United States
Environmental Protection
Agency

Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

September 2002

U.S. Environmental Protection Agency
Office of Water (4303T)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

EPA-821-R-02-024

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The content of this method version is identical to the February 2000 version of *Membrane Filter Method for the Simultaneous Detection of Total Coliforms and Escherichia coli in Drinking Water* (EPA-600-R-00-013) with one exception, the addition of MI broth. Since MI broth was approved on November 6, 2001, as a minor modification of the MI agar method, it has also been included in this document.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this method or its application should be addressed to:

Robin K. Oshiro
Engineering and Analysis Division (4303T)
U.S. EPA Office of Water, Office of Science and Technology
1200 Pennsylvania Avenue, NW
Washington, DC 20460
oshiro.robin@epa.gov
202-566-1075
202-566-1053 (facsimile)

Table of Contents

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1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using MI agar or MI broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl-β-D-galactopyranoside (MUGal) and a chromogen Indoxyl-β-D-glucuronide (IBDG), are included in the medium to detect the enzymes β-galactosidase and β-glucuronidase, respectively, produced by TC and *E. coli*, respectively.

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. coli* are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (References 16.8 - 16.10), will be used primarily by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human or veterinary), other environmental samples (*e.g.*, aerosols, soil, runoff, or sludge) and/or isolation and separation of transformants though the use of *E. coli lac* Z or *gus* A/*uid* reporter genes (Reference 16.11).

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

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35°C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β-glucuronidase and fluorescence under longwave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β-galactosidase (Reference 16.8).

3.0 Definitions

3.1 Total coliforms (TC) - In this method, TC are those bacteria that produce fluorescent colonies upon exposure to longwave ultraviolet light (366 nm) after primary culturing on MI agar or broth (See Figure 1.). The fluorescent colonies can be completely blue-white (TC other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-

green *E. coli* colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG (Reference 16.8).

3.2 *Escherichia coli* - In this method, the *E. coli* are those bacteria that produce blue colonies under ambient light after primary culturing on MI agar or broth (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under longwave ultraviolet light (366 nm) (Reference 16.8).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies. However, the blue *E. coli* colonies can often be counted on plates with heavy particulates or high concentrations of total bacteria (See Figures 2 and 3.) (Reference 16.8).

4.2 The presence of some lateral diffusion of blue color away from the target *E. coli* colonies can affect enumeration and colony picking on plates with high concentrations of *E. coli*. This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples (Reference 16.8).

4.3 Tiny, flat or peaked pinpoint blue colonies ($\leq$ 0.5-mm in diameter on filters containing $\leq$ 200 colonies) may be due to species other than *E. coli*. These colonies occur occasionally in low numbers and should be excluded from the count of the *E. coli* colonies, which are usually much larger in size (1-3-mm in diameter). The small colonies have never been observed in the absence of typical *E. coli*, but, if such should occur, the sample should not be considered *E. coli*-positive unless at least one colony has been verified by another method [*e.g.*, EC medium with 4-Methylumbelliferyl-β-D-glucuronide (MUG) or API 20E strips] (Reference 16.8).

4.4 Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent TC colonies, may be species other than coliforms. These colonies, which generally occur in low numbers ($\leq$ 5%) and can usually be distinguished from the TC, should be eliminated from the TC count. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium (Reference 16.8).

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.

5.3 Avoid prolonged exposure to longwave or germicidal ultraviolet light.

5.4 Autoclave all contaminated plates and materials at the end of the analysis.

6.0 Equipment and Supplies

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

6.2 Stereoscopic microscope, with magnification of 10-15x, wide-field type.

6.3 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.

6.4 Hand tally.

6.5 Pipet container of stainless steel, aluminum, or Pyrex glass, for pipets.

6.6 Graduated cylinders (100-mL for drinking water), covered with aluminum foil or kraft paper and sterilized.

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

6.8 Germicidal ultraviolet (254 nm) light box for sanitizing the filter funnels is desirable, but optional.

6.9 Line vacuum, electric vacuum pump, or aspirator is used as a vacuum source. In an emergency, a hand pump or a syringe can be used. Such vacuum-producing devices should be equipped with a check valve to prevent the return flow of air.

