



Ground Water Rule Source Water Monitoring Methods Guidance

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1. Introduction

1.1 Background

The 1996 Amendments to the Safe Drinking Water Act (SDWA) required the United States Environmental Protection Agency (EPA) to promulgate a Ground Water Rule (GWR) that assures public health protection for people served by ground water sources. More than 147,000 public water systems in the United States use ground water as their primary water source (GWSs); these GWSs serve more than 100 million people. Ground water occurrence studies and outbreak data show that pathogenic viruses and bacteria can occur in GWSs and that people may become ill due to exposure to contaminated ground water. Pathogens found in GWSs may include enteric viruses such as Echovirus, Coxsackieviruses, Hepatitis A and E, Rotavirus and Noroviruses (i.e., Norwalk-like viruses) and enteric bacterial pathogens such as *Escherichia coli*, *Salmonella*, *Campylobacter* and *Shigella*. Ingestion of these pathogens can cause gastroenteritis or serious illnesses such as hemolytic uremic syndrome, meningitis, hepatitis, or myocarditis. Health implications in sensitive subpopulations (e.g., children, elderly, immunocompromised) may be severe and may cause death. The primary goal of the GWR is to improve public health by identifying public ground water systems that are susceptible to fecal contamination and ensure that these systems take corrective action to eliminate the source of contamination or to remove or inactivate pathogens in the drinking water they provide to the public.

The control of microbial contaminants in drinking water supplies using ground water is complicated, as there are a substantial number of microbial contaminants of concern and pathways of contamination, and no single approach for controlling pathogens is universally applicable. The risk of fecal contamination of ground water sources and the subsequent threat to public health is addressed under the GWR through implementation of a risk-targeted approach. This risk-targeted approach uses the following elements to identify and mitigate potential fecal contamination of ground water sources:

- Periodic sanitary surveys of GWSs requiring the evaluation of eight critical elements and the identification of significant deficiencies (discussed in Section 2.1.1)
- Triggered source water monitoring of systems that do not achieve 4-log inactivation or removal of viruses (discussed in Section 2.1.2)
- Corrective actions to eliminate significant deficiencies and fecal contamination (discussed in Section 2.1.3)
- Compliance monitoring to ensure that disinfection treatment for drinking water is reliably operated where it is used and achieves a 4-log inactivation or removal of viruses (discussed in Section 2.1.4)

In addition, assessment source water monitoring is recommended for systems that are determined by the State to be high risk systems (discussed in Section 2.1.2).

1.2 Document Objectives and Organization

The main objective of this document is to provide guidance on triggered and assessment source water monitoring issues such as selection of fecal indicators, sample collection and shipping, source water monitoring methods, laboratory quality assurance (QA) and quality control (QC), and evaluation of fecal indicator data. This manual also provides an overview of GWR requirements and includes frequently asked questions regarding source water monitoring. Several appendices provide example forms that may assist in performing the procedures described in this guidance manual. However, it is important to note that these forms are provided as guidance and are NOT required for compliance with GWR requirements.

Regarding the selection of fecal indicators, this manual provides guidance, when possible, based on aquifer type, historical data, environmental elements, and whether assessment monitoring is required by the State. The guidance provided in this manual is definitive to the extent that the available literature provided sufficient data for EPA to evaluate and make recommendations on a national level. An overview of literature pertaining to the selection of fecal indicators is provided in Section 4.1.

This document is organized into nine chapters and 10 appendices. A description of each remaining chapter and the appendices is provided below.

Chapter 2—Ground Water Rule (GWR) Summary and Source Water Monitoring Methods Requirements: Summarizes GWR components and method requirements.

Chapter 3—Basis for Ground Water Monitoring for Fecal Indicators: Discusses the rationale for indicator monitoring and describes the indicators considered and selected for GWR monitoring.

Chapter 4—Determining the Appropriate Fecal Indicator for Source Water Monitoring: Provides guidance on determination of the most appropriate fecal indicator.

Chapter 5—Collecting and Shipping Ground Water Samples: Provides guidance on GWR monitoring sample collection and shipping procedures.

Chapter 6—Understanding Ground Water Rule Fecal Indicator Methods: Summarizes and discusses the analytical methods approved for use under the GWR.

Chapter 7—Evaluating Fecal Indicator Data: Provides information on how to evaluate fecal indicator data including guidance regarding reporting, archiving, and evaluating data.

Chapter 8—Frequently Asked Questions: Provides answers to frequently asked questions pertaining to GWR requirements, collecting and shipping samples, indicator analyses, and data evaluation.

Chapter 9—References: Provides a list of the references cited within the manual.

Appendix A—Glossary: Provides definitions, acronyms, and abbreviations cited within the manual.

Appendix B—Procedure for Collecting Ground Water Samples for *E. coli* and Enterococci Analyses: Provides detailed sampling guidance for collection of *E. coli* and enterococci samples.

Appendix C—Procedure for Collecting Ground Water Samples for Coliphage Analyses: Provides detailed sampling guidance for collection of coliphage samples.

Appendix D—*E. coli* Method Bench Sheets: Provides example bench sheets for the *E. coli* methods approved for use under the GWR.

Appendix E—Enterococci Method Bench Sheets: Provides example bench sheets for the enterococci methods approved for use under the GWR.

Appendix F—Coliphage Method Bench Sheets: Provides example bench sheets for the coliphage methods approved for use under the GWR.

Appendix G—Ground Water Rule Source Water Monitoring Quality Control Checklist for Presence Absence and Most Probable Number *E. coli* or Enterococci Sample Results: Provides a checklist of quality control procedures and descriptions for the evaluation of *E. coli* and enterococci data from presence/absence or most probable number method formats.

Appendix H—Ground Water Rule Source Water Monitoring Quality Control Checklist for Membrane Filtration *E. coli* or Enterococci Sample Results: Provides a checklist of quality control procedures and descriptions for the evaluation of *E. coli* and enterococci data from membrane filtration method formats.

Appendix I—Ground Water Rule Source Water Monitoring Quality Control Checklist for Method 1601: Two-Step Enrichment Coliphage Sample Results: Provides a checklist of quality control procedures and descriptions for the evaluation of data from Method 1601.

Appendix J—Ground Water Rule Source Water Monitoring Quality Control Checklist for Method 1602: Single Agar Layer (SAL) Coliphage Sample Results: Provides a checklist of quality control procedures and descriptions for the evaluation of data from Method 1602.

1.3 Other Guidance Manuals Available

Several additional guidance manuals are under development which EPA expects to be published to help water systems comply with the requirements of the Ground Water Rule.

- *Complying with the Ground Water Rule: Small Entity Compliance Guide* (EPA 815-R-07-018, July 2007)
- *Consecutive System Guide for the Ground Water Rule* (EPA 815-R-07-020, July 2007)
- *Ground Water Rule Corrective Action Guidance Manual*
- *Ground Water Rule Source Water Assessment Guidance Manual*
- *Ground Water Rule Sanitary Survey Guidance Manual*
- *The Ground Water Rule Implementation Guidance*

Further information about the status of these guidance documents is available on EPA's website (<http://www.epa.gov/safewater/disinfection/gwr/compliancehelp.html>).

2. Ground Water Rule (GWR) Summary and Source Water Monitoring Methods Requirements

The GWR applies to all public water systems that use ground water, except public water systems that combine all of their ground water with surface water or with ground water under the direct influence of surface water prior to treatment under subpart H (“Filtration and Disinfection”). The GWR also applies to consecutive systems receiving finished ground water. Ground water systems (GWSs) must comply, unless otherwise noted, with the GWR beginning December 1, 2009.

This chapter provides an overview of the general requirements of the final ground water rule (2.1) and analytical method requirements (2.2).

2.1 Ground Water Rule Summary

The final GWR establishes a risk-targeted approach to target ground water systems that are susceptible to fecal contamination and then further target those systems that must take corrective action to protect public health. Key components of the GWR are:

1. Sanitary surveys,
2. Triggered source water monitoring,
3. Corrective actions, and
4. Compliance monitoring.

Each of these components is discussed further below and Exhibit 2.1 provides a summary flowchart of the final GWR requirements.

2.1.1 Sanitary Surveys

The final GWR requires regular (every three years for CWSs and every five years for NCWSs) comprehensive sanitary surveys of 8 critical components: (1) source; (2) treatment; (3) distribution system; (4) finished water storage; (5) pumps, pump facilities, and controls; (6) monitoring and reporting, and data verification; (7) system management and operation; and (8) operator compliance with State requirements. The State may reduce the frequency of sanitary surveys for CWSs to at least once every five years if the water system has an outstanding performance record as determined by the State (e.g., no significant deficiencies documented in previous assessments and no history of total coliform MCL or monitoring violations under the Total Coliform Rule (TCR)) or the system maintains 4-log treatment of viruses using inactivation, removal, or State-approved combination of virus inactivation and removal. If a significant deficiency is identified, corrective action is required or a treatment technique violation is incurred.

2.1.2 Source Water Monitoring

In the final GWR, systems not achieving, or not performing compliance monitoring for, 4-log treatment of viruses (using inactivation, removal, or a State-approved combination of these technologies) must conduct triggered source water monitoring for the presence of at least one of the following fecal indicators: *E. coli*, enterococci, or somatic coliphage. The triggered monitoring requirements apply to systems that are notified that a TCR routine sample is total coliform-positive. Within 24 hours of receiving the total coliform-positive notice, GWSs must collect a source water sample and test it for the presence of a GWR fecal indicator.

If the State does not require corrective action (see Corrective Action section below) for the initial fecal indicator-positive source water sample immediately, the system must collect five additional source water samples within 24 hours of being notified of the initial fecal indicator-positive source water sample. The GWR requires systems to take corrective action if any of the five additional source water samples are fecal-indicator positive.

The GWR provides States with the option to require systems to conduct assessment source water monitoring as needed and require systems to take corrective action for a fecal indicator-positive sample found during assessment monitoring. The purpose of this optional assessment source water monitoring requirement is to target source water monitoring to systems that the State determines are at risk for fecal contamination.

2.1.3 Corrective Action

The GWR requires that systems implement corrective action for;

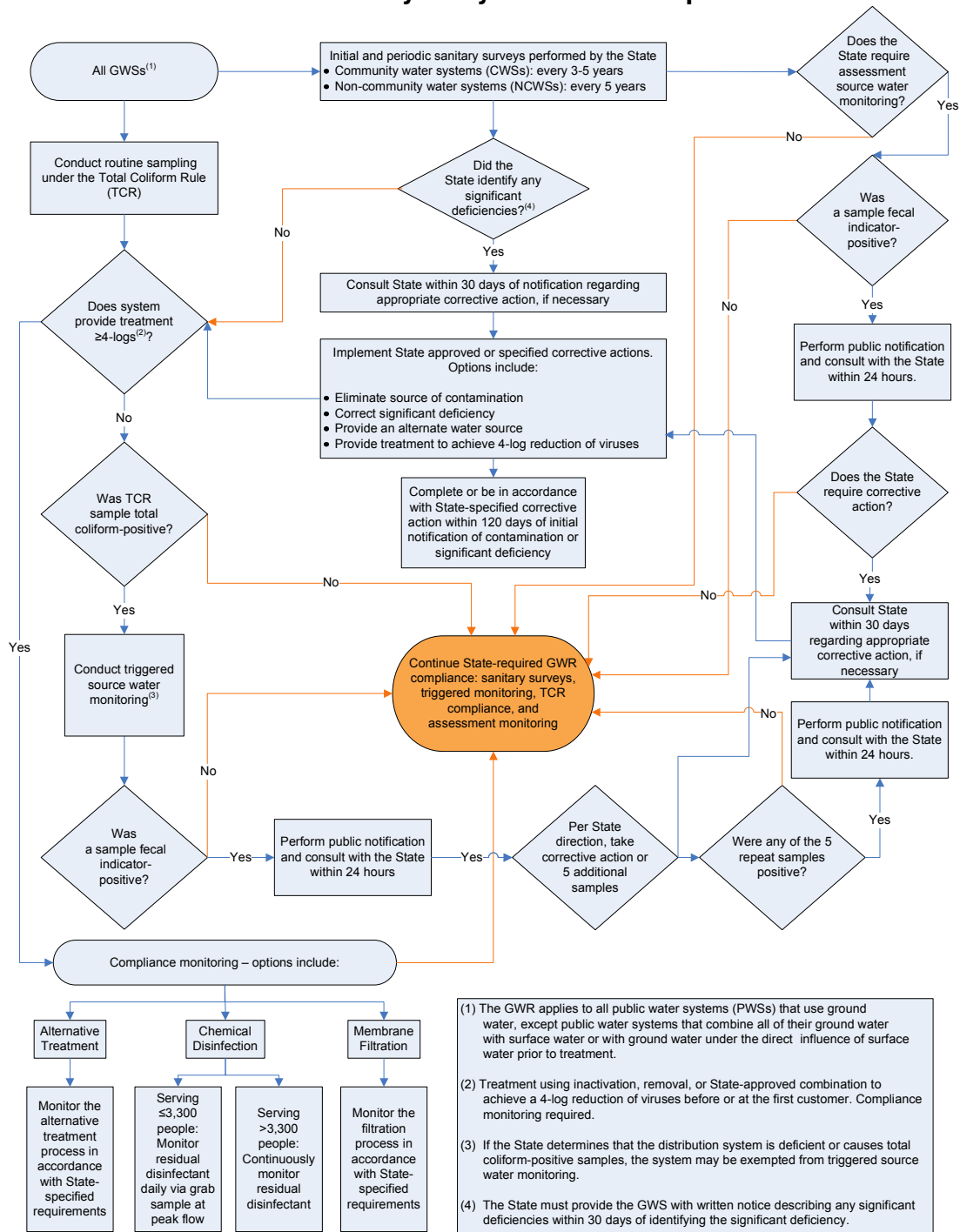
- 1) significant deficiencies,
- 2) fecal-indicator positive samples if directed by the State after the initial fecal indicator-positive in triggered monitoring or for a fecal indicator-positive found during assessment monitoring, or
- 3) a fecal indicator-positive sample in any of the five additional source water samples collected after the initial fecal indicator-positive source water sample during triggered monitoring.

