1.0	A	A	A	I	I
	5/8/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	10/25/2000	<1	A	A	A	A	<1.0	A	I	--	--	--

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	3/20/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/20/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
8	7/27/1999	<1	--	--	--	--	<1.0	I	A	I	I	I
	11/30/1999	<1	--	--	--	--	--	A	A	A	A	A
	4/18/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	4/18/2000[b]	--	--	--	--	--	PS	PS	A	A	A	A
	8/29/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	2/13/2001	<1	A	A	A	A	--	A	A	--	--	--
9	***9/1/1999***	<1	--	--	--	--	<1.0	A	A	A	A	A
	11/16/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	3/27/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	8/14/2000	<1	A	A	A	A	<1.0	A	I	A	I	I
	2/26/2001	<1	A	A	A	A	--	A	I	--	--	--
	2/26/2001[b]	--	--	--	--	--	--	PS	I	--	--	--
10	7/28/1999	<1	--	--	--	--	**P**	A	A	A	A	I
	12/16/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	5/15/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	10/31/2000	<1	A	A	A	A	<1.0	A	I	--	--	--
	10/31/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	1/31/2001	<1	A	A	A	A	--	A	A	--	--	--
11	6/30/1999	<1	--	--	--	--	<1.0	I	**P**	A	A	I
	11/15/1999	<1	--	--	--	--	<1.0	**P**	A	A	A	I
	3/28/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	3/28/2000[b]	--	--	--	--	--	PS	PS	A	A	A	A
	8/9/2000	<1	A	A	A	A	<1.0	A	I	A	I	I
	11/6/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
12	6/29/1999	<1	--	--	--	--	<1.0	I	A	A	A	I
	11/17/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	3/29/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	9/5/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	1/22/2001	<1	A	A	A	A	--	A	A	--	--	--

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	1/22/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
13	11/1/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	2/22/2000	<1	--	--	A	A	--	A	A	A	I	I
	2/22/2000[b]	--	--	--	--	--	PS	PS	A	A	I	I
	7/12/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	2/20/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/24/2001	<1	A	A	A	A	--	A	A	--	--	--
14	8/18/1999	<1	--	--	--	--	<1.0	A	A	I	I	I
	1/12/2000	<1	--	--	A	A	--	A	A	I	I	I
	6/12/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/28/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/28/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	3/26/2001	<1	A	A	A	A	--	A	A	--	--	--
15	7/21/1999	<1	--	--	--	--	<1.0	I	A	I	I	I
	12/14/1999	<1	--	--	--	--	--	A	A	A	I	I
16	8/16/1999	<1	--	--	--	--	<1.0	A	A	I	I	I
	1/10/2000	<1	--	--	A	A	--	A	A	I	I	I
	5/16/2000	<1	A	A	A	A	<1.0	A	I	A	A	A
	10/23/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	6/11/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/11/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
17	8/2/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	2/9/2000	<1	--	--	A	A	--	A	A	--	--	--
	8/21/2000	<1	A	A	A	A	<1.0	A	A	A	I	I
	11/15/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/15/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	3/28/2001	<1	A	A	A	A	--	A	A	--	--	--
18	8/9/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	12/20/1999	<1	--	--	--	--	--	A	A	A	A	A
	5/17/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	11/1/2000	<1	A	A	A	A	<1.0	A	I	--	--	--

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	11/1/2000[b]	--	--	--	--	--	--	PS	I	--	--	--
	5/23/2001	<1	A	A	A	A	--	A	A	--	--	--
19	10/5/1999	<1	--	--	--	--	<1.0	A	A	I	I	I
	2/2/2000	<1	--	--	A	A	<1.0	A	A	I	I	I
	2/2/2000[b]	--	--	--	--	--	PS	PS	A	I	I	I
	6/13/2000	<1	A	A	A	A	<1.0	A	I	--	--	--
	2/14/2001	<1	A	A	A	A	--	A	I	--	--	--
	4/10/2001	<1	A	A	A	A	--	A	**P**	--	--	--
20	8/17/1999	<1	--	--	--	--	<1.0	A	A	I	I	I
	1/11/2000	<1	--	--	A	A	--	A	A	I	I	I
	5/30/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	11/29/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/29/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	3/27/2001	<1	A	A	A	A	--	A	A	--	--	--
21	11/9/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	4/24/2000	<1	A	A	A	A	--	A	A	A	A	A
	8/1/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	12/6/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	3/12/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/12/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
22	10/6/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	1/18/2000	<1	--	--	A	A	--	A	A	A	A	A
	5/31/2000	<1	A	A	A	A	<1.0	A	I	--	--	--
	8/15/2000	<1	A	A	A	A	<1.0	A	I	A	I	I
	3/21/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/21/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
23	10/25/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	2/15/2000	<1			A	**P**	<1.0	A	A	A	I	I
	2/15/2000[b]	--	--	--	--	--	PS	PS	A	A	I	I
	6/27/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	2/7/2001	<1	A	A	A	A	--	A	A	--	--	--

