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Research And Development Cincinnati, OH

Test Methods For *Escherichia Coli* In Drinking Water

EC Medium With Mug Tube Procedure

Nutrient Agar With Mug Membrane Filter Procedure

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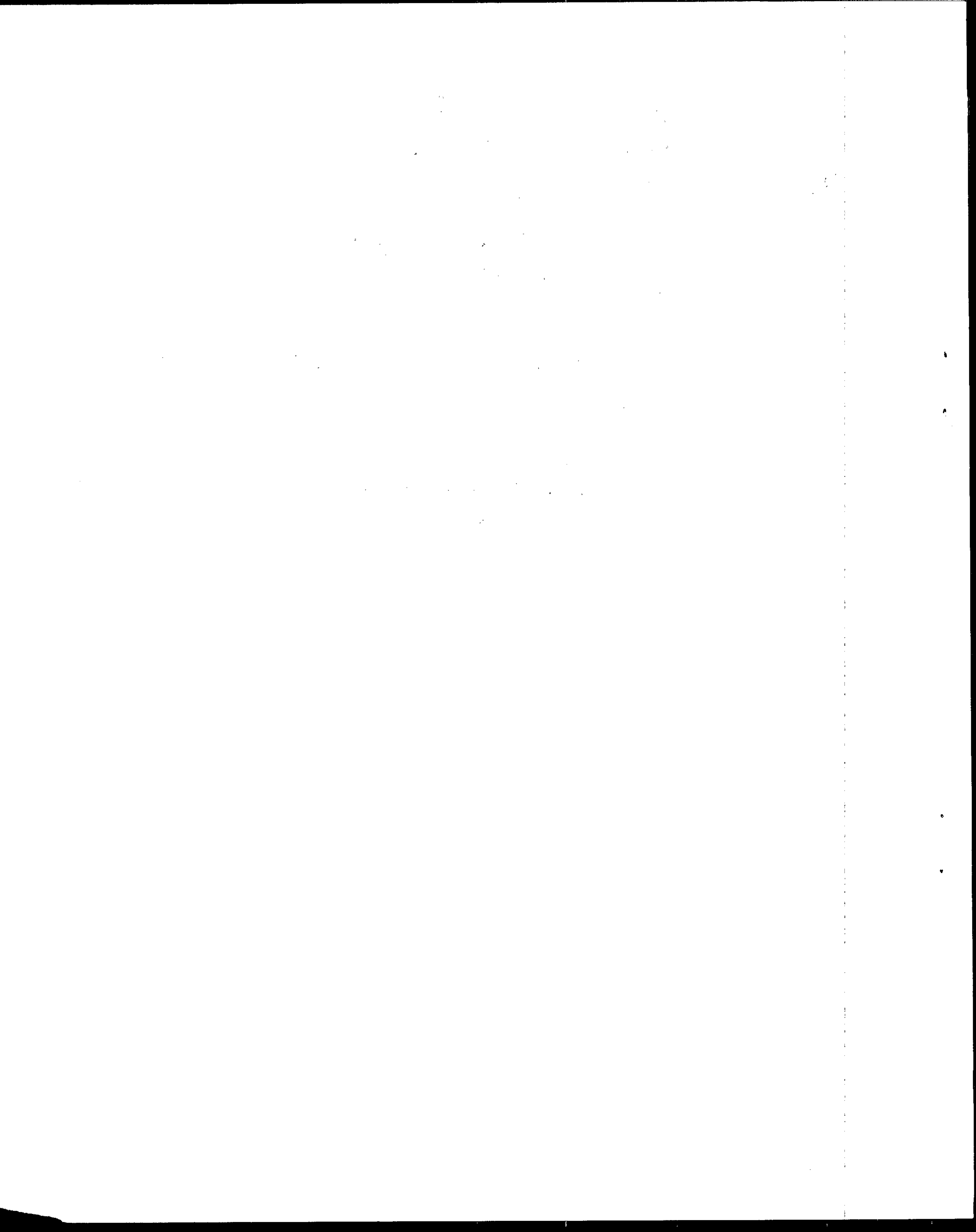

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- o Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.
- o Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.
- o Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.

