

10A NCAC 42D .0248 MICROBIOLOGY GENERAL LABORATORY PRACTICES

- (a) The general laboratory practices for microbiological analyses shall be in accordance with those listed in the EPA "Manual for the Certification of Laboratories Analyzing Drinking Water", Chapter 5, Section 4, General Laboratory Practices, which is hereby incorporated by reference including any subsequent amendments and editions, except that Sections 4.6.2-4.6.11 are not incorporated by reference. A copy is available for inspection at the Division of Public Health, 1915 Mail Service Center, Raleigh, North Carolina 27699-1915. Nonprofit organizations or government agencies may obtain a copy by contacting the EPA Drinking Water Hotline at 800-426-4791. Other organizations may obtain a copy from the National Technical Information Service at 800-336-4700 for thirty five dollars (\$35.00).
- (b) The laboratory shall follow the additional laboratory practices:

(1) Membrane Filter Media:

Use m-Endo broth or agar or LES Endo broth or agar in the single step or enrichment techniques. Ensure that ethanol used in rehydration procedure is not denatured. Prepare medium in a sterile flask and use a boiling water bath or, if constantly attended, a hot plate with a stir bar to bring medium to the boiling point. Do not boil medium. Final pH shall be 7.2 0.2.

(2) Multiple Tube Fermentation (MTF) Media:

- (A) Use double strength lauryl sulfate broth or lactose broth in the presumptive test and single strength brilliant green lactose bile (BGLB) broth in the confirmed test. Autoclave media at 121°C for 12 minutes. Final pH shall be 6.8 0.2 or 7.2 0.2 for BGLB broth.
- (B) If MTF media are refrigerated after sterilization, incubate overnight at 35°C 0.5°C before use. Discard tubes showing growth or bubbles. Use MTF media prepared in tubes with loose fitting closures within one week. Store broth media in screw cap tubes or bottles no longer than three months, provided media are stored in the dark. Discard media if evaporation exceeds 10 percent of original volume.
- (C) LES Endo agar shall be used for the completed test. Refrigerate medium and use within two weeks.

(3) Clark's Total Coliform Medium:

- (A) Autoclave for 12 minutes at 121°C. Allow space between bottles.
- (B) Final pH shall be 6.8 0.2.
- (C) Store prepared medium in screw capped culture bottle no longer than three months; discard if evaporation exceeds 10 percent of original volume.

(4) EC Medium (for fecal coliforms):

- (A) Autoclave for 12 minutes at 121°C.
- (B) Examine tubes after sterilization to insure that inverted inner tubes are free of air bubbles and that the vials are at least partially covered with medium.
- (C) Incubate refrigerated sterilized medium overnight at 35°C 0.5°C; discard tubes that show growth or bubbles.
- (D) Store prepared medium in screw cap tubes.
- (E) Final pH shall be 6.9 0.2.

(5) EC + MUG Medium (for detection of fecal coliforms-E. coli):

- (A) Autoclave medium at 121°C (gas tubes shall not be used).
- (B) Final pH shall be 6.9 0.2.
- (C) Store prepared medium in screw cap tubes no longer than three months.

(6) MMO-MUG Test Medium (for Total Coliform and E. coli):

- (A) The laboratory shall not prepare this medium from basic ingredients.
- (B) Each lot purchased shall be tested for performance by inoculation with three control bacteria: Escherichia coli, a total coliform other than E. coli (e.g., Klebsiella pneumoniae) and a non-coliform (e.g. Pseudomonas aeruginosa).

These control organisms shall be stock cultures or commercially available discs impregnated with the organism. Incubate these controls at 35°C 0.5°C for 24 hours, and read and record result.

- (C) Do not autoclave.

(7) Fecal Coliform Membrane Filter Medium (for enumeration of fecal coliform in source water) .

- (A) Rehydrate medium in reagent water containing 10 ml of 1 percent rosolic acid in 2N NaOH. Bring it to the boiling point; do not autoclave.
- (B) Autoclave for 12 minutes at 121°C.
- (C) Final pH shall be 7.4 0.2.

- (D) Refrigerate unused prepared medium; discard after 96 hours.
- (8) Heterotrophic Plate Count (HPC) Medium:
- (A) Autoclave HPC agar at 121°C for 15 minutes.
 - (B) Final pH shall be 7.0 ± 0.2.
 - (C) Temper melted agar at 44°-46°C before pouring.
 - (D) Hold melted agar no longer than four hours. Do not melt sterile agar medium more than once.

History Note: Authority G.S. 130A-315;
Eff. December 1, 1991;
Amended Eff. January 1, 1996; April 1, 1993;
Pursuant to G.S. 150B-21.3A, rule is necessary without substantive public interest Eff. December 23, 2017.