

Enterobacteria Enrichment Broth, Mossel

MH287

Intended use

Recommended for selective enrichment of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP .

Composition**

Ingredients	Gms / Litre
Gelatin peptone #	10.000
Glucose monohydrate	5.000
Dehydrated bile ##	20.000
Disodium hydrogen phosphate, dihydrate	8.000
Potassium dihydrogen phosphate	2.000
Brilliant green	0.015
pH after heating (at 25°C)	7.2±0.2

Pancreatic digest of gelatin

Dehydrated ox-bile

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.93 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Dispense into tubes or flasks as desired. Heat in free flowing steam or boiling water (100°C) for 30 minutes and cool immediately. **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 (2). This medium is prepared in accordance with the harmonized method of USP/EP/BP/JP/IP (3,4,5,6,7). Gelatin peptone and glucose monohydrate allows the growth of most of the members of *Enterobacteriaceae*. Brilliant green and dehydrated bile 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 (MH011) 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 (MH581). A loopful of the enriched sample from EE Broth. is subcultured onto Violet Red Bile Glucose Agar (MH581) after an initial incubation at 30-35°C for 24 hours. Typical pink colonies from MH581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (11). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (MH287).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (3,4,5,6,7). 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. Further isolation has to be carried out for confirmation.
2. 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

pH

7.00-7.40

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. 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 ≥ 100 cfu (at 30-35°C for ≥ 48 hours).

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Acid	Incubation temperature	Incubation period
Growth Promoting					
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	≤ 24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant	-	30 -35 °C	≤ 24 hrs
Inhibitory					
<i>Staphylococcus aureus subsp.aureus</i> ATCC 6538 (00032*)	$\geq 10^3$	inhibited		30 -35 °C	≥ 48 hrs
Additional Microbiological testing					
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs

<i>Escherichia coli</i> NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
<i>Pseudomonas aeruginosa</i> ATCC 27853# (00025*)	50 -100	luxuriant	-	30 -35 °C	24 -48 hrs
<i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
<i>Proteus mirabilis</i> ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
<i>Shigella boydii</i> ATCC 12030	50 -100	luxuriant	negative reaction	30 -35 °C	24 -48 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ³	inhibited		30 -35 °C	>=48 hrs

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

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 (12,13).

Reference

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Revision : 06/2022

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