The two analytical methods described in this report can be used to measure the bacteriological quality of potable water. They are approved for determining compliance of public water systems with the National Primary Drinking Water Regulations. The presence of *Escherichia coli* in drinking water is an indication of fecal pollution and the possible presence of enteric pathogens.

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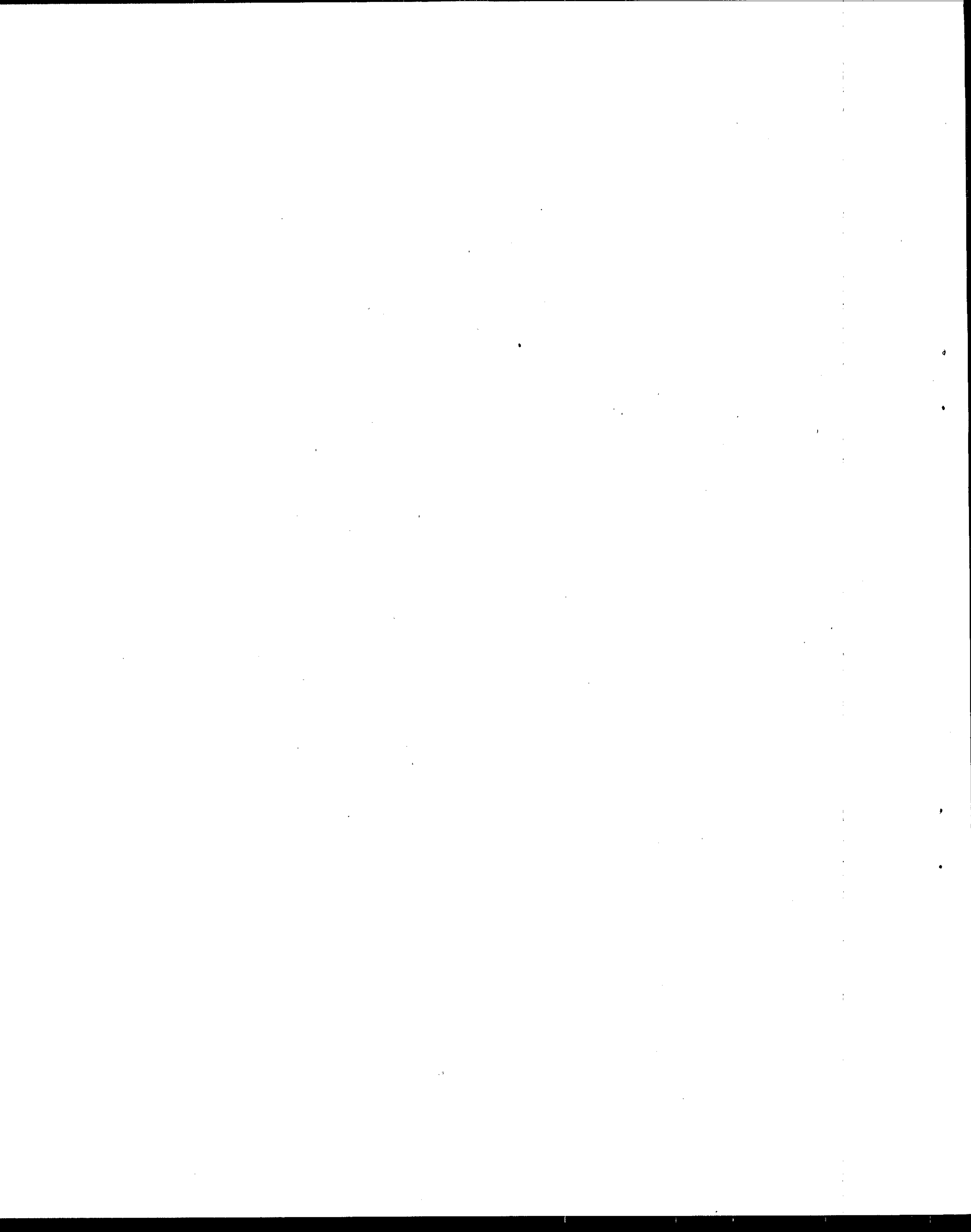

