

ENVIRONMENTAL PROTECTION
AGENCY

40 CFR Part 136

[FRL-7529-7]

RIN 2040-AD71

Guidelines Establishing Test
Procedures for the Analysis of
Pollutants; Analytical Methods for
Biological Pollutants in Ambient Water

AGENCY: Environmental Protection
Agency (EPA).

ACTION: Final rule.

SUMMARY: By today's action, EPA approves test methods for the analysis of *Escherichia coli* (*E. coli*), enterococci, *Cryptosporidium* and *Giardia* in fresh ambient water matrices. In addition, EPA approves test methods for the analysis of enterococci in marine ambient water matrices. The test methods approved in today's rule have been published by the following organizations: EPA, American Public Health Association, American Water Works Association, Water Environment Federation, Association of Official Analytical Chemists International, and American Society for Testing and Materials, or commercial vendors. EPA's approval of these methods will help States, Tribes, communities, and environmental laboratories better assess public health risks from microbiological pollutants.

DATES: This regulation is effective August 20, 2003. The incorporation by reference of these methods is approved by the Director of the Federal Register

on August 20, 2003. For judicial review purposes, this final rule is promulgated as of 1 p.m. (Eastern time) on August 4, 2003 as provided at 40 CFR 23.2.

FOR FURTHER INFORMATION CONTACT:
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SUPPLEMENTARY INFORMATION:

A. Potentially Regulated Entities

EPA Regions, as well as States, Tribes, and Territories authorized to implement the National Pollutant Discharge Elimination System (NPDES) program, issue permits to implement the technology-based and water quality-based requirements of the Clean Water Act (CWA). Forty five States and one Territory are currently authorized to issue NPDES permits. EPA retains permit issuance authority in non-authorized jurisdictions. NPDES permitting authorities make a number of discretionary choices associated with permit writing, including the selection of pollutants to be measured and, in many cases, limited in permits. If EPA has "approved" (*i.e.*, promulgated through rulemaking) standardized testing procedures for a given pollutant, the NPDES permitting authority must specify one of the approved testing procedures or an approved alternate test procedure for the measurements required under the permit. Although

EPA is including test methods for four biological pollutants in 40 CFR 136.3, it recommends their use for ambient water quality monitoring only. EPA is not approving these test methods for effluent matrices. Therefore, EPA expects entities operating under an NPDES permit would be affected by the promulgation of these ambient methods only where their permit specifies ambient monitoring requirements for the specified parameters.

EPA developed and recommended ambient recreational water quality criteria for *E. coli* and enterococci bacteria and is considering criteria for *Cryptosporidium* and *Giardia*. The States, Territories, and Tribes may adopt these criteria into their water quality standards and may issue water quality-based permits that require monitoring for these pollutants in ambient waters. If the NPDES permitting authority requires ambient water monitoring in the permit for the specified parameters, dischargers could be affected by the standardization of testing procedures in this rulemaking. Generally, the permitting authority requires the use of methods approved at 40 CFR part 136 for compliance with such monitoring requirements. If no approved methods are available at 40 CFR part 136, then the permitting authority has discretion to specify the use of suitable methods.

In addition, when a State, Territory, or authorized Tribe provides certification of Federal licenses under the CWA section 401, approved testing procedures generally must be used where applicable. Categories and entities that may be regulated include:

Category	Examples of potentially regulated entities
State, Territorial and Indian Tribal Governments.	States, Territories, and Tribes authorized to administer the NPDES permitting program.
Municipalities	Publicly-owned treatment works with ambient monitoring requirements for the specified parameters in their NPDES permits.
Industry	Industrial facilities with ambient monitoring requirements for the specified parameters in their NPDES permits.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists the types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table could also be regulated. To determine whether your facility or organization is regulated by this action, you should carefully examine the applicability criteria in parts 122 and 136 of title 40 of the Code of Federal Regulations. If you have questions regarding the applicability of

this action to a particular entity, consult the person listed in the preceding **FOR FURTHER INFORMATION CONTACT** section.

B. How Can I Get Copies of This Document and Other Related Information?

1. *Docket.* EPA has established an official public docket for this action under Docket ID No. OW-2002-0010. The official public docket consists of the documents specifically referenced in this action, any public comments received, and other information related to this action. Although a part of the

official docket, the public docket does not include Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. The official public docket is the collection of materials that is available for public viewing at the Water Docket in the EPA Docket Center, EPA West, Room B102, 1301 Constitution Avenue, NW., Washington, DC. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m. Eastern Time, Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is

202-566-1744, and the telephone number for the Water Docket is 202-566-2426.

2. *Electronic Access.* You may access this **Federal Register** document electronically through the EPA Internet under the **Federal Register** listings at <http://www.epa.gov/fedrgstr/>.

An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at <http://www.epa.gov/edocket/> to view public comments, access the index listing of the contents of the official public docket, and to access those documents in the public docket that are available electronically. Once in the system, select "search," then key in the appropriate docket identification number. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in section B.1.

3. *Copies of Consensus Standards.* Copies of the consensus standards may be obtained from the Docket (see section B.1.). Copies of the consensus standards may also be obtained from the following sources, depending on the standard. Copies of final methods published by American Society for Testing and Materials (ASTM) are available for a nominal cost through ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. Copies of "Standard Methods" are available for a nominal cost from the American Public Health Association, 1015 Fifteenth Street, NW., Washington, DC 20005. Copies of Association of Official Analytical Chemists International (AOAC) methods are available for a nominal cost from the Association of Official Analytical Chemists International, 481 N. Frederick Ave., Suite 500, Gaithersburg, MD 20877.

