

## Enterobacteria Enrichment Broth Mossel

LQ119D

### Intended use

For the enrichment of bile tolerant organisms in accordance with harmonized methods of USP, EP, BP, JP and IP.

### Composition\*\*

Ingredients	g/ L
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

Label the ready to use LQ119D bottle. Inoculate the sample and Incubate at specified temperature and time.

### Principle And Interpretation

EE Broth, Mossel, formulated by Mossel et. al.(1-5) is recommended as an enrichment medium for bile tolerant gram-negative bacteria in the biological examination of foods (1), animal feed stuffs (6) and is in accordance with the harmonized method of USP/EP/BP/JP & IP (7-11).

Gelatin peptone and glucose monohydrate allows the growth of most of the members of *Enterobacteriaceae*. Brilliant green and dehydrated 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 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 (12). EE Broth is an enrichment broth and a loopful of the enriched sample is then subcultured onto Violet Red Bile Glucose Agar (MPH581) after an initial incubation at 30-35°C for 24 hours. Typical pink colonies from MPH581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (10).

### Type of specimen

Pharmaceutical samples

### Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (7-11). 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 on selective media is required to be carried out for confirmation.

### 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

Sterile clear Enterobacteria Enrichment Broth Mossel in bottle

### Colour

Emerald green coloured solution.

### Quantity of medium

500ml of medium in glass bottle

### pH

7.00- 7.40

### Sterility Check

Passes release criteria

### 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  $\leq 100$  cfu (at 30-35°C for  $\leq 24$  hours).

### Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating  $\geq 100$ cfu (at 30-35°C for  $\geq 48$  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
<b>Growth Promoting</b>					
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	$\leq 24$ hrs
<sup>^</sup> <i>Pseudomonas paraeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant	-	30 -35 °C	$\leq 24$ hrs
<b>Inhibitory</b>					
<i>Staphylococcus aureus</i> subsp.aureus ATCC 6538 (00032*)	$\geq 10^3$	inhibited		30 -35 °C	$\geq 48$ hrs

Key : (\*) Corresponding WDCM numbers,

<sup>^</sup> Formerly known as *Pseudomonas aeruginosa*

## Storage and Shelf Life

Store between 15-30°C. Use before expiry date on the label. 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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