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	6/5/2001	<1	A	A	**P**	A	--	A	A	--	--	--
24	6/22/1999	<1	--	--	--	--	<1.0	I	A	A	A	I
	11/10/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	4/17/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	8/28/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	2/27/2001	<1	A	A	A	A	--	A	A	--	--	--
	2/27/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
25	10/4/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	1/26/2000	<1	--	--	A	A	<1.0	A	I	--	--	--
	1/26/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	6/14/2000	<1	A	A	A	A	**P**	A	A	--	--	--
	2/21/2001	<1	A	A	A	A	--	A	I	--	--	--
	4/11/2001	<1	A	A	A	A	--	A	A	--	--	--
26	8/31/1999	<1	--	--	--	--	<1.0	A	A	I	I	I
	1/3/2000	<1	--	--	A	A	<1.0	A	I	I	I	I
27	7/20/1999	<1	--	--	--	--	<1.0	I	I	I	I	I
	12/9/1999	<1	--	--	--	--	--	A	A	A	A	A
28	11/22/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	2/29/2000	<1	--	--	A	A	--	A	**P**	A	A	A
	2/29/2000[b]	--	--	--	--	--	PS	PS	A	A	A	A
	7/19/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	3/6/2001	<1	A	A	A	A	--	**P**	A	--	--	--
	6/27/2001	<1	A	A	A	A	--	A	A	--	--	--
29	6/23/1999	<1	--	--	--	--	<1.0	I	A	A	A	I
	12/1/1999	<1	--	--	--	--	--	A	A	A	I	I
	4/19/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	9/20/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/20/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	4/4/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/4/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
30	8/11/1999	<1	--	--	--	--	<1.0	A	A	I	I	I

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	1/5/2000	<1	--	--	A	A	--	A	A	I	I	I
	5/23/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	11/27/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	11/27/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	5/22/2001	<1	A	A	A	A	--	A	A	--	--	--
31	8/4/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	12/15/1999	<1	--	--	--	--	<1.0	A	A	A	I	I
32	10/27/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	2/16/2000	<1	--	--	A	A	--	A	A	A	I	I
	6/28/2000	<1	A	A	A	A	<1.0	A	A	--	--	--
	1/16/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/4/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/4/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
33	1/23/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/5/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/5/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
	4/26/2001	<1	A	A	A	A	--	A	A	--	--	--
	5/29/2001	<1	--	--	--	--	--	I	A	--	--	--
	6/25/2001	<1	A	A	A	A	--	A	A	--	--	--
34	2/6/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/19/2001	<1	A	A	A	A	--	A	A	--	--	--
	3/19/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
35	3/14/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/3/2001	<1	A	A	A	A	--	A	A	--	--	--
	4/3/2001[b]	--	--	--	--	--	--	PS	I	--	--	--
	5/7/2001	<1	A	A	A	A	--	**P**	A	--	--	--
	6/12/2001	<1	A	A	A	A	--	A	A	--	--	--
	7/9/2001	<1	A	A	A	A	--	A	A	--	--	--
36	11/8/1999	<1	--	--	--	--	<1.0	A	A	A	A	I
	3/8/2000	<1	--	--	A	A	--	A	A	A	A	A
	7/17/2000	<1	A	A	A	A	<1.0	A	A	--	--	--

Table 11. Field water-quality measurements and microbiological data for small-public-supply wells sampled in July 1999 through July 2001—*Continued.*

[Dates in bold and italics indicate detection of bacterial indicators and (or) coliphage on more than one occasion at the same well; shaded areas indicate replicate pairs, the regular and replicate spike sample collected on the same date at the same well. Abbreviations: col/100mL, colonies per 100 milliliters; plaques/100mL, plaques per 100 milliliters; SAL, single agar layer; P/A, presence/absence; --, not done; A, absent; P, present; NTU, Nephelometric Turbidity Units; K, results based on colony count outside the ideal range of 20-80 colonies per 100 milliliters for total coliforms and *Escherichia coli* and 20-60 colonies per 100 milliliters for enterococci; E, estimate; <, less than; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; PS, present in replicate spike sample; I, inconclusive because of failure to pass quality-assurance checks]

