SO 0191-5

Revisions

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Notes:

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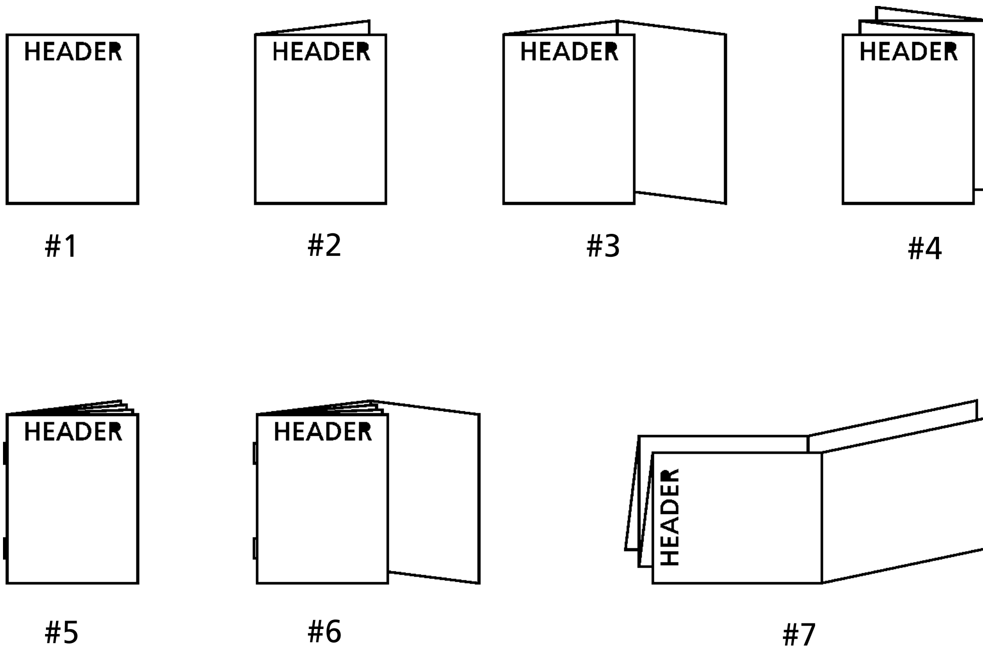
3. Style (see illustrations below): # 1



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Category and Description
Package Insert
BBL MI Agar

Scale: N/A

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BD BBL™ MI Agar

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INTENDED USE

BBL™ MI Agar is a chromogenic/fluorogenic medium used to detect and enumerate *Escherichia coli* and total coliforms in drinking water by the membrane filtration technique. It conforms with the U.S. Environmental Protection Agency (USEPA) Approved Method 1604: *Total Coliforms and* Escherichia coli *in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium).*

SUMMARY AND EXPLANATION

Coliform bacteria are species that 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 these bacteria are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of other pathogens. *E. coli* is one species in this group of coliform bacteria. Since it is always found in feces, it is a more direct indicator of fecal contamination and the possible presence of enteric pathogens.

Chromogens or fluorogens have been used for many years to detect and identify total coliforms (TC) and *E. coli*. Some methods use liquid media in a multiple-tube-fermentation (MTF) test, a presence-absence (PA) format or other tube tests. Agar media are also used for direct plating or membrane filtration (MF) technology. However, standard MF technology for the detection of TC and fecal coliforms requires the use of several different types of media and two different incubation temperatures.[1]

The newest technology developed by the USEPA for testing drinking water is a single membrane filtration technique where no membrane filter transfers are required.[1-4] The medium is named after the two enzyme substrates included in the formulation: a fluorogen, 4-Methylumbelliferyl-ß-D-galactopyranoside (MUGal) and a chromogen, Indoxyl-ß-D-glucuronide (IBDG). MI Agar can simultaneously detect and enumerate both TC and *E. coli* in water samples in 24 hours or less based on their specific enzyme activities. MI Agar detects the presence of the bacterial enzymes ß-galactosidase and ß-glucuronidase produced by TC and *E. coli*, respectively.

MI Agar is approved for use 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.[5]

As referenced in USEPA method 1604, this method has a detection limit of one *E. coli* and/or one total coliform per sample volume or dilution tested.[5] The false-positive and false-negative rates for *E. coli* are both 4.3%.[5] Specificity for *E. coli* is 95.7% and for total coliforms is 93.1%.[5] The single lab recovery of *E. coli* is 97.9% of the heterotrophic plate count (pour plate) and 115% of the R2A spread plate count.[5] For *Klebsiella pneumoniae* and *Enterobacter aerogenes*, recoveries are 87.5% and 85.7% of the heterotrophic plate count and 89.3% and 85.8% of the R2A spread plate method, respectively.[5]

PRINCIPLES OF THE PROCEDURE

BBL MI Agar contains peptone as a source of nitrogen, carbon and amino acids. Yeast extract provides trace elements, vitamins and amino acids. Lactose is a fermentable carbohydrate and carbon source. Sodium chloride maintains osmotic equilibrium. Monopotassium and dipotassium phosphates offer buffering capabilities. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. *E. coli* that produce the enzyme ß-D-glucuronidase cleave the chromogen indoxyl-ß-D-glucuronide (IBDG) to form a blue- or indigo-colored compound. The ß-galactosidase produced by total coliforms cleaves the fluorogen 4-methylumbelliferyl-ß-D-galactopyranoside (MUGal), producing 4-methylumbelliferone, a fluorescent compound when exposed to long-wave UV light (366 nm). Agar is the solidifying agent. Cefsulodin is added to inhibit gram-positive bacteria and some non-coliform gram-negative bacteria that may cause false positives.