6.10 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.

6.11 Safety trap flask, placed between the filter flask and the vacuum source.

6.12 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.

6.13 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.

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

6.15 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mL volumes).

6.16 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ± 0.02-µm pore size, presterile or sterilized for 10 minutes at 121°C (15-lb pressure).

6.17 Longwave ultraviolet lamp (366 nm), handheld 4-watt (preferred) or 6-watt, or microscope attachment.

6.18 *Dilution water*: Sterile phosphate-buffered dilution water, prepared in large volumes (*e.g.*, 1 liter) for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks [Section 9050C in *Standard Methods* (Reference 16.2)].

6.19 Indelible ink marker for labeling plates.

6.20 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.

6.21 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids, or 15 x 60 mm, glass or plastic, with loose-fitting lids; 15 x 100 mm dishes may also be used.

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

6.23 Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, for agar preparation.

6.24 Waterbath maintained at 50°C for tempering agar.

6.25 Syringe filter, sterile, disposable, 25-mm diameter, 0.22-µm pore size, to filter cefsulodin for MI agar.

6.26 Syringe, sterile, plastic, disposable, 20-cc capacity. Autoclaved glass syringes are also acceptable.

6.27 Test tubes, sterile, screw-cap, 20 x 150-mm, borosilicate glass or plastic, with lids.

6.28 Sterilization filter units, presterile, disposable, 500- or 1000-mL capacity, 0.2-µm pore size, to filter stock buffer solutions.

6.29 Sterile 47-mm diameter absorbent pads (used with MI broth).

Note: *Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

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 on Analytical Reagents of the American Chemical Society (Reference 16.1). 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 *Purity of Water*: Reagent-grade distilled water conforming to Specification D1193, Type II water or better, *ASTM Annual Book of Standards* (Reference 16.3).

7.4 Buffered Dilution Water (Reference 16.2)

7.4.1 *Stock Phosphate Buffer Solution* (Reference 16.2):

Potassium Dihydrogen Phosphate (KH_2PO_4)	34.0	g
Reagent-Grade Distilled Water	500	mL

7.4.2 *Preparation of Stock Buffer Solution*: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring volume to 1000 mL with reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).

7.4.3 *$MgCl_2$ Solution* (Reference 16.2): Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2 \cdot 6H_2O$) in one liter of reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).

7.4.4 *Storage of Stock Buffer and $MgCl_2$ Solutions*: After sterilization of the stock solutions, store in the refrigerator until used. Handle aseptically. If evidence of mold or other

contamination appears in either stock, the solution should be discarded, and a fresh solution should be prepared.

7.4.5 *Working Solution* (Final pH 7.0 ± 0.2): Add 1.25 mL phosphate buffer stock (Section 7.4.2) and 5 mL $MgCl_2$ stock (Section 7.4.3) for each liter of reagent-grade distilled water prepared. Mix well, and dispense in appropriate amounts for dilutions in screw-cap dilution bottles or culture tubes, and/or into larger containers for use as rinse water. Autoclave at 121°C (15-lb pressure) for 15 minutes. Longer sterilization times may be needed depending on the container and load size and the amount of time needed for the liquid to reach 121°C.

7.5 MI Agar (Reference 16.8)

7.5.1 *Composition:*

Proteose Peptone #3	5.0	g
Yeast Extract	3.0	g
β-D-Lactose	1.0	g
4-Methylumbelliferyl-β-D-Galactopyranoside (MUGal) (Final concentration 100µg/mL)	0.1	g
Indoxyl-β-D-Glucuronide (IBDG) (Final concentration 320 µg/mL)	0.32	g
NaCl	7.5	g
K_2HPO_4	3.3	g
KH_2PO_4	1.0	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
Agar	15.0	g
Reagent-Grade Distilled Water	1000	mL

7.5.2 *Cefsulodin Solution* (1 mg / 1 mL): Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-µm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time. Do not save the unused portion.