Test Method 1104
Detection of Escherichia Coli in Drinking Water by the EC Medium with Mug Tube Procedure

1. Scope and Application

1.1 This method describes a procedure for the detection and enumeration of Escherichia coli (E. coli) in drinking water by transfer from total coliform-positive presumptive tests to EC + MUG medium. Because this species is a natural inhabitant only of the intestinal tract of warm-blooded animals, its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens.

1.2 This method can be applied to positive presumptive results from the multiple tube fermentation (MTF), presence-absence (P-A), and membrane filter (MF) procedures for total coliforms.

1.3 The detection limit of this procedure is one micro-organism per 100 mL.

1.4 The Total Coliform Rule[1] requires that all total coliform-positive cultures be tested for either fecal coliforms or E. coli. This method for E. coli is approved in the National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria[2].

2. Summary

2.1 Culture from total coliform-positive tubes or bottles of lauryl tryptose, lactose or P-A medium, or from total coli form MF colonies or entire surface growth in the presumptive phase of these procedures for total coliforms is inoculated into EC broth containing 4-methylumbelliferyl-ß-D-glucuronide (EC + MUG) and incubated at 44.5 ± 0.2°C for 24 hours[3]. Observance of bright blue fluorescence when subjected to long-wave (366 nm) ultraviolet (UV) light indicates a positive test for E. coli.

3. Definition

3.1 In this method E. coli are defined as those bacteria which produce bright blue fluorescence in EC + MUG medium after initial culture in lauryl tryptose, lactose or P-A broth or on Endo MF plates.

4. Interferences

4.1 Certain brands of test tubes fluoresce under long-wave UV light and may interfere with test results. Tubes should be examined before use.

4.2 Do not use an inverted vial; gas production is not relevant to the test and observation for this reaction may cause confusion in test interpretation.

5. Safety Precautions

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents and materials and while operating sterilization equipment.

6. Apparatus and Equipment

6.1 Water bath, gable-covered incubator that maintains a 44.5 ± 0.2°C temperature.

6.2 Lamp, ultraviolet, long-wave, 366 nm, preferably with a 6-watt bulb.

6.3 Inoculation loop, 3 mm diameter or needle of nichrome wire, 26 B&S gauge, in suitable holder. Sterile applicator sticks are a suitable alternative to inoculation loops or needles. Sterile cotton-tipped applicator sticks are used for the transfer of growth from the entire MF surface by the swab technique.

6.4 Bunsen or Fisher-type burner or electric incinerator unit.

6.5 Thermometer, glass/mercury or dial calibrated in 0.2°C increments or less, checked against a National Bureau of Standards (NBS) certified thermometer, or one traceable to an NBS thermometer.

6.6 Ethanol, methanol or isopropanol in small, wide-mouth container, for flame-sterilizing forceps.

6.7 Pyrex test tubes, 150 x 20 mm.

6.8 Culture tube racks to hold 20 mm diameter tubes.

6.9 Flasks, borosilicate glass, screw-cap, 250 - 1000 mL volume.

7. Media and Reagents

7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. The agar used in preparation of culture media must be of microbiological grade.

7.2 Whenever possible, use commercial culture media as a means of quality control.

7.3 EC Medium with MUG (Difco 0022-17), EC Broth with MUG (BBL 12332) or equivalent.

Composition:

Tryptose or Pancreatic Digest of Casein Peptone	20.0 g
Lactose	5.0 g
Bile Salts No. 3 or Bile Salts Mixture	1.5 g
Dipotassium Phosphate	4.0 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5.0 g
4-methyl-ß-D-umbelliferyl glucuronide (MUG)	0.05 g

Reagent Grade Water 1 L
Final pH: 6.9 ± 0.2

Preparation: Add 37.1 grams (Difco) or 37.0 grams (BBL) of EC with MUG medium or equivalent to 1 liter of water and warm slightly to dissolve completely. Dispense into tubes (150 x 20 mm). Sterilize for 12-15 minutes at 121°C (15 lbs. pressure).
Alternatively, 0.05g MUG per liter may be added to EC Medium (Difco 0314.02), EC Broth (BBL 11187) or equivalent before autoclaving.