Well number	Sampling date	Coliphage, somatic, SAL (plaques/ 100mL)	Coliphage, F-specific (P/A per 100mL)	Coliphage, somatic (P/A per 100mL)	Coliphage, F-specific (P/A per 1L)	Coliphage, somatic (P/A per 1L)	Culturable virus (P/A per 500L)	Enterovirus (P/A per 50L)	Hepatitis A virus (P/A per 50L)	Reovirus (P/A per 50L)	Rotavirus (P/A per 50L)	Norwalk (P/A per 50L)
	1/3/2001	<1	--	--	--	--	--	A	A	--	--	--
	1/3/2001[b]	--	--	--	--	--	--	PS	A	--	--	--
	7/23/2001	<1	A	A	A	A	--	A	A	--	--	--
37	9/29/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	2/8/2000	<1	--	--	A	A	--	A	A	--	--	--
	2/8/2000[b]	--	--	--	--	--	--	PS	A	--	--	--
	6/26/2000	<1	A	A	A	A	<1.0	**P**	A	--	--	--
	5/21/2001	<1	A	A	A	A	--	A	A	--	--	--
	7/11/2001	<1	A	A	A	A	--	A	A	--	--	--
38	9/28/1999	<1	--	--	--	--	<1.0	A	A	A	A	A
	1/4/2000	<1	--	--	A	A	--	I	A	I	I	I
	5/22/2000	<1	A	A	A	A	<1.0	A	A	A	A	A
	8/22/2000	<1	A	A	A	A	<1.0	A	I	A	I	I
	6/18/2001	<1	A	A	A	A	--	A	A	--	--	--
	6/18/2001[b]	--	--	--	--	--	--	PS	A	--	--	--

[a] Italics indicate the detection of more than one bacterial and (or) viral indicator in the same sample.

[b] Replicate samples were spiked with enterovirus.

Table 12. Chemical-quality data for small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001.

[USGS, U.S. Geological Survey; --, no data; <, less than; mg/L, milligrams per liter; µg/L, micrograms per liter; °C, degrees Celsius; E, estimate]

Well number	Sampling date	Bicarbonate (mg/L)	Nitrogen, ammonia (mg/L)	Nitrogen, nitrite (mg/L)	Nitrogen, ammonia + organic (mg/L)	Nitrogen, nitrate plus nitrite (mg/L)	Phosphorus (mg/L)	Phosphorus, ortho (mg/L)	Carbon, dissolved organic (mg/L)	Calcium (mg/L)
1	7/26/2000	329	0.095	<.010	0.13	<.050	<.006	<.010	0.81	63.3
2	--	--	--	--	--	--	--	--	--	--
3	8/2/2000	306	<.020	<.010	E.07	<.050	<.006	<.010	1.0	81.9
4	--	--	--	--	--	--	--	--	--	--
5	6/13/2001	400	0.185	<.006	0.21	<.050	<.006	<.020	0.77	74.1
6	7/25/2000	381	<.020	<.010	<.10	0.343	E.003	<.010	<.33	117
7	10/25/2000	452	<.041	E.003	<.10	E.033	<.006	<.018	0.72	93.8
8	8/29/2000	391	0.178	<.010	0.24	<.050	0.008	<.010	1.6	99.9
9	8/14/2000	321	4.65	<.010	5.4	<.050	0.027	0.038	4.0	77.7
	2/26/2001	--	4.31	<.006	5.0	<.047	0.029	0.025	3.8	--
10	10/31/2000	404	E.039	E.003	0.17	E.029	<.006	<.018	1.7	128
11	11/6/2000	311	E.040	E.004	0.11	<.047	E.004	<.081	0.53	84.0
12	9/5/2000	308	0.030	<.010	<.10	<.050	<.006	<.010	0.57	77.4
13	7/12/2000	353	0.099	<.010	0.16	<.050	<.006	<.010	0.77	96.4
14	6/12/2000	356	0.131	<.010	0.21	<.050	<.006	0.012	0.78	71.9
15	--	--	--	--	--	--	--	--	--	--
16	10/23/2000	306	0.133	<.006	0.16	<.047	<.006	<.018	0.86	69.7
17	3/28/2001	250	0.112	<.006	0.16	<.047	0.010	E.011	0.94	96.4
18	11/1/2000	378	<.041	<.006	E.06	0.103	<.006	<.018	0.67	87.2
19	6/13/2000	336	0.282	<.010	0.37	<.050	<.006	<.010	1.6	71.8
20	5/30/2000	252	--	--	--	--	--	--	1.1	63.5
21	8/1/2000	237	0.192	<.010	0.27	<.050	<.006	0.016	1.2	61.7
22	5/31/2000	578	0.100	<.010	0.20	<.050	<.006	<.010	1.2	68.8
23	6/27/2000	353	0.174	<.010	0.25	<.050	E.003	<.010	1.4	74.8
24	8/28/2000	271	0.355	<.010	0.42	<.050	0.010	<.010	0.92	51.5
25	6/14/2000	304	0.089	<.010	0.14	<.050	<.006	<.010	0.82	64.2
26	--	--	--	--	--	--	--	--	--	--
27	--	--	--	--	--	--	--	--	--	--
28	7/19/2000	329	<.020	<.010	<.10	0.071	<.006	<.010	0.51	69.7
	6/27/2001	337	--	--	--	--	--	--	--	72.5