I. Statutory Authority

Today's rule is promulgated pursuant to the authority of sections 303(c), 304(a), 304(h), and 501(a) of the Clean Water Act (CWA or "the Act"), 33 U.S.C. 1314(a), 1314(h), 1361(a). Section 303(c) of the Act establishes the basis for the current water quality standards program. This section requires EPA to review and approve or disapprove State-adopted water quality standards. Section 304(a) of the Act requires the EPA Administrator to develop and publish water quality criteria associated with specific ambient water uses. When these criteria are adopted as State water quality standards under section 303(c), they become the enforceable maximum

acceptable levels of pollutants in ambient waters. Section 304(h) of the Act requires the EPA Administrator to "promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to section 401 of this Act or permit applications pursuant to section 402 of this Act." Section 501(a) of the Act authorizes the Administrator to "prescribe such regulations as are necessary to carry out his functions under this Act." EPA publishes CWA analytical method regulations at 40 CFR part 136.

II. Background

A. The Role of Methods for Biological Pollutants

To fulfill the CWA's mandate to maintain "fishable and swimmable" waters, EPA develops ambient water quality criteria based on a scientific assessment of the relationship between pollutant concentrations and environmental and human health effects. Ambient water refers to any fresh, marine, or estuarine surface water used for recreation, propagation of fish, shellfish, or wildlife, agriculture, industry, navigation, or as source water for drinking water facilities. Ambient water quality criteria become enforceable water quality standards when adopted by State, Territorial, Tribal, and local governments and approved by EPA.

For bacterial pollution in ambient water designated for recreational use, EPA has developed water quality criteria for *E. coli* in freshwater and for enterococci in both freshwater and marine waters (51 FR 8012, March 7, 1986). There are a number of zoonotic diseases of concern to humans (diseases transferred from animals to humans) if ambient waters are contaminated with fecal material from non-human animal species. *E. coli* species are a subset of the coliform bacteria group that is part of the normal intestinal flora of humans and animals and are direct indicators of fecal contamination from these sources in water. Enterococci, which include *Enterococcus faecalis* and *Enterococcus faecium*, are enteric bacteria used to indicate fecal contamination and the possible presence of pathogens in water. Based on previous EPA guidance, total and fecal coliform bacteria are included in many water quality standards as indicators of bacterial contamination (EPA, 1976). More recent epidemiological studies (Cabelli 1983, Dufour 1984) described in Ambient Water Quality Criteria for Bacteria—1986 (EPA, 1986a), indicate that *E. coli*

and enterococci show a direct correlation with swimming-associated gastrointestinal illness rates, while fecal coliforms do not. As the concentration of *E. coli* and/or enterococci increase(s), the illness rates also increase. Thus, using these indicators as part of the bacterial water quality standards will enhance the protection of human health and the environment.

In addition to bacterial pollution, EPA is concerned about waterborne parasites and developed test methods for *Cryptosporidium* and *Giardia* in freshwater. These waterborne parasites have been found to be the causative agent of human gastroenteritis in some contaminated waters and are responsible for cases of severe and widespread human illness when present in drinking water supplies as a result of contamination of source waters. Because one of the designated uses of some ambient waters may be use of the water body as a drinking water source, EPA may develop ambient water quality criteria for *Cryptosporidium* and *Giardia* in the future. EPA would expect to use the test methods discussed in this action to support these future criteria. By doing so, EPA desires to promote consistency in the methods used for these future criteria to ensure that the data collected are of good quality and are comparable for all freshwater. EPA also wishes to make these methods available for use by the States for general risk assessments.

By today's action, EPA is promulgating test methods for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* for use in freshwaters, and enterococci for use in marine waters. Promulgation of the bacterial methods supports the use of *E. coli* and enterococci as indicators of fecal contamination in addition to fecal coliform indicators in State, Territorial, Tribal, and local water quality-based monitoring. States may use the test methods for *Cryptosporidium* and *Giardia* for different monitoring purposes, such as evaluating surface water occurrence of these organisms and the associated watershed vulnerability for waterbodies designated as potential drinking water sources.

This rule provides uniform methodology to assist State, Territorial, Tribal, and local implementation of water quality standards, ambient water monitoring programs, and public notification programs to reduce public health risks posed by biological pollutants in ambient water. Today's rule supports several EPA initiatives: The Beaches Environmental Assessment Closure and Health (BEACH) Program, the Beach Action Plan (EPA-600-R-98-079), the Beach Watch Program, the

Beaches Environmental Monitoring for Public Access and Community Tracking (EMPACT) Program (EPA 905-R-98-002), and the Water Quality Criteria and Standards Plan (EPA-822-R-98-003). Additionally, this rule is expected to satisfy requests from governments, regulated entities, and environmental laboratories that EPA publish analytical test procedures that were evaluated through interlaboratory validation for enumerating *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* in ambient waters.

As previously noted, EPA developed water quality criteria for enterococci in both freshwater and in marine waters. Today's action approves methods for measuring enterococci in both freshwater and marine waters. EPA has not developed marine criteria for *E. coli*, *Cryptosporidium*, and *Giardia* because these pollutants do not generally survive in marine conditions. Thus, EPA has not identified any programmatic need to promulgate methods for these pollutants in marine waters.

EPA is aware of the importance of having methods for measuring these pollutants in wastewater effluent. The Agency does not currently have validated methods for use in this matrix and thus was unable to propose any such methods with the methods for ambient waters. The Agency is currently in the process of trying to validate *E. coli* and enterococci methods for use with wastewater effluent and plans to propose them by the end of 2004.

