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Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)

September 2002

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Method 1103.1 *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)

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1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Escherichia coli*. Because the bacterium 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 *E. coli* test is recommended as a measure of recreational fresh water quality. Epidemiological studies have led to the development of criteria which can be used to promulgate recreational water standards based on established relationships between health effects and water quality. The significance of finding *E. coli* in recreational fresh water samples is the direct relationship between the density of *E. coli* and the risk of gastrointestinal illness associated with swimming in the water (Reference 18.3).

1.3 The test for *E. coli* can be applied to fresh, estuarine, and marine waters.

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 The MF method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter (Reference 18.4). A water sample is filtered through the membrane which retains the bacteria. After filtration, the membrane containing the bacterial cells is placed on a selective and differential medium, mTEC, incubated at 35°C for 2 h to resuscitate injured or stressed bacteria, and then incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 min, yellow, yellow-green or yellow-brown colonies are counted with the aid of a fluorescent lamp and a magnifying lens.

3.0 Definitions

3.1 In this method, *E. coli* are those bacteria which produce colonies that remain yellow, yellow-green or yellow-brown on a filter pad saturated with urea substrate broth after primary culturing on mTEC medium.

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter and prevent filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies.

5.0 Safety

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.

5.2 Mouth-pipetting is prohibited.

6.0 Equipment and Supplies

6.1 Glass lens with magnification of 2-5x , or stereoscopic microscope.

6.2 Lamp, with a cool, white fluorescent tube.

6.3 Hand tally or electronic counting device.

6.4 Pipet container, stainless steel, aluminum or borosilicate glass, for glass pipets.

6.5 Pipets, sterile, T.D. bacteriological or Mohr, glass or plastic, of appropriate volume.

6.6 Graduated cylinders, 100-1000 mL, covered with aluminum foil or kraft paper and sterile.

6.7 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel, wrapped with aluminum foil or kraft paper and sterilized.

6.8 Ultraviolet unit for sanitization of the filter funnel between filtrations (optional).

6.9 Line vacuum, electric vacuum pump, or aspirator for use as a vacuum source. In an emergency or in the field, a hand pump or a syringe equipped with a check valve to prevent the return flow of air, can be used.

6.10 Flask, filter, vacuum, usually 1 L, with appropriate tubing. A filter manifold to hold a number of filter bases is optional.

6.11 Flask for safety trap placed between the filter flask and the vacuum source.

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

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

6.14 Burner, Bunsen or Fisher type, or electric incinerator unit for sterilizing loops and needles.

6.15 Thermometer, checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.

6.16 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids; or 15 x 60 mm, glass or plastic, with loose-fitting lids; or 15 x 100 mm.

6.17 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions. Dilution bottles marked at 90 mL or tubes marked at 9 mL may be used for 1:10 dilutions.

6.18 Flasks, borosilicate glass, screw-cap, 250-2000 mL volume.

6.19 Membrane filters, sterile, white, grid marked, 47 mm diameter, with 0.45 ± 0.02 µm pore size.

6.20 Absorbent pads, sterile, 47 mm diameter (usually supplied with membrane filters).

6.21 Inoculation loops, at least 3 mm diameter, and needles, nichrome or platinum wire, 26 B & S gauge, in suitable holders. Sterile disposable applicator sticks or plastic loops are alternatives to inoculation loops.

Note: *A platinum loop is required for the cytochrome oxidase test in the verification procedure.*

6.22 Incubator maintained at 35 ± 0.5°C, with approximately 90% humidity if loose-lidded petri dishes are used.

6.23 Waterbath maintained at 44.5 ± 0.5°C.

6.24 Waterbath maintained at 50°C for tempering agar.

6.25 Test tubes, 20 x 150 mm, borosilicate glass or plastic.

6.26 Test tubes, 10 x 75 mm, borosilicate glass.

6.27 Caps, aluminum or autoclavable plastic, for 20 mm diameter test tubes.

6.28 Test tubes screw-cap, borosilicate glass, 16 x 125 mm or other appropriate size.

6.29 Filter Paper.

6.30 Whirl-Pak® bags.

7.0 Reagents and Standards

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 of Analytical Reagents of the American Chemical Society (Reference 18.6). The agar used in preparation of the culture media must be of microbiological grade.

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

7.3 Purity of Water: Reagent water conforming to Specification D1193, Type II water, ASTM Annual Book of Standards (Reference 18.1).

