EE Broth, Mossel

M287

Intended Use:

Recommended for the selective enrichment of *Enterobacteriaceae* in bacteriological examination of foods.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate	6.450
Potassium dihydrogen phosphate	2.000
Bile, purified#	20.000
Brilliant green	0.0135
Final pH (at 25°C)	7.2±0.2

Equivalent to Ox bile, purified
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.46 grams in 1000 ml purified/distilled water. Dispense in tubes or flasks as desired. Stopper with cotton plugs or loose fitting caps. Heat in free flowing steam or boiling water for 30 minutes. Avoid overheating of the medium. **DO NOT AUTOCLAVE.**

Principle And Interpretation

The family *Enterobacteriaceae* consists of *Salmonella*, *Shigella* and other enteric pathogens. These organisms find entry into the food system through faecally contaminated water. Majority of these organisms may be eliminated under the stringent food processing parameters. But some of these organisms may become sublethally injured during the changes in pH, exposure to steam or heat and other unfavourable conditions (1). Therefore the important aspect of food monitoring depends upon the identification and enumeration of these injured cells, after resuscitation. EE Broth, Mossel, formulated by Mossel et. al. (2) is recommended as an enrichment medium for *Enterobacteriaceae* in the biological examination of foods (2) and animal feed stuffs (3).

Peptone and dextrose provide the essential nutrients required for the growth of most of the members of *Enterobacteriaceae.* Brilliant green and Bile, purified, purified inhibit growth of gram-positive bacteria. Lactose-negative, anaerogenic lactose-positive or late lactose-fermenting *Enterobacteriaceae* are often missed by the standard coli-aerogenes test. To overcome this problem, lactose is replaced by dextrose in these media. Phosphates form the buffering system of the medium.The cells damaged while drying or low pH are resuscitated in well-aerated Tryptone Soya Broth (M011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (4), animal feeds (5) and semi-preserved foods (6). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (M581).

Subcultures must be made onto lactose differential media such as MacConkey Agar (M081), Deoxycholate Citrate Agar (M065) or Brilliant Green Agar (M016) for the detection of lactose negative or delayed lactose fermenters. This is used to inoculate MPN tubes prepared using EE Broth. Inoculate a loopful from these tubes onto Violet Red Bile Glucose Agar (M581) after an initial incubation at 35-37°C for 24 hours. Typical pink colonies from M581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (7). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (M287).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Avoid overheating of the medium as media is heat sensitive.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate

Reaction

pH of 4.35% w/v aqueous solution at 25°C. pH 7.00-7.40

pH

7.00-7.40

Cultural Response

Cultural response was observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Acid
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 9027(00026*)	50 -100	luxuriant	Negative reaction, no colour change
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	positive reaction, yellow colour
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	Negative reaction, no colour change
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	positive reaction, yellow colour
Proteus mirabilis ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	variable reaction
Shigella boydii ATCC 12030	50 -100	luxuriant	negative reaction
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers.
(#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15 - 25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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