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EPA

Membrane Filter Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Drinking Water

MEMBRANE FILTER METHOD FOR THE SIMULTANEOUS DETECTION OF TOTAL COLIFORMS AND *ESCHERICHIA COLI* IN DRINKING WATER

1. Scope and Application

1.1 This test method describes a membrane filter (MF) medium, MI Agar, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl-*b*-D-galactopyranoside (MUGal) and a chromogen Indoxyl-*b*-D-glucuronide (IBDG), are included in the medium to detect the enzymes *b*-galactosidase and *b*-glucuronidase, respectively, produced by TCs 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 (12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (8-10), 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 through the use of *E. coli* *lac Z* or *gus A/uid* reporter genes (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. Summary of the Method

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, a selective and differential medium, 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 (8).

3. Definitions

3.1 Total Coliforms (TC) - In this method, TCs are those bacteria that produce fluorescent colonies upon exposure to longwave ultraviolet light (366 nm) after primary culturing on MI agar (See Figure 1.). The fluorescent colonies can be completely blue-white (TCs 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 (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 (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under longwave ultraviolet light (366 nm) (8).

4. Interferences

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.)(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 (8).

4.3 Tiny, flat or peaked pinpoint blue colonies (≤ 0.5 mm in diameter on filters containing ≤ 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-*b*-D-glucuronide (MUG) or API 20E strips] (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 (≤ 5%) and can usually be distinguished from the TCs, 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 (8).

5. Safety and Health

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. Apparatus, Equipment, and Supplies

6.1 Incubator set at 35° ± 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 mm $\pm$ 0.02-mm pore size, presterile or sterilized for 10 min 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 (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.

7. **Reagents and Materials**

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 (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 (3).

7.4 Buffered Dilution Water (2)

7.4.1 Stock Phosphate Buffer Solution (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 lbs pressure).

7.4.3 $MgCl_2$ Solution (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 min 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 $\pm$ 0.2): Add 1.25 ml phosphate buffer stock and 5 ml $MgCl_2$ stock 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 min. 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 (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 100 μg/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 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B) (TSA)

7.6.1 Composition:

Tryptone	15	g
Soytone	5	g
NaCl	5	g
Agar	15	g

7.6.2 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 min. Dispense the agar into 9 x 50 mm petri dishes (5 ml/plate). Incubate the plates for 24-48 hr at 35°C to check for contamination. Discard any plates with growth. If $\geq$ 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 $\pm$ 0.2.

8. Sample Collection, Preservation and Holding Times

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 (2) or in the USEPA Microbiology Methods Manual, Section II, A (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 is 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 (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. Calibration and Standardization

9.1 Check temperatures in incubators twice daily to insure operation within stated limits (14).

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

10. Quality Control (QC)

10.1 Pretest each batch of MI agar 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 (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 Controls: Place one or more TSA plates and one or more MI agar plates in the incubator for 24 hours at 35°C. Absence of growth indicates sterility of the agar 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" (14) and the USEPA Microbiology Methods Manual, Part IV, C (6).

11. Procedure

11.1 Prepare MI Agar and TSA as described in 7.5 and 7.6, respectively. 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 (8).

11.2 Label the bottom of the MI Agar plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar 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 on 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 about 30 ml of sterile dilution water in the bottom of the funnel.

11.6 Shake the sample container <u>vigorously</u> 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. Slide the filter onto the agar, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar. Reseat the membrane if non-wetted areas occur due to air bubbles.

11.10 Invert the petri dish, and incubate the plate at 35^{o}C for 24 hours.

11.11 Count all blue colonies on each MI agar plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the E. coli count.

11.12 Expose each MI agar plate to longwave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TCs 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 (8).

12. Calculation of Results

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 filters 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 con-fluent, 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 formulae:

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

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

12.2 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules (6).

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

13. Performance Characteristics

13.1 The detection limits of this method are one E. coli

and/or one total coliform per sample volume or dilution tested (8).

13.2 The false-positive and false-negative rates for E. coli are both reported to be 4.3% (8).

13.3 The single lab recovery of E. coli is reported (8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (2) and 115% of the R2A spread plate (2). For Klebsiella pneumoniae and Enterobacter aerogenes, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (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% (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% (8), respectively, for a variety of water types.

13.6 In a collaborative study (4,5,9), 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 (5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (9) results.

14. **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. **Waste Management**:

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.

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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] (%)	X[7]	S_R[8]	RSD_R[9] (%)	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 (5).

[2] The samples were grouped by their E. coli count on MI agar into the following categories: Low ($\leq$ 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 (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] 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.

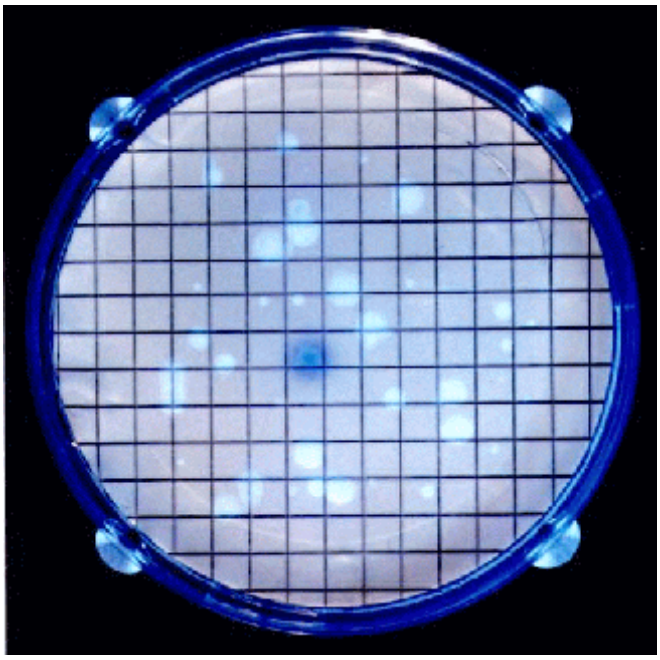

FIGURE 1. This photograph shows Escherichia coli (blue/green fluorescence) and total coliforms other than E. coli (blue/white fluorescence) on MI agar under longwave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