B. Summary of Proposed Rule

EPA published a proposed rule in the **Federal Register** on August 30, 2001 (66 FR 45811) to amend 40 CFR part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants," by approving several analytical test procedures for enumerating the bacteria *Escherichia coli* (*E. coli*) and enterococci and the protozoans *Cryptosporidium* and *Giardia* in ambient water. The proposal described a suite of Most Probable Number (MPN) (*i.e.*, multiple-tube, multiple-well) and membrane filter (MF) methods for enumerating *E. coli* and enterococci bacteria in ambient water, and improved filtration/immunomagnetic separation/fluorescent antibody methods for *Cryptosporidium* and *Giardia* protozoans. These test methods were proposed for use by States, Territories, and Tribes, for use in water quality monitoring programs.

A summary of the major comments to the proposal is presented in Section V.

III. Summary of Final Rule

EPA is approving the use of test methods for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* for ambient fresh water quality monitoring. In addition, EPA is approving the use of test methods for enterococci for ambient marine water quality monitoring. Although EPA believes that these methods are appropriate for ambient water quality monitoring, the Agency has not determined that these methods are acceptable for application to matrices other than ambient waters.

Today's action promulgates the test methods described in the proposed rule (66 FR 45811, August 30, 2001) for the analysis of *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* in ambient water. For *E. coli*, approved methods include most probable number methods (LTB→EC-MUG, ONPG-MUG) and membrane filtration methods (mENDO→NA-MUG, LES-ENDO→NA-MUG, mFC→NA-MUG, mTEC agar, Modified mTEC agar, MI agar, m-ColiBlue 24 broth). For enterococci, approved methods include most probable number methods (Azide-Dextrose/PSE/BHI, MUG) and membrane filtration methods (mE→EIA agar, mEI agar). For *Cryptosporidium*, EPA approves Methods 1622 and 1623. For *Giardia*, EPA approves Method 1623.

The proposed rule indicated that EPA intended to issue guidance on the assessment of method comparability in conjunction with the final rule. In the record for today's rule, EPA is making available the latest version of the guidance document, EPA *Microbiological Alternate Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods, Guidance* (EPA-821-B-03-004). The guidance is a result of the Agency's desire to develop a guidance document to describe the process for seeking EPA approval of alternate test procedures (ATPs) for microbiological methods or new microbiological methods for use in monitoring drinking water, ambient water, and wastewater. Under EPA's ATP program, any person may apply for approval of the use of an ATP or new method to test for a regulated analyte. EPA anticipates that the standardized ATP procedures described in the guidance should generally expedite the approval of ATPs and encourage the development of innovative methods for compliance monitoring under the National Pollution Discharge Elimination System (NPDES) permit program. In addition to the ATP process, the guidance describes the

process for conducting side-by-side method comparisons and for conducting quality control (QC) acceptance criteria-based method studies for EPA-designated reference methods with QC acceptance criteria. The guidance document serves as a supplement to the ATP program requirements specified at 40 CFR 136.4, 136.5, and 141.27. The guidance document may be revised in the future based on comments received from persons using the guidance, as appropriate.

IV. Changes From the Proposed Rule

A. Revision of Method Titles

To ensure consistency with other EPA microbiological methods, EPA revised some of the EPA methods' titles and added some method numbers. The technical content of these methods did not change from the versions of the methods included in the proposed rule. Specifically, EPA adopted the following modified titles:

- Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)
- Method 1106.1: Enterococci in Water by Membrane Filtration using membrane-Enterococcus-Esculin Iron Agar (mE-EIA)
- Method 1600: Enterococci in Water by Membrane Filtration using membrane-Enterococcus Iron Agar (mEI)
- Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration using Modified Membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)
- Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration using a Simultaneous Detection Technique (MI Medium)

B. Colisure

EPA included this method in the proposal because it anticipated that new validation data for ambient waters would be provided to the Agency prior to this final rule. EPA requested such data from the manufacturer, but the manufacturer declined to conduct the study. Therefore EPA declines to approve this method and did not include it in today's final rule.

C. Table II Protozoan Test Holding Time

The proposal incorrectly indicated that the maximum sample holding time for the protozoan tests (*Cryptosporidium* and *Giardia*) was 72 hours. This has been changed to the correct holding time of 96 hours, as indicated in the Methods, which were included in the docket for the proposal. The correct

holding time of 96 hours is clearly indicated in the Methods and can be found on page 10, section 8.2.1 of the April 2001 versions of Method 1622 and Method 1623.

Although footnote 17 of the proposal inaccurately stated the technique for calculating holding time, the underlying methods themselves described this technique correctly. The footnote has been corrected to indicate that holding time is properly calculated from the time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

V. Response to Major Comments

EPA encouraged public participation in this rulemaking and requested comments on the methods proposed for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia*. EPA also requested any data that would support comments on specific test methods. Fourteen stakeholders provided comments addressing over 25 issues. These stakeholders included four laboratories, seven regulatory authorities, and three industries/industry groups.

The following sections summarize major comments received on the proposed rule and EPA's response. The complete Response to Comments document can be found in the Docket for today's final rule.

A. *E. coli* and Enterococci Methods for Wastewater Analysis

Several commenters requested that the methods for *E. coli* and enterococci be approved for the analysis of wastewater samples. Since these methods were not validated in wastewater, they are not approved for use in that matrix. EPA is in the process of validating methods for the analysis of *E. coli* and enterococci in wastewater and plans to propose test methods for these bacterial indicators by the end of 2004.

B. *Cryptosporidium* and *Giardia* Methods for Wastewater and Biosolids Analysis

Several comments advocated the use of EPA Method 1622 and 1623 for the analysis of wastewater and biosolids samples; other comments requested that EPA modify and approve the methods for use in those matrices. EPA has not validated these methods for those uses. Thus this final rule applies only to ambient water. If EPA develops water quality criteria for *Cryptosporidium* and *Giardia* at a future time, EPA may validate EPA Methods 1622 and/or 1623 for use in the NPDES Program.