7.4 Phosphate buffered saline

7.4.1 Composition:

Sodium Dihydrogen Phosphate	0.58 g
Sodium Monohydrogen Phosphate	2.5 g
Sodium Chloride	8.5 g
Reagent-Grade Distilled Water	1.0 L

7.4.2 Preparation: Dissolve the ingredients in 1 L of reagent-grade distilled water in a flask, and dispense in appropriate amounts for dilutions in screw-cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave at 121°C (15 lb pressure) for 15 min. Final pH should be 7.4 ± 0.2.

7.5 Phosphate buffered dilution water (Reference 18.2)

7.5.1 Composition of Stock Phosphate Buffer Solution:

Phosphate Dihydrogen Phosphate	34.0 g

Reagent-Grade Distilled Water	500.0 mL

Preparation: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring the volume to 1 L with reagent-grade distilled water. Sterilize by filtration or autoclave at 121°C (15 lb pressure) for 15 min.

7.5.2 Preparation of Stock Magnesium Chloride Solution: Add 38 g anhydrous $MgCl_2$ or 81.1 g $MgCl_2$Ć$6H_2O$ to 1 L reagent-grade distilled water. Sterilize by filtration or autoclave at 121°C (15 lb pressure) for 15 min.

7.5.3 Storage of Stock Solutions: After sterilization, store the stock solutions in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears, the affected stock solution should be discarded and a fresh solution should be prepared.

7.5.4 Working Phosphate Buffered Dilution Water: Mix 1.25 mL of the stock phosphate buffer and 5 mL of the $MgCl_2$ stock per liter of reagent-grade distilled water. Dispense in appropriate amounts for dilutions in screw-cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave at 121°C (15 lb pressure) for 15 min. Final pH should be 7.0 ± 0.2.

7.6 mTEC Agar

7.6.1 Composition:

Protease Peptone #3	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
NaCl	7.5 g
Dipotassium Phosphate	3.3 g
Monopotassium Phosphate	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Brom Cresol Purple	0.08 g
Brom Phenol Red	0.08 g
Agar	15.0 g
Reagent-Grade Distilled Water	1.0 L

7.6.2 Preparation: Add dry ingredients to 1 L of reagent-grade distilled water in a flask, and heat to boiling until the ingredients dissolve. Autoclave at 121°C (15 lb pressure) for 15 min, and cool in a 50°C waterbath. Pour the medium into each 9 x 50 mm culture dish to a 4-5 mm depth (approximately 4-6 mL), and allow to solidify. Final pH should be 7.3 ± 0.2. Store in a refrigerator.

7.7 Urea Substrate Medium

7.7.1 Composition:

Urea	2.0 g
Phenol Red	0.01 g
Reagent-Grade Distilled Water	100.0 mL

7.7.2 Preparation: Add dry ingredients to 100 mL reagent-grade distilled water in a flask. Stir to dissolve, and adjust to pH 3-4 with 1 N HCl. The substrate solution should be a straw-yellow color at this pH (See Photo 1.).

Photo 1. Urea Substrate Medium. After adjusting the pH of the medium to 3-4, the Urea Substrate Medium should be straw-yellow in color.

7.8 Nutrient Agar

7.8.1 Composition:

Peptone	5.0 g
Beef Extract	3.0 g
Agar	15.0 g
Reagent-Grade Distilled Water	1.0 L

7.8.2 Preparation: Add dry ingredients to 1 L of reagent-grade distilled water, and mix well. Heat to boiling to dissolve the agar completely. Dispense in screwcap tubes, and autoclave at 121°C (15 lb pressure) for 15 min. Remove the tubes and slant. Final pH should be 6.8 ± 0.2.

7.9 Tryptic Soy Broth; Trypticase Soy Broth

7.9.1 Composition:

Tryptone or Trypticase	17.0 g
Soytone or Phytone	3.0 g
Sodium Chloride	5.0 g
Dextrose	2.5 g
Dipotassium Phosphate	2.5 g
Reagent-Grade Distilled Water	1.0 L

7.9.2 Preparation: Add dry ingredients to 1 L of reagent-grade distilled water. Warm the broth, and mix gently to dissolve the medium completely. Dispense in screwcap tubes, and autoclave at 121°C (15 lb pressure) for 15 min. Final pH should be 7.3 ± 0.2.

