

APPENDIX G
ENHANCED TREATMENT FOR *CRYPTOSPORIDIUM*

2401-G

A. ANALYTICAL METHODS

1. **CRYPTOSPORIDIUM** Systems must analyze for Cryptosporidium using *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA, 2005*, United States Environmental Protection Agency, EPA-815-R-05-002 or *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA, 2005*, United States Environmental Protection Agency, EPA-815-R-05-001, which are incorporated by reference. You may obtain a copy of these methods online from <http://www.epa.gov/safewater/disinfection/lt2> or from the United States Environmental Protection Agency, Office of Ground Water and Drinking Water, 1201 Constitution Ave., NW, Washington, DC 20460 (Telephone: 800-426-4791). You may inspect a copy at the Water Docket in the EPA Docket Center, 1301 Constitution Ave., NW, Washington, DC, (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.
 - a Systems must analyze at least a 10 L sample or a packed pellet volume of at least 2 mL as generated by the methods listed in paragraph (A) of this section. Systems unable to process a 10 L sample must analyze as much sample volume as can be filtered by two filters approved by EPA for the methods listed in paragraph (A) of this section, up to a packed pellet volume of at least 2 mL.
 - b
 1. Matrix spike (MS) samples, as required by the methods in paragraph (A) of this section, must be spiked and filtered by a laboratory approved for Cryptosporidium analysis under §2406.
 2. If the volume of the MS sample is greater than 10 L, the system may filter all but 10 L of the MS sample in the field, and ship the filtered sample and the remaining 10 L of source water to the laboratory. In this case, the laboratory must spike the remaining 10 L of water and filter it through the filter used to collect the balance of the sample in the field.
 - c Flow cytometer-counted spiking suspensions must be used for MS samples and ongoing precision and recovery (OPR) samples.
2. **E. COLI.** Systems must use methods for enumeration of E. coli in source water approved in 40 CFR §136.3(a) of this title.
 - a The time from sample collection to initiation of analysis may not exceed 30 hours unless the system meets the condition of paragraph (B)(2) of this section.
 - b The Director may approve on a case-by-case basis the holding of an E. coli sample for up to 48 hours between sample collection and initiation of analysis if the Director determines that analyzing an E. coli sample within 30 hours is not feasible. E. coli samples held between 30 to 48 hours must be analyzed by the Colilert reagent version of Standard Method 9223B as listed in 40 CFR §136.3(a) of this title.
3. Systems must maintain samples between 0°C and 10°C during storage and transit to the laboratory.
4. **TURBIDITY.** Systems must use methods for turbidity measurement approved in Appendix D - 800-D (A)(1).

2402-G

A. Approved laboratories.

1. Cryptosporidium. Systems must have Cryptosporidium samples analyzed by a laboratory that is approved under EPA's Laboratory Quality Assurance Evaluation Program for Analysis of Cryptosporidium in Water or a laboratory that has been certified for Cryptosporidium analysis by an equivalent State laboratory certification program.
2. E. coli. Any laboratory certified by the EPA, the National Environmental Laboratory

Accreditation Conference or the State for total coliform or fecal coliform analysis under Appendix D - 800-D is approved for E. coli analysis under this subpart when the laboratory uses the same technique for E. coli that the laboratory uses for Appendix D - 800-D.

3. Turbidity. Measurements of turbidity must be made by a party approved by the Director.

2403 - G

A. E. coli sample analysis.

1. The analysis of E. coli samples must meet the analytical method and approved laboratory requirements of Appendix G -2401 - G through 2402 - G.

2404 -G

A. Cryptosporidium sample analysis. The analysis of Cryptosporidium samples must meet the criteria in this paragraph.

1. Laboratories analyzed Cryptosporidium samples using one of the analytical methods in paragraphs (C)(1)(a) through (f) of this section, which are incorporated by reference. You may obtain a copy of these methods on-line from the United States Environmental Protection Agency, Office of Ground Water and Drinking Water, 1201 Constitution Ave, NW, Washington, DC 20460 (Telephone: 800-426-4791). You may inspect a copy at the Water Docket in the EPA Docket Center, 1301 Constitution Ave., NW, Washington, DC, (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.
 - a *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA, 2005*, United States Environmental Protection Agency, EPA-815-R-05-002.
 - b *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA, 2005*, United States Environmental Protection Agency, EPA-815-R-05-001.
 - c *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA, 2001*, United States Environmental Protection Agency, EPA-821-R-01-025.
 - d *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA, 2001*, United States Environmental Protection Agency, EPA-821-R-01-026.
 - e *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA, 1999*, United States Environmental Protection Agency, EPA-821-R-99-006.
 - f *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA, 1999*, United States Environmental Protection Agency, EPA-821-R-99-001.
2. For each Cryptosporidium sample, the laboratory analyzed at least 10 L of sample or at least 2 mL of packed pellet or as much volume as could be filtered by 2 filters that EPA approved for the methods listed in paragraph (C)(1) of this section.

**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 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 using one of the analytical methods listed in the following table 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 ³ Colisure ³ Membrane Filter Method with MI Agar m-ColiBlue24 Test ⁵ E*Colite Test ⁶ EC-MUG ⁷ NA-MUG ⁷ | 9223B. ² 9223B. ² EPA Method 1604. ⁴ 9221F. ² 9222G. ² |
| Enterococci | Multiple-Tube Technique | 9230B. ² |
| Coliphage | Two-Step Enrichment Presence-Absence Proce | 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 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.

³ Medium is available through IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

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-ColiBlue24® 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 §141.21(F)(6)(i) or (ii) after use of Standard Methods 9221 B, 9222 B, or 9222 C.

⁸ 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.

⁹ 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.

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.