7.5.3 *Preparation*: Autoclave the medium for 15 minutes at 121°C (15-lb pressure), and add 5 mL of the freshly-prepared solution of Cefsulodin (5 µg/mL final concentration) per liter of tempered agar medium. Pipet the medium into 9 x 50-mm Petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks. The final pH should be 6.95 ± 0.2.

7.6 MI Broth: The composition of MI broth is the same as MI agar, but without the agar. The final pH of MI broth should be 7.05 ± 0.2. The broth is prepared and sterilized by the same methods described for MI agar in Sections 7.5.1, 7.5.2, and 7.5.3, except that absorbent pads are placed in 9 x 50 mm Petri dishes and saturated with 2-3 mL of MI broth containing 5 µg/mL final concentration of Cefsulodin. Alternately, the broth can be filter-sterilized. Excess broth is poured off before using the plates. Plates should be stored in the refrigerator and discarded after 96 hours (Reference 16.15).

7.7 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or equivalent) (TSA)

7.7.1 *Composition*:

Tryptone	15.0 g
Soytone	5.0 g
NaCl	5.0 g
Agar	15.0 g

7.7.2 *Preparation*: Add the dry ingredients listed above to 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121°C (15-lb pressure) for 15 minutes. Dispense the agar into 9 x 50-mm petri dishes (5 mL/plate). Incubate the plates for 24 - 48 hours at 35°C to check for contamination. Discard any plates with growth. If > 5% of the plates show contamination, discard all plates, and make new medium. Store at 4°C until needed. The final pH should be 7.3 ± 0.2.

8.0 Sample Collection, Preservation, and Storage

8.1 Water samples are collected in sterile polypropylene sample containers with leakproof lids.

8.2 Sampling procedures are described in detail in Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Reference 16.2) or in the *USEPA Microbiology Methods Manual*, Section II, A (Reference 16.6). Residual chlorine in drinking water (or chlorinated effluent) samples should be neutralized with sodium thiosulfate (1 mL of a 10% solution per liter of water) at the time of collection. Adherence to sample preservation procedures and holding time limits are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.2.1 *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 from melted ice during transit or storage.

8.2.2 *Holding Time Limitations:* Analyze samples as soon as possible after collection. Drinking water samples should be analyzed within 30 h of collection (Reference 16.13). Do not hold source water samples longer than 6 h between collection and initiation of analyses, and the analyses should be complete within 8 h of sample collection.

9.0 Calibration and Standardization

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits (Reference 16.14).

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

10.0 Quality Control (QC)

10.1 Pretest each batch of MI agar or broth for performance (*i.e.*, correct enzyme reactions) with known cultures (*E. coli*, TC, and a non-coliform).

10.2 Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (Reference 16.7).

10.3 Perform specific filtration control tests each time samples are analyzed, and record the results.

10.3.1 *Filter Control*: Place one or more membrane filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the filter(s).

10.3.2 *Phosphate-Buffered Dilution Water Controls*: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the dilution water.

10.3.3 *Agar or Broth Controls*: Place one or more TSA plates and one or more MI agar plates or MI broth pad plates in the incubator for 24 hours at 35°C. Broth pad plates should be incubated *grid-side up*, not inverted like the agar plates. Absence of growth indicates sterility of the plates.

10.4 See recommendations on quality control for microbiological analyses in the "*Manual for the Certification of Laboratories Analyzing Drinking Water*: *Criteria and Procedures; Quality Assurance*" (Reference 16.15) and the *USEPA Microbiology Methods Manual*, part IV, C (Reference 16.6).

11.0 Procedure

11.1 Prepare MI agar or MI broth and TSA as described in Sections 7.5, 7.6, and 7.7. If plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. The crystals that form on MI agar after refrigeration will disappear as the plates warm up (Reference 16.8).

11.2 Label the bottom of the MI agar or MI broth plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar or MI broth sterility control plate(s).

11.3 Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.

11.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.

11.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.