7.10 Simmons Citrate Agar

7.10.1 Composition:

Magnesium Sulfate	0.2 g
Monoammonium Phosphate	1.0 g
Dipotassium Phosphate	1.0 g
Sodium Citrate	2.0 g
Sodium Chloride	5.0 g
Brom Thymol Blue	0.08 g
Agar	15.0 g
Reagent-Grade Distilled Water	1.0 L

7.10.2 Preparation: Add the dry ingredients to 1 L of reagent-grade distilled water. Heat to boiling to dissolve completely. Dispense into screw-cap tubes, and autoclave at 121°C (15 lb pressure) for 15 min. Cool the tubes and slant. Final pH should be 6.8 ± 0.2.

7.11 Tryptone 1%; Tryptophane Broth

7.11.1 Composition:

Tryptone or Trypticase Peptone	10.0 g
Reagent-Grade Distilled Water	1.0 L

7.11.2 Preparation: Add the tryptone or trypticase peptone to 1 L or reagent-grade distilled water, and heat, mixing until dissolved. Dispense in 5-mL volumes into tubes, and autoclave at 121°C (15 lb pressure) for 15 min. Final pH should be 7.2 ± 0.2.

7.12 EC Broth

7.12.1 Composition*:*

Tryptose or Trypticase 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
Reagent-Grade Distilled Water	1.0 L

7.12.2 Preparation: Add dry ingredients to 1 L of reagent-grade distilled water, and warm to dissolve completely. Dispense into fermentation tubes (20 x 150 mm tubes containing inverted 10 x 75 mm vials). Autoclave at 121°C (15 lb pressure) for 15 min. Final pH should be 6.9 ± 0.2.

7.13 Oxidase Reagent

7.13.1 Composition*:*

N, N, N', N'-tetramethyl-D-phenylenediamine dihydrochloride, 1% aqueous solution (1 g per 100 mL *sterile* reagent-grade distilled water).

7.14 Kovacs Indole Reagent

7.14.1 Composition*:*

D-dimethylaminobenzaldehyde	10.0 g
Amyl or Isoamyl Alcohol	150.0 mL

Concentrated (12 M) Hydrochloric Acid 50.0 mL

7.14.2 Preparation: Dissolve D-dimethylaminobenzaldehyde in alcohol, slowly add hydrochloric acid, and mix.

8.0 Sample Collection, Preservation, and Storage

8.1 Sampling procedures are described in detail in the USEPA microbiology methods manual, Section II, A (Reference 18.2). Adherence to sample preservation procedures and holding time limits is critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.1.1 Storage Temperature and Handling Conditions

Ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water during transit or storage.

8.1.2 Holding Time Limitations

Examine samples as soon as possible after collection. Do not hold samples longer than 6 h between collection and initiation of analyses.

9.0 Quality Control

9.1 See recommendations on quality control for microbiological analyses in the USEPA microbiology methods manual, Part IV, C (Reference 18.2).

10.0 Calibration and Standardization

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

10.2 Check thermometers at least annually against a NIST certified thermometer or one that meets the requirements of NIST monograph SP 250-23. Check mercury columns for breaks.

11.0 Procedure

11.1 Prepare mTEC Agar and Urea Substrate Medium as directed in Sections 7.6 and 7.7, respectively.

11.2 Mark the petri dish and report form with the sample identification and volume.

11.3 Place a sterile membrane filter on the filter base, grid side up, and attach the funnel to the base so that the membrane filter is held between the funnel and the base.

11.4 Shake the sample bottle vigorously at least 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel.

11.5 Select sample volumes based on previous knowledge of the pollution level, to produce 20-80 *E. coli* colonies on the membranes. Sample volumes of 1-100 mL are normally tested at half-log intervals (*e.g.*, 100, 30, 10, 3 mL).

11.6 Smaller sample sizes or sample dilutions can be used to minimize the interference of turbidity or for high bacterial densities. Multiple volumes of the same sample or sample dilutions may be filtered, and the results may be combined.

11.7 Filter the sample, and rinse the sides of the funnel at least twice with 20-30 mL of sterile buffered rinse water. Turn off the vacuum, and remove the funnel from the filter base.

11.8 Use sterile forceps to aseptically remove the membrane filter from the filter base, and roll it onto the mTEC Agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane if bubbles occur. Run the forceps around the edge of the filter to be sure that the filter is properly seated on the agar. Close the dish, invert, and incubate 35 ± 0.5°C for 2 h.

11.9 After a 2 h incubation at 35 ± 0.5°C, transfer the plate to a Whirl-Pak® bag, seal the bag, place the bag with the plate inverted in a test-tube rack, and put the rack in a 44.5 ± 0.2°C waterbath for 22-24 h.