The system must implement at least one of the following corrective actions: correct all significant deficiencies; provide an alternate source of water; eliminate the source of contamination; or provide treatment that reliably achieves at least 4-log treatment of viruses. Furthermore, the system is required to notify the public served by the water system of any uncorrected significant deficiencies and/or source water contamination. (The State may also require notification of corrected significant deficiencies.)

2.1.4 Compliance Monitoring

Compliance monitoring requirements are the final defense against microbial contaminants provided by the final GWR. All GWSs that provide 4-log treatment of viruses, either as a corrective action or in lieu of GWR triggered source water monitoring, must conduct compliance monitoring to demonstrate continual treatment effectiveness.

Exhibit 2.1 Summary of System GWR Requirements



2.2 Source Water Monitoring Methods Requirements

Ground water systems conducting source water monitoring under the GWR must collect and analyze at least 100 mL of source water for one of three fecal indicators (*E. coli*, enterococci, or coliphage) using one of the following analytical methods:

E. coli Methods:

- Colilert (Standard Methods 9223 B) (APHA, 1998)
- Colisure (Standard Methods 9223 B) (APHA, 1998)
- Membrane Filter Method with MI Agar (EPA Method 1604) (USEPA 2002)
- m-ColiBlue24 (Hach Company, Inc., Revision 2, 1999)
- E*Colite Test (Charm Sciences, Inc., 1997)
- EC-MUG (Standard Methods 9221 F) (APHA, 1998)
- NA-MUG (Standard Methods 9222 G) (APHA, 1998)

Enterococci Methods:

- Multiple-Tube Technique - Azide Dextrose/BEA/BHI (Standard Methods 9230 B) (APHA, 1998)
- Membrane Filter Technique with mE-EIA (Standard Methods 9230 C) (APHA, 1998)
- Membrane Filter Technique with mEI Agar (EPA Method 1600) (USEPA 2006a)
- Enterolert (Budnick, G.E. et al., 1996)

Coliphage Methods:

- Two-Step Enrichment Presence-Absence Procedure (EPA Method 1601) (USEPA 2001a)
- Single Agar Layer Procedure (EPA Method 1602) (USEPA 2001b)

Sample analysis must be initiated within 30 hours of sample collection for all analytical methods recognized by the GWR. Systems are encouraged but not required to hold samples below 10°C during transit. All analyses must be conducted by a laboratory certified by the State or EPA in accordance with specified analytical method requirements. Chapter 6 describes these methods in greater detail.

3. Basis for Ground Water Monitoring for Fecal Indicators

Fecally contaminated ground water can be identified by monitoring for either pathogenic microorganisms or for non-pathogenic fecal indicator microorganisms whose presence suggests fecal contamination or a pathway for contamination and, therefore, the potential presence of pathogens. Monitoring for indicators is generally more practical than monitoring for actual pathogens. Only the more advanced water laboratories currently have the analytical capabilities to analyze water samples directly for pathogens. In addition, pathogen concentrations in water tend to be low, thereby requiring the analysis of larger sample volumes and increasing analytical costs; and many of the viruses associated with waterborne disease are either difficult or impossible to culture. For example, some viruses such as infectious norovirus and wild-type Hepatitis A virus are not culturable, while other viruses, such as enteroviruses, have variable, limited recovery and culturability. Some bacteria are also difficult to culture. Finally, laboratory analytical methods for fecal indicators are typically more widely available, more widely used, and significantly less expensive than methods for monitoring for individual enteric pathogens.

Indicator data are important because illness can result from consuming ground water with fecal contamination in the absence of identified pathogens. EPA recognizes that any indicator organism may or may not co-occur with pathogens and that co-occurrence could be intermittent.

The evaluation of fecal indicators for monitoring under the GWR is briefly discussed in Section 3.1, with more detail on the bacterial (*E. coli* and enterococci) and viral (coliphage) fecal indicators being provided in Sections 3.2 and 3.3, respectively.

3.1 Indicators Evaluated

Prior to proposal of the GWR, EPA carefully evaluated the existing scientific literature to identify the most appropriate indicators of fecal contamination in ground water. EPA considered a number of issues including, but not limited to, distribution, transport, and fate of fecal organisms in ground water (Pedley et al., 2006, DeBorde et al., 1998 and 1999). Detailed information on the scientific literature reviewed in support of fecal indicator selection for the rule can be found in the Occurrence and Monitoring Document for the Ground Water Rule (USEPA, 2006b). The organisms selected as the most reliable indicators of fecal contamination in ground water were *E. coli*, enterococci, and coliphage (male-specific and somatic). Other organisms that were considered for use as indicators are discussed in the bacterial (Section 3.2) and viral (Section 3.3) indicator sections below. For source water monitoring, the final GWR requires the use of *E. coli*, enterococci, or coliphage as fecal indicators in ground water based on the following:

- *E. coli*, enterococci, and coliphage are closely associated with recent fecal contamination.
- *E. coli*, enterococci, and coliphage are frequently found, sometimes in high concentrations, in sewage and septage.
- *E. coli*, enterococci, or coliphage presence implies that other fecal pathogens (including enteric viruses) or a pathway for fecal pathogens could be present.
- *E. coli*, enterococci, and coliphage are present in higher concentrations than other fecal pathogens (including enteric viruses), and are therefore easier to detect.
- Approved analytical methods for *E. coli*, enterococci, and coliphage are simple, reliable, and inexpensive.

- The TCR allows for *E. coli* monitoring, therefore many laboratories are familiar with the methods used for the detection of *E. coli*.
- Enterococci are recommended as indicators for fecally contaminated recreational waters and are commonly used as fecal indicators.

3.2 Bacterial Indicators

Bacteria that have been used as indicators of fecal contamination include the total coliform bacterial species, many of which are free-living in the environment, and fecal bacteria including *E. coli*. Fecal coliforms are coliform bacteria found in animal feces. Because total coliform bacteria are primarily free-living in the environment, when identified at the tap they are considered to be indicators of chlorine demand and distribution system contamination, as well as possible fecal contamination of source water. When total coliforms are identified in source ground water they may be indicators of surface or near-surface inflow to ground water as well as possible fecal contamination. However, they may not be representative of fecal contamination specifically, and therefore are not considered an appropriate fecal indicator for source water monitoring. Other bacteria that are used as indicators of fecal contamination include enterococci and *Clostridium perfringens*, a spore-forming anaerobic organism. Some indicator bacteria have specialized uses. For example, heterotrophic plate count (HPC) bacteria may be used to track treatment efficiency, and *Bacillus* is used as an indicator of surface or near-surface water inflow to ground water (Rice et al, 1996). Both HPC and total coliform bacteria are used to identify the presence of biofilm or other distribution system problems (Geldreich 1996, Carter et al. 2000).

As indicated above, *E. coli* (Section 3.2.1) and enterococci (Section 3.2.2) were selected for use as bacterial indicators of fecal contamination.

3.2.1 *E. coli*

E. coli bacteria are a subgroup of the coliform group that can be found in high numbers in the intestines and feces of warm-blooded animals. *E. coli* is considered the most appropriate group of the coliform bacteria to indicate fecal contamination and the possible presence of enteric pathogens because it is generally believed that there are no significant non-fecal sources of *E. coli*, and *E. coli* generally does not grow extensively in the environment. However, exceptions have been reported in areas with little human impact and in warm, moist tropical/subtropical environments where *E. coli* has been shown to be part of the normal soil environment and grow in both soil and surface water (Hardina and Fujioka, 1991, Jimenez et al, 1989, and Rivera et al., 1988). This issue is discussed further in Section 4 in the context of selecting the most appropriate indicator for ground water monitoring.

3.2.2 Enterococci

Enterococci bacteria initially were a subgroup of fecal streptococci and consist of several species of bacteria in the genus *Streptococcus*. The current taxonomic approach separates enterococci into a separate genus (Hardie and Whiley, 1997). Enterococci are commonly found in relatively high numbers in the feces of humans and other warm-blooded animals. Although some strains are ubiquitous and not related to fecal contamination, the presence of enterococci in water is an indication of fecal contamination and the possible presence of enteric pathogens. Epidemiological studies conducted in fresh and marine waters have demonstrated there is a direct relationship between the density of enterococci and the risk of gastrointestinal illness associated with swimming (Cabelli, 1979). The risk would also be applicable to drinking water sources.

3.3 Viral Indicators: Coliphage

Bacteriophages (“phages”) are viruses that infect bacteria. They can replicate only in a living host bacterial cell. While some phages are considered to be indicators of human enteric viruses (Curry 1999; Grabow 2001), some are more frequently associated with fecal contamination than others. Feces-specific bacteriophages evaluated for potential use under the GWR included somatic coliphage, male-specific coliphage, and *Bacteroides fragilis* bacteriophages. Bacteriophages that infect and replicate in *Bacteroides fragilis* were determined not to be a useful fecal indicator because counts are usually low in United States waters. Coliphage are a group of bacteriophages that infect and replicate predominantly in *E. coli* and are considered to be indicators of human enteric viruses (Curry 1999; Grabow 2001). The two types of coliphage, somatic and male-specific, differ in the mechanism by which they infect host bacterial cells.

- Somatic coliphage are viruses that infect host cells (*E. coli*) via receptors on the outer cell membrane. The majority of the somatic coliphage detected in water are host-specific, i.e., they can only replicate in *E. coli*. However, under certain conditions, closely related bacterial species may support the growth of somatic coliphage in water environments.
- Male-specific coliphage (also referred to as FRNA coliphage) are viruses that infect host cells (*E. coli*) via the receptor sites on the F-pilus, a minute “fiber-like” structure produced by some bacteria for the exchange of genetic material. F-pili are only produced by actively growing bacteria under optimal conditions, usually at temperatures near 30°C or higher. This characteristic is important because in environmental waters, conditions rarely exist for the replication of male-specific coliphage. For all practical purposes, it is highly unlikely that male-specific coliphage can replicate in ground water environments (typically 8°C to 16°C).

Conditions for coliphage replication typically exist in the gastrointestinal tract of humans and other warm blooded animals. Since human enteric viruses are released into the environment almost exclusively through human feces, coliphage reflect the potential origin and release of human viruses. Coliphage may be better indicators of fecal contamination than bacterial fecal indicators when viruses are the most likely pathogen of concern. The GWR specifically authorizes the use of coliphage as viral fecal indicators in ground water for the following reasons:

- Coliphage more closely resemble human enteric viruses in shape, size, morphology, and composition (Grabow 2001).
- Coliphage respond to water treatment and natural environments similarly to human enteric viruses.
- Coliphage may be similar to the enteric viruses in transport efficiency through soil and aquifer materials due to similar size and shape.
- Coliphage generally do not infect non-fecal bacteria and it is unlikely that they can reproduce in water environments due to strict conditions (e.g., temperature, log phase growth of their hosts) needed for replication (Grabow 2001).

The process of selection by a State of the most appropriate source water monitoring indicator from those described above will take into consideration the chemical and physical elements of that state’s environment, as discussed in Chapter 4 of this document.

4. Determining the Appropriate Fecal Indicator for Source Water Monitoring

A State's selection of the most appropriate indicator(s) should consider factors such as aquifer type, historical water sampling data of the region, climate and other environmental factors. Based on available data, this chapter provides guidance regarding these factors, discussed in the following sections: Viral and Bacterial Transport (Section 4.2); Use of Multiple Fecal Indicators (Section 4.3); Tropical/Subtropical Environments (Section 4.4); Use of Historical Data (Section 4.5); and Coliphage Matrix Spike Sample Results (Section 4.6).

4.1 Literature Overview

As indicated in the USEPA Occurrence and Monitoring Document for the Final Ground Water Rule (USEPA, 2006b), EPA does not have a single preferred choice of indicator because no single indicator can definitively determine whether pathogens are present. With regard to occurrence, studies reviewed by the Agency observed both bacterial and viral indicators in various ground water systems surveyed. Some researchers observed that bacteria are more prevalent in ground water (e.g., Doherty, 1998; Francy et al., 2004; Femmer et al., 2000), while other researchers recovered viruses more frequently (e.g., Atherholt et al., 2003; Davis and Witt, 2000).

In a comprehensive review of the current knowledge about distribution of pathogens in ground water and the factors that control their transport and attenuation, Pedley et al. (2006) provide information on both waterborne pathogens and indicator organisms, and describe the impact that system characteristics may have on the survival and migration of microorganisms in the subsurface. According to Pedley et al. (2006), while the current knowledge offers a number of guiding principles about the transport and attenuation of pathogens in ground water, the complex interaction of factors controlling the fate of pathogens is poorly understood and difficult to predict in some environments. The literature suggests that the properties of a ground water system that affect the transport and/or attenuation of microorganisms include but are not limited to: hydrogeologic conditions; flow mechanisms; light; temperature; pH; and soil properties, including moisture content, organic matter, iron content, nutrient content, and salt concentration. Additionally, characteristics of the microorganism, such as type, size, aggregation, microbial activity, heterogeneity of the population, predation and/or antagonism, and potential association of the microorganism with soil, will affect transport and attenuation (Pedley et al., 2006; Foppen and Schijven, 2006; USEPA, 2006b).

