

**APPENDIX H
GROUNDWATER ANALYTICAL METHODS**

2501-A GROUNDWATER SOURCE MICROBIAL MONITORING AND ANALYTICAL METHODS

- A. A groundwater system subject to the source water monitoring requirements of § 2503(a) must collect a standard sample volume of at least 100 mL for fecal indicator analysis regardless of the fecal indicator or analytical method used.
- B. A groundwater system must analyze all ground water source samples collected under §2503(a) using one of the analytical methods listed in the following table, or one of the alternative methods listed in Appendix A to subpart C of 40 C.F.R. Part. 141, for the presence of E. coli, enterococci, or coliphage:

Table 2501-A-1 ANALYTICAL METHODS FOR SOURCE WATER MONITORING

Fecal Indicator ¹	Methodology	Method Citation
E.coli	Colilert ² Colisure ² Membrane Filter Method with MI Agar m-ColiBlue24 Test ⁵ E*Colite Test ⁶ EC-MUG ⁷ NA-MUG ⁷	9223B. ³ 9223B. ³ EPA Method 1604. ⁴ 9221F. ³ 9222G. ³
Enterococci	Multiple-Tube Technique Membrane Filter Technique Membrane Filter Technique Enterolert ⁹	9230B. ³ 9203C. ³ EPA Method 1600. ⁸
Coliphage	Two-Step Enrichment Presence-Absence Procedure Single Agar Layer Procedure	EPA Method 1601. ¹⁰ EPA Method 1602. ¹¹

Analyses must be conducted in accordance with the documents listed below. Copies of the documents may be obtained from the sources listed below. Copies may be inspected in EPA's Drinking Water Docket, EPA West, 1301 Constitution Avenue, NW., EPA West, Room B102, Washington DC 20460 (Telephone: 202-566-2426); or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to:

http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

¹ The time from sample collection to initiation of analysis may not exceed 30 hours. The ground water system is encouraged but is not required to hold samples below 10°C during transit.

² Medium is available through IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

³ Methods are described in Standard Methods for the Examination of Water and Wastewater 20th edition (1998) and copies may be obtained from the American Public Health Association, 1015 Fifteenth Street, NW., Washington, DC, 20005-2605.

⁴ EPA Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium); September 2002, EPA 821-R-02-024. Method is available at <http://www.epa.gov/nerlcwww/1604sp02.pdf> or EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460.

⁵ A description of the mColiBlue24 Test, "Total Coliforms and E.coli Membrane Filtration Method with m-ColiBlue247 Broth," Method No. 10029 Revision 2, August 17, 1999, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010 or from EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460.

⁶ A description of the E*Colite Test, "Charm E*Colite Presence/Absence Test for Detection and Identification of Coliform Bacteria and Escherichia coli in Drinking Water," January 9, 1998, is available from Charm Sciences, Inc., 659 Andover St., Lawrence, MA 01843-1032 or from EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460.

⁷ EC-MUG(Method 9221F) or NA-MUG (9222G) can be used for E.coli testing step as described in Appendix A 402-A (C)(1) or (2) after use of Standard Methods 9221 B, 9222 B, or 9222 C.

⁸ Medium is available through IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

Preparation and use of the medium is set forth in the article "Evaluation of Enterolert for Enumeration of Enterococci in Recreational Waters," by Budnick, G.E., Howard, R.T., and Mayo, D.R., 1996, Applied and Environmental Microbiology, 62:3881-3884.

⁹ EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl- β -D-Glucoside Agar (mEI) EPA 821-R-02-022 (September 2002) is an approved variation of Standard Method 9230C. The method is available at <http://www.epa.gov/nerlcwww/1600so02.pdf> or from EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460. The holding time and temperature for ground water samples are specified in footnote 1 above, rather than as specified in Section 8 of EPA Method 1600.

¹⁰ EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure; April 2001, EPA 821-R-01-030. Method is available at <http://www.epa.gov/nerlcwww/1601ap01.pdf> or from EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460.

¹¹ EPA Method 1602: Male-specific (+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure; April 2001, EPA 821-R-01-029. Method is available at <http://www.epa.gov/nerlcwww/1601ap01.pdf> or from EPA's Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW., Washington, DC 20460.