11.10 After 22-24 h, remove the plate from the waterbath. Place an absorbent pad in a new petri dish or the lid of the same petri dish, and saturate the pad with Urea Substrate Medium. Aseptically transfer the membrane from mTEC Agar to the absorbent pad saturated with Urea Substrate Medium, and allow to sit at room temperature for 15-20 min. (See Photo 2.).

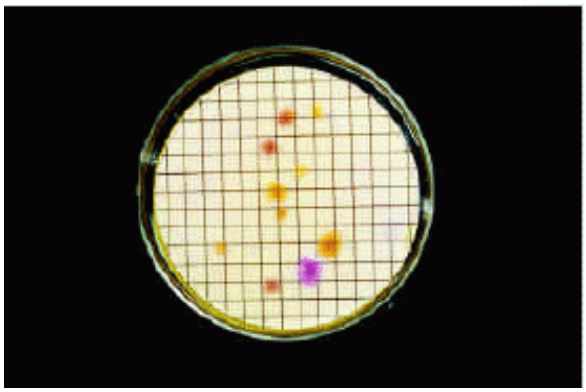

Photo 2. *Escherichia coli* colonies on mTEC agar. Colonies that are yellow, yellow-green, or yellow-brown are *E. coli*.

11.11 After incubation on the urea substrate at room temperature, count and record the number of yellow, yellow-green, or yellow-brown colonies on the membrane filters, ideally containing 20-80 colonies (See Photo 3.).

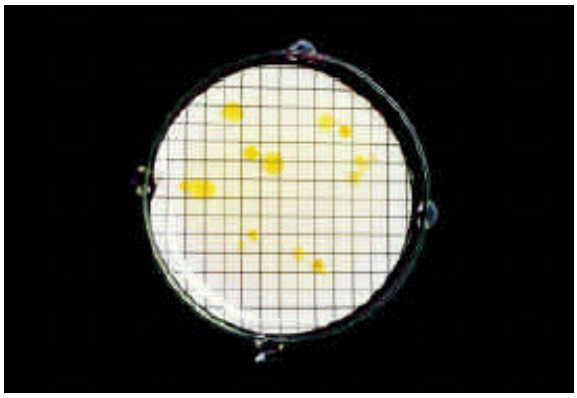

Photo 3. *Escherichia coli* colonies on an absorbent pad saturated with Urea Substrate Medium. *E. coli* colonies remain yellow, yellow-green, or yellow-brown when the filter is placed on the Urea Substrate Medium, while nontarget colonies turn pink or purple.

12.0 Data Analysis and Calculations

Use the following general rules to calculate the *E. coli* count per 100 ml of sample:

12.1 Select the membrane filter with an acceptable number of yellow, yellow-green, or yellow-brown colonies (20-80) on the urea substrate, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$\textit{E. coli}/100\ \text{mL} = \frac{\text{Number of } \textit{E. coli} \text{ colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules (Reference 18.2).

13.0 Method Performance

13.1 Performance characteristics

13.1.1 Precision – The degree of agreement of repeated measurements of the same parameter expressed quantitatively as the standard deviation or as the 95% confidence limits of the mean computed from the results of a series of controlled determinations. The mTEC method precision was found to be fairly representative of what would be expected from counts with a Poisson distribution (Reference 18.4).

13.1.2 Bias – The persistent positive or negative deviation of the average value of the method from the assumed or accepted true value. The bias of the mTEC method has been reported to be -2% of the true value (Reference 18.4).

13.1.3 Specificity – The ability of a method to select and or distinguish the target bacteria under test from other bacteria in the same water sample. The specificity characteristic of a method is usually reported as the percent of false positive and false negative results. The

false positive rate reported for mTEC medium averaged 9% for marine and fresh water samples. Less than 1% of the *E. coli* colonies observed gave a false negative reaction (Reference 18.4).

13.1.4 Upper Counting Limit (UCL) – That colony count above which there is an unacceptable counting error. The error may be due to overcrowding or antibiosis. The UCL for *E. coli* on mTEC medium has been reported as 80 colonies per filter (Reference 18.4).

13.2 Collaborative Study Data

13.2.1 A collaborative study was conducted among eleven volunteer laboratories, each with two analysts who independently tested local fresh and marine recreational waters and sewage treatment plant effluent samples, in duplicate. The data were reported to the Environmental Monitoring and Support Laboratory - Cincinnati, U.S. Environmental Protection Agency, for statistical calculations.