7.4 Check test tubes before use with a 366 nm UV light to ensure they do not fluoresce.

7.5 For quality control of the medium, include a MUG-positive (E. coli) and MUG-negative (e.g., uninoculated) control for each analysis or set of analyses. Check medium before use with a 366 nm UV light to ensure it does not fluoresce.

7.6 Incubate positive and negative control cultures at 35 ± 0.5°C for 24 hours in lauryl tryptose broth. Transfer a loopful to EC medium with MUG and incubate at 44.5 ± 0.2°C for 24 hours. Read and record results.

7.7 Use prepared medium in tubes with loose-fitting closures within one week. Store sterile refrigerated medium for up to three months in screw-cap tubes/containers, and incubate stored medium overnight at 35°C before use; discard tubes with growth.

8. Sample Collection, Preservation and Holding Time

8.1 This test method is a transfer procedure from a preceeding sample analysis; therefore, it does not involve direct analysis of the water sample. Consequently, sample collection, preservation and holding time are not procedures specifically applicable to this method. However, adherence to sample collection and preservation procedures and holding time limits for the original water sample is critical to the production of valid data.

9. Calibration and Standardization

9.1 Check temperatures in incubators daily to ensure operation within stated limits.

9.2 Check thermometers at least annually against an NBS certified thermometer or one traceable to NBS. Check mercury columns for breaks.

10. Quality Control

10.1 Verify at least 5% of both MUG-positive results and turbid total coliform-positive, MUG-negative results. Verification of a pure culture may be performed by the use of API 20 E or an equivalent bacterial identification system; standard biochemical tests (e.g. citrate, indole and urease tests); serotyping after biochemical identification if desired; or the indole test at 44 °C and growth in citrate.

10.2 See recommendations on quality control for microbiological analyses in Standard Methods for the Examination of Water and Wastewater.[4]

11. Procedure

11.1 Gently swirl the presumptive total coliform tube or bottle. Using a sterile inoculating loop or wooden applicator, transfer inocula from total coliform-positive presumptive phase tubes or bottles at 24 hours (or 48 hours if needed)to EC + MUG tubes.
Transfer inocula from total coliform MF colonies with a sterile needle or wooden applicator stick. Alternatively, use a sterile cotton-tipped swab to transfer the entire surface growth from a total coliform-positive MF plate to EC + MUG tubes. Do not leave the cotton swab in the medium. Gently swirl inoculated EC + MUG tubes to ensure mixing of inoculum with medium.

11.2 Incubate inoculated EC + MUG tubes at 44.5 ± 0.2°C for 24 ± 2 hours. Tubes must be placed in the incubator within 30 minutes after inoculation. The water depth in the water bath incubator must come to the top level of the culture medium in the tube.

11.3 Detect fluorescence using an ultraviolet lamp (366 nm), preferably with a 6-watt bulb. Ensure that weak autofluorescence of medium, if present, is not misinterpreted as positive for E. coli. A MUG-positive (E. coli) and MUG-negative (e.g., uninoculated) control are necessary for each analysis. The observation of bright blue fluorescence in the EC + MUG tubes after 24 ± 2 hours constitutes a positive test for E. coli.