11.6 Shake the sample container vigorously 25 times.

11.7 Measure an appropriate volume (100 mL for drinking water) or dilution of the sample with a sterile pipette or graduated cylinder, and pour it into the funnel. Turn on the vacuum, and leave it on while rinsing the funnel twice with about 30 mL sterile dilution water.

11.8 Remove the funnel from the base of the filter unit. A germicidal ultraviolet (254 nm) light box can be used to hold and sanitize the funnel between filtrations. At least 2 minutes of exposure time is required for funnel decontamination. Protect eyes from UV irradiation with glasses, goggles, or an enclosed UV chamber.

11.9 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the MI agar plate or MI broth pad plate. Slide the filter onto the agar or pad, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar or absorbent pad. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar or pad. Reseat the membrane if non-wetted areas occur due to air bubbles.

11.10 Invert the agar petri dish, and incubate the plate at 35°C for 24 hours. Pad plates used with MI broth should be incubated *grid-side up* at 35°C for 24 hours. If loose-lidded plates are used for MI agar or broth, the plates should be placed in a humid chamber.

11.11 Count all blue colonies on each MI plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the *E. coli* count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete (Reference 16.14).

11.12 Expose each MI plate to longwave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent *E. coli*, blue/white fluorescent TC other than *E. coli*, and blue/green with fluorescent edges (also *E. coli*)] (See Figure 1.). Record the data.

11.13 Add any blue, non-fluorescent colonies (if any) found on the same plate to the TC count (Reference 16.8).

12.0 Data Analysis and Calculations

12.1 Use the following general rules to calculate the *E. coli* or TC per 100 mL of sample:

12.1.1 Select and count filters with ≤ 200 total colonies per plate.

12.1.2 Select and count filter with ≤ 100 target colonies (ideally, 20-80).

12.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^+ (TNTC)" and count the number of *E. coli*. If both target organisms are ≥ 200, record the results as "TC^+ EC^+ (TNTC)".

12.1.4 Calculate the final values using the formula:

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

$$\text{TC}/100\text{ mL} = \frac{\text{Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the *USEPA Microbiology Manual*, Part II, Section C, 3.5, for general counting rules (Reference 16.6).

12.3 Report results as *E. coli* or TC per 100 mL of drinking water.

13.0 Method Performance

13.1 The detection limits of this method are one *E. coli* and/or one total coliform per sample volume or dilution tested (Reference 16.8).

13.2 The false-positive and false-negative rates for *E. coli* are both reported to be 4.3% (Reference 16.8).

13.3 The single lab recovery of *E. coli* is reported (Reference 16.8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (Reference 16.2) and 115% of the R2A spread plate (Reference 16.2). For *Klebsiella pneumoniae* and *Enterobacter aerogenes*, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (Reference 16.8), respectively, and 89.3% and 85.8% of the R2A spread plate, respectively.

13.4 The specificities for *E. coli* and total coliforms are reported to be 95.7% and 93.1% (Reference 16.8), respectively.

13.5 The single lab coefficients of variation for *E. coli* and total coliforms are reported to be 25.1% and 17.6% (Reference 16.8), respectively, for a variety of water types.

13.6 In a collaborative study (References 16.4, 16.5, and 16.9), 19 laboratories concurrently analyzed six wastewater-spiked Cincinnati tap water samples, containing 3 different concentrations of *E. coli* ($\leq$ 10, 11-30, and > 30 per 100 mL).

13.6.1 The single laboratory precision (coefficient of variation), a measure of the repeatability, ranged from 3.3% to 27.3% for *E. coli* and from 2.5% to 5.1% for TC for the six samples tested, while the overall precision (coefficient of variation), a measure of reproducibility, ranged from 8.6% to 40.5% and from 6.9% to 27.7%, respectively. These values are based on $\log_{10}$-transformed data (Reference 16.5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (Reference 16.9) results.