13.2.2 The results of the study are shown in Figure 1 where S_O equals the pooled standard deviation among replicate counts from a single analyst for three groupings (counts less than 30, counts from 30 to 50, and counts greater than 50) and S_B equals the pooled standard deviation between means of duplicates from analysts in the same laboratory for the same groupings. The precision estimates from this study did not show any difference among the water types analyzed.

13.2.3 By linear regression, the precision of the method can be generalized as:

$S_O = 0.028$ count/100 mL + 6.11 (dilution factor) and
$S_B = 0.233$ count/100 mL + 0.82 (dilution factor)

$$\text{Where dilution factor} = \frac{100}{\text{Volume of Original Sample Filtered}}$$

13.2.4 Because of the instability of microbial populations in water samples, each laboratory analyzed its own sample series and no full measure of recovery or bias was possible. However, all laboratories analyzed a single surrogate sample prepared from a freeze-dried culture of *E. coli*. The mean count ($\bar{O}$) and the overall standard deviation of the counts (S_T) (which includes the variability among laboratories for this standardized *E. coli* sample) were 31.6 colonies/membrane and 7.61 colonies/membrane, respectively.

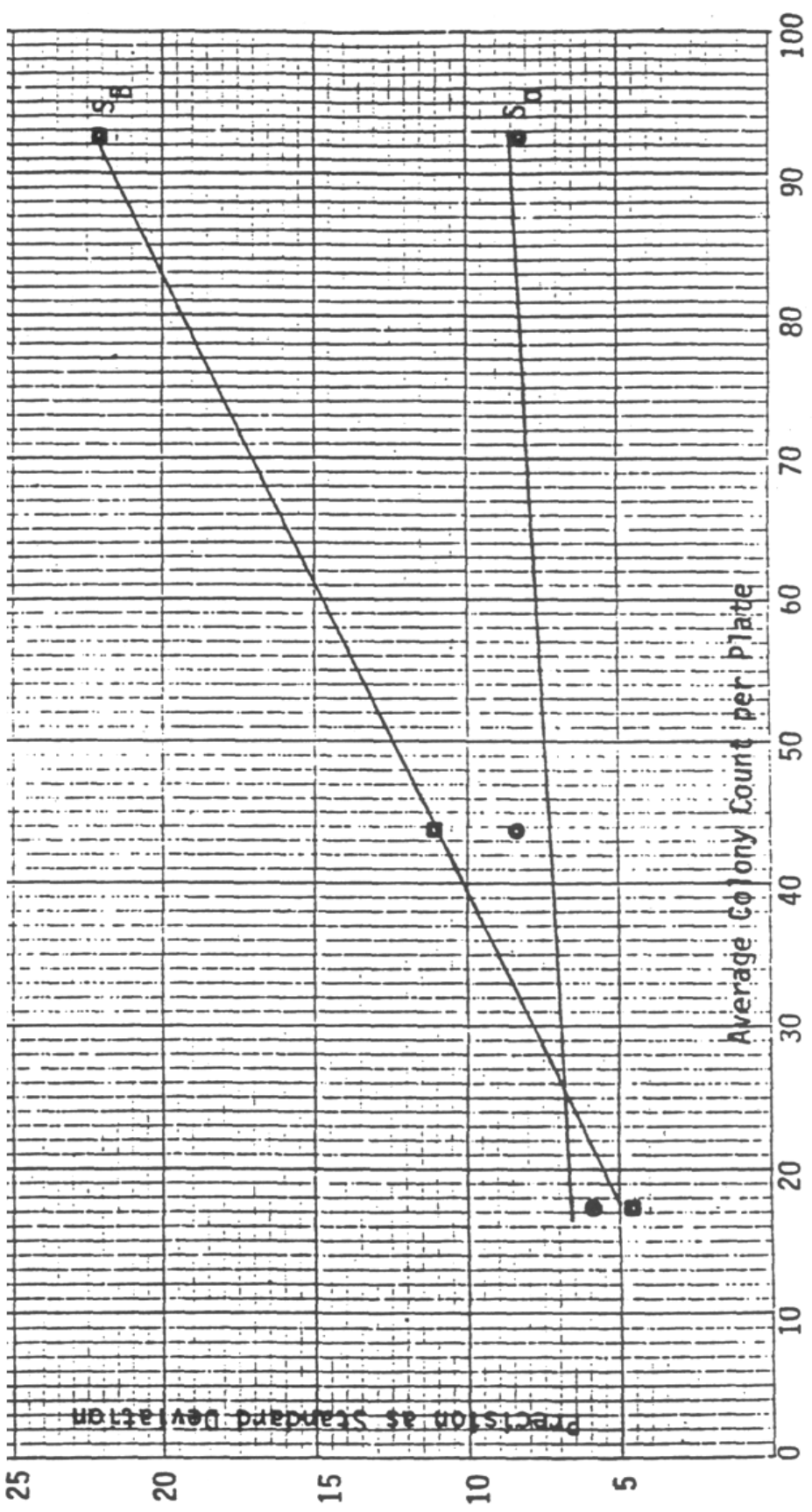

FIGURE 1. Precision Estimates for E. coli in Water by the Membrane Filter/mTEC Procedure.

14.0 Reporting Results

14.1 There should be at least three volumes filtered per sample. Report the results as *E. coli* per 100 mL of sample.

15.0 Verification Procedure

15.1 Yellow, yellow-green, or yellow-brown colonies from the urease test can be verified as *E. coli*. Verification of colonies may be required in evidence gathering and is also recommended as a means of quality control for the initial use of the test and for changes in sample sites, lots of commercial media, or major ingredients in media compounded in the laboratory. The verification procedure follows.

15.1.1 Using a sterile inoculation loop, transfer growth from the centers of at least 10 well-isolated colonies to Nutrient Agar plates or slants and to Trypticase Soy Broth. Incubate the agar and broth cultures for 24 h at 35 ± 0.5°C.

15.1.2 After incubation, remove a loopful of growth from the Nutrient Agar slant *with a platinum loop*, and deposit it on the surface of a piece of filter paper that has been saturated with freshly prepared Cytochrome Oxidase Reagent. If the spot where the bacteria were deposited turns deep purple within 15 seconds, the test is positive.

15.1.3 Transfer growth from the Trypticase Soy Broth tube to Simmons Citrate Agar, Tryptone Broth, and an EC Broth fermentation tube.