12. Reporting

12.1 Report the presence or absence of E. coli.

References

1. Drinking Water; National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and E. Coli); Final Rule. 40 Code of Federal Regulations (CFR) Parts 141 and 142. Federal Register 54: p. 27544, June 29, 1989.
2. National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria. 40 Code of Federal Regulations (CFR), Part 141, Federal Register 56, p. 636, January 8, 1991.
3. Rippey, S. R., L. A. Chandler and W. D. Watkins. 1987. Fluorometric Method for Enumeration of Escherichia coli in Molluscan Shellfish. J. Food Protection 50:685-690.
4. American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater. 16th edition. American Public Health Association, Washington, D.C.

Test Method 1105
Detection of Escherichia Coli in Drinking Water by the Nutrient Agar with Mug Membrane Filter Procedure

1. Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of Escherichia coli (E. coli) in drinking water by transfer of a total coliform-positive membrane containing sheen colonies from m-Endo medium to another substrate selective for E. coli. Because this species is a natural inhabitant only of the intestinal tract of warm-blooded animals, its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens.

1.2 The detection limit of this procedure is one E. coli per 100 mL.

1.3 The Total Coliform Rule[1] requires that all total coliform-positive cultures be tested for either fecal coliforms or E. coli. This method for E. coli detection is approved in the National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria[2].

2. Summary

2.1 The MF method determines the presence or absence or provides a direct count of E. coli in water based on the development of fluorescent colonies on the surface of the MF[2,3]. A 100 mL water sample is filtered through the membrane which retains the bacteria. After filtration, the membrane containing the bacterial cells is placed on Endo-type medium and incubated at 35 ± 0.5°C for 24 ± 2 hours. Following incubation, the total coliform-positive filter is transferred to a nutrient agar substrate containing 4-methylumbelliferyl-ß-D-glucuronide (NA + MUG). Production of bright blue fluorescence around the sheen colonies after an additional 4 hours incubation at 35°C indicates E. coli. Fluorescent halo colonies are observed and/or counted with a long-wave (366 nm) ultraviolet (UV) lamp.

3. Definition

3.1 In this method, E. coli are those bacteria which produce bright blue fluorescence around the periphery of total coliform colonies.

4. Interferences

4.1 Dull fluorescence on the entire surface of some colonies or green fluorescence produced by some pseudomonads capable of growing on Endo media may be mistaken for the bright blue fluorescence typical of E. coli.

5. Safety Precautions

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents and materials and while operating sterilization equipment.

6. Apparatus and Equipment

6.1 Stereoscopic microscope, wide-field type with magnification of 10-15X.

6.2 Microscope lamp producing diffuse light from a cool, white fluorescent tube and diffuser.

6.3 UV lamp, long-wave, 366 nm, preferably with a 6-watt bulb.

6.4 Forceps, straight or curved, with smooth tips to handle filters without damage.

6.5 Ethanol, methanol or isopropanol in a small, wide-mouth container, for flame-sterilizing forceps.

6.6 Burner, Bunsen or Fisher type, or electric incinerator unit for sterilizing inoculation needles.

6.7 Thermometer, glass/mercury or dial calibrated in 0.5°C increments or less, checked against a National Bureau of Standards (NBS) certified thermometer, or one traceable to an NBS thermometer.

6.8 Flask, borosilicate glass, screw-cap, 250-1000 mL volume.

6.9 Needles, nichrome wire, 26 B & S gauge, in suitable holders. Disposable applicator sticks are alternatives.

6.10 Incubator maintained at 35 ± 0.5°C.

6.11 Petri dishes, plastic, sterile, with tight-fitting lids. 50 X 12 mm.

7. Media and Reagents

7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. The agar used in preparation of culture media must be of microbiological grade.

7.2 Whenever possible, use commercial culture media as assurance of quality control.

7.3 Nutrient Agar with MUG (NA + MUG) (Difco 0023-15-6)or equivalent, or
Nutrient Agar (Difco 0001-02, BBL 11471) or equivalent with MUG added separately.

