EE Broth Mossel Enrichment

Intended Use

EE Broth Mossel Enrichment is used for selectively enriching and detecting *Enterobacteriaceae*, particularly from foods.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

EE Broth Mossel Enrichment is prepared according to the formula of Mossel, Visser and Cornelissen.[4] The formula contains dextrose to facilitate growth of most *Enterobacteriaceae*, thus ensuring the detection of *Salmonella* and other lactose-negative organisms. EE Broth Mossel Enrichment should be used as an enrichment broth, followed by a selective medium; e.g., Violet Red Bile Agar.

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[5] Recovery relies on proper resuscitation of damaged cells. EE Broth Mossel Enrichment is used to detect and enumerate *Enterobacteriaceae* found per milliliter or per gram of test sample of food when performing the Most Probable Number (MPN) technique with pre-enrichment.[6,7]

EE Broth Mossel Enrichment is listed in the *USP* as one of the recommended media for the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products.[1]

Principles of the Procedure

Peptones provide nitrogen, vitamins and amino acids. Dextrose is a carbon source. Disodium phosphate and monopotassium phosphate are buffering agents. Brilliant green and oxgall are selective agents.

Formulae

Difco™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	10.0	g
Dextrose	5.0	g
Disodium Phosphate	8.0	g
Monopotassium Phosphate	2.0	g
Brilliant Green	15.0	mg
Oxgall	20.0	g

BBL™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	10.0	g
Dextrose	5.0	g
Oxgall	20.0	g
Disodium Phosphate	8.0	g
Monopotassium Phosphate	2.0	g
Brilliant Green	15.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water.
2. Heat with frequent agitation until dissolved. DO NOT OVERHEAT. Media is heat sensitive.
3. Dispense into tubes or bottles as required.
4. Heat at 100°C in water bath or flowing steam for 30 minutes. DO NOT AUTOCLAVE.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[6,7]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For food samples, refer to appropriate standard references for details on test methods for performing MPN technique with enrichment using EE Broth Mossel Enrichment.[6,7]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Enterobacteriaceae* using EE Broth Mossel Enrichment.[1]

Expected Results

Acid production causes the color of EE Broth Mossel Enrichment to become yellow. A negative reaction results in no color change and the medium remains green.

Uninoculated Tube

Escherichia coli ATCC™ 25922

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Light green, free flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water. Solution is emerald green, clear.
Prepared Appearance:	Emerald green, clear.
Reaction of 4.5% Solution at 25°C:	7.2 ± 0.2

Cultural Response

Difco™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 9 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
Enterobacter aerogenes	13048	30-100	Good	+ (yellow)
Escherichia coli	25922	30-100	Good	+ (yellow)
Shigella boydii	12030	30-100	Good	–
Staphylococcus aureus	25923	30-100	Marked to complete inhibition	–

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
Escherichia coli	8739	<100	30-35°C	24	Growth
Escherichia coli	8739	<100	35-37°C	18-48	Growth
Pseudomonas aeruginosa	9027	<100	30-35°C	24	Growth
Staphylococcus aureus	6538	>100	30-35°C	48	No growth

Identity Specifications

BBL™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Fine, homogeneous and free of extraneous material.
Solution:	4.5% solution, soluble in purified water. Solution is medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.
Prepared Appearance:	Medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.
Reaction of 4.5% Solution at 25°C:	pH 7.2 ± 0.2

BBL™ EE Broth Mossel Enrichment (prepared)

Appearance:	Medium to dark green and clear to trace hazy.
Reaction at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 10 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
Escherichia coli	25922	10^3-10^4	Good	+ (yellow)
Pseudomonas aeruginosa	10145	10^3-10^4	Good	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	+ (yellow)
Shigella sonnei	9290	10^3-10^4	Good	– to reduced (yellow green)

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
Escherichia coli	8739	<100	30-35°C	24	Growth
Escherichia coli	8739	<100	35-37°C	18-48	Growth
Pseudomonas aeruginosa	9027	<100	30-35°C	24	Growth
Staphylococcus aureus	6538	>100	30-35°C	48	No growth

BBL™ EE Broth Mossel Enrichment (prepared)

Inoculate 90 mL bottles and incubate as directed below.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY	ACID
Enterobacter aerogenes	13048	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Escherichia coli	25922	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Salmonella enterica subsp. *enterica* serotype Typhimurium	13311	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Escherichia coli	8739	10-100	30-35°C	18-24	Growth	+ (yellow) to – or weak
Pseudomonas aeruginosa	9027	10-100	30-35°C	18-24	Growth	N/A
Staphylococcus aureus	6538	10^2-10^3	30-35°C	48	No growth	–

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Mossel, Vissar and Cornelisen. 1963. J. Appl. Bacteriol. 26:444.
5. Hartman and Minnich. 1981. J. Food Prot. *44*:385.
6. International Organization for Standardization. 2004 Microbiology of food and animal feeding stuffs – horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 1: Detection and enumeration by MPN technique with pre-enrichment. ISO 21528-1, 1st ed., 2004-08-15. International Organization for Standardization, Geneva, Switzerland.
7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 256620 Dehydrated – 500 g†

BBL™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 297005 Dehydrated – 500 g†
292627 Prepared Bottles, 90 mL (wide mouth) – Pkg. of 10†

† QC testing performed according to USP/EP/JP performance specifications.

mEI Agar

Intended Use

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 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 filter (MF) method[4] was developed by Levin et al. to measure enterococci in fresh and marine recreational waters. Using mE 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 mE agar formulation by reducing the triphenyltetrazolium chloride component and adding the chromogen, indoxyl-β-D-glucoside. The new medium, mEI Agar,[1,5] 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

User Quality Control

Identity Specifications

Difco™ mEI Agar

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	7.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 7.2% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ mEI Agar

Prepare the medium per label directions. Inoculate and incubate at 41± 0.5°C for 24 ± 2 hours. Count all colonies with blue halos.

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

Enterococcus faecalis
ATCC™ 19433