14.0 Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

16.0 References

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16.15 U.S. Environmental Protection Agency. 1992. Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, Third Edition. EPA-814B-92-002, Office of Ground Water and Drinking Water, Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

17.0 Tables and Figures

Table 1. Statistical Summary of the Collaborative Study Results[1]

Target Organism	Sample Number	*E. coli* Count Category (Range)[2]	Initial n[3]	Final n[4]	S_r[5]	RSD_r[6] (%)	$\bar{\chi}$[7]	S_R[8]	RSD_R[9] (%)	$\frac{RSD_R}{RSD_r}$ Ratio
Escherichia coli	1	Low (≤ 10)	63	63	0.17	27.3	0.64	0.26	40.5	1.49
	2		63	63	0.21	25.0	0.84	0.33	39.0	1.56
	3	Medium (11-30)	63	63	0.10	7.9	1.27	0.15	12.1	1.52
	4		63	60	0.07	5.6	1.32	0.12	9.2	1.65
	5	High (> 30)	63	60	0.06	3.3	1.87	0.16	8.6	2.62
	6		63	63	0.09	4.3	1.99	0.25	12.6	2.91
Total Coliforms	1	Low (≤ 10)	63	63	0.10	4.3	2.35	0.62	26.4	6.11
	2		63	63	0.09	3.8	2.31	0.64	27.7	7.25
	3	Medium (11-30)	63	63	0.11	5.1	2.17	0.47	21.8	4.28
	4		63	57	0.10	3.3	3.07	0.21	6.9	2.08
	5	High (> 30)	63	63	0.15	4.8	3.10	0.43	14.0	2.96
	6		63	63	0.08	2.5	3.14	0.46	14.7	5.97

[1] The values are based on $\log_{10}$ transformed data (Reference 16.5).

[2] The samples were grouped by their *E. coli* count on MI agar into the following categories:

Low (≤ 10 *E. coli* / 100 mL, samples 1 and 2),

Medium (11-30 *E. coli* / 100 mL, samples 3 and 4), and

High (> 30 *E. coli* / 100 mL, samples 5 and 6).

[3] These values are based on triplicate analyses by each laboratory. The reference laboratory analyzed three sets of samples: the initial and final samples prepared and a sample shipped along with the other 18 lab samples.

[4] These values were obtained after removing outliers by the AOAC procedure (Reference 16.5).

[5] S_r, Single Operator Standard Deviation, a measure of repeatability.

[6] RSD_r, Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability.

[7] $\bar{\chi}$, The mean of the replicate analyses for all laboratories.

[8] S_R, Overall Standard Deviation, a measure of reproducibility.

[9] RSD_R, Overall Relative Standard Deviation (Coefficient of Variation), a measure of reproducibility.

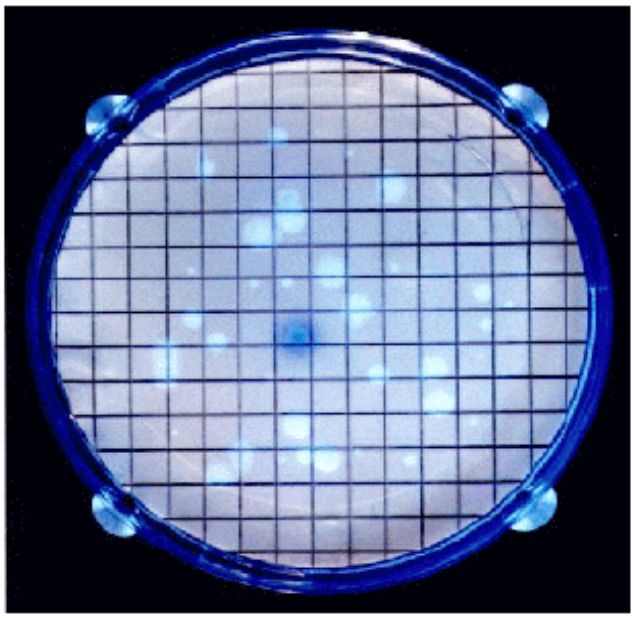

Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under longwave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

