MI Broth, 2 mL
(6510)

Intended Use

Ampouled MI Broth, 2 mL is used for the detection of Total Coliforms and *E. coli* in water testing using the membrane filtration method.

Product Summary and Explanation

Ampouled MI Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing for the detection of Total Coliforms and *E. coli,* following the EPA approved method for drinking water.[1] This test method describes a sensitive and differential membrane filter medium for the concurrent detection and enumeration of Total Coliforms and *E. coli* in water samples in 24 hours or less, dependent upon the enzyme activity. 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 fecal-polluted water and are often associated with disease outbreaks. The Total Coliform test is the primary indicator of bacteriological quality for potable water, distribution system water, and public water supplies because it is a larger measure of pollution than the Fecal Coliform Test.[2,3]

This EPA Method (1604)[1] is used mainly by certified drinking water laboratories for the microbial analysis of potable water. The bacterial colonies that grow on the plate are inspected for the presence of a blue color from the breakdown of IBDG by the *E.coli* enzyme β-glucuronidase and fluorescence under long wave ultraviolet light (366 nm) from the breakdown of MUGal by the Total Coliform enzyme β-galactosidase.[1]

Principles of the Procedure

Enzymatic Digest of Animal Tissue is the nitrogen source in MI Broth. Yeast Extract supplies vitamins, and Lactose is the carbon energy source. Sodium Chloride maintains the osmotic balance of the medium, while Potassium Phosphates are used as buffers. Sodium Lauryl Sulfate and Sodium Deoxycholate are selective agents to inhibit non-coliform bacteria and Gram-positive organisms. The two enzyme substrates are 4-Methylumbelliferyl-β-D-galactopyranoside (MUGal, fluorogen) and Indoxyl-β-D-glucuronide, (chromogen) used for the detection of the enzymes β-galactosidase and β-glucuronidase for Total Coliforms and *E. coli* respectively. *E. coli* and the coliforms produce the enzyme galactosidase that hydrolyzes MUGal to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light. *E. coli* produces glucuronidase that hydrolyses the IBDG and allows deposition of the chromogen in the colonies, forming blue colonies. A positive reaction for E. coli is a blue colony which fluoresceces under long wavelength UV light.

Medium Composition:

	Per Liter
Enzymatic Digest of Animal Tissue	5.0 g
Yeast Extract	3.0 g
Lactose	1.0 g
Sodium Chloride	7.5 g
Potassium Phosphate, Monobasic	1.0 g
Potassium Phosphate, Dibasic	3.3 g
Sodium Lauryl Sulfate	0.2 g
Sodium Deoxycholate	0.1 g
Indoxyl-Beta-d-Glucuronide Cyclohexyalmmonium Salt (IBDG)	0.32 g
4-Methylumbelliferyl-Beta-d-Galactopyranoside (MUGal)	0.1 g
Cefsulodin	0.005 g

Physical Characteristics

Appearance of medium:	Trace to slightly hazy, light amber
pH at 25ºC:	7.05 ± 0.2

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.

2. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
3. Using the same gentle twisting motion, secure the Neogen Filter onto the funnel adapter.

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter (if using a manifold, open only one valve at a time.)
3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: This step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the rubber stopper to release any remaining vacuum pressure, and then re-secure into the stopper.
5. Add the MI-Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: The media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a Petri dish for sample incubation.
8. Remove the filter from the funnel adapter, and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubate at 35 ± 2ºC. Read and record results after 18 – 24 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response:

Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampoulized MI Broth and the filtration housing removed. Plates were incubated aerobically at 35 ± 2 ºC and examined for growth at 18 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth (Chromagen)	Fluorescence (MUG)
Uninoculated Media	NA	No Growth	NA
Aeromonas hydrophila ATCC 7966	10 - 300	Suppressed to Inhibited	Negative
Citrobacter freundii ATCC 8090	10 - 300	≥ 85% recovery, white to clear colonies	Positive
Enterobacter aerogenes ATCC 13048	10 - 300	≥ 85% recovery, yellow to clear colonies	Positive
Enterococcus faecalis ATCC 29212	10 - 300	Suppressed to Inhibited	Negative
Escherichia coli ATCC 25922	10 - 300	≥ 85% recovery, blue colonies	Positive
Escherichia coli ATCC 11775	10 - 300	≥ 85% recovery, blue colonies	Positive
Klebsiella pneumonia ATCC 13883	10 - 300	≥ 85% recovery, yellow to clear colonies	Positive
Proteus mirabilis ATCC 12453	10 - 300	Suppressed to Inhibited	Negative
Pseudomonas aeruginosa ATCC 10145	10 - 300	Suppressed to Inhibited	Negative

Results[1]

In this method, Total Coliforms are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light and primary culturing on MI Broth. The fluorescent colonies can be completely blue-white (Total Coliforms 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.

E. coli Count

Count all blue colonies on each MI plate under normal/ambient light; record results. This is the *E. coli* count. Note: 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.

Total Coliform Count

Expose each MI plate to long wave ultraviolet light (366 nm); count all fluorescent colonies.

E. coli = colonies fluoresce blue/green and/or fluoresce blue/green with fluorescent edges.
Total Coliforms (other than *E. coli*) = fluoresce blue/white, and/or any blue, non-fluorescent colonies are considered Total Coliforms. These colonies should be added to the Total Coliform count.

Storage

Store Ampouled MI Broth, 2 mL at 2 - 8 ºC.

Expiration

Refer to expiration date printed on the front of the box container.

Limitations of the Procedure[1]

1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. Tiny, flat or peaked pinpoint blue 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 much larger in size (1 – 3 mm in diameter).
4. Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent Total Coliform colonies may be species other than coliforms. These colonies, which generally occur in low numbers, can usually be distinguished from Total Coliform colonies.

Packaging

MI Broth, 2 mL	**Code No.**	**6510**	**Box of 50**
Neogen Filter "White"	**Code No.**	**6550**	**Box of 50**
Neogen Filter "Black"	**Code No.**	**6555**	**Box of 50**

References

1. **U. S. Environmental Protection Agency.** 2002. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI Medium). EPA- 821-R-02-024. Office of Water. Washington DC, 20460.
2. **U. S. Environmental Protection Agency.** 2007. R9 Laboratory SOP1101. Membrane filtration coliform analysis.
3. **U. S. Environmental Protection Agency.** 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Technical Information

Contact Neogen Corporation for Technical Service or questions involving Ampouled Media at (517)372-9200 or (800)-234-5333 or fax us at (517)372-2006.

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