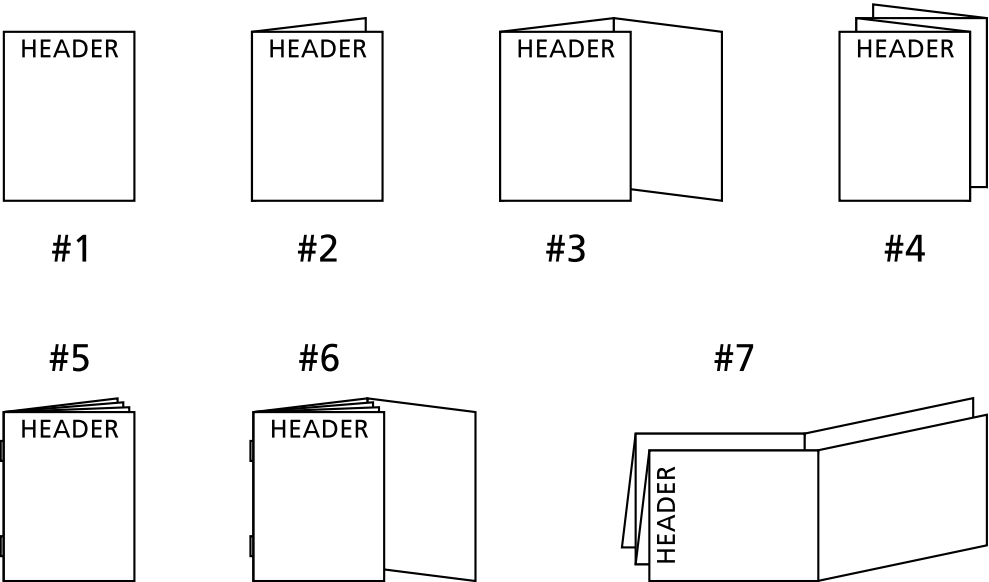



Revisions				SO 0046-2
Rev From	Rev To	ECO #	Date	Appr.
New	0703	1909-03		

Notes

- BD Cat. No. 215045, 215047
- Blank (Sheet) Size : Length: 11" Width: 8.5"
 Number of Pages: 2 Number of Sheets: 1
 Page Size: Length 11" Width 8.5" Final Folded Size: 5 1/2 x 4 1/4
- Style (see illustrations below): #1



- See Specification Control No. n/a for Material Information
- Ink Colors: Printed two sides ☒ Yes ☐ No
 No. of Colors: 1 PMS# Black
- Graphics are approved by Becton, Dickinson and Company. Supplier has the responsibility for using the most current approved revision level.

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Part Number: 8011771		Category and Description Package Insert BBL mEI Agar	Sheet: 1 of 3		A
			Scale: 1:1		

BD BBL™ mEI Agar

8011771
2003/07

INTENDED USE

BBL™ mEI Agar is a selective culture medium used for the chromogenic detection and enumeration of enterococci in water by the single-step membrane filtration technique. It conforms with the U.S. Environmental Protection Agency (USEPA) Approved Method 1600: *Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI)*.

SUMMARY AND EXPLANATION

Enterococci are found in the feces of humans and other warm-blooded animals. Although some strains are ubiquitous and are not related to fecal pollution, the presence of enterococci in water is an indication of fecal pollution and the possible presence of enteric pathogens.¹ In epidemiological studies conducted by the USEPA, it was found that the presence of enterococci had a higher correlation with swimming-associated gastroenteritis in fresh and marine water environments than fecal coliforms.² In 1986, the USEPA recommended that both *Escherichia coli* and enterococci be used as bacterial water quality indicators to monitor recreational waters.³

A two-step membrane filtration (MF) method⁴ was developed by Levin et al. to measure enterococci in fresh and marine recreational waters. Using mEI agar, the method required a 48-hour incubation and a transfer of the membrane to another substrate medium, Esculin Iron Agar, to differentiate enterococci.