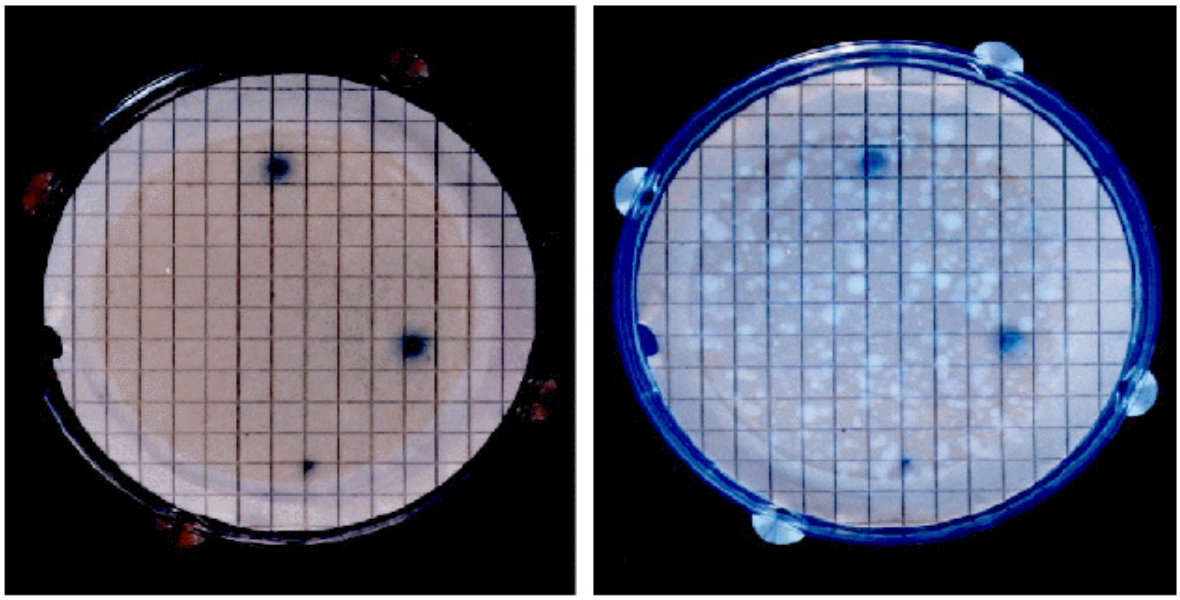

Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and longwave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other than *E. coli* and non-coliforms are their natural color. Under longwave UV light, all total coliforms, including *E. coli*, are fluorescent, and non-coliforms are non-fluorescent (*i.e.*, they are not visible).

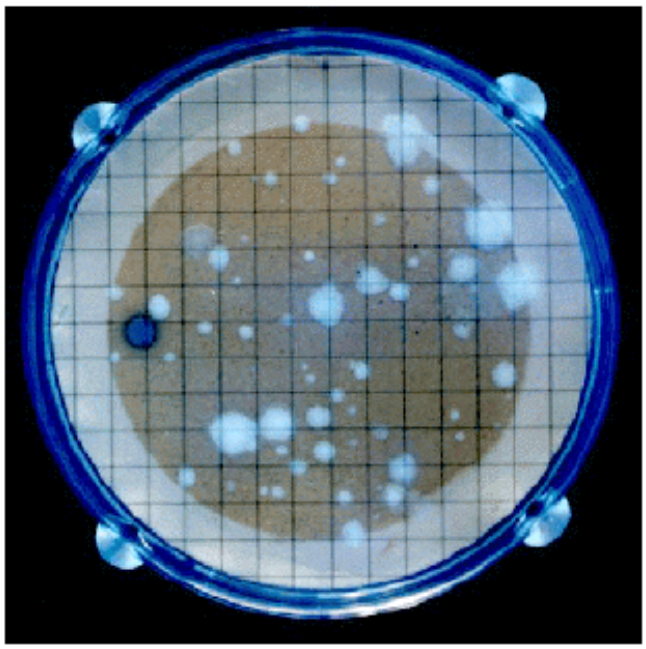

Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.