While the available literature is variable and dependent on the ground water system, some factors may warrant the use of a specific indicator. Guidance provided in subsequent sections of this chapter is prescriptive to the extent that the available literature provides sufficient data to evaluate and make recommendations on a national level. Specific literature associated with these recommendations is cited below. For additional information on the current knowledge regarding pathogens in ground water, refer to Pedley et al. (2006) and to the Occurrence and Monitoring Document for the Final Ground Water Rule (USEPA, 2006b), which includes a review of the literature considered in development of the final GWR.

4.2 Viral and Bacterial Transport

Aquifers are broadly classified into two categories; porous and non-porous. In this document, non-porous aquifers (e.g., karst limestone, fractured igneous or metamorphic rock aquifers) as well as gravel aquifers are defined as sensitive aquifers. Non-porous aquifers are those that transmit ground water through large, well

connected openings such as fractures, solution enhanced fractures, conduits or caverns. The Source Assessment Guidance Manual provides additional explanation on what constitutes a sensitive aquifer and how best to identify one, and identifies pathways to obtain additional hydrogeologic information.

Among the remaining (excluding gravel) porous aquifers (e.g., sand, or sand and gravel, aquifers), sand and gravel aquifers more efficiently transmit fecal contaminants than sand aquifers because average ground water velocity is higher. In general, for porous media aquifers the greater the grain size or grain size heterogeneity, the more efficiently pathogenic microorganisms pass through the aquifer.

All subsurface particles, including microbes, may be transported by flowing ground water. Particles may be removed from flow or be retarded. That is, they may permanently or temporarily become associated with the solid aquifer materials in either porous or non-porous aquifers. Microbial transport in porous media aquifers is an active research area and consensus is difficult in many issues in this field. It is generally agreed that microbe size is an important element in determining mobility in porous media, although many other factors, such as surface charge, may also have significant influence. Given the importance of microbe size, the significant (one-thousand fold) size difference between viruses (measured in nanometers) and bacteria (measured in micrometers) increases the likelihood that an infectious virus, rather than an infectious bacterium, will reach a GWS well in a porous aquifer.

4.2.1 Sand Aquifers

The thousand-fold size difference between viruses and bacteria may be particularly significant in sand aquifers for two reasons: 1) Viruses are less likely to be subject to removal or retardation at pore margins by straining, wedging, or micro-straining; and 2) viruses may be more likely to be excluded from the smaller pores where ground water velocities are slower. As a result of this pore-size exclusion (which is due indirectly to size because charge effects predominate for smaller particles), viruses may be favored over bacteria because the viruses remain in faster flowing ground water for longer periods. As the result of straining and pore-size exclusion, sand aquifers may facilitate virus transport as compared with bacterial transport. All other factors such as average ground water velocity being equal, this manual assumes that infectious viruses are more likely than infectious bacteria to be found in GWS well source water in sand aquifers because the viruses are smaller and thus more mobile in the subsurface. Some sand aquifers appear to more efficiently transmit viruses as compared with bacteria, thus, if targeted for assessment source water monitoring, sand aquifers should be monitored using coliphage rather than *E. coli* or enterococci.

4.2.2 High Population Density Combined with On-site Wastewater Treatment Systems.

Each aquifer has some risk that at any site, the natural attenuation capability may be overwhelmed by a combination of large wastewater discharge to the subsurface, high rate pumping and reduced recharge (and dilution). For example, areas with a high density population, using septic tanks and other on-site wastewater treatment systems discharge fecal contamination to the subsurface combined with restricted areal extent of an aquifer is an especially risky combination. This is because aquifer recharge by septage discharge in such environments is significant as compared to infiltrating precipitation. Some aquifers, such as barrier island or marine island aquifers, are capable of supplying only limited yield because over-pumping will result in seawater intrusion, permanently damaging the aquifer. Where population density is high and yield is limited, dilution and other natural attenuation processes are limited and fecal contamination is more likely. Barrier island aquifers are typically sand aquifers and, like all sand aquifers, may be more susceptible to viral rather than bacterial contamination. If targeted for additional monitoring, barrier island sand aquifers should be monitored using coliphage rather than *E. coli* or enterococci.

4.2.3 Other Aquifers

In other aquifers, such as non-porous aquifers (e.g., fractured igneous or metamorphic rock aquifers) and gravel aquifers, average ground water velocities are exceptionally fast, and straining and pore-size exclusion are much less significant and bacteria and viruses are assumed to travel at equal rates. In general, straining and pore-size exclusion effects are more significant in sand aquifers than in sand and gravel aquifers. In sand aquifers, ground water velocity is moderate because mean grain size is moderate. As ground water velocities increase because of increasing gravel content or increasing proximity to a pumping well, the differences between virus and bacterial transport efficiency become less important, and either a viral or bacterial indicator may be recommended.

On the other hand, ground water velocities through the finest grained porous aquifers, such as shale and clay beds, are generally very slow. Because ground water velocity is very slow, these aquifers do not readily produce water in quantities sufficient to supply a PWS and thus are not considered further in this guidance.

4.2.4 Non-Fecal Contamination and Proximity of Contamination to the Well

Some microbial pathogens such as *Legionella pneumophila* (Costa et al., 2005; Riffard et al., 2004), *Helicobacter pylori*, *Naegleria fowleri* (Blair and Gerba, 2006), and perhaps *Toxoplasma gondii* (Sroka et al, 2006) are not associated with fecal contamination and, instead, may be resident members of aquifer ecosystems. For these microbes, transport from the surface or near surface is not an important risk element because the microbes can colonize the well gravel pack or the aquifer immediately surrounding the gravel pack. In these instances, the bacterial versus virus size difference and associated subsurface mobility differences become much less important.

4.3 One Versus Two or More Fecal Indicators

Although EPA's Science Advisory Board (SAB) and the National Drinking Water Advisory Council (NDWAC) recommended that systems should monitor for coliphage and either *E. coli* or enterococci for source water monitoring, the available data did not provide for evaluation of such a measure on a national level. While coliphage data are available for many of the occurrence studies used to estimate national occurrence for *E. coli*, the methods used to measure coliphage were often based on high volume analysis and a variety of methods different than those specified under the final GWR. Thus, EPA could not determine whether the SAB/NDWAC proposal would provide additional effectiveness. Furthermore, EPA was concerned with the increase in sampling burden and cost relative to the additional number of fecally contaminated wells that would be identified using two indicators compared to the use of one indicator. Therefore, based on the data available, EPA requires all GWSs to monitor for a single fecal indicator under the final GWR, but encourages States to consider the use of multiple indicators where a net benefit seems likely.

Pathogen and indicator occurrence in wells is intermittent. However, when indicators occur frequently in source water samples, then sampling for one fecal indicator is sufficient. When indicator occurrence is rare, then sampling for multiple fecal indicators may be more likely to recognize fecal contamination. For example, a well in Oregon (Lieberman et al, 2002, well number 31) was enteric virus positive in four of twelve monthly samples. It was also *E. coli* positive in six of twelve monthly samples. Thus, for this well *E. coli* sampling only is probably sufficient to indicate the fecal contamination hazard. In contrast, a well in North Carolina (Lieberman et al, 2002 well number 99) was enteric virus positive in one of twelve monthly samples. None of the twelve *E. coli* samples were positive but one of the twelve enterococci and one of the twelve male-specific coliphage samples were positive. In this site with infrequent pathogen and indicator occurrence, assaying for multiple indicators will increase the likelihood that the well is identified as fecally contaminated.

4.4 Tropical/Subtropical Environments

The GWR-approved bacterial indicators of fecal contamination, *E. coli* and enterococci, may not be reliable for assessing fecal contamination in tropical environments. There is a growing consensus among researchers that: (1) soil, sediments, water, and plants may be indigenous sources of *E. coli* and enterococci in tropical waters; (2) fecal indicators can multiply and persist in soil, sediment and water in some tropical and subtropical environments; and (3) tropical environments change the relationship between the presence of the indicators and potential health effects.

The current literature suggests that *E. coli* and enterococci can be found in soil and surface waters of tropical environments in the absence of warm-blooded animals and, therefore, are endemic in tropical ecosystems and not simply indicators of fecal origin (Hardina and Fujioka, 1991; Rivera et al., 1988). In a study of Hawaiian freshwater streams, Hardina and Fujioka (1991) observed that *E. coli* was capable of replicating in stream water samples and that both *E. coli* and enterococci were present in soil samples free of fecal contamination. In subtropical environments, such as Florida, *E. coli* has been shown to grow in soil with high moisture content, (Solo-Gabrielle et al., 2000). As a result, positive bacterial indicator results for wells in these environments may not be due to fecal contamination.

There are also non-fecal species of enterococci, such as *Enterococcus casseliflavus* that may grow in environmental settings (Niemi et al., 1993). Environmental proliferation of non-fecal enterococci further complicates the assessment of fecal contamination when detecting enterococci. Research is still needed to better define the parameters such as soil, nutrients, moisture, temperature, time of year, and latitudes that may promote natural proliferation of fecal indicator bacteria in the soil.

Based on the literature, it appears that the presence of *E. coli* or enterococci from a ground water source in a tropical or subtropical ecosystem may not always be indicative of fecal contamination. As a result, selection of coliphage as an indicator in these environments merits consideration. However, as noted in Section 4.2 above, some microbial pathogens (e.g., *Legionella pneumophila*, *Helicobacter pylori*) are not associated with fecal contamination, and instead may be resident members of aquifer ecosystems. As such, detection of endemic, non-fecal *E. coli* and enterococci would not necessarily confirm that water from that source is safe.

4.5 Use of Historical Source Water Monitoring Data

Some GWSs may have historical source water data for total coliforms, *E. coli*, enterococci, or coliphage. Although historical data is unlikely to be useful in determining whether an indicator is appropriate, it may help determine that an indicator is not appropriate. With regard to fecal indicator selection, the following considerations may be useful regarding historical source water data:

- If a GWS has a history of negative source water results for one of the GWR-approved indicators (*E. coli*, enterococci, or coliphage), the State may consider requiring that an alternate fecal indicator be selected for GWR monitoring.
- A GWS may have a history of total coliform-negative source water samples, indicating that *E. coli* (a subset of total coliforms) is most likely not present at detectable levels in the source water. Since the GWS is unlikely to detect *E. coli* under GWR monitoring, another indicator may be more appropriate.

- A GWS may have a history of total coliform-positive source water samples that are not positive for *E. coli*, where data indicate that although coliform bacteria are present at detectable levels at the sampling location, *E. coli* are not present, or are present at levels below detection. Because the GWS is unlikely to detect *E. coli* under GWR monitoring, another indicator may be more appropriate.

5. Collecting and Shipping Ground Water Samples

The GWR requires systems to collect source water samples and analyze for the presence of GWR fecal indicator(s) through triggered, and in some cases assessment, monitoring. This section provides an overview of recommendations for sample location and monitoring frequency (Section 5.1), sample containers and volume (Section 5.2), shipping regulations and documentation (Section 5.3), holding time (Section 5.4), and holding temperature and temperature monitoring (Section 5.5). Detailed sample collection protocols and sample packing and shipping procedures are provided in Appendix B for *E. coli* and enterococci samples, and Appendix C for coliphage samples.

5.1 Sample Collection Location and Monitoring Frequency

As indicated in Section 2.2.5, ground water samples used for triggered or assessment monitoring must be collected at a location prior to any treatment of the ground water source, unless the State approves a sampling location after treatment. If the system's configuration does not allow for sampling at the well itself, the system may collect a sample at a State-approved location, if the sample is representative of the water quality of that well.

With State approval, a system that uses more than one source of ground water may conduct source water monitoring at a representative ground water source or a subset of sources. Sample collection location and monitoring frequency information specific to triggered and assessment source water monitoring are detailed in Sections 5.1.1 and 5.1.2, respectively.

5.1.1 Triggered Source Water Monitoring

If a routine sample collected in accordance with §141.21(a) (TCR) is total coliform-positive a GWS that does not provide 4-log treatment of virus, as determined by the State, for its ground water source(s) must conduct triggered source water monitoring within 24 hours of receiving notification. A GWS is not required to comply with triggered source water monitoring if the cause of the total coliform-positive sample directly relates to the distribution system, as determined by the State. The GWS must collect at least one ground water source sample from each ground water source in use at the time the total coliform-positive sample was collected and test for *E. coli*, enterococci, or coliphage. The State may extend the 24-hour limit for triggered source water monitoring on a case-by-case basis, if the State determines that the system cannot collect the ground water source sample within 24 hours due to circumstances beyond its control. If the State approves the use of *E. coli* as a fecal indicator for triggered source water monitoring, GWSs serving 1,000 people or fewer may use a TCR repeat sample collected from a ground water source to simultaneously meet the requirements of the TCR and satisfy the GWR's triggered source water monitoring requirements for that ground water source only.

If any initial triggered source water sample is fecal indicator-positive, the system must collect five additional water samples within 24 hours at the site, unless the State requires immediate corrective action to address contamination at that site.

Consecutive and wholesale systems must comply with triggered source water monitoring provisions for their own sources. A consecutive GWS with a total coliform-positive sample must notify the wholesale system(s) within 24 hours of being notified of the test result. The wholesale system must, within 24 hours of notification, conduct triggered source water monitoring by collecting a sample from its ground water source(s) and analyzing for the presence of a GWR fecal indicator.

5.1.2 Assessment Source Water Monitoring

The GWR provides States with the option to require systems to conduct assessment source water monitoring at any time and require systems to take corrective action based on the results of these analyses. Assessment source water monitoring is not a requirement of the GWR but a recommended tool for States when targeting high-risk GWSs. States may identify high risk GWSs and require assessment source water monitoring based on information from hydrogeologic sensitivity assessments (HSAs), triggered monitoring results, or historical data from the system(s).