Composition:

Peptone	5.0 g
Beef Extract	3.0 g
Agar	15.0 g
4-Methyl-ß-d-umbelliferyl glucuronide (MUG)	0.1 g
Reagent Grade Water	1 L

Final pH: 6.8 ± 0.1

Preparation: Add 23 grams of NA + MUG agar per liter of reagent grade water and mix well. Heat in boiling water bath to dissolve the agar completely. Dispense in bottles or flasks and sterilize for 15 minutes at 121°C (15 lbs. pressure). Pour into 50 mm petri plates (4 mL per plate).

7.4 If medium in petri dishes is not used immediately, store refrigerated for up to two weeks. Incubate stored medium overnight at 35°C before use; inspect and discard plates with growth.

7.5 For quality control of the medium spot inoculate control culture(s) onto a MF placed on m-Endo agar and incubate at 35 ± 0.5°C for 18-24 hours. Then transfer the MF to NA + MUG and incubate at 35°C for 4 hours. Read for fluorescence around periphery of growth and record results. Fluorescence over the entire colony or growth area usually does not confirm as E. Coli. Test each commercial lot or laboratory batch prepared from basic ingredients.

8. Sample Collection, Preservation and Holding Time

8.1 This test method is a transfer procedure from a preceeding sample analysis; therefore, it does not involve direct analysis of the water sample. Consequently, sample collection, preservation and holding time are not procedures specifically applicable to this method. However, adherence to sample collection and preservation procedures and holding time limits for the original water sample is critical to the production of valid data.

9. Calibration and Standardization

9.1 Check temperatures in incubators daily to insure operation within stated limits.

9.2 Check thermometers at least annually against an NBS certified thermometer or one traceable to NBS. Check mercury columns for breaks.

10. Quality Control

10.1 Verify at least 5% of both MUG-positive results and MUG-negative, total coliform-positive results. Also verify any non-sheen colonies that fluoresce. Verification of a pure culture may be performed by the use of API 20 E or an equivalent bacterial identification system; standard biochemical tests (e.g. citrate, indole and urease tests); serotyping after biochemical identification if desired; or the indole test at 44 °C and growth in citrate.

10.2 See recommendations on quality control for microbiological analyses in Standard Methods for the Examination of Water and Wastewater.[4]

11. Procedure

11.1 Record the total coliform-positive MF results, and count and record the sheen colonies on Endo medium, if desired.

11.2 Prior to transfer of the membrane, transfer a small portion of each sheen colony to the appropriate total coliform verification media using a sterile needle. Alternatively, transfer a small portion of the sheen fluorescent colonies or swab the surface growth on the entire membrane after transfer of the membrane and incubation on NA + MUG.

11.3 Use sterile forceps to transfer the MF from a total coliform positive Endo plate to the NA + MUG medium. Mark each sheen colony with a fine-tipped marker or by puncturing a hole in the MF adjacent to the colony with a needle.

11.4 Incubate at 35 ± 0.5°C for 4 hours.

11.5 Observe individual colonies for fluorescence using a long wave (366 nm) UV light source, preferably with a 6-watt bulb. Any amount of bright blue fluorescence on the periphery (outer edge) of the colony is considered positive for E. coli. Dull fluorescent sheen on the entire colony is not typical of E. coli. Read fluorescence on the membrane surface, not from the bottom of the plate; the fluorescent halo cannot always be read clearly from the underside. The analyst must distinguish the blue fluorescence of a MUG-positive reaction from the green fluorescence caused by some Pseudomonas species. The number of E. coli colonies with fluorescent halo may be counted if desired.

12. Reporting

12.1 Report the presence or absence of E. coli.

References

1. Drinking Water; National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and E. Coli); Final Rule. 40 Code of Federal Regulations (CFR) Parts 141 and 142. Federal Register 54: p. 27544, June 29, 1989.
2. National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria. 40 Code of Federal Regulations (CFR), Part 141, Federal Register 56, p. 636, January 8, 1991.
3. Mates, A. and M. Shaffer. 1989. Membrane Filtration Differentiation of E. coli from Coliforms in the Examination of Water. Jour. Appl. Bacteriol. 67, 343-346.
4. American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater. 16th edition. American Public Health Association, Washington, D.C.