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Procedural Modification for Drinking Water Total Coliform	01/17/12	1 of 3	198.3

I. Most Probable Number (*Standard Methods,* 18th – 21st Ed. (99), 9221, A, B and 40 CFR 141.21 (F) 6i)

A. Lauryl Tryptose (or Lactose Broth) Presumptive Test

1. A **full 100.0 ml** must be inoculated for each sample. Thus **ten (10)** double strength lauryl tryptose (or lactose broth) must each be inoculated with **ten (10) ml** of sample. Other inoculum quantities and media concentrations may be used following Table 9221:I, (*Standard Methods,* **18 – 21st Ed. (99), 9221B**).

Alternatively, the Presence-Absence (P-A) Coliform Test (*Standard Methods,* **18th – 21st Ed.**, 9221D and **40 CFR 141.21 (F) 6i**) may be used for presumptive testing. A positive P-A test is then handled as a positive Lauryl Tryptose tube.

2. Tubes displaying gas production after 24 ± 2 hours or after 48 ± 3 hours of incubation at 35 ± 0.5 C are to be confirmed using **both** brilliant green lactose bile broth (BGB) **and** EC media with *MUG* added (EC/MUG)[1]. Invalidate samples which produce a turbid culture in the absence of gas production (lauryl tryptose) or are not yellow (P-A).

3. Using a sterile loop or applicator stick, transfer material from each positive presumptive tube to:
   a. a fermentation tube containing BGB **and**
   b. a test tube containing 10. ml of EC/MUG.

4a. Incubate the BGB tube(s) at 35 ± 0.5 C. Examine for gas production after 24 ± 2 hours. Gas production in **ANY** tube indicates that **TOTAL COLIFORM ARE PRESENT**. If no gas has been produced continue incubation for an additional 24 hours (for a total time of 48 ± 3 hours). Again, examine tubes for gas production. If gas has been produced, **TOTAL COLIFORM ARE PRESENT**. If no gas has been produced, total coliform are absent.

4b. Incubate the EC/MUG tube(s) at 44.5 ± 0.2 C in a mechanically stirred water bath[2] for 24 ± 2 hours. Examine tube(s) for fluorescence under *UV* (366 nm, 6 Watt) light[3]. Tubes exhibiting fluorescence are positive for E. coli regardless of the outcome of the LT and BGB tests conducted in 4a

[1] Difco catalog number 0022-15-7 or equivalent

[2] For example *Blue M* model MW-110A-1.

[3] Using, for example, *UVP Black-Ray* Model ML 49, 6 Watt ultraviolet lamp

above. A sterile EC/MUG tube must also be included to serve as a negative control since the EC/MUG media does fluoresce to some extent.

II. Membrane Filtration Method (*Standard Methods,* **18th – 21st Ed. (97)**, 9222A, B, C and **40 CFR 141.21 (F) 6i/ii**).

A. Filter 100 ml of sample in the usual manner and incubated.

1. If typical sheen colonies develop, verify total coliforms and check for E. coli using step 2 or 3 below. MF cultures without sheen colonies but with confluent or "TNTC" growth are to be invalidated.

2. Swab the entire filter with a sterile applicator stick[4] and transfer this material to MPN tubes of Lauryl Tryptose and Brilliant Green Lactose Bile and a tube containing 10. ml of EC/MUG medium for verification. In the event a heavy growth is observed, such as in proficiency test samples, the filter surface should be divided into sections with material from each section transferred, using a new applicator, to its own set of tubes. The material is transferred by swirling the cotton tip swab in the medium in the following order: LT, EC/MUG, BGB. DO NOT leave the applicator in any tube. Incubate the LT and BGB tubes at 35 ± 0.5 C as described above. Incubate the EC/MUG tube at 44.5 ± 0.2 C for 24 ± 2 hours.

Examine the EC/MUG tube for fluorescence using a *UV* light (see I.4b above). If the tube fluoresces, E. COLI ARE PRESENT. If the EC/MUG tube does not fluoresce, the LT and BGB tubes will serve for the required total coliform verification.

3. Fish a portion of each colony (at least 5 colonies) into Lauryl Tryptose and Brilliant Green Lactose Bile MPN tubes for verification. Transfer the filter to petri dish containing nutrient agar supplemented with *MUG*[5]. Incubate at 35 ± 0.5 C for 4 hours. Observe the filter under *UV* light (note 3), if the colony(ies) fluoresce **E. COLI ARE PRESENT.**

III. Chromogenic/Fluorogenic Methods (***Standard Methods,* 18th – 21st Ed. (97), 9223**).

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[4] For example Thomas Scientific Catalogue Number 1132-H05

[5] See *Standard Methods,* 16th Ed. p. 874. Nutrient agar is available from Difco (cat. no. 0001-02-7). Supplemented with 100 mg/L 4-methylumbelliferyl-*beta*-D-glucuronide (*MUG*), Sigma Chemical product number M 9130

Fill bottle containing dehydrated medium to the 100 mL mark with sample. Alternatively, if quantitation is desired, ten MPN tubes containing ONPG-MUG media could be inoculated with 10 mL of sample each and proceed as follows.
The Colilert Quanti-Tray and Quanti-Tray 2000 are acceptable.

1. Incubate container(s) at 35.0 $\pm$ 0.5 degrees C for 24 hrs.. Examine media for change in color to yellow for Colilert and to red for Colisure which indicates **TOTAL COLIFORM PRESENCE.** Color comparators are available from the manufacturers. Examine positive containers under UV light (note 2). Containers that fluoresce indicate **E. coli PRESENCE.**

Colilert containers that are yellow, but lighter than the comparator should be re-incubated for an additional four hours, for a maximum total incubation time of 28 hours. If the containers are still lighter than the comparator then record the sample as **Total Coliform negative and E. coli negative.** If the color reaction occurs after inoculation but before incubation then the media batch cannot be used and the sample must be invalidated.

NOTE: A confirmation step is intrinsic in these methods; no further confirmation is required.

OPTIONAL:

2. Using sterile technique transfer 0.1 mL ONPG-MUG media displaying a yellow color, but failing to fluoresce after 28 hours of incubation to each a tube of EC/MUG media and to MPN tubes of lauryl tryptose and brilliant green lactose bile broth. Incubate and examine as described in I.A.2., I.A.4a, and I.A.4b respectively, above. Positive LT and BGB reactions confirm **TOTAL COLIFORM PRESENCE.** A positive EC/MUG reaction confirms E. coli **PRESENCE** regardless of LT and BGB reactions.