If assessment monitoring is required, EPA recommends that States require collection of a minimum of 12 ground water source samples that represent each month the system provides ground water to the public. Collection of samples from each well is also recommended, unless the system obtains written State approval to conduct monitoring at one or more wells within the GWS that are representative of multiple wells used by that system.

5.2 Sample Containers and Volume

Samples should be collected in sterile, plastic or glass containers with a leak-proof lid. The GWR requires GWSs conducting source water monitoring to analyze at least a 100-mL sample volume. However, EPA recommends that the GWS collect and ship more than 100-mL of sample to ensure that a minimum of 100 mL is available for analysis. The capacity of sample containers should be sufficient to allow at least a 1-inch headspace to facilitate mixing of the sample by shaking prior to analysis. Sample volume and container size recommendations are provided below.

- ***E. coli* and Enterococci Samples.** The GWS should collect at least 120 mL of sample to ensure sufficient volume for sample analysis is available in the event of spillage at the laboratory. The capacity of sample containers should be at least 150 mL to allow at least a 1-inch headspace to facilitate mixing of the sample by shaking prior to analysis.
- **Coliphage Samples.** If Method 1601 is used for coliphage sample analyses, either 100-mL or 1-L sample volumes may be analyzed (Method 1602 only accommodates 100-mL volumes). While the minimum sample volume requirement for the GWR is 100 mL, systems may wish to collect and analyze a 1-L sample volume to increase the sensitivity of the Method 1601 analysis.

For 100-mL sample analyses, the GWS should collect 250 mL of sample for each of the two coliphage types to allow for sample re-analysis, if necessary.

The capacity of sample containers should allow at least a 1-inch headspace to facilitate mixing of the sample by shaking prior to analysis. Alternatively, samples for male-specific and somatic sample analyses can be collected in separate containers.

For 1-L sample analyses, the GWS should collect 2.5 L of sample for each of the two coliphage types to allow for sample re-analysis, if necessary.

The capacity of sample containers should allow at least a 1-inch headspace to facilitate mixing of the sample by shaking prior to analysis. Alternatively, samples for male-specific and somatic sample analyses can be collected in separate containers.

Note: While samples may still be analyzed if spillage occurs at the laboratory, if spillage or leakage occurs during shipment, there is an opportunity for sample contamination to occur and the sample should not be analyzed.

5.3 Shipping Regulations and Documentation

Unless the sample is known or suspected to contain infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. U.S. Department of Transportation (DOT) regulations (49 CFR 172) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. If an outbreak is suspected, ship less than 4 L per shipment.

Sample Tracking Information. The GWS should record the following information on the sample collection form:

- Name of system (public water system site identification number, if available)
- Sample identification (number)
- Sample site location
- Sample type (e.g., triggered monitoring, assessment monitoring)
- Date and time of collection
- Analysis requested
- Name of sampler
- Any remarks

Sample Container Information. The sample container should indicate the following:

- Sample number
- Name of system (public water system site identification number, if available)
- Date and time of collection
- Sample collection location
- Analysis requested

Chain-of-Custody. Sample collectors and laboratories should follow applicable State regulations pertaining to chain-of-custody (COC). Appendix A of the Manual for the Certification of Laboratories Analyzing Drinking Water (USEPA, 2005) provides detailed guidance on COC procedures, including the following:

- Sample collection, handling, and identification

- Transfer of custody and shipment
- Laboratory sample control procedures
- Sample ID tag examples
- Example COC record

5.4 Holding Time

The analytical holding time is defined as the time between sample collection and the start of sample analysis. During the time between sample collection and analysis, it is possible for stressed bacteria and/or bacterial viruses to suffer die-off or further injury due to adverse conditions during transit or the presence of substances in the water which can be toxic (e.g., heavy metals). Additionally, the presence of nutrients in the sample may lead to growth of background bacterial populations which could interfere with the analysis. As a result of these concerns, while the GWR requires that fecal indicator samples be analyzed within a 30-hour hold time period, EPA highly recommends that samples be analyzed as soon as possible after collection.

5.5 Holding Temperature and Temperature Monitoring

While not required, EPA strongly recommends that GWR monitoring samples that are not analyzed immediately following sample collection be chilled below 10°C to reduce biological activity and preserve the condition of ground water samples between collection and analysis. Samples for all analyses should remain above freezing at all times. Samples that arrive frozen should not be analyzed. Several options are available that mitigate the risk of sample contamination while providing an indication of sample temperature upon receipt at the laboratory and, in some cases, during shipment:

- *Temperature sample.* Using this option, the GWS would fill a small, inexpensive sample bottle with water and pack this “temperature sample” next to the source water monitoring sample. The temperature of this extra sample volume is measured upon receipt to estimate the temperature of the source water monitoring sample. Temperature sample bottles are not appropriate for use with bulk samples (i.e., 1-L samples) because of the potential affect that the difference in sample volume may have in temperature equilibration in the sample cooler. *Example product:* Cole Parmer catalog number U-06252-20.
- *Minimum/maximum thermometer.* A minimum/maximum thermometer not only provides the current temperature of the shipping cooler, which is read upon receipt at the laboratory, but indicates the minimum and maximum temperatures that the sample experienced during shipment. Ideally, if the minimum/maximum thermometer is water-immersible, it should be placed in a temperature sample in the cooler, rather than placed directly in the cooler, where it may be affected by close contact with the coolant. This additional information may be used to determine whether the sample froze during shipment or exceeded the maximum temperature, even if the sample is received at an acceptable temperature. *Example product:* Cole Parmer catalog number U-08107-30.
- *iButton.* A Thermocron® iButton is a small, waterproof device that contains a computer chip to record temperature at different time intervals. The information is then downloaded from the iButton onto a computer. The iButton should be placed in a temperature sample in the cooler, rather than

placed directly in the cooler, where it may be affected by close contact with the coolant. Information on Thermocron® iButtons is available from <http://www.maxim-ic.com/products/ibutton/>.

- *Stick-on temperature strips.* A stick-on temperature strip may be applied to the outside of the sample container upon receipt at the laboratory. This option does not measure temperature as precisely as the other options, but still mitigates the risk of sample contamination while providing an indication of sample temperature upon receipt at the laboratory. *Example product:* Cole Parmer catalog number U-90316-00.
- *Infrared thermometers.* An infrared thermometer may be used to measure the temperature of the surface of the sample container upon receipt at the laboratory. The thermometer is pointed at the sample, and the temperature is measured without coming in contact with the sample volume. *Example product:* Cole Parmer catalog number EW-39641-04.

As with other laboratory equipment, all temperature measurement devices should be calibrated routinely to ensure accurate measurements. See the U.S. EPA *Manual for the Certification of Laboratories Analyzing Drinking Water* (USEPA, 2005) for more information.

6. Understanding Ground Water Rule Fecal Indicator Methods

Section 6.1 describes the formats available for the analysis of *E. coli*, enterococci, and coliphage. Sections 6.2, 6.3, and 6.4 provide descriptions of the analytical methods approved for use under the GWR for the analysis of *E. coli*, enterococci, and coliphage, respectively.

6.1 Selection of Method Format

Three formats, presence/absence (Section 6.1.1), most probable number (Section 6.1.2), and direct plating (Section 6.1.3) are approved for use in the analysis of *E. coli*, enterococci, and coliphage samples under the Ground Water Rule (GWR). The type of format selected may be influenced by water sample type, quality, and character (e.g., organism density, turbidity).

6.1.1 Presence/Absence

A presence/absence procedure may be performed using a single vessel. Depending on the method and analyte (e.g., *E. coli*, coliphage), medium is added to the sample and thoroughly mixed. The mixture is incubated for the method-specified time at the method-specified temperature. Positive tubes/bottles are then confirmed/verified according to method-specific protocols.

6.1.2 Most Probable Number (MPN)

Multiple-Well

A multiple-well procedure may be performed with sterilized disposable packets. The commercially available Quanti-Tray[®] or Quanti-Tray[®]/2000 multiple-well tests use Colilert[®] or Colisure[®] media to detect the presence of *E. coli* and Enterolert[™] to detect the presence of enterococci. In these tests, the medium is added to a 100-mL sample, mixed thoroughly, and poured into the tray. A tray sealer separates the sample into 51 wells (Quanti-Tray[®]) or 97 wells (Quanti-Tray[®]/2000) and seals the package which is subsequently incubated according to method-specific requirements. A single positive well constitutes a positive sample under the GWR. If enumeration is desired, MPN tables provided by the manufacturer can be used to estimate the number of bacteria per 100 mL of sample.

Multiple-Tube

In multiple-tube tests, serial dilutions may be used to obtain estimates of bacterial density over a range of concentrations, with replicate tubes analyzed at each ten-fold dilution/volume. The multiple-tube methodology is useful for detecting organisms in samples containing heavy particulate matter, toxic compounds (e.g., metals), or injured or stressed organisms. Generally, for nonpotable water samples, 5 replicate tubes at a minimum of 3 dilutions/volumes (for a total of 15 tubes) are used. However, since the GWR requires 100 mL of sample to be analyzed, 10 tubes containing 10 mL of sample per tube should be analyzed. Tubes are incubated and positive results are reported and confirmed. Positive results are determined under specified conditions (e.g., production of acid and/or gas using multiple-tube fermentation tests, color change or fluorescence using enzyme substrate tests). A single positive tube constitutes a positive result for GWR compliance monitoring requirements.

6.1.3 Direct Plating

Membrane Filtration

Membrane filtration (MF) is a direct-plating method in which sample dilutions/volumes are filtered through one or more 0.45- μm membrane filters that are subsequently transferred to Petri plates containing selective primary isolation agar or an absorbent pad saturated with selective broth. A second substrate medium is used in two-step MF procedures to confirm and/or differentiate the target organisms. The total sample volume to be analyzed may be distributed among multiple filters and diluted as necessary based on the anticipated water sample type, quality, and character (e.g., organism density, turbidity). Target colonies are detected by observing the presence of colonies that meet a specific morphology, color, or fluorescence under specified conditions. Membrane filtration results can be subject to interferences caused by water samples with high turbidity, toxic compounds, or large numbers of non-coliform (background) bacteria, and organisms damaged by chlorine or toxic compounds.

Pour Plate (Coliphage Only)

Pour plate is a direct-plating method in which 100 mL of sample is added to 100 mL of molten agar, mixed and poured into Petri plates. Following incubation plates are examined for plaques (circular lysis zones). A single plaque forming unit (PFU) constitutes a positive result for the GWR compliance monitoring requirements.

6.2 *E. coli* Methods Approved for Ground Water Rule Monitoring

The *E. coli* methods approved for use under the GWR are specified in Exhibit 6.1 and discussed in Sections 6.2.1 - 6.2.7, below. Exhibit 6.1 lists approved methods for GWR monitoring as of the date of this guidance document. Additional methods may have been approved for GWR monitoring since this date. Additional information, including additional approved analytical methods for GWR monitoring, can be found at the following URL: <http://www.epa.gov/safewater/methods/methods.html>.

6.2.1 Colilert (Standard Methods 9223)

Colilert[®] simultaneously detects total coliforms and *E. coli* in water. Commercially prepared media formulations are available in packets for presence-absence and multiple-well procedures, and disposable tubes for the multiple-tube procedure. The use of commercially prepared media is required for quality assurance and uniformity. Incubate the sample at $35.0^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ for 24 hours. If the response is unclear after the specified incubation period, the sample is incubated for up to an additional 4 hours at $35.0^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$. After the appropriate incubation period, compare each bottle/tube/well to the reference color “comparator” provided by the manufacturer. A yellow color greater or equal to the comparator indicates the presence of total coliforms in the sample, and the bottle/tube/well is then checked for fluorescence under long-wavelength UV light (365-nm). The presence of fluorescence greater than or equal to the comparator is a positive result for *E. coli*. The concentration in MPN/100 mL, although not required under the GWR, can then be calculated based on the number of positive tubes or wells using MPN tables provided by the manufacturer.

Exhibit 6.1: *E. coli* Methods Approved for Use under the Ground Water Rule

Media	Method Reference	Description of Positive Result	Section
Colilert®	SM ¹ 9223	Yellow, fluorescent	6.2.1
Colisure®	SM ¹ 9223	Red/magenta, fluorescent	6.2.2
E*Colite	—	Blue/green, fluorescent	6.2.3
(LTB/P-A →) EC-MUG	(SM ¹ 9221B/ SM ¹ 9221D→) SM ¹ 9221F	(Growth and the presence of acid and/or gas in LTB/P-A), fluorescence in EC-MUG	6.2.4
(mEndo or LES Endo →) NA-MUG	(SM ¹ 9222B/C →) SM ¹ 9222G	(Pink to red colonies with metallic (golden-green) sheen); fluorescence after transfer to NA-MUG	6.2.5
MI Medium	EPA Method 1604	Blue colonies	6.2.6
m-ColiBlue24®	—	Blue colonies	6.2.7

¹Standard Methods for the Examination of Water and Wastewater, 20th edition.

6.2.2 Colisure® (Standard Methods 9223)

Colisure® simultaneously detects total coliforms and *E. coli* in water. Similar to the Colilert® method, Colisure® can be used in presence/absence, multiple-tube, or multiple-well procedures. The reagent is added to the sample and incubated at 35.0°C±0.5°C for 24 hours. In this method, coliform bacteria are those bacteria which produce a red or magenta color and *E. coli* also produce a fluorescent signal under a 6-watt, 365nm UV light after incubation 35±0.5°C for 24 hours. If after 24 hours the sample is a pink to orange color, incubate for an additional 4 hours, to determine the appropriate result. Samples may be incubated for a maximum of 48 hours if necessary. A red/magenta color greater or equal to the comparator indicates the presence of total coliforms in the sample, and the presence of fluorescence greater than or equal to the comparator is a positive result for *E. coli*. The concentration in MPN/100 mL, although not required under the GWR, can then be calculated based on the number of positive tubes or wells using MPN tables provided by the manufacturer.