15.1.3.1 Incubate the Simmons Citrate Agar and Tryptone Broth for 48 h at 35 ± 0.5°C.

15.1.3.2 Incubate the EC Broth at 44.5 ± 0.2°C in a waterbath for 24 h. The water level must be above the level of the EC Broth in the tube.

15.1.3.3 Add 0.5 mL of Kovacs Indole Reagent to the 48 h Tryptone Broth culture, and shake the tube gently. A positive test for indole is indicated by a deep red color which develops in the alcohol layer on top of the broth.

15.1.3.4 *E. coli* is EC gas-positive, indole-positive, and oxidase-negative, and does not utilize citrate (*i.e.*, the medium remains green).

15.1.4 Alternately, commercially available multi-test identification systems may be used to verify colonies. Inoculate the colonies into an identification system for *Enterobacteriaceae* that includes lactose fermentation, F-nitrophenyl-$-D-galactopyranoside (ONPG), and cytochrome oxidase test reactions.

16.0 Pollution Prevention

16.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

16.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

17.0 Waste Management

17.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

17.2 Samples, reference materials, and equipment known or suspected to have viable *E. coli* attached or contained must be sterilized prior to disposal.

17.3 Samples preserved with HCl to pH <2 are hazardous and must be neutralized before being disposed, or must be handled as hazardous waste.

17.4 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less Is Better: Laboratory Chemical Management for Waste Reduction," both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

18.0 References

18.1 Annual Book of ASTM Standards, Vol. 11.01, American Society for Testing and Materials, Philadelphia, PA 19103.

18.2 Bordner, R., J.A. Winter and P.V. Scarpino (eds.), Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA.

18.3 Cabelli, V.J., A.P. Dufour, M.A. Levin, L.J. McCabe, and P.W. Haberman. 1979. Relationship of Microbial Indicators to Health Effects at Marine Bathing Beaches. *Am. J. Public Health*. 69: 690-696.

18.4 Dufour, A.P., E.R. Strickland, V.J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli*. *Appl. Environ. Microbiol*. 41:1152-1158.

18.5 Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*. 2000. EPA/821/R-97/004. Office of Science and Technology, Washington D.C.

18.6 Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions of the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, UK and the United States Pharmacopeia.

18.7 Test methods for *Escherichia coli* and enterococci in water by the membrane filter procedure. 1985. EPA-600/4-85/076. Environmental Monitoring and Support Laboratory, Cincinnati, USEPA.