In 1997, the USEPA improved on the mEI agar formulation by reducing the triphenyltetrazolium chloride component and adding the chromogen indoxyl β-D-glucoside. The new medium, mEI Agar,¹ was developed as a single-step procedure that does not require the transfer of the membrane filter to another substrate. Observation of a blue halo around colonies in 24 hours is confirmatory for the presence of enterococci. A wide range of sample volumes or dilutions can be tested by this single-step MF procedure for the detection and enumeration of enterococci in potable, fresh, estuarine, marine and shellfish-growing waters. The USEPA published false-positive rate is 6.0% and false-negative rate is 6.5%.¹

BBL mEI Agar conforms to the 1986 revisions to the bacteriological ambient water quality criteria, that included the indicator bacteria *E. coli* and enterococci, which provide better correlation with swimming-associated gastrointestinal illness. In response to this health risk, the USEPA established the Beaches Environmental Assessment Closure and Health (BEACH) Program. This method is published for use in the BEACH Program.¹

Colonies having a blue halo can be verified as enterococci by appropriate biochemical procedures in instances where required in evidence gathering or for performing quality control for the initial use of the test.¹

PRINCIPLES OF THE PROCEDURE

BBL mEI Agar contains peptone that supplies nitrogen and carbon compounds. Sodium chloride maintains osmotic equilibrium. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. Cycloheximide inhibits fungi. Sodium azide acts as a selective agent to inhibit gram-negative bacteria. Yeast extract provides trace elements, vitamins and amino acids. The addition of the chromogen indoxyl β-D-glucoside results in the production of an insoluble indigo blue complex by β-D-glucosidase-positive enterococci, which diffuses into the surrounding media, forming a blue halo around the colony.⁵ Agar is incorporated into the medium as a solidifying agent. Nalidixic acid inhibits gram-negative bacteria. Triphenyltetrazolium chloride helps to differentiate enterococci from other gram-positive cocci and inhibits other organisms.

REAGENTS

BBL™ mEI Agar

Approximate Formula* Per Liter Purified Water

Peptone	10.0 g
Sodium Chloride	15.0 g

Esculin	1.0 g
Cycloheximide	0.05 g
Sodium Azide	0.15 g
Yeast Extract	30.0 g
Indoxyl β-D-glucoside	0.75 g
Agar	15.0 g
Nalidixic Acid	0.24 g
Triphenyltetrazolium Chloride	0.02 g
*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.

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.^{6,7}

PROCEDURE

Materials Provided: BBL mEI 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 sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.⁶ Select sample volumes to produce 20-80 colonies on the membrane filter.
2. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto mEI Agar to avoid the formation of bubbles between the membrane and the agar surface.
3. Invert inoculated plates and incubate for 24 ± 2 h at 41 ± 0.5°C.
4. After incubation, count and record the number of colonies with a blue halo using an illuminated lens with a 2-5X magnification.
5. Calculate and report the number of enterococcal colonies per 100 mL of sample.

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 41 ± 0.5°C for 24 ± 2 h. Count all colonies with blue halos.

ORGANISM	INOCULUM		RECOVERY	APPEARANCE
	ATCC™	CFU		
<i>Enterococcus faecalis</i>	19433	20 - 80	Good	Blue halo
<i>Enterococcus faecium</i>	19434	20 - 80	Good	Blue halo
<i>Escherichia coli</i>	25922	20 - 80	Marked to complete inhibition	–

3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2 .

4. Incubate uninoculated representative plates at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

EXPECTED RESULTS

Colonies with a blue halo regardless of color may be confirmatively identified as enterococci. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.⁸

LIMITATIONS OF THE PROCEDURE

1. Choose a water sample size that will result in 20-80 colonies per filter.
2. Minimize the exposure of mEI Agar to light before and during incubation, as light may destroy the chromogen.

REFERENCES

1. U.S. Environmental Protection Agency. 2002. Method 1600: Enterococci in water by membrane filtration using membrane-*Enterococcus* indoxyl- β -D-glucoside agar (mEI). Publication EPA-821-R-02-022. Office of Water, USEPA, Washington, D.C.
2. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: enterococci and *Escherichia coli*. Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.
3. U.S. Environmental Protection Agency. 1986. Bacteriological ambient water quality criteria: availability. Fed. Regist. 51(45):8012-8016.
4. Levin, M.A., J.R. Fischer and V.J. Cabelli. 1975. Membrane filter technique for enumeration of enterococci in marine waters. Appl. Microbiol. 20:66-71.
5. Messer, J.W., and A.P. Dufour. 1998. A rapid, specific membrane filtration procedure for enumeration of enterococci in recreational water. Appl. Environ. Microbiol. 64:678-680.
6. Clesceri, L.S., A.E. Greenberg and A.D. Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
7. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM 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
215045	BBL™ mEI Agar Prepared Plates, 60 x 15 mm, Pkg. of 20*
215047	BBL™ mEI Agar Prepared Plates, 60 x 15 mm, Ctn. of 100*

*Store at 2-8°C

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