6.2.3 E*Colite

The E*Colite test simultaneously detects total coliforms and *E. coli*. The E*Colite test involves a dehydrated medium to which a 100-mL water sample is added. The test consists of a sterile burst-a-seal bag divided into three compartments. A 100-mL water sample is added to the bag and sealed. Then the water sample is pushed through the burst-a-seal into the medium, and the two are mixed thoroughly. The bag is then incubated for 28 hours at 35.0°C±0.5°C. The bag may first be placed in a 35.0°C±0.5°C water bath for 10 minutes to bring the sample up to incubation temperature quickly. After incubation, the bag is observed for the presence of a blue/green color, which indicates a total coliform-positive sample. If the blue/green sample does not fluoresce after 28 hours, the sample should be incubated an additional 20 hours for a maximum of 48 hours and re-checked for fluorescence. If a blue/green sample is also fluorescent under an ultraviolet light (366 nm), the sample is *E. coli*-positive.

6.2.4 EC-MUG (Standard Methods 9221B/9221D → 9221F)

Positive total coliform tests from the presumptive medium (SM 9221B/9221D) are inoculated into EC broth supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG) EC-MUG, a differential medium used for the detection of *E. coli*. Growth from the presumptive total coliform test is transferred using a sterile loop or applicator stick to tubes containing EC-MUG broth. Inoculated tubes are incubated at 44.5°C±0.2°C for 24±2 hours in a water bath. All tubes exhibiting growth are examined for bright blue fluorescence under long-wavelength UV light (366-nm). If the sample exhibits growth and fluorescence, the sample is positive for *E. coli*.

6.2.5 NA-MUG (Standard Methods 9222B/9222C → 9222G)

Following initial isolation of total coliforms on mEndo or LES-Endo media, the filter is transferred to nutrient agar containing 4-methylumbelliferyl-β-D-glucuronide (NA-MUG) and incubated for 4 hours at 35.0°C±0.5°C. Presumptive colonies (pink to red colonies with a metallic (golden-green) sheen) from mEndo that fluoresce under a long-wavelength UV light (366-nm) on NA-MUG are considered *E. coli* positive.

6.2.6 MI Medium (EPA Method 1604)

The MI medium method is a single-step membrane filtration procedure used to simultaneously detect total coliforms and *E. coli* (USEPA, 2002). In this method, a water sample is filtered through a 0.45-μm membrane filter, the filter is placed on an MI agar or broth saturated pad, and the medium is incubated at 35.0°C±0.5°C for 24 hours. *E. coli* colonies exhibit a blue color. The plates can also be observed under long-wavelength UV light (366-nm) for the presence of total coliform species that fluoresce. Because the blue color from the breakdown of IBDG can mask fluorescence, non-fluorescent blue colonies are included in the total coliform count. Blue colonies regardless of fluorescence are considered *E. coli*.

6.2.7 m-ColiBlue24®

The m-ColiBlue24® method is a single-step membrane filtration procedure that simultaneously detects total coliforms and *E. coli*. A water sample is filtered through a 0.45-μm membrane filter, and the filter is transferred to a plate containing an absorbent pad saturated with m-ColiBlue24® broth. The filter is incubated at 35.0°C±0.5°C for 24 hours and examined for colony growth (Hach Co., 1999). Total coliforms are indicated by red colonies. The presence of *E. coli* is indicated by blue colonies.

6.3 Enterococci Methods Approved for Ground Water Rule Monitoring

The enterococci methods approved for use under the GWR are specified in Exhibit 6.2 and discussed below, in Sections 6.3.1 - 6.3.4.

6.3.1 Multiple-Tube Technique (Standard Methods 9230B)

The Azide Dextrose/BEA/BHI protocol for detecting enterococci in water employs a three-step procedure (presumptive fecal streptococcus, confirmed fecal streptococcus, and enterococcus) to determine the presence of enterococci bacteria (APHA, 1998). In the presumptive phase, azide dextrose broth is inoculated incubated at $35.0 \pm 0.5^{\circ}\text{C}$. Growth within 48 ± 3 hours constitutes a positive presumptive reaction for fecal streptococci.

Note: Although Standard Methods 9230B indicated the use of Pfizer selective enterococcus (PSE) agar for confirmation, it is no longer commercially available. Bile esculin agar (BEA) is an accepted alternative plating medium for confirmation testing.

After enrichment during the presumptive phase, positive azide dextrose tubes are subjected to a fecal streptococci confirmation step. A portion of growth from each positive azide dextrose tube is streaked onto bile esculin agar (BEA). Inverted plates are incubated at $35.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours and observed for the presence of brownish-black colonies with a brown halo. Such colonies are confirmed as fecal streptococci. Target colonies from the BEA medium are transferred to a tube of brain heart infusion (BHI) broth and incubated at $45^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 48 hours. Simultaneously, target colonies from the BEA are transferred to BHI broth containing 6.5% NaCl and incubated at $35.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 48 hours. Growth at 45.0°C in BHI broth and in BHI broth containing 6.5% NaCl at 35.0°C is indicative of enterococci.

Exhibit 6.2 Enterococci Methods Approved for Use under the GWR

Media	Method Reference	Description of Positive Results	Section
Azide Dextrose / BEA / BHI	SM ¹ 9230B	Growth at 45°C in BHI and growth in BHI with 6.5% NaCl at 35°C	6.3.1
mE-EIA	SM ¹ 9230C	Pink to red colonies that form black or reddish-brown precipitate on underside of filter	6.3.2
mEI	EPA Method 1600	All colonies with a blue halo	6.3.3
Enterolert™	Budnick, G.E. et al., 1996	Presence of blue-white fluorescence	6.3.4

¹Standard Methods for the Examination of Water and Wastewater 20th edition.

6.3.2 mE-EIA (Standard Methods 9230C)

The mE-EIA agar method is a two-step membrane filtration procedure that detects enterococci based on the development of colonies on the surface of a filter when placed on a selective medium. In this method, a water sample is filtered through a 0.45-µm membrane filter and the filter is placed on mE agar. After the plate is incubated at 41°C±0.5°C for 48±3 hours, the filter is transferred to an esculin iron agar (EIA) plate and incubated at 41°C±0.5°C for 20-30 minutes. After incubation, all pink to red colonies on mE agar that form a black or reddish-brown precipitate on the underside of the filter when placed on EIA are considered enterococci. Plates should be examined upside down to determine the presence of enterococci colonies.

6.3.3 mEI (EPA Method 1600)

The mEI agar method is a single-step membrane filtration procedure that detects enterococci in water samples (USEPA, 2006a). In this method, a water sample is filtered through a 0.45-µm membrane filter. The filter is placed on mEI agar and incubated at 41°C±0.5°C for 24 hours. Following incubation, all colonies with a blue halo, regardless of colony color, are considered enterococci.

6.3.4 Enterolert™ (Budnick, G.E. et al., 1996)

Enterolert™ is a commercially available enzyme-substrate test for the determination of enterococci in water (Budnick, G.E. et al, 1996). In this method, the sample is mixed with the Enterolert™ medium and incubated for 24 hours at 41°C±0.5°C. After incubation, the presence of blue fluorescence is a positive result for enterococci. If enumeration is desired, the concentration in MPN/100 mL can be calculated from the number of positive tubes or wells using MPN tables provided by the manufacturer.

6.4 Coliphage Methods Approved for Ground Water Rule Monitoring

The coliphage methods approved for use under the GWR are specified in Exhibit 6.3 and discussed below, in Sections 6.4.1 - 6.4.2.

Exhibit 6.3 Coliphage Methods Approved for Use under the GWR

Media	Method Reference	Description of Positive Result	Section
Two-Step Enrichment	EPA Method 1601	Presence of plaques (circular lysis zones)	6.4.1
Single Agar Layer	EPA Method 1602	Presence of plaques (circular lysis zones)	6.4.2

6.4.1 Two-Step Enrichment (Method 1601)

The two-step enrichment procedure detects the presence or absence of somatic or male-specific coliphage based on the formation of plaques after enrichment. A 100-mL or 1-L ground water sample is supplemented with MgCl₂ (magnesium chloride), log-phase host bacteria (*E. coli*_{Famp} for male-specific coliphage or *E. coli* CN-13 for somatic coliphage), and tryptic soy broth (TSB) in an enrichment step. After overnight incubation, samples are “spotted” onto a lawn of host bacteria specific for each type of coliphage, incubated, and examined for plaque forming units, which indicate the presence of coliphage.

6.4.2 Single Agar Layer (SAL) (Method 1602)

The single agar layer procedure is a single-step procedure to detect somatic or male-specific coliphage. If desired, this method can be used to provide a direct count of somatic or male-specific coliphage. A 100-mL ground water sample is assayed by adding MgCl₂ (magnesium chloride), log-phase host bacteria (*E. coli*_{Famp} for male-specific coliphage or *E. coli* CN-13 for somatic coliphage), and 100 mL of double-strength molten tryptic soy agar to the sample. The sample is thoroughly mixed and the total volume is poured into 5 to 10 plates (dependent on plate size). After overnight incubation, plaques are counted and summed for all plates from a single sample. The quantity of coliphage in a sample can be expressed as plaque forming units (PFU)/100 mL, if enumeration is desired.

7. Evaluating Fecal Indicator Data

Data on sample measurements, sample processing times, and sample results are recorded at the laboratory when fecal indicator samples are processed and analyzed by the laboratory. Although only final results (e.g., presence/absence, CFU/100 mL, MPN/100 mL, PFU/100 mL) may be reported by the laboratory to the GWS, the “primary” data elements recorded by the laboratory will likely need to be consulted if questions on the data arise. This chapter provides an overview of the data recording and reporting processes and provides guidance on how to review and interpret the data. This information will be useful in the event that a GWS requests that a sample result be invalidated.

7.1 Data Recording at the Laboratory

The laboratories performing fecal indicator analyses under the Ground Water Rule (GWR) should record the following general types of information:

- Sample identification information
- The incubation start/read times and temperatures for each method to verify that method requirements were met
- The name of the analyst performing the sample analysis
- Primary measurements performed by the laboratory during sample analysis to determine the presence/absence/concentration of the fecal indicator
- QC analysis results (e.g., positive/negative controls, blanks, OPR, etc.)

Example bench sheets for each of the methods approved for use under the GWR (Section 6) can be found in Appendix D - Appendix F.

7.1.1 Sample Identification Information

Sample identification information is used to track the sample through sample collection, analysis, and data reporting. At a minimum, the laboratory should record the GWS PWSID, sample collection date, sample collection time, sample collection location, sample ID, and the fecal indicator(s) (e.g., enterococci, *E. coli*, somatic coliphage, male-specific coliphage) being analyzed.

7.1.2 Primary Data

The laboratory records all primary measurements necessary to determine the presence or absence of the GWR fecal indicator(s). Primary measurements may include the sample volume analyzed and the results of preliminary steps for the determination of the fecal indicator. If the GWS chooses to determine the concentration of the fecal indicator (rather than simply the presence or absence of the indicator), primary measurements may also include the number of positive tubes or wells for each volume analyzed (multiple-tube and multiple-well methods), the number of colony-forming units (membrane filtration methods), or the number of plaque forming units (Method 1602).

7.1.3 Sample Results

The final result of fecal indicator analyses will be reported as “present” or “absent” per volume analyzed unless the GWS elects to perform quantitative analyses. If the GWS requests the laboratory to determine the concentration of the fecal indicator in the sample, the final result will be reported as CFU/100 mL (for membrane filtration methods), MPN/100 mL (for multiple-tube and multiple-well methods), or PFU/100 mL (for EPA Method 1602).

7.2 Data Archiving

GWR source water monitoring data must be maintained by the GWS for a period of five years. The requirements of The NELAC Institute (TNI) (formerly known as the National Environmental Laboratory Accreditation Conference (NELAC)) require also require data to be maintained by the laboratory for a period of five years.

7.3 Evaluating Fecal Indicator Data

If questions arise regarding the validity of *E. coli* or enterococci results submitted by the GWS, the information recorded by the laboratory on the data reporting forms should be carefully evaluated. Sections 8.3.1 through 8.3.3 provide guidance on how to review the data reporting forms and verify the accuracy.

7.3.1 Data Completeness Check

The first step in evaluating hard copy sample results for a GWR monitoring sample is to verify that the information below is included. If information is missing, incomplete, or incorrect, the State should request that the GWS contact the laboratory to request the missing information.

- **Sample result summary sheet.** This form should include the following information:
 - Sample identification information
 - Sample result
 - Laboratory QC checklist or other verification from the laboratory that all QC requirements were met
- **Sample collection form.** This form should have been completed by the GWS at the time of sample collection, indicating when and where the sample was collected.
- **Method bench sheet.** This form should have been completed by the laboratory with primary sample processing and analysis data associated with the monitoring sample
- **Laboratory comments.** If the laboratory encountered problems with the sample (e.g., receipt, processing, or analysis), they should be documented with the sample results; any of these issues may be associated with a GWS’s request to invalidate data. Possible issues include the following:
 - Sample arrived at the laboratory in unacceptable condition (i.e., leaking).
 - Sample holding time was exceeded.
 - Sample was frozen.
 - Sample holding temperature was not within acceptable range.
 - Unacceptable blank sample result.
 - Unacceptable positive or negative control result.