Table 12. Chemical-quality data for small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; --, no data; <, less than; mg/L, milligrams per liter; µg/L, micrograms per liter; °C, degrees Celsius; E, estimate]

Well number	Sampling date	Bicarbonate (mg/L)	Nitrogen, ammonia (mg/L)	Nitrogen, nitrite (mg/L)	Nitrogen, ammonia + organic (mg/L)	Nitrogen, nitrate plus nitrite (mg/L)	Phosphorus (mg/L)	Phosphorus, ortho (mg/L)	Carbon, dissolved organic (mg/L)	Calcium (mg/L)
29	9/20/2000	282	0.055	<.010	E.06	<.050	<.006	<.010	0.97	103
	11/20/2000	272	0.064	<.006	E.07	<.047	<.006	<.018	1.0	95.6
30	5/23/2000	316	0.228	<.010	0.31	<.050	<.006	<.010	1.6	50.7
31	--	--	--	--	--	--	--	--	--	--
32	6/28/2000	311	0.276	<.010	0.35	<.050	E.004	<.010	1.2	49.4
33	5/29/2001	375	<.040	E.003	<.10	0.057	<.006	<.020	0.86	102
34	3/19/2001	--	0.084	<.006	0.11	<.047	0.011	<.018	0.58	72.1
	3/19/2001	298	0.080	<.006	E.09	<.047	0.011	<.081	0.62	71.6
35	6/12/2001	305	0.085	<.006	0.16	E.033	<.006	<.020	2.2	74.4
36	7/17/2000	331	0.316	<.010	0.49	<.050	<.006	0.013	2.9	83.9
37	6/26/2000	391	0.057	<.010	0.23	<.050	<.006	0.011	1.3	111
38	5/22/2000	316	0.350	<.010	0.40	<.050	<.006	<.010	1.2	64.2
	6/18/2001	315	--	--	--	--	--	--	--	67.6

Table 12. Chemical-quality data for small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; --, no data; <, less than; mg/L, milligrams per liter; µg/L, micrograms per liter; °C, degrees Celsius; E, estimate]

