E E Broth, Mossel, Granulated

GM287

E E Broth, Mossel, granulated is used for selective enrichment of *Enterobacteriaceae* in bacteriological examination of foods.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Dextrose	5.000
Disodium phosphate	6.450
Monopotassium phosphate	2.000
Ox bile, purified	20.000
Brilliant green	0.0135
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.46 grams in 1000 ml distilled water. Dispense 120 ml amounts in 250 ml flasks or 9 ml amounts in tubes. 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 foodsystem 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).

Peptic digest of animal tissue and dextrose provide the essential nutrients required for the growth of most of the members of *Enterobacteriaceae*. Brilliant green and ox bile, 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 (GM011/M011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (5), animal feeds (6) and semi-preserved foods (7). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (GM581/M581). Subcultures must be made onto lactose differential media such as MacConkey Agar (GM081/M081), Deoxycholate Citrate Agar (GM065/M065) or Brilliant Green Agar (GM016A/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 (GM581/ M581) after an initial incubation at 35-37°C for 24 hours. Typical pink colonies from GM581/M581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (4). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used.EE Broth, Mossel (GM287/M287).

Quality Control

Appearance

Light yellow to greenish coloured granular medium

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate

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

pH
7.00-7.40

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Acid
Cultural Response			
Escherichia coli ATCC 8739	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant	-
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	
Escherichia coli ATCC 25922	50 -100	luxuriant	positive reaction, yellow colour
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant	-
Enterobacter aerogenes ATCC 13048	50 -100	luxuriant	positive reaction, yellow colour
Proteus mirabilis ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	variable reaction
Shigella boydii ATCC 12030	50 -100	luxuriant	negative reaction
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Mossel D. A. A., and Harrewijn G. A., 1972, Alimenta II, 29-30
2. Mossel D. A. A., Vissar M. and Cornellisen A. M. R., 1963, J. Appl.Bacteriol., 26(3):444.
3. Van Schothurst M. et al, 1966, Vet Med., 13(3):273.
4. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 7402.
5. Mossel D. A. A. and Ratto M. A., 1970, Appl. Microbiol., 20:273.
6. Mossel D. A. A., Shennan J. L. and Clare V., 1973, J. Sci. Fd. Agric., 24: 499.
7. Mossel D. A. A. and Ratto M. A., 1973, J. Food Technol., 8: 97.

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