REAGENTS

BBL™ MI Agar

Approximate Formula* Per Liter Purified Water

Ingredient	Amount
Proteose Peptone No. 3	5.0 g
Yeast Extract	3.0 g
Lactose	1.0 g
4-Methylumbelliferyl-ß-D-Galactopyranoside (MUGal)	0.1 g
Indoxyl-ß-D-Glucuronide (IBDG)	0.32 g
Sodium Chloride	7.5 g
Dipotassium Phosphate	3.3 g
Monopotassium Phosphate	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Agar	15.0 g
Cefsulodin	5.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions

For Laboratory Use.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates and other contaminated materials must be sterilized by autoclaving.

Storage: On receipt, store plates in the dark with top side up (agar bed at bottom) at 2-8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light.

Prepared plates stored in their original wrapping at 2-8°C should be warmed to room temperature prior to use.

NOTE: Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is accepted.

Plates may be inoculated up to their expiration date and incubated for recommended incubation times. Discard the unused portion of all packages.

Do not use packages if they show evidence of damage, microbial contamination, drying or other signs of deterioration.

SAMPLE COLLECTION

Collect and prepare water samples in accordance with recommended guidelines.[5,6]

PROCEDURE

Materials Provided: BBL MI Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Test Procedure

1. Test recommended sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[6] For drinking water, test 100 mL samples. For other samples, select sample volumes to produce 20-80 colonies of the target organism on the membrane filter. With water of good quality, the occurance of coliforms generally will be minimal. Therefore, count all coliform colonies (disregarding the lower limit of 20 cited above) and use the formula given below to obtain coliform density.[6]
2. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto MI Agar to avoid the formation of bubbles between the membrane and the agar surface.
3. Invert inoculated plates and incubate for 20-24 hours at 35 ± 0.5°C.
4. After incubation, count and record the number of blue or indigo colonies under normal/ambient light to obtain *E. coli* count.

5. Expose each MI Agar plate to long-wave ultraviolet light (366 nm) and count and record all fluorescent colonies plus all blue, non-fluorescent colonies to obtain a total coliform count.

For drinking water, calculate and report the number of *E. coli* and total coliform colonies per 100 mL of sample using the formula:

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

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

User Quality Control

1. Examine plates for signs of deterioration.
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. Inoculate and incubate the plates at 35°C ± 0.5 for 20-24 hours. Count all blue or indigo colonies under ambient light. Expose MI Agar plates to long-wave ultraviolet light (366 nm) and count all fluorescent colonies.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR/ FLUORESCENCE
Enterobacter aerogenes	13048	20 - 80	Good	Tan/blue- white
Escherichia coli	25922	20 - 80	Good	Blue/blue-green
Pseudomonas aeruginosa	27853	20 - 80	Partial to complete inhibition	Tan/none

3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.95 ± 0.2.
4. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

EXPECTED RESULTS

E. coli produces blue or indigo colonies under normal/ambient light. Total coliforms produce blue/green fluorescent colonies (*E. coli*), blue/white fluorescent colonies (total coliforms other than *E. coli*) and blue/green colonies with fluorescent edges. In addition, any blue, non-fluorescent colonies (also *E. coli*) found should be added to the total coliform count.[1]

Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[7]

LIMITATIONS OF THE PROCEDURE

1. For water samples other than drinking water, choose a water sample size that will result in 20-80 colonies per filter. The ideal volume for *E. coli* enumeration may not be optimum for total coliform enumeration, and vise versa. However, since blue *E. coli* colonies were found to be clearly visible on a total coliform background that was too numerous to count, this should be of minor importance for drinking water compliance purposes, and multiple volumes or dilutions of other water types should provide accurate enumeration.[1]
2. Water samples containing colloidal or suspended particulate material can clog the membrane filter, preventing adequate filtration or causing the spread of bacterial colonies. This could interfere with identification of the target colonies. However, the blue *E. coli* colonies can often be counted on plates with heavy particulate matter or high concentrations of total bacteria.[1]
3. 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.[1]
4. Colonies that are tiny, flat or peaked pinpoint blue, with a size of less than 0.5 mm in diameter on filters containing less than 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 *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 they should occur, the sample should not be considered *E. coli*-positive unless at least one colony has been verified by another method.[1]
5. 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 generally occur in low numbers (≤ 5%) and can usually be distinguished from the total coliforms. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium.[1]
6. Minimize the exposure of MI Agar to light before and during incubation, as light may destroy the chromogen.

REFERENCES

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7. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASMT International, West Conshohocken, Pa.
8. Bordner, R., J. Winter and P. Scarpino. 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

AVAILABILITY

Cat. No.	Description
214986	**BBL™** MI Agar Prepared Plates, 60 x 15 mm, Pkg. of 20
214985	**BBL™** MI Agar Prepared Plates, 60 x 15 mm, Ctn. of 100

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