- Media sterility checks were not acceptable.
- Method incubation times or temperatures were not within acceptable range.
- Membrane filtration: Too much sediment on the filter.
- Membrane filtration: Confluent growth of non-target organism.
- Membrane filtration: Pre- or post- filtration series sterility check not acceptable (e.g., contamination with *E. coli* or enterococci).
- Quanti-Tray[®] was damaged or leaked.
- Sample was not distributed to all wells in Quanti-Tray[®].
- Positive presumptive tubes prepared for multiple-tube analyses were not transferred into the appropriate confirmatory medium.
- All rows of tubes prepared for multiple-tube analyses were not inoculated.

Any of the above data qualifiers may result in the sample being considered invalid for GWR monitoring requirements.

7.3.2 Evaluation of Data Against Method Quality Control Requirements

The following items may be reviewed to verify that the laboratory analyzed the GWR *E. coli* or enterococci sample within the analytical controls specified by the method:

- **Sample condition upon receipt.** If the sample was shipped to the laboratory, the completed sample collection form will reflect that the sample was received in acceptable condition (e.g., not leaking, etc.), and was not frozen. The temperature of the sample upon receipt should be noted. Systems are encouraged, but not required, to maintain the sample at <10°C during transport. If the sample is >10°C upon arrival, the sample result may be affected.
- **QC samples associated with source water samples.** Verification can be made that the required QC samples were run with the field sample(s). The frequency of analysis of quality control samples, including method blanks and positive and negative controls, depends on the method-specified requirements and the requirements in the *Manual for the Certification of Laboratories Analyzing Drinking Water* (USEPA, 2005).
- **Holding time.** The sample collection date and time on the data collection form and the date and time of the initiation of sample analysis, recorded by the laboratory on the method bench sheet, can be used to verify that the laboratory began sample analysis within 30 hours of sample collection.
- **Incubation times and temperatures.** The dates, times, and temperatures for the incubation initiation and completion of all method steps (recorded by the laboratory on the method bench sheet) can be used to verify consistency with the incubation times and temperatures..

8. Frequently Asked Questions

8.1 Collecting and Shipping Ground Water Samples

Does the sampling procedure differ for bacterial and viral indicators?

Separate guidance for the collection of bacterial and viral indicators is provided in Section 5.2, as well as Appendix B (for bacterial indicators) and Appendix C (for viral indicators). The primary difference in the sample collection procedures is the flexibility to collect larger sample volumes for the analysis of coliphage using EPA Method 1601.

If the GWS is within driving distance of a certified laboratory, is the system required to monitor the temperature of the samples in the same manner as samples shipped overnight?

Under the GWR, systems are encouraged, but not required, to hold samples at <10°C regardless of whether samples are shipped overnight or delivered to a certified laboratory within driving distance. Samples for all analyses should remain above freezing at all times and samples that arrive frozen should not be analyzed. Please refer to Section 5.5 for detailed guidance on holding temperature and temperature monitoring.

Can a ground water system analyze > 100 mL for bacterial indicators?

Yes, more than 100 mL of sample may be analyzed. It should be noted that larger sample volumes may increase the analytical sensitivity of the method. However, certain conditions (e.g., high turbidity) may prohibit the analysis of larger sample volumes.

8.2 Understanding Ground Water Rule Fecal Indicator Analyses

If the method format allows for enumeration, can the GWS report the numerical value or do they have to report presence/absence?

Unless otherwise specified by the State, the GWS should report the results to the State as “present” or “absent” regardless of method format.

*If the GWS initially selects membrane filtration for the analysis for *E. coli* or enterococci, can they switch to another format?*

Yes. Generally *E. coli* and enterococci samples should be analyzed using the same method during source water monitoring. However, if it is necessary to switch methods, Exhibits 6.1 and 6.2 lists methods approved for *E. coli* and enterococci, respectively. Additional methods may have been approved for GWR monitoring since this date. Additional information, including additional approved analytical methods for GWR monitoring, can be found at the following URL: <http://www.epa.gov/safewater/methods/methods.html>.

Which methods are better for the analyses of the bacterial indicators?

All bacterial methods approved under the GWR are considered comparable for the evaluation of the target analyte (*E. coli* or enterococci).

Can a GWS use the methods in SM 9224 to analyze for coliphage?

No. SM 9224 has not been approved for use under the GWR. The only methods approved for coliphage monitoring under the GWR are EPA Method 1601 and EPA Method 1602. See Section 6.4 of this manual for a detailed discussion of the methods approved for coliphage monitoring. Section 6.4 discusses approved methods for GWR monitoring as of the date of this guidance document. Additional methods may have been approved for GWR monitoring since this date. Additional information, including additional approved analytical methods for GWR monitoring, can be found at the following URL: <http://www.epa.gov/safewater/methods/methods.html>.

Will the certification program be expanded to include enterococci and coliphage analyses?

The Drinking Water Certification Manual (5th Edition) has been revised to include GWR-approved enterococci and coliphage methods. In addition, EPA's training course "Laboratory Certification Officer's Training Course" is providing auditors with training that will allow them to audit laboratories using both enterococci and coliphage methods.

Are the methods recently approved for use under the Total Coliform Rule (e.g., ReadyCult, Chromocult) approved for E. coli monitoring under the GWR?

No. Exhibit 6.1 lists approved methods for GWR monitoring as of the date of this guidance document. Additional methods may have been approved for GWR monitoring since this date. Additional information, including additional approved analytical methods for GWR monitoring, can be found at the following URL: <http://www.epa.gov/safewater/methods/methods.html>.

8.3 Evaluating Fecal Indicator Data

Is it up to the GWS to validate the data/final results submitted by the laboratory?

Yes. The GWS should evaluate/validate the data submitted by the laboratory prior to submitting the results to the State. Data review and interpretation guidance are provided in Chapter 8 of this manual. In the event the GWS requests that a sample be considered invalid, the State should request the primary data from the GWS for evaluation.

Can the State invalidate a positive source water sample result?

Yes. The State may invalidate a positive source water sample if a laboratory establishes that improper sample analyses caused the positive result or if the State has substantial grounds to believe that a positive result was attributable to a circumstance or condition that did not reflect source water quality. The State must document this in writing.

9. References

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Appendix A

Glossary

Revised March 2008

Glossary

Analytical holding time - The time between the collection of the sample and the start of analysis.

Aquifer - A geologic formation, a group of formations, or a part of a formation that is water bearing. A geological formation or structure that stores or transmits water, or both, such as to wells and springs.

Coliphage - Viruses that infect *E. coli* in Methods 1601 and 1602.

Community Water System (CWS) - A public water system that serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

DAL - Double agar layer plate used to enumerate coliphage in Methods 1601 and 1602.

E. coli - A bacteria of the fecal coliform group that can be used as an indicator of fecal contamination in water.

Enteric pathogens - Pathogens that infect the gastrointestinal tract.

Enterococci - A bacteria in the fecal streptococci group that can be used as an indicator of fecal contamination in water.

Fecal indicators - Organisms that can be used as indicators of fecal contamination of water.

Fractured bedrock aquifers - Under the Ground Water Rule all igneous and metamorphic aquifers.

Gravel aquifer - Unconsolidated water-bearing deposits of well-sorted pebbles, cobbles, and boulders.

Ground Water System (GWS) - For the purposes of the GWR and this guidance document, a GWS is defined as any public water system that uses ground water as a source unless that ground water source is combined with surface water, or ground water under direct influence of surface water, prior to treatment under subpart H (“Filtration and Disinfection”).

Hydrogeologic sensitivity assessment (HSA) - A determination of whether a ground water system obtains water from a hydrogeologically sensitive setting.

Igneous aquifer - An aquifer consisting of rock formed by the cooling and solidification of magma.

Karst aquifer - An aquifer with geologic terrain within which flowing ground water has dissolved significant portions of the area's soluble (usually carbonate) rocks.

Lysis zone - Typically a circular zone of clearing indicating a sample is positive for coliphages in Method 1601.

Male-specific coliphage - Viruses (bacteriophages) that infect coliform bacteria only via the F-pilus.

Matrix spikes (MS) - A sample prepared by adding a known quantity of organisms to a specified amount of sample matrix for which an independent estimate of target analyte concentration is available. A matrix spike is used to determine the effect of the matrix on a method's recovery

Method blank - An aliquot of reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, and procedures that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Membrane filtration - Direct plating of a sample for detection and estimation of coliform densities.

Metamorphic aquifer - Aquifer formed from rock that has been transformed under extreme pressure or heat from sedimentary, igneous, or other older metamorphic rock.

Most probable number (MPN) format - A method format uses an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by laboratory examination. It is not an actual enumeration.

Multiple-well format - A method format that uses the number of positive wells to determine a result using an MPN index.

National Environmental Laboratory Accreditation Conference (NELAC) - A cooperative association of States and Federal Agencies, formed to establish and promote mutually acceptable performance standards for the operation of environmental laboratories.

Negative control culture - A culture that, when analyzed exactly like a field sample, will produce a negative result for a given type of media.

Non-community water system (NCWS) - A public water system that is not a community water system. A non-community water system is either a transient non-community water systems or a non-transient non-community water system.

Ongoing demonstration of capability (ODC) - Reagent water samples spiked with known quantities of analytes and analyzed exactly like a field sample. The purpose of this test is to assure that the results produced by the laboratory remain within limits specified in this method.

Ongoing precision and recovery (OPR) - A reagent water sample method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified within this method for precision and recovery.

Plaque - Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria in DAL or SAL plates after incubation (Method 1601 and 1602) or SAL (Method 1601).

Plaque forming units (PFU) - The units for reporting the concentration of coliphage in Method 1602.

Positive control - For Method 1601 and 1602 a reagent water sample spiked with sewage filtrate or pure coliphage stock culture that is used to assess the stock coliphage suspensions, host bacterial cultures, and growth media are performing properly.

Primacy - Primary enforcement responsibility-primary responsibility for administration and enforcement of primary drinking water regulations and related requirements applicable to public water systems within a State.

Presence/absence format - A qualitative method format for detection of a microorganism where the result indicates whether or not the microorganism is present in the sample. The presence/absence format method will not

give the numbers of a microorganism present.

Quality assurance (QA) - The system of management controls that cover planning, implementation, and review of data collection activities and data use.

Quality control (QC) - The technical functions that include all scientific precautions, such as calibrations, controls, and duplications, that are needed to acquire data of a known and adequate quality.

Quantitative format - A method format for detection of a microorganism where the result indicates the concentration of microorganism in the sample.

SAL - Single agar layer plate used to enumerate coliphage in Method 1601.

Sanitary survey - An on-site review of the water source, facilities, equipment, operation, and maintenance of the public water system for the purpose of evaluating the adequacy of such source, facilities, equipment, operation, and maintenance for producing and distributing safe drinking water.

Somatic coliphage - Those coliphage that infect host cells via the outer cell membrane but do not infect host cells via the F-pilus.

Total Coliform Rule (TCR) - EPA rule that sets monitoring and compliance requirements for coliform bacteria in all PWSs.

Turbidity - Unit of measurement quantifying the degree to which light traveling through a water column is scattered by the suspended organic (including algae) and inorganic particles.

Appendix B

Procedure for Collecting Ground Water Samples for *E. coli* and Enterococci Analyses

Revised March 2008

Procedure for Collecting Ground Water Samples for *E. coli* and Enterococci Analyses

1.0 Materials

1.1 Check to make sure the following materials are available prior to collecting sample(s):

- Several pairs of new, powder-free latex gloves (Lab Safety Supply, cat. number 16285XL, or equivalent)
- 250 mL, Sterile glass or plastic containers with a leak-proof lids (Nalgene 2105-0008 or equivalent)
- Labels

1.2 The following additional materials will be necessary if the sample will be shipped off-site for analysis:

- Sample collection form
- Plastic bags (1 gallon) (Ziplock, or equivalent)
- Cooler, approximately 9-quart (Coleman, cat. number, 6209-703, or equivalent)
- Two large plastic trash bags
- One 8-lb. bag of ice or Gel ice packs (VWR, cat. number, 15715-105, or equivalent)
- Strapping tape
- Two, self-adhesive plastic airbill sleeves
- Airbill for shipment
- Duct tape

2.0 Collecting the Sample

2.1 If the sample will be analyzed on-site, record the sample number, sample location, samplers name, observations, and sampling date and time in a sampling log book.

If the sample will be shipped off-site, record the following information on the sample collection form:

- Name of system (e.g., Public Water System Identification number)
- Sample site location
- Sample type (assessment, triggered)
- Sampler's name
- Sample number
- Date of sample collection
- Time of sample collection
- Analysis requested

2.2 Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

2.3 Adjust the flow of water out of the tap or hose so the water will not splash out when it is collected.

- 2.4 Using aseptic technique (i.e., sanitize tap, do not touch the inside of the sample container), fill each of the *E. coli* and enterococci sample containers, leaving at least 1 inch of head space. Do not expose leave a container open for any longer than necessary. Record the system name, sampler's name, sample number, sample type, date and time of sample collection, sample location, and analysis requested on the sample container.
- 2.5 Immediately following sample collection, tighten the sample container lid. If the sample will be shipped off-site for analysis, and will not be shipped for several hours, place the sample container upright in a refrigerator to maintain the sample at a temperature of <math><10^{\circ}\text{C}</math> prior to shipment. If a refrigerator is not available, wrap the sample with insulation such as bubble wrap or paper towels (to prevent freezing), place the sample in a ziplock bag, and place the bag containing the sample in the shipping cooler with wet ice or ice packs. Replace with fresh ice or ice packs immediately prior to shipment.