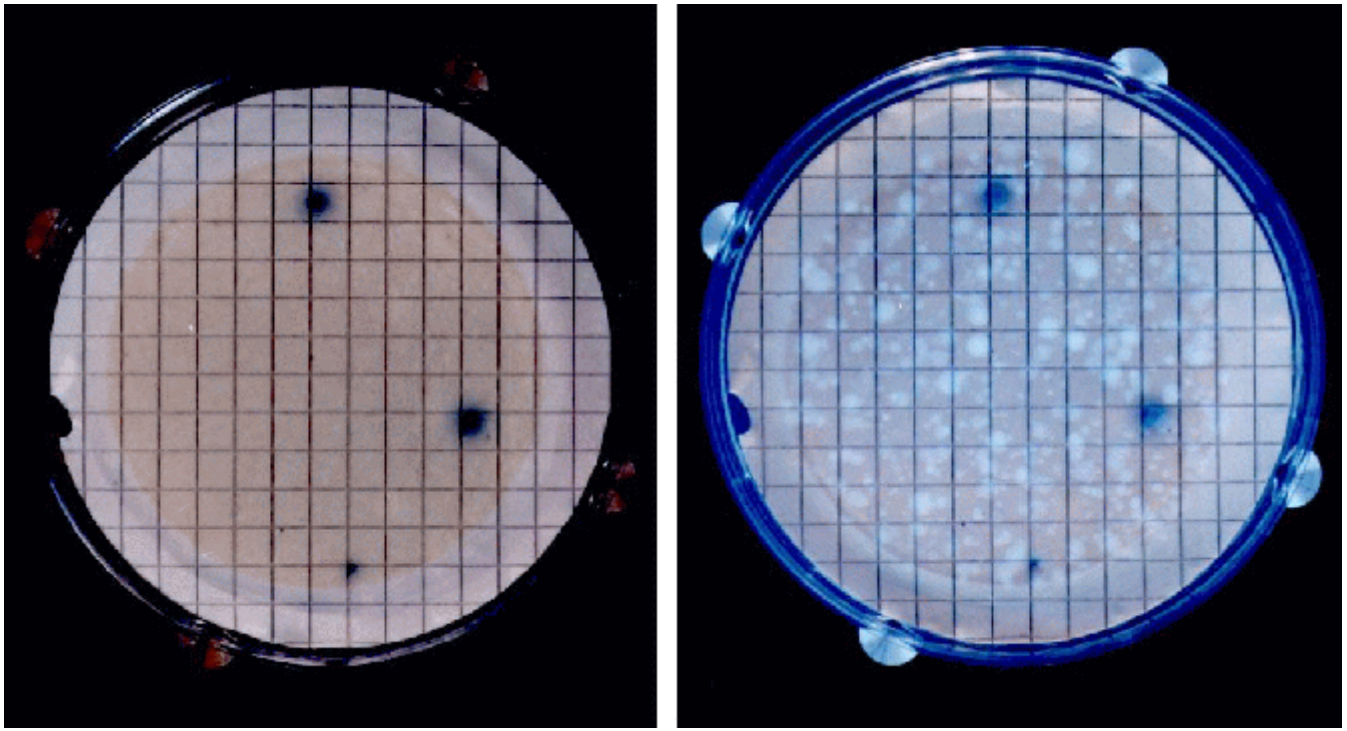

FIGURE 2. These photographs show Escherichia coli and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and longwave UV light (366 nm) on the right. Under ambient light, E. coli are blue, and total coliforms other 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).

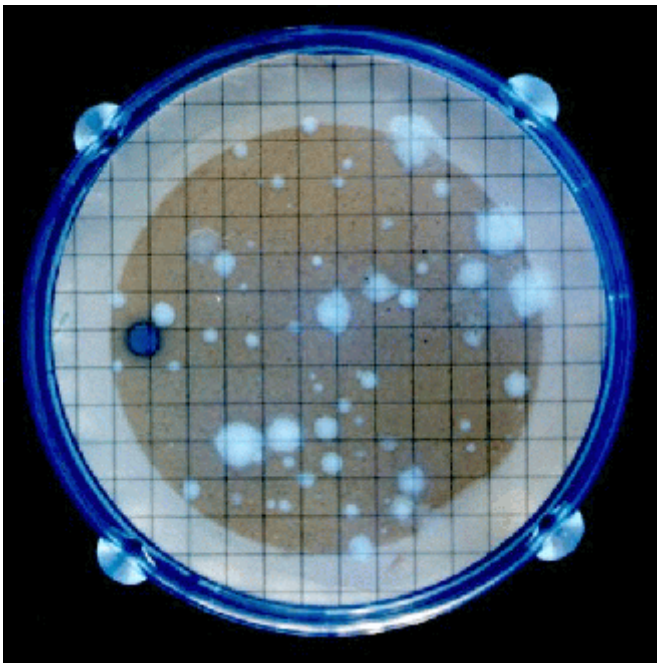

FIGURE 3. This photograph shows that Escherichia coli (blue/green fluorescence) and total coliforms other than E. coli (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was a surface water-spiked Cincinnati, Ohio tap water.