Well number	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	Fluoride (mg/L)	Silica (mg/L)	Boron (µg/L)	Iron (µg/L)	Manganese (µg/L)	Alkalinity (mg/L)	Solids, residue at 180°C (mg/L)	Bromide (mg/L)
1	19.7	10.8	0.95	7.90	0.40	0.4	16.7	32	310	15.6	269	264	0.02
2	--	--	--	--	--	--	--	--	--	--	--	--	--
3	23.3	26.1	1.59	61.6	33.3	0.2	11.3	25	680	60.1	251	416	0.06
4	--	--	--	--	--	--	--	--	--	--	--	--	--
5	27.6	13.4	1.23	4.10	14.8	0.6	19.6	60	1,300	19.2	328	339	0.11
6	35.8	50.6	1.80	123	48.2	0.1	13.3	37	160	130	312	622	0.24
7	31.4	8.30	1.84	34.3	56.8	E.2	11.5	29	100	104	371	422	0.29
8	31.5	12.9	1.50	28.4	53.5	0.4	18.3	39	1,950	34.0	320	457	0.07
9	17.2	21.4	1.44	63.2	1.30	<.1	12.2	28	4,120	121	263	373	0.04
	--	--	--	--	--	--	--	--	--	--	--	--	--
10	36.6	45.4	2.48	117	78.5	0.3	16.0	29	2,270	63.6	331	662	0.07
11	27.9	8.30	1.49	22.0	37.4	0.3	15.5	19	1,260	29.6	255	364	0.06
12	24.7	3.70	0.80	13.7	34.9	0.3	15.1	19	1,000	25.1	252	329	0.06
13	32.1	51.1	2.87	113	67.0	0.1	10.9	64	970	56.9	290	579	0.15
14	26.4	15.5	1.34	10.7	23.4	0.5	16.2	51	1,260	31.2	292	346	0.09
15	--	--	--	--	--	--	--	--	--	--	--	--	--
16	22.6	9.70	1.00	8.60	13.8	0.3	15.4	33	610	12.7	251	310	0.06
17	29.1	5.70	1.12	11.3	48.4	0.3	18.4	27	1,840	50.8	205	431	0.11
18	24.5	6.10	1.13	4.50	16.5	0.3	18.5	23	900	27.0	310	348	0.03
19	27.5	14.8	1.12	17.9	22.1	0.6	17.6	51	940	22.0	276	346	0.05
20	22.5	8.50	1.20	9.10	51.0	0.6	15.4	50	940	19.6	207	309	0.06
21	20.6	17.7	1.72	52.0	26.6	0.2	11.3	29	1,520	31.5	194	328	0.08
22	21.6	4.90	0.78	7.20	24.4	0.2	16.0	25	1,220	20.7	474	295	0.04
23	20.3	7.20	0.97	2.20	4.80	0.3	17.5	42	1,550	11.4	290	309	0.04
24	22.1	17.5	1.21	7.70	18.3	0.8	15.3	82	730	14.3	222	279	0.07
25	21.4	12.1	1.12	13.4	14.2	0.6	16.2	37	640	20.0	249	306	0.04
26	--	--	--	--	--	--	--	--	--	--	--	--	--
27	--	--	--	--	--	--	--	--	--	--	--	--	--
28	25.1	12.0	1.19	16.2	20.5	0.5	17.1	30	<10	12.4	269	335	0.04
	25.8	14.2	1.41	19.0	21.7	0.5	17.6	--	<10	12.9	276	335	0.04

Table 12. Chemical-quality data for small-public-supply wells in southeastern Michigan sampled from July 1999 through July 2001—*Continued.*

[USGS, U.S. Geological Survey; --, no data; <, less than; mg/L, milligrams per liter; µg/L, micrograms per liter; °C, degrees Celsius; E, estimate]

Well number	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	Fluoride (mg/L)	Silica (mg/L)	Boron (µg/L)	Iron (µg/L)	Manganese (µg/L)	Alkalinity (mg/L)	Solids, residue at 180°C (mg/L)	Bromide (mg/L)
29	32.1	5.00	1.05	10.1	147	0.2	15.9	37	1,600	26.4	231	473	0.14
	31.2	5.10	1.06	9.50	144	0.3	15.2	22	1,510	24.3	223	465	0.10
30	20.7	49.5	1.29	61.3	12.4	0.8	16.1	78	420	9.30	259	363	0.13
31	--	--	--	--	--	--	--	--	--	--	--	--	--
32	22.0	18.9	1.08	5.70	10.1	0.7	16.5	92	810	9.20	255	284	0.05
33	31.4	18.1	1.45	41.8	43.5	E.1	12.4	44	<10	17.4	308	462	0.06
34	22.0	4.60	0.85	13.6	35.9	0.2	13.8	16	1,780	37.9	--	318	0.09
	22.0	4.70	0.84	13.5	35.9	0.2	13.8	27	1,730	37.9	244	318	0.22
35	20.1	3.50	1.08	4.90	18.6	0.2	16.1	24	1,890	41.1	250	300	0.16
36	20.6	14.1	1.54	33.9	24.1	0.2	13.3	54	1,620	114	271	391	0.07
37	31.9	5.00	1.36	29.2	66.3	0.3	18.0	30	2,290	48.7	320	491	0.09
38	18.7	190	3.40	201	122	0.8	12.8	494	850	22.2	259	778	0.25
	20.1	198	3.64	217	131	0.8	13.3	506	630	24.1	258	836	0.24