C. Limitations of Determinative Technique of Proposed *Cryptosporidium* and *Giardia* Methods and Potential for False Positives

Several comments expressed concern regarding the subjectivity and limitations of the immunofluorescence assay (IFA)-based determination procedure in EPA Methods 1622 and 1623 and the related potential for false positives. EPA acknowledges that IFA relies on analyst training and experience for reliable results. However, EPA Methods 1622 and 1623 provide the analyst with three microscopy tools to aid in the identification of potential target particulates during microscopic examination. The methods provide detailed, progressive criteria for determining whether a particulate is a *Cryptosporidium* oocyst or a *Giardia* cyst based on the use of these tools and include the use of immunomagnetic separation (IMS) as the sample cleanup procedure to minimize the transfer of non-target particulates to the slide. Nonetheless, the inherent technical judgement involved in the determinative step in EPA Methods 1622 and 1623, combined in some cases with interfering materials and/or cross-reactivity of the antibody stain, may still lead to false positives or false negatives. Although other determinative techniques that are currently under development have the promise of providing less-subjective assessments of the presence of *Cryptosporidium* oocysts and *Giardia* cysts in a sample, these techniques are not yet validated and are therefore not yet appropriate for EPA approval for ambient water monitoring. Extensive details on the performance of EPA Methods 1622 and 1623, including inter- and intra-laboratory precision and recovery of the methods at multiple laboratories and on a variety of ambient water types (*i.e.*, validation), are provided in the Results of the Interlaboratory Validation Study of EPA Method 1622 (EPA-821-R-01-027), the Results of the Interlaboratory Validation Study of EPA Method 1623 (EPA-821-R-01-028) and the Implementation and Results of the Information Collection Rule Supplemental Surveys (EPA-815-R-01-003), which were included in the docket for the proposal. Given the robustness of the validation procedure, the Agency is confident that although the IFA technique requires specialized training, overall, the methods will provide for valid *Cryptosporidium* and *Giardia* precision and recovery for use in ambient waters.

D. Application of Performance-Based Measurement System (PBMS) Concept to EPA Methods 1622 and 1623

Several commenters recommended that the performance of alternate antibody reagents be evaluated for EPA Methods 1622 and 1623 using a quantitative PBMS approach. EPA agrees with the comments, and considers the PBMS Tier 2 validation approach described in Methods 1622 and 1623, Section 9, to be appropriate for antibody stains and IMS. However, EPA does not believe that the PBMS Tier 2 validation approach is adequate to assess the comparability of methods with different determinative techniques, such as comparing a polymerase chain reaction (PCR)-based method to an IFA-based method. Use of a different determinative technique is generally considered to be a different method, rather than a modified version of a method because it is usually very difficult to compare methods that use different determinative techniques. For example, the filtration/IMS/IFA technique employed in Methods 1622 and 1623 differs considerably from genetic tests because the former measures the infective form of *Cryptosporidium* and *Giardia*, while the latter measures genetic material (DNA or RNA). Similarly, the membrane filtration method for bacteria differs from an MPN method for bacteria because the former is a direct quantitative method, whereas the latter employs a qualitative statistical index rather than an actual enumeration of the number of organisms present in the sample. An appropriate approach for these comparisons would be to perform side-by-side tests. This approach is outlined in the draft guidance document, EPA Microbiological Alternate Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods, Guidance (EPA-821-B-03-004).

VI. Statutory and Executive Order Reviews

A. Executive Order 12866: Regulatory Planning and Review

Under Executive Order 12866 (58 FR 51735 (October 4, 1993)), the Agency must determine whether the regulatory action is "significant" and therefore subject to Office of Management and Budget (OMB) review and the requirements of the Executive Order. The Executive Order defines "significant regulatory action" as one that is likely to result in a rule that may:

(1) Have an annual effect on the economy of \$100 million or more, or adversely affect in a material way the

economy, a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or tribal governments or communities;

(2) Create a serious inconsistency or otherwise interfere with an action taken or planned by another agency;

(3) Materially alter the budgetary impact of entitlements, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or

(4) Raise novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in the Executive Order.

It has been determined that this rule is not a "significant regulatory action" under the terms of Executive Order 12866 and is therefore not subject to OMB review.

B. Paperwork Reduction Act

This action does not impose an information collection burden under the provisions of the Paperwork Reduction Act, 44 U.S.C. 3501 *et seq.* This action promulgates new test methods for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* for use in ambient water monitoring programs. If the regulating authority replaces the indicator organism from fecal coliforms to one of the bacterial organisms (*E. coli* or enterococci) and the relevant NPDES permit requires ambient water monitoring, then the permittee would be required to use one of these approved methods for these organisms. Currently, permittees generally are not required to monitor for *Cryptosporidium* or *Giardia* because EPA has not developed water quality criteria for these protozoans. Burden means that the total time, effort, or financial resources expended by persons to generate, maintain, retain or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install and utilize technology and systems for the purpose of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

An agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control

numbers for EPA's regulations are listed in 40 CFR part 9 and 48 CFR chapter 15.

C. Regulatory Flexibility Act

The RFA generally requires an agency to prepare a regulatory flexibility analysis of any rule subject to notice and comment rulemaking requirements under the Administrative Procedure Act or any other statute unless the agency certifies that the rule will not have a significant economic impact on a substantial number of small entities. Small entities include small businesses, small organizations, and small governmental jurisdictions.

For purposes of assessing the impacts of today's rule on small entities, small entity is defined as: (1) A small business as defined by the U.S. Small Business Administration definitions at 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district or special district with a population of less than 50,000; and (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of today's final rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities. This regulation promulgates testing procedures for the measurement of *E. coli* and enterococci bacteria, and *Cryptosporidium* and *Giardia* protozoa in ambient water. EPA anticipates that the methods will be used by some State regulatory authorities for evaluating attainment of water quality standards or ambient monitoring requirements. EPA NPDES regulations do not require monitoring of ambient water conditions in NPDES permits. In a few instances, ambient water monitoring requirements may be included in an EPA-issued permit where site-specific circumstances warrant. EPA regulations, do however, require NPDES permittees to use EPA-approved test methods for all monitoring data reported to the Agency (40 CFR 122.21). Consequently, to the extent that an NPDES permit requires monitoring and reporting of ambient water for *E. coli*, enterococci, *Cryptosporidium*, or *Giardia*, EPA approval of these test methods arguably may impose costs on NPDES permit holders, including small entities. EPA is unaware, however, of any EPA-issued NPDES permits that currently require monitoring of ambient water for such pollutants. Hence, EPA does not expect approval of these methods to impose any additional costs as a result of their applicability to EPA issued permits. As

noted above, EPA's NPDES regulations do not require monitoring of ambient water conditions. Consequently, to the extent that a State requires such monitoring, those requirements are imposed under State, rather than Federal, authority. Because States have the discretion not to require such monitoring, any increased costs to small entities arising from use of the methods approved by EPA today that are imposed as a result of State law are not attributable to this regulation.

Nonetheless, EPA evaluated these potential costs to determine whether EPA approval of the methods will have a significant impact on a substantial number of small entities. As previously noted, States may require ambient water monitoring to evaluate attainment of water quality standards. A few States currently require NPDES permit holders to monitor ambient waters. Thus, some NPDES permittees are already testing ambient water for these parameters. The impact of using EPA approved methods for such dischargers may represent little or no increased burden since, for these permittees, the replacement of fecal coliforms with *E. coli* or enterococci would simply require different methods.

The small entities that might be affected by this rule include small governmental jurisdictions that have publicly-owned treatment works (POTWs) and small businesses with water quality-based discharge permits. The average costs for total and fecal coliform were comparable to those for *E. coli* and enterococci (\$35) because the analytical procedures generally employ similar techniques, media, equipment, and require comparable laboratory time and effort. Some States are already using the methods for *E. coli* and enterococci in State ambient water quality monitoring programs. This rule would formalize current practice in those States. Furthermore, EPA expects that any modest potential increase in costs for enterococci analyses will be reduced once the promulgated methods are broadly implemented by environmental laboratories and State water quality monitoring programs.

EPA also reviewed the costs for testing for *Cryptosporidium* and *Giardia*. The costs for Methods 1622 and 1623 analysis of *Cryptosporidium* and *Giardia* range from \$400 to \$500 for each sample (with matrix spikes being assessed as individual samples) for each method. Because of the relatively high costs, EPA does not anticipate that these test methods will be used for daily or ongoing monitoring, but they may be used for program-specific occurrence assessments.

The purpose of this rule is only to make these methods available to States, Tribes, and municipalities that may want to use them for ambient water monitoring. The costs associated with *Cryptosporidium* and *Giardia* analysis would not be a Federally-mandated cost, but rather would emanate from a State's adoption of ambient monitoring requirements or other identified needs such as evaluation of Best Management Practices (BMPs) or downstream impacts of wastewater treatment plant effluents or other identified needs. The inclusion of these test methods in 40 CFR 136.3 is intended to make these test methods available to States and others for use in water quality monitoring programs. While monitoring for these protozoans may be beneficial since these organisms may be ingested from recreational and source waters, EPA is not establishing any compliance monitoring requirements for these pollutants. Therefore, EPA believes that this rule will not have a significant economic impact on a substantial number of small entities.

D. Unfunded Mandates Reform Act

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Public Law 104-4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, Tribal, and local governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with "Federal mandates" that may result in expenditures to State, Tribal, and local governments, in the aggregate, or to the private sector, of \$100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205 of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation of why that alternative was not adopted.

Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including Tribal governments, it must have developed under section 203 of the UMRA a small government agency plan. The plan must provide for the

notification of potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

EPA has determined that this rule does not contain a Federal mandate for State, Tribal, and local governments or the private sector that may result in expenditures of \$100 million or more for State, Tribal, and local governments, in the aggregate, or the private sector in any one year. This rule makes available testing procedures for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia* that may be used by a State, Territorial, Tribal or local authority for compliance with water quality standards or ambient monitoring requirements when testing is otherwise required by these regulatory authorities. Thus, today's rule is not subject to the requirements of sections 202 and 205 of the UMRA.

EPA has also determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. As discussed above, under the Regulatory Flexibility Act, the economic impact on small entities is anticipated to be small. It would not significantly affect them because any incremental costs incurred are small, and it would not uniquely affect them because it would affect entities of all sizes depending upon whether testing for these bacteria or protozoa is otherwise required by a regulatory authority. Further, monitoring for small entities is generally expected to be less frequent than monitoring for larger entities. Thus, today's rule also is not subject to the requirements of section 203 of the UMRA.

E. Executive Order 13132: Federalism

Executive Order 13132, entitled "Federalism" (64 FR 43255, August 10, 1999), requires EPA to develop an accountable process to ensure "meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications." "Policies that have federalism implications" is defined in the Executive Order to include regulations that have "substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government."

This final rule does not have federalism implications. It will not have

substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132. Today's rule promulgates new analytical methods for conducting analysis of ambient water for enumeration of *E. coli*, enterococci, *Cryptosporidium*, or *Giardia*. EPA does not, however, require use of these methods under this rule. Thus, Executive Order 13132 does not apply to this rule.

Although Executive Order 13132 does not apply to this rule, EPA did consult with representatives of State and local governments in developing the proposed regulation. In fact, it was State representatives who requested that EPA include test methods for these biological pollutants in 40 CFR 136.3 because they want to use EPA approved test methods for ambient water monitoring. EPA included a number of test methods currently being used by States for these pollutants in today's rulemaking. In the spirit of Executive Order 13132, and consistent with EPA policy to promote communications between EPA and State and local governments, EPA specifically solicited comment on the proposed rule from State and local officials. No significant concerns were raised by commenters about these methods.

F. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments

Executive Order 13175, entitled "Consultation and Coordination with Indian Tribal Governments" (65 FR 67249, November 9, 2000), requires EPA to develop an accountable process to ensure "meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications." "Policies that have tribal implications" is defined in the Executive Order to include regulations that have "substantial direct effects on one or more Indian tribes, on the relationship between the Federal government and the Indian tribes, or on the distribution of power and responsibilities between the Federal government and the Indian tribes."

This final rule does not have tribal implications. It will not have substantial direct effects on tribal governments, on the relationship between the Federal government and Indian tribes, or on the distribution of power and responsibilities between the Federal government and Indian tribes, as specified in Executive Order 13175. Today's rule promulgates new analytical methods for conducting analysis of

ambient water for enumeration of *E. coli*, enterococci, *Cryptosporidium*, or *Giardia*. EPA does not, however, require use of these methods under this rule. Thus, Executive Order 13175 does not apply to this rule. Moreover, in the spirit of Executive Order 13175, and consistent with EPA policy to promote communications between EPA and tribal governments, EPA specifically solicited comment on the proposed rule from tribal officials. EPA did not receive comments from Tribal officials. Thus, Executive Order 13175 does not apply to this rule.

G. Executive Order 13045: Protection of Children From Environmental Health Risks and Safety Risks

Executive Order 13045 (62 FR 19885, April 23, 1997) applies to any rule that: (1) Is determined to be “economically significant” as defined under Executive Order 12866, and (2) concerns an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. If the regulatory action meets both criteria, the Agency must evaluate the environmental health or safety effects of the planned rule on children, and explain why the planned regulation is preferable to other potentially effective and reasonably feasible alternatives considered by the Agency. This rule is not subject to the Executive Order because it is not “economically significant” as defined in Executive Order 12866. Further, it does not concern an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children.

H. Executive Order 13211: Actions That Significantly Affect Energy Supply, Distribution, or Use

This rule is not subject to Executive Order 13211, “Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use” (66 FR 28355 (May 22, 2001)) because it is

not a significant regulatory action under Executive Order 12866.

I. National Technology Transfer and Advancement Act

As noted in the proposed rule, section 12(d) of the National Technology Transfer and Advancement Act of 1995, (“NTTAA”), Public Law 104–113, section 12(d) (15 U.S.C. 272 note), directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (e.g., material specifications, test methods, sampling procedures, and business practices) that are developed or adopted by voluntary consensus standards bodies. The NTTAA directs EPA to provide Congress, through the Office of Management and Budget (OMB), explanations when the Agency decides not to use available and applicable voluntary consensus standards.

This rulemaking involves technical standards. Therefore, the Agency conducted a search to identify potentially applicable voluntary consensus standards. EPA’s search of the technical literature revealed several consensus methods appropriate for enumerating *E. coli* and enterococci in ambient waters. Accordingly, methods for *E. coli* and enterococci published by Standard Methods for the Examination of Water and Wastewater, ASTM, and AOAC-International are included for promulgation and are listed in Table IA at the end of this document (see footnotes 4, 10, and 11, respectively, for the complete citations). No voluntary consensus standards were found for *Cryptosporidium* or *Giardia*.

J. Congressional Review Act

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996 (SBREFA), generally provides that before a rule may take effect, the agency

promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of the rule in the **Federal Register**. A major rule cannot take effect until 60 days after it is published in the **Federal Register**. This action is not a “major rule” as defined by 5 U.S.C. 804(2). This rule will be effective on August 20, 2003.

List of Subjects in 40 CFR Part 136

Environmental protection, Incorporation by reference, Reporting and recordkeeping requirements, Water pollution control.

Dated: July 11, 2003.

Linda J. Fisher,
Acting Administrator.

■ For the reasons set out in the preamble, title 40, chapter I of the Code of Federal Regulations, is amended as follows:

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

■ 1. The authority citation for part 136 continues to read as follows:

Authority: Secs. 301, 304(h), 307, and 501(a), Pub. L. 95–217, 91 Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977.)

■ 2. Section 136.3 is amended:

■ a. In paragraph (a) by revising Table IA.

■ b. In paragraph (b) by revising references (10), (34), (38) and (39) and adding references (52) through (62).

■ c. In Table II to paragraph (e) by revising entries to the Section labeled “Table IA—Bacteria Tests,” to read as follows:

* * * * *

(a) * * *

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method ¹	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
Bacteria:							
1. Coliform (fecal), number per 100 mL.	Most Probable Number (MPN), 5 tube 3 dilution, or	p. 132 ³	9221C E ⁴				
	Membrane filter (MF) ² , single step.	p. 124 ³	9222D ⁴			B–0050–85 ⁵	

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method ¹	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 132 ³	9221C E ⁴				
3. Coliform (total), number per 100 mL.	MF, single step ⁶ .. MPN, 5 tube, 3 dilution, or	p. 124 ³ p. 114 ³	9222D ⁴ 9221B ⁴				
4. Coliform (total), in presence of chlorine, number per 100 mL.	MF ² , single step or two step. MPN, 5 tube, 3 dilution, or	p. 108 ³ p. 114 ³	9222B ⁴ 9221B ⁴			B-0025-85 ⁵	
5. <i>E. coli</i> , number per 100 mL ²⁸ .	MF ² with enrichment. MPN ^{7,9,15} , multiple tube, .. multiple tube/multiple well, MF ^{2,6,7,8,9} two step, or single step	p. 111 ³ 1103.1 ²⁰ 1603 ²¹ 1604 ²²	9222(B+B.5c) ⁴ 9221B.1/9221F ^{4,12,14} 9223B ^{4,13} 9222B/9222G ^{4,19} 9213D ⁴	 D5392-93 ¹⁰	 991.15 ¹¹	 	Colilert® ^{13,17} Colilert-18® ^{13,16,17} mColiBue ^{24 18}
6. Fecal streptococci, number per 100 mL.	MPN, 5 tube, 3 dilution, MF ² , or	p. 139 ³ p. 136 ³	9230B ⁴		 B-0055-85 ⁵		
7. Enterococci, number per 100 mL.	Plate count	p. 143 ⁴	9230B ⁴				
	MPN ^{7,9} multiple tube. multiple tube/multiple well. MF ^{2,6,7,8,9} two step. single step, or Plate count	 1106.1 ²⁴ 1600 ²⁵ p. 143 ³	 9230C ⁴	 D6503-99 ¹⁰ D5259-92 ¹⁰			Enterolert® ^{13,23}
Protozoa:							
8. <i>Cryptosporidium</i> ²⁸ .	Filtration/IMS/FA ..	1622 ²⁶ 1623 ²⁷					
9. <i>Giardia</i> ²⁸ ..	Filtration/IMS/FA ..	1623 ²⁷					
Aquatic Toxicity:							
10. Toxicity, acute, fresh water organisms, LC50, percent effluent.	<i>Ceriodaphnia dubia</i> acute. <i>Daphnia pulex</i> and <i>Daphnia magna</i> acute.	2002.0 ²⁹ 2021.0 ²⁹					

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method ¹	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
11. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC50, percent effluent.	Fathead Minnow, <i>Pimephales promelas</i> , and Bannerfin shiner, <i>Cyprinella leedsii</i> , acute.	2000.0 ²⁹					
	Rainbow Trout, <i>Oncorhynchus mykiss</i> , and brook trout, <i>Salvelinus fontinalis</i> , acute.	2019.0 ²⁹					
	Mysid, <i>Mysidopsis bahia</i> , acute.	2007.0 ²⁹					
	Sheepshead Minnow, <i>Cyprinodon variegatus</i> , acute.	2004.0 ²⁹					
	Silverside, <i>Menidia beryllina</i> , <i>Menidia menidia</i> , and <i>Menidia peninsulae</i> , acute.	2006.0 ²⁹					
12. Toxicity, chronic, fresh water organisms, NOEC or IC25, percent effluent.	Fathead minnow, <i>Pimephales promelas</i> , larval survival and growth.	1000.0 ³⁰					
	Fathead minnow, <i>Pimephales promelas</i> , embryo-larval survival and teratogenicity.	1001.0 ³⁰					
	Daphnia, <i>Ceriodaphnia dubia</i> , survival and reproduction.	1002.0 ³⁰					
	Green alga, <i>Selenastrum capricornutum</i> , growth.	1003.0 ³⁰					
13. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC25, percent effluent.	Sheepshead minnow, <i>Cyprinodon variegatus</i> , larval survival and growth.	1004.0 ³¹					

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method ¹	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , embryo-larval survival and teratogenicity.	1005.0 ³¹					
	Inland silverside, <i>Menidia beryllina</i> , larval survival and growth.	1006.0 ³¹					
	Mysid, <i>Mysidopsis bahia</i> , survival, growth, and fecundity.	1007.0 ³¹					
	Sea urchin, <i>Arbacia punctulata</i> , fertilization.	1008.0 ³¹					

Notes to Table IA:

¹ The method must be specified when results are reported.

² A 0.45 µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

³ USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/8-78/017.

⁴ APHA. 1998, 1995, 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 20th, 19th, and 18th Editions. Amer. Publ. Hlth. Assoc., Washington, D.C.

⁵ USGS. 1989. U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of Interior, Reston, Virginia.

⁶ Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

⁷ Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

⁸ When the MF method has not been used previously to test ambient waters with high turbidity, large number of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

⁹ To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

¹⁰ ASTM. 2000, 1999, 1996. Annual Book of ASTM Standards—Water and Environmental Technology. Section 11.02. American Society for Testing and Materials. 100 Barr Harbor Drive, West Conshohocken, PA 19428.

¹¹ AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. Association of Official Analytical Chemists International. 481 North Frederick Avenue, Suite 500, Gaithersburg, Maryland 20877-2417.

¹² The multiple-tube fermentation test is used in 9221B.1. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

¹³ These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.

¹⁴ After prior enrichment in a presumptive medium for total coliform using 9221B.1, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F. Commercially available EC-MUG media or EC media supplemented in the laboratory with 50 µg/mL of MUG may be used.

¹⁵ Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® or Quanti-Tray® 2000, and the MPN calculated from the table provided by the manufacturer.

¹⁶ Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35°C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

¹⁷ Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

¹⁸ A description of the mColiBlue24™ test, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010.

¹⁹ Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA-MUG media.

²⁰ USEPA. 2002. Method 1103.1: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-020.

