

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