3.0 Packing the Sample (Applicable to Samples Shipped Off-Site for Analysis)

- 3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, disperse 6 pounds of ice into 3 to 4 plastic, ziplock bags. Gel packs or blue ice may be used in lieu of wet ice, as long as the sample is maintained at the appropriate temperature range. Seal the ziplock bags, expelling as much air as possible, and secure top with tape.

Note: Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will help prevent leakage and delays.

- 3.2 Place the bag containing the samples into the shipping container. Place the ice or ice packs around, but not directly on, the sample bag to help prevent freezing. Seal each liner bag by twisting top of bag and tying in a knot.
- 3.3 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid. Sign and date the sample collection form and fold the completed sample collection form and place it inside the plastic sleeve.
- 3.4 Close the cooler lid, seal the joints with duct tape, and secure the lid with strapping tape by taping the cooler at each end, perpendicular to the seal.

Note: Be sure to seal the cooler joints as shipping companies may delay sample shipments if leakage occurs.

- 3.5 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

- 4.1 Contact the laboratory to notify them prior to sampling. This will allow the labs to ensure that they have the appropriate media ready for the samples.
- 4.2 Ship samples on the day of collection and use a reliable shipping service for priority overnight delivery

- 4.3 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.
- 4.4 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.
- 4.5 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix C

Procedure for Collecting Ground Water Samples for Somatic and/or Male-Specific Coliphage Analyses

Revised March 2008

Procedure for Collecting Ground Water Samples for Somatic and/or Male-Specific Coliphage Analyses

1.0 Materials

1.1 Check to make sure the following materials are available prior to collecting sample:

- Several pairs of new, powder-free latex gloves (Lab Safety Supply, cat. number 16285XL, or equivalent)
- Sterile glass or plastic containers with leak proof lids
 - 250 mL (Nalgene 2105-0008 or equivalent) for 100 mL samples
 - 4 L (Nalgene 2121-0010 or equivalent) for 1 L samples
- Labels

1.2 The following additional materials will be necessary if the sample will be shipped off-site for analysis:

- Sample collection form
- Plastic bags (1 gallon) (Ziplock, or equivalent)
- For 100 mL samples: cooler, approximately 9-quart (Coleman, cat. number, 6209-703, or equivalent)
- For 1 L samples: cooler, approximately 28-quart (Coleman, cat. number, 5277-718, or equivalent)
- Two large plastic trash bags
- One 8-lb. bag of ice or Gel ice packs (VWR, cat. number, 15715-105, or equivalent)
- Strapping tape
- Two, self-adhesive plastic airbill sleeves
- Airbill for shipment
- Duct tape

2.0 Collecting the Sample

2.1 If the sample will be analyzed on-site, record the sample number, sample location, samplers name, observations, and sampling date and time in a sampling log book.

If the sample will be shipped off-site, record the following information on the sample collection form:

- Name of system (e.g., Public Water System Identification number)
- Sample site location
- Sample type (assessment, triggered)
- Sampler's name
- Sample number
- Date of sample collection
- Time of sample collection
- Analysis requested

2.2 Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. The service line should be cleared before sampling by maintaining a steady water

flow for at least two minutes (until the water changes temperature). *Note:* Sample bottles should not be pre-rinsed with sample when collecting somatic and/or male-specific coliphage samples.

- 2.3 Adjust the flow of water out of the tap or hose so the water will not splash out when it is collected.
- 2.4 Using aseptic techniques (i.e., sanitize tap, do not touch the inside of the sample container, etc.), fill each of the somatic and/or male-specific coliphage sample containers, leaving at least 1 inch of head space. Do not leave a container open for any longer than necessary. Record the system name, sampler's name, sample number, sample type, date and time of sample collection, sample location, and analysis requested on the sample container.
- 2.5 Immediately following sample collection, tighten the sample container lid. If the sample will be shipped off-site for analysis, and will not be shipped for several hours, place the sample container upright in a refrigerator to maintain the sample at a temperature of $< 8^{\circ}\text{C}$ prior to shipment. If a refrigerator is not available, wrap the sample with insulation such as bubble wrap or paper towels (to prevent freezing), place the sample in a ziplock bag, and place the bag containing the sample in the shipping cooler with wet ice or ice packs. Replace with fresh ice or ice packs immediately prior to shipment.

3. Packing the Sample (Applicable to Samples Shipped Off-Site for Analysis)

- 3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, disperse 6 pounds of ice into 3 to 4 plastic, ziplock bags. Gel packs or blue ice may be used in lieu of wet ice, as long as the sample is maintained at the appropriate temperature range. Seal the ziplock bags, expelling as much air as possible, and secure top with tape.

Note: Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will help prevent leakage and delays.

- 3.2 Place the bag containing the samples into the shipping container. Place the ice or ice packs around, but not directly on, the sample bag to help prevent freezing. Seal each liner bag by twisting top of bag and tying in a knot.
- 3.3 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid. Sign and date the sample collection form. Fold the completed sample collection form and place it inside the plastic sleeve.
- 3.4 Close the cooler lid, seal the joints with duct tape, and secure the lid with strapping tape by taping the cooler at each end, perpendicular to the seal.

Note: Be sure to seal the cooler joints as shipping companies may delay sample shipments if leakage occurs.

- 3.5 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

- 4.1 Contact the laboratory to notify them prior to sampling. This will allow the labs to ensure that they have the appropriate media and, in the case of coliphage, host organisms ready for the samples.
- 4.2 Ship samples on the day of collection and use a reliable shipping service for priority overnight delivery.
- 4.3 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.
- 4.4 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.
- 4.5 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix D

***E. coli* Method Bench Sheets**

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

**Ground Water Rule Source Water Monitoring
Presence/Absence:
E. coli (Colilert® and Colisure)
Presence/Absence (SM 9223)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Please circle the method used for analysis:	
Colilert	Colisure

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Additional incubation start	
Date	
Time	
Temperature (°C)	

Additional incubation end	
Date	
Time	
Temperature (°C)	

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume analyzed (mL)	Total coliforms "+" = present "-." = absent	<i>E. coli</i> "+" = present "-." = absent

Comments

**Ground Water Rule Source Water Monitoring
Multiple-Tube:
E. coli (Colilert® and Colisure)
Most Probable Number (SM 9223)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Please circle the method used for analysis:	
Colilert	Colisure

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Additional incubation start	
Date	
Time	
Temperature (°C)	

Additional incubation end	
Date	
Time	
Temperature (°C)	

	Analyst initials	Start Temp.	Read Temp.	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
Total coliforms/ <i>E. coli</i>	/	/	/	/	/	/	/	/	/	/	/	/	/

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS	
"+" = present "-" = absent	
Total coliforms	<i>E. coli</i>

QUANTITATIVE RESULTS		
	Total coliforms	<i>E. coli</i>
Number of positive tubes		
Organisms per 100 mL		

Comments

**Ground Water Rule Source Water Monitoring
Multiple-Well (51 Wells):
E. coli (Colilert® and Colisure)
Quanti-Tray 51 (SM 9223)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Please circle the method used for analysis:	
Colilert	Colisure

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Additional incubation start	
Date	
Time	
Temperature (°C)	

Additional incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume analyzed (mL)	Total coliforms "+" = present "- " = absent	E. coli "+" = present "- " = absent

QUANTITATIVE RESULTS					
Analyst initials	Volume analyzed (mL)	Total coliforms		E. coli	
		Number of positive wells	Total coliforms per 100 mL	Number of positive wells	E. coli per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Multiple-Well (97 Wells):
E. coli (Colilert® and Colisure)
Quanti-Tray 2000 (SM 9223)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Please circle the method used for analysis:	
Colilert	Colisure

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Additional incubation start	
Date	
Time	
Temperature (°C)	

Additional incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume analyzed (mL)	Total coliforms "+" = present "." = absent	E. coli "+" = present "." = absent

QUANTITATIVE RESULTS							
Analyst initials	Volume analyzed (mL)	Total Coliforms			E. coli		
		Number of large positive wells	Number of small positive wells	Total coliforms per 100 mL	Number of large positive wells	Number of small positive wells	E. coli per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Presence/Absence: *E. coli* (E*Colite)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete the presence/absence results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume analyzed (mL)	Total coliforms "+" = present "- " = absent	<i>E. coli</i> "+" = present "- " = absent

Comments

**Ground Water Rule Source Water Monitoring
Multiple-Tube Fermentation: *E. coli* (LTB/EC-MUG)
(SM 9221B/9221F)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

	LTB incubation start	LTB 24 hour incubation read	LTB 48 hour incubation read
Date			
Time			
Temperature (°C)			

EC-MUG incubation 24 hour read (from 24 hour LTB)	
Date	
Time	
Temperature (°C)	

EC-MUG incubation 24 hour read (from 48 hour LTB)	
Date	
Time	
Temperature (°C)	

LTB

LTB read	Analyst initials	Start Temp.	Read Temp.	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
24 hr LTB/ 48 hr LTB													

EC-MUG

EC-MUG read	Analyst initials	Start Temp.	Read Temp.	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
from 24 hr LTB													
from 48 hr LTB													

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS
<i>E. coli</i> "+" = present "-" = absent

QUANTITATIVE RESULTS	
Number of positive tubes	<i>E. coli</i> per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Membrane Filtration: *E. coli* (mEndo or LES-Endo/NA-MUG)
(SM 9222B to 9222G)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

mEndo or LES-Endo incubation start	
Date	
Time	
Temperature (°C)	

mEndo or LES-Endo incubation end	
Date	
Time	
Temperature (°C)	

NA-MUG incubation start	
Date	
Time	
Temperature (°C)	

NA-MUG incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume filtered	Total coliforms "+" = present "- " = absent	<i>E. coli</i> "+" = present "- " = absent

QUANTITATIVE RESULTS					
Analyst initials	Volume filtered	Total coliforms		<i>E. coli</i>	
		Number of colonies	Total coliforms per 100 mL	Number of colonies	<i>E. coli</i> per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Membrane Filtration: *E. coli* (MI Medium)
(EPA Method 1604)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

MI Medium incubation start	
Date	
Time	
Temperature (°C)	

MI Medium incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume filtered	Total coliforms "+" = present "- " = absent	<i>E. coli</i> "+" = present "- " = absent

QUANTITATIVE RESULTS					
Analyst initials	Volume filtered	Total coliforms		<i>E. coli</i>	
		Number of colonies	Total coliforms per 100 mL	Number of colonies	<i>E. coli</i> per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Membrane Filtration: *E. coli* (m-ColiBlue24®)
(m-ColiBlue24)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

m-ColiBlue24 incubation start	
Date	
Time	
Temperature (°C)	

m-ColiBlue24 incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS			
Analyst initials	Volume filtered	Total coliforms "+" = present "-" = absent	<i>E. coli</i> "+" = present "-" = absent

QUANTITATIVE RESULTS					
Analyst initials	Volume filtered	Total coliforms		<i>E. coli</i>	
		Number of colonies	Total coliforms per 100 mL	Number of colonies	<i>E. coli</i> per 100 mL

Comments

Appendix E

Enterococci Method Bench Sheets

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

**Ground Water Rule Source Water Monitoring
Multiple-Tube: Enterococcus (Azide Dextrose/BEA/BHI/BHI-6.5% NaCl)
(SM 9230B)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

	ADB incubation start	ADB 24 hour incubation read	ADB 48 hour incubation read
Date			
Time			

24 hour BEA read (from 24 hour ADB)	
Date	
Time	
Temperature (°C)	

24 hour BEA read (from 48 hour ADB)	
Date	
Time	
Temperature (°C)	

48 hour BHI (at 45°C) read (from 24 hour BEA)	
Date	
Time	
Temperature (°C)	

48 hour BHI-6.5% NaCl read (from 24 hour BEA)	
Date	
Time	
Temperature (°C)	

ADB

ADB read	Analyst initials	Start Temp.	Read Temp.	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
24 hr ADB/ 48 hr ADB	/	/	/	/	/	/	/	/	/	/	/	/	/

BEA Plates

BEA read	Analyst initials	Start Temp.	Read Temp.	Plate 1 (10 mL)	Plate 2 (10 mL)	Plate 3 (10 mL)	Plate 4 (10 mL)	Plate 5 (10 mL)	Plate 6 (10 mL)	Plate 7 (10 mL)	Plate 8 (10 mL)	Plate 9 (10 mL)	Plate 10 (10 mL)
from 24 hr ADB/ from 48 hr ADB	/	/	/	/	/	/	/	/	/	/	/	/	/

BHI/BHI-6.5% NaCl READ

BHI/ BHI-6.5% NaCl	Analyst initials	Start Temp.	Read Temp.	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
BHI (at 45°C)/ BHI-6.5% NaCl	/	/	/	/	/	/	/	/	/	/	/	/	/

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS
Enterococci "+" = present "-" = absent

QUANTITATIVE RESULTS	
Number of positive tubes	Enterococci per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Membrane Filtration: Enterococcus (mE-EIA)
(SM 9230C)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

mE incubation start	
Date	
Time	
Temperature (°C)	

mE incubation end	
Date	
Time	
Temperature (°C)	

EIA incubation start	
Date	
Time	
Temperature (°C)	

EIA incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS		
Analyst initials	Volume filtered	Enterococcus "+" = present "- " = absent

QUANTITATIVE RESULTS			
Analyst initials	Volume filtered	Number of colonies	Enterococci per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Membrane Filtration: Enterococcus (mEI)
(EPA Method 1600)**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

mEI incubation start	
Date	
Time	
Temperature (°C)	

mEI incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete either the presence/absence results table or the quantitative results table based on the type of analysis requested by the PWS.

