

## ENTEROBACTERIACEAE (EE) BROTH MOSSEL

Selective enrichment medium for the detection of *Enterobacteriaceae*

### Typical formula (g/L)

Peptone	10.000
Glucose	5.000
Disodium Hydrogen Phosphate	6.450
Potassium Dihydrogen Phosphate	2.000
Oxgall	20.000
Brilliant Green	0.014

### Directