Enterobacteria Enrichment Broth, Mossel

MU287

Enterobacteria Enrichment Broth, Mossel is used for selective enrichment of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	10.000
Glucose monohydrate	5.000
Dehydrated ox-bile	20.000
Disodium dihydrogen phosphate, dihydrate	8.000
Potassium dihydrogen phosphate	2.000
Brilliant green	0.015
pH after heating (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.93 grams of dehydrated medium in 1000 ml purified/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 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 sub lethally 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 bile tolerant gram-negative bacteria in the biological examination of foods (2), animal feed stuffs (3). This medium is prepared as per USP (4) and is in accordance with the harmonized method of USP/ EP/BP/JP/IP(4,5,6,7,11).

Pancreatic digest of gelatin and glucose monohydrate allows the growth of most of the members of *Enterobacteriaceae* . Brilliant green and ox-bile, purified are the inhibitory agents for gram-positive bacteria. Phosphates act as a good buffering agent and neutralizes acids produced by lactose fermenters that otherwise would adversely affect the growth of the organism. 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 glucose in this medium. Phosphates form the buffering system of the medium. The cells damaged while drying or low pH are resuscitated in well-aerated Soybean Casein Digest Broth (MU011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (8), animal feeds (9) and semi-preserved foods (10). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (MU581). A loopful of the enriched sample from EE Broth. is subcultured onto Violet Red Bile Glucose Agar (MU581) after an initial incubation at 30-35°C for 24 hours. Typical pink colonies from MU581 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 (MU287)

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

pH of 4.29% w/v aqueous solution at 25°C (after heating).

pH
7.00-7.40

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP. Cultural response was observed after an incubation at 30-35°C for specified time.

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=24 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >= 100cfu (at 30-35°C for >= 48 hours).

Cultural Response
MU287: Cultural characteristics observed after incubation at 30-35 °C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	Incubation temperature	Incubation period
Growth Promoting					
Escherichia coli ATCC 8739	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	<=24 hrs
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	<=24 hrs
Inhibitory					
Staphylococcus aureus ATCC 6538	>=10^3	luxuriant		30 -35 °C	>=48 hrs
Additional Microbiological testing					
Escherichia coli ATCC 25922	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Enterobacter aerogenes ATCC 14028	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Proteus mirabilis ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Shigella boydii ATCC 12030	50 -100	luxuriant	negative reaction	30 -35 °C	24 -48 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited		30 -35 °C	>=48 hrs

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.The United States Pharmacopoeia, 2011, The United States Pharmacopeial Convention. Rockville, MD.
5.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
6.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
7.Japanese Pharmacopoeia, 2008.
8.Mossel D.A.A. and Ratto M.A., 1970, Appl. Microbiol., 20:273.
9.Mossel D.A.A. and Shennan J.L. and Clare V., 1973, J. Sci. Fd. Agric., 24 : 499.
10.Mossel D.A.A., Ratto M.A., 1973, J. Fd. Technol., 8 : 97.
11.The Indian Pharmacopoeia 2010, Govt. of India, 2010. The Controller of Publication, Delhi.

Revision : 1 / 2011

CE