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Notes

1. BD Cat. No. 215044, 215046
2. Blank (Sheet) Size : Length: 11" Width: 8.5"
   Number of Pages: 2 Number of Sheets: 1
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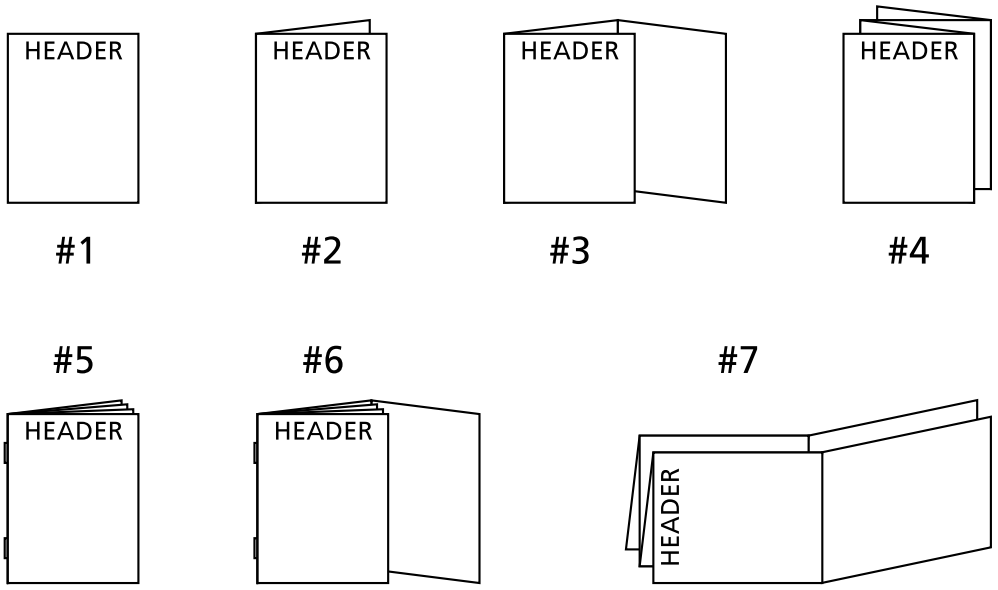

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Label Design Date Proofer Date Checked By Date Part Number: 8011773		BD Becton, Dickinson and Company 250 Schilling Circle Cockeysville, MD. 21030-0243 USA	
	Category and Description Package Insert BBL Modified mTEC Agar	Sheet: 1 of 3 Scale: 1:1	A

BD BBL™ Modified mTEC Agar

8011773
2003/07

INTENDED USE

BBL™ Modified mTEC Agar is a selective culture medium used for the chromogenic detection and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique. It conforms with U.S. Environmental Protection Agency (USEPA) Approved Method 1603: Escherichia coli (E. coli) *in Water by Membrane Filtration Using Modified membrane-Thermotolerant* Escherichia coli *Agar (modified mTEC)*.

SUMMARY AND EXPLANATION

mTEC is an acronym for "membrane Thermotolerant *E. coli*." *E. coli* is widely used as an indicator of fecal pollution in water. This organism has a high correlation with gastroenteritis in fresh water environments.[1] In 1986, the USEPA recommended that *E. coli* be used as a bacterial water quality indicator to monitor recreational waters.[2]

Many procedures have been developed for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan.[3,4] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

Dufour developed a membrane filtration procedure using mTEC agar for the rapid enumeration of *E. coli*.[5,6] This alternative two-step test procedure quantified *E. coli* within 24 hours without requiring subculture and identification of isolates. However, the membrane filter had to be transferred after the initial incubation at an elevated temperature to a urea substrate/phenol red-saturated pad.

The modified mTEC method was developed by the USEPA in 1998[7,8] as a single-step procedure that does not require the transfer of the membrane filter to another substrate. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-ß-D-glucuronide. This chromogen is catabolized to glucuronic acid by *E. coli* strains that produce the enzyme ß-D-glucuronidase to form red- or magenta-colored colonies, enabling confirmatory identification of *E. coli* in 24 hours. Red or magenta colonies can be verified as *E. coli* in instances where required in evidence gathering or for performing quality control for the initial use of this test.[8] As referenced in USEPA method 1603, the false-positive rate is <1% and the false-negative rate is 4% from a variety of environmental water samples.[8]

This medium is recommended for testing the presence of *E. coli* as an indicator organism for fecal contamination in fresh recreational water. This allows for a wide range of sample volumes or dilutions to be analyzed by membrane filtration for the detection and enumeration of *E. coli* levels in water.

PRINCIPLES OF THE PROCEDURE

BBL Modified mTEC Agar contains sufficient nutrients to support the growth of *E. coli*. Peptone is 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. The chromogen, 5-bromo-6-chloro-3-indolyl-ß-D-glucuronide, is catabolized to form glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme ß-D-glucuronidase. Agar is the solidifying agent.

REAGENTS

BBL™ Modified mTEC Agar

Approximate Formula* Per Liter Purified Water

Proteose Peptone No. 3	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 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
5-Bromo-6-Chloro-3-Indolyl-ß-D-Glucuronide	0.5 g
Agar	15.0 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 2-8ºC. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light.

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

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

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.[8,9]

PROCEDURE

Materials Provided: BBL Modified mTEC 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 required sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[9] 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 Modified mTEC Agar to avoid the formation of bubbles between the membrane and the agar surface.
3. Invert inoculated plates and incubate for 2 hours at 35 ± 0.5ºC to resuscitate injured cells.
4. After a 2-h incubation at 35 ± 0.5°C, transfer the plates to a plastic bag, seal the bag, and place it onto a rack in a 44.5 ± 0.2ºC water bath for 22-24 h.
5. After the 22-24 hour incubation, remove the plates from the water bath and count and record the number of red or magenta colonies using an illuminated lens with a 2-5x magnification or a stereoscopic microscope.
6. Calculate and report the number of *E. coli* 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 35ºC ± 0.5 for 2 h. Transfer plates and incubate at 44.5 ± 0.2ºC for 22-24 h. Count all red or magenta colonies

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	20 - 80	Marked to complete inhibition	–
Escherichia coli	13762	20 - 80	Good	red to, magenta
Proteus mirabilis	25933	20 - 80	Good	Tan

3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
4. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

EXPECTED RESULTS

Red to magenta colonies may be confirmatively identified as *E. coli*. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[11]

LIMITATIONS OF THE PROCEDURE

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. Choose a water sample size that will result in 20-80 colonies per filter. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate test results.
3. Minimize the exposure of Modified mTEC Agar to light before and during incubation, as light may destroy the chromogen.

REFERENCES

1. U.S. Environmental Protection Agency. 1986. Ambient water quality criteria for bacteria. Publication EPA-440/5-84/002. Office of Water, Regulations and Standards. Criteria and Standards Division, USEPA, Washington, D.C.
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4. Pugsley, A.P., L.J. Evision and A. James. 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water Res. *7*:1431-1437.
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7. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicator: enterococci and *Escherichia coli*. Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.
8. U.S. Environmental Protection Agency. 2002. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). Publication EPA-821-R-02-023. USEPA Office of Water, Office of Science and Technology, Washington, D.C.
9. 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.
10. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM International, West Conshohocken, Pa.
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AVAILABILITY

Cat. No.	Description
215044	**BBL™** Modified mTEC Agar Prepared Plates, 60 x 15 mm, Pkg. of 20*
215046	**BBL™** Modified mTEC Agar Prepared Plates, 60 x 15 mm, Ctn. of 100*

*Store at 2-8°C

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