PRESENCE/ABSENCE RESULTS		
Analyst initials	Volume filtered	Enterococcus "+" = present "-" = absent

QUANTITATIVE RESULTS			
Analyst initials	Volume filtered	Number of colonies	Enterococci per 100 mL

Comments

**Ground Water Rule Source Water Monitoring
Multiple-Tube:
Enterococcus (Enterolert™)
Most Probable Number**

Laboratory: _____ Sample collection date: _____ Sample collection time: _____

PWS ID: _____ Sampling point: _____

Incubation start	
Date	
Time	
Temperature (°C)	

Incubation end	
Date	
Time	
Temperature (°C)	

Note: Complete the most probable number results table based on the type of analysis requested by the PWS.

MOST PROBABLE NUMBER RESULTS

	Analyst initials	Tube 1 (10 mL)	Tube 2 (10 mL)	Tube 3 (10 mL)	Tube 4 (10 mL)	Tube 5 (10 mL)	Tube 6 (10 mL)	Tube 7 (10 mL)	Tube 8 (10 mL)	Tube 9 (10 mL)	Tube 10 (10 mL)
Enterococci											

QUANTITATIVE RESULTS	
Number of positive tubes	Enterococci per 100 mL

Comments

Appendix F

Coliphage Method Bench Sheets

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

Method 1601: Two-Step Enrichment, Presence/Absence

Please complete one report form for each spot plate

Section 1: General Information

Laboratory name:	Analyst(s):
Phage type (circle one): Male-specific Somatic	Volume analyzed (circle one): 100 mL 1 L
PWS ID:	Sample collection:
Batch ID:	Spot plate ID:

Section 2: Sample Analysis

Indicate "+" = presence "-" = absence									
Sample ID	Sample collection date	Sample collection time	Enrichment start date	Enrichment start time	Enrichment start temp	Enrichment end date	Enrichment end time	Enrichment end temp	Results
Positive Control									
Method Blank									

Spot plate	Spot plate
Start date: _____	End date: _____
Start time: _____	End time: _____
Start temp: _____	End temp: _____
Analysts initials: _____	Analysts initials: _____

Section 3: Comments

• PFU =

• V =

Method 1602: Single Agar Layer (SAL), Quantitative

Section 1: General Information

Laboratory name:	Analyst(s):
PWS ID:	Sample collection point:
Coliphage type (circle one): Male-specific Somatic	Batch ID:

Section 2: Sample Analysis

Start date: _____	End date: _____
Start time: _____	End time: _____
Start temp: _____	End temp: _____
Analysts initials: _____	Analysts initials: _____

Sample ID	Sample collection date	Sample collection time	No. PFU per Plate 1	No. PFU per Plate 2	No. PFU per Plate 3	No. PFU per Plate 4	No. PFU per Plate 5	Total PFU per 100 mL sample

Section 3: Comments

Coliphage Enumeration by the Double Agar Layer Procedure (DAL, Section 11.0)

Section 1: General

Laboratory name: _____	Filtrate collection date: _____	Filtrate collection time: _____
------------------------	---------------------------------	---------------------------------

Section 2: Coliphage enumeration

Start date:	Filtrate Concentration	Dilution Factor	Inoculation volume	Number of Plaques	PFU/mL
	Undiluted	1	0.5 mL		
Start time:	Undiluted	1	0.5 mL		
Analyst initials:	0.1 dilution	0.1	0.5 mL		
	0.1 dilution	0.1	0.5 mL		
End date:	0.01 dilution	0.01	0.5 mL		
	0.01 dilution	0.01	0.5 mL		
End time:					
	0.001 dilution	0.001	0.5 mL		
Analyst initials:	0.001 dilution	0.001	0.5 mL		

Section 3: Comments

***Undiluted spiking suspension PFU / mL = (PFU1 + PFU2... PFUn)/(V1 + V2.... Vn)**

Where:

- PFU = number of plaque forming units from plates of all countable sample dilutions (dilutions with 1 or more PFU per plate, excluding dilutions with all TNTC or all zeros)
- V = volume of undiluted sample in all plates with countable plaques
- n = number of useable counts

Appendix G

Ground Water Rule Source Water Monitoring Quality Control Checklist for Presence Absence and Most Probable Number *E. coli* or Enterococci Sample Results

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

**Ground Water Rule Source Water Monitoring
Quality Control Checklist for Presence Absence and Most Probable Number
E. coli or Enterococci Sample Results**

No.	Quality Control (QC) Procedure	Description	✓
Sample Condition			
1	Sample condition upon receipt	The sample was not frozen or leaking upon receipt	
Holding Time			
2	Holding time	The sample was analyzed within 30 hours of sample collection	
General Quality Control			
3	Dilution/rinse water sterility check	The dilution/rinse water sterility check was acceptable (target organism or potentially interfering materials were not found in the sterility check)	
4	Media sterility check	The media sterility check was acceptable (target organism or potentially interfering materials were not found in the sterility check)	
5	Positive/negative controls	The positive/negative controls were acceptable	
6	Media storage requirements	The media storage requirements were not exceeded	
7	Autoclave sterilization verification	Autoclave sterilization verifications were acceptable	
8	Incubator/waterbath temperature checks	Incubator/waterbath temperatures were measured and recorded 2 times per day, 4 hours apart, and were within the temperature ranges specified in the method	
9	Refrigerator/freezer temperature checks	Refrigerator/freezer temperatures were measured and recorded once per day and were within acceptable temperature ranges	
10	Sample processing equipment sterility checks	Sample processing equipment sterility checks were acceptable (target organism or potentially interfering materials were not found in the sterility check)	

Presence Absence and Most Probable Number Specific QC			
11	Incubation time and temperature	<p>The following incubation time and temperature requirements were not exceeded:</p> <p><u>E. coli</u></p> <p>Colilert 24 - 28 hours at 35.0°C±0.5°C</p> <p>Colisure 24 hours (up to 48 hours) at 35.0°C±0.5°C</p> <p>LTB 24±2 or 48±3 hours at 35.0°C±0.5°C</p> <p>EC-MUG 24±2 hours at 44.5°C±0.2°C</p> <p>E*Colite 28 hours (up to 48 hours) at 35.0°C±0.5°C</p> <p><u>Enterococci</u></p> <p>Enterolert 24 hours at 41.0°C ± 0.5°C</p> <p>ADB 24±2 or 48±3 hours 35.0°C±0.5°C</p> <p>BEA 24±2 hours at 35.0°C±0.5°C</p> <p>BHI 48 hours at 45.0°C±0.5°C</p> <p>BHI - 6.5% NaCl 48 hours at 35.0°C±0.5°C</p>	
12	Preparation blank	The preparation blank was not contaminated with the target organism	
13	Verification of positive results	The verifications, performed in accordance with method-specific requirements, were acceptable	

Note: Please see Sections 6.5.1 and 6.5.3 for detailed descriptions of quality control procedures for *E. coli* and enterococci methods.

Appendix H

Ground Water Rule Source Water Monitoring Quality Control Checklist for Membrane Filtration *E. coli* or Enterococci Sample Results

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

**Ground Water Rule Source Water Monitoring
Quality Control Checklist for Membrane Filtration
E. coli or Enterococci Sample Results**

No.	Quality Control (QC) Procedure	Description	✓
Sample Condition			
1	Sample condition upon receipt	The sample was not frozen or leaking upon receipt	
Holding Time			
2	Holding time	The sample was analyzed within 30 hours of sample collection	
General Quality Control			
3	Dilution/rinse water sterility check	The dilution/rinse water sterility check was acceptable (target organism or potentially interfering materials were not found in the sterility check)	
4	Media sterility check	The media sterility check was acceptable (target organism or potentially interfering materials were not found in the sterility check)	
5	Positive/negative controls	The positive/negative controls were acceptable	
6	Media storage requirements	The media storage requirements were not exceeded	
7	Autoclave sterilization verification	Autoclave sterilization verifications were acceptable	
8	Incubator/waterbath temperature checks	Incubator/waterbath temperatures were measured and recorded 2 times per day, 4 hours apart and were within the temperature ranges specified in the method	
9	Refrigerator/freezer temperature checks	Refrigerator/freezer temperatures were measured and recorded once per day and were within acceptable temperature ranges	
10	Sample processing equipment sterility checks	Sample processing equipment sterility checks were acceptable (no <i>E. coli</i> / enterococci or potentially interfering materials were found in the sterility checks)	
Membrane Filtration Specific QC			
11	Incubation time and temperature	<p>The following incubation time and temperature requirements were not exceeded:</p> <p><u>E. coli</u> mEndo→NA-MUG 24±2 hours at 35.0°C±0.5°C 4 hours at 35.0°C±0.5°C LES-Endo→NA-MUG 24±2 hours at 35.0°C±0.5°C 4 hours at 35.0°C±0.5°C MI Medium 24 hours at 35.0°C±0.5°C m-ColiBlue24 24 hours at 35.0°C±0.5°C</p> <p><u>Enterococci</u> mE→EIA 48 ± 3 hours at 41.0°C±0.5°C→ 20-30 minutes at 41.0°C±0.5°C mEI 24±2 hours at 35.0°C±0.5°C</p>	

Membrane Filtration Specific QC (continued)			
12	Filtration unit sterility check	The filtration unit sterility check was acceptable (target organism or potentially interfering materials were not found in the sterility check)	
13	Preparation blank	The preparation blank was not contaminated with the target organism	
14	Colony verification	The verifications, performed in accordance with method-specific requirements, were acceptable	

Note: Please see Sections 6.5.1 and 6.5.2 for detailed descriptions of quality control procedures for *E. coli* and enterococci methods.

Appendix I

Ground Water Rule Source Water Monitoring Quality Control Checklist for Method 1601: Two-Step Enrichment Coliphage Sample Results

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

Ground Water Rule Source Water Monitoring Quality Control Checklist for Method 1601: Two-Step Enrichment *Coliphage* Sample Results

No.	Quality Control (QC) Procedure	Description	✓
Sample Condition			
1	Sample condition upon receipt	The sample was not frozen or leaking upon receipt	
Holding Time			
2	Holding time	The sample was analyzed within 48 hours of sample collection	
General Quality Control			
3	Media sterility checks	The media sterility checks were acceptable (no coliphage or any potentially interfering organisms were found in the sterility check) for all media	
4	Media storage requirements	Media storage time and temperature requirements were met	
Coliphage Specific QC			
5	Matrix spikes	Matrix spike sample was spiked at the appropriate level for volume analyzed (100 mL or 1 L) and the results were acceptable (at least 1 of 3 were positive)	
6	Ongoing demonstration of capability (ODC)	The ODC sample was spiked at the appropriate level for volume analyzed (100 mL or 1 L) and the results were acceptable (at least 1 of 3 were positive)	
7	Incubation time and temperature	The following incubation time and temperature requirements were met: 10X TSB 16 - 24 hours at 36.0°C±1.0°C TSA (DAL plates) 16 - 24 hours at 36.0°C±1.0°C TSA (spot plates) 16 - 24 hours at 36.0°C±1.0°C	
8	Positive controls	The positive controls were acceptable	
9	Method blank	The method blank was acceptable (no coliphage or any potentially interfering organisms were found in the sterility check)	
10	Spot plates	A positive control and method blank were analyzed for each spot plate used for field samples	
11	ODC and MS analyses	ODC and MS analyses were conducted at the method-specified frequency	
Verification Specific QC			
12	Spot plates	A positive control and method blank were analyzed for each spot plate	
13	Incubation time and temperature	The following incubation time and temperature requirements were met: TSA (spot plates) 16 - 24 hours at 36.0°C±1.0°C	

Note: Please see Sections 6.5.1 and 6.5.3 for detailed descriptions of quality control procedures for Method 1601.

Appendix J

Ground Water Rule Source Water Monitoring Quality Control Checklist for Method 1602: Single Agar Layer (SAL) Coliphage Sample Results

Note: Forms provided in this appendix are presented as guidance and are NOT required for compliance with GWR requirements.

Revised March 2008

**Ground Water Rule Source Water Monitoring
Quality Control Checklist for Method 1602: Single Agar Layer (SAL)
Coliphage Sample Results**

No.	Quality Control (QC) Procedure	Description	✓
Sample Condition			
1	Sample condition upon receipt	The sample was not frozen or leaking upon receipt	
Holding Time			
2	Holding time	The sample was analyzed within 48 hours of sample collection	
General Quality Control			
3	Media sterility checks	The media sterility checks were acceptable (no coliphage or any potentially interfering organisms found in the sterility check) for all media	
4	Media storage requirements	Media storage time and temperature requirements were met	
Coliphage Specific QC			
5	Matrix spikes	Matrix spike was spiked at the appropriate level (80 PFU) and the results were acceptable (range for male-specific and somatic)	
6	Ongoing precision and recovery (OPR)	The OPR sample was spiked at the appropriate level (80 PFU) and the results were acceptable (range for male-specific and somatic)	
7	Incubation time and temperature	The following incubation time and temperature requirements were met: TSA (DAL plates) 16 - 24 hours at 36.0°C±1.0°C TSA (SAL plates) 16 - 24 hours at 36.0°C±1.0°C	
8	Method blank	The method blank was acceptable (no coliphage or any potentially interfering organisms were found in the sterility check)	
9	Positive controls	The positive controls were acceptable	
Verification Specific QC			
10	Spot plates	A positive control and method blank were analyzed for each spot plate	
11	Incubation time and temperature	The following incubation time and temperature requirements were met: TSA (spot plates) 16 - 24 hours at 36.0°C±1.0°C	

Note: Please see Section 6.5.1 and 6.5.4 for detailed descriptions of quality control procedures for Method 1602