²¹ USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-023.

²² Preparation and use of MI agar with a standard membrane filter procedure is set forth in the article, Brenner *et al.* 1993. "New Medium for the Simultaneous Detection of Total Coliform and *Escherichia coli* in Water." Appl. Environ. Microbiol. 59:3534-3544 and in USEPA. 2002. Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA 821-R-02-024.

²³ A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

²⁴ USEPA. 2002. Method 1106.1: Enterococci In Water By Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE-EIA). U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA-821-R-02-021.

²⁵ USEPA. 2002. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA-821-R-02-022.

²⁶ Method 1622 uses filtration, concentration, immunomagnetic separation of oocysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the detection of *Cryptosporidium*. USEPA. 2001. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA-821-R-01-026.

²⁷ Method 1623 uses filtration, concentration, immunomagnetic separation of oocysts and cysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the simultaneous detection of *Cryptosporidium* and *Giardia* oocysts and cysts. USEPA. 2001. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA-821-R-01-025.

²⁸ Recommended for enumeration of target organism in ambient water only.

²⁹ USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/821/R-02/012.

³⁰ USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/821/R-02/013.

³¹ USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/821/R-02/014.

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(b) * * *

REFERENCES, SOURCES, COSTS, AND TABLE CITATIONS

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(10) Annual Book of ASTM Standards, Water, and Environmental Technology, Section 11, Volumes 11.01 and 11.02, 1994, 1996, 1999, and Volume 11.02, 2000 in 40 CFR 136.3, Tables IA, IB, IC, ID, and IE.

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(34) USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA 821-R-02-012. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB2002-108488. Table IA, Note 29.

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(38) USEPA. October 2002. Short-Term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA-821-R-02-013. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB2002-108489. Table IA, Note 30.

(39) USEPA. October 2002. Short-Term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA-821-R-02-014. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia

22161, Pub. No. PB2002-108490. Table IA, Note 31.

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(52) IDEXX Laboratories, Inc. 2002. Description of Colilert®, Colilert-18®, Quanti-Tray®, Quanti-Tray®/2000, Enterolert® methods are available from IDEXX Laboratories, Inc., One Idexx Drive, Westbrook, Maine 04092. Table IA, Notes 17 and 23.

(53) Hach Company, Inc. Revision 2, 1999. Description of m-ColiBlue24® Method, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010. Table IA, Note 18.

(54) USEPA. 2002. Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. September 2002, EPA-821-R-02-020. Available at NTIS, PB2003-100125. Table IA, Note 20.

(55) USEPA. 2002. Method 1106.1: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE-EIA). U.S. Environmental Protection Agency, Office of Water, Washington D.C. September 2002, EPA-821-R-02-021. Available at NTIS, PB2003-100126. Table IA, Note 24.

(56) USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC September 2002, EPA-821-R-02-023. Available at NTIS, PB2003-100128. Table IA, Note 21.

(57) Brenner *et al.* 1993. New Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water. *Appl. Environ. Microbiol.* 59:3534-3544.

Available from the American Society for Microbiology, 1752 N Street NW., Washington, DC 20036. Table IA, Note 22.

(58) USEPA. 2002. Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington D.C.. September 2002, EPA 821-R-02-024. Available from NTIS, PB2003-100129. Table IA, Note 22.

(59) USEPA. 2002. Method 1600: Enterococci in Water by Membrane Filtration using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington D.C. September 2002, EPA-821-R-02-022. Available from NTIS, PB2003-100127. Table IA, Note 25.

(60) USEPA. 2001. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC April 2001, EPA-821-R-01-026.

Available from NTIS, PB2002-108709. Table IA, Note 26.

(61) USEPA. 2001. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC April 2001, EPA-821-R-01-025. Available from NTIS, PB2002-108710. Table IA, Note 27.

(62) AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. AOAC International. 481 North Frederick Avenue, Suite 500, Gaithersburg, Maryland 20877-2417. Table IA, Note 11.

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TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter No./name	Container ¹	Preservation ^{2,3}	Maximum holding time ⁴ (hours)
Table IA—Bacteria Tests:			
1–5 Coliform, total, fecal, and <i>E. coli</i>	PP, G	Cool, <10°C, 0.008% Na ₂ S ₂ O ₃ ⁵	6
6 Fecal <i>streptococci</i>	PP, G	Cool, <10° 0.008% Na ₂ S ₂ O ₃ ⁵	6
7 <i>Enterocci</i>	PP, G	Cool, <10° 0.008% Na ₂ S ₂ O ₃ ⁵	6
Table IA—Protozoa Tests:			
8 <i>Cryptosporidium</i>	LDPE	0–8°C	96 ¹⁷
9 <i>Giardia</i>	LDPE	0–8°C	96 ¹⁷
*	*	*	*

¹ Polyethylene (P) or glass (G). For bacteria, plastic sample containers must be made of sterilizable materials (polypropylene [PP] or other autoclavable plastic). For protozoa, plastic sample containers must be made of low-density polyethylene (LDPE).

² Sample preservation should be performed immediately upon sample collection. For composite chemical samples, each aliquot should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4°C until compositing and sample splitting is completed.

³ When any sample is to be shipped by common carrier or sent through the United States Mails, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Transportation Bureau, Department of Transportation, has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO₃) in water solutions of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H₂SO₄) in water solutions of concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

⁴ Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analyses and still be considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory, has data on file to show that for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under § 136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the samples for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See § 136.3(e) for details. The term "analyze immediately" usually means within 15 minutes or less of sample collection.

⁵ Should only be used in the presence of residual chlorine.

¹⁶ Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the 4°C temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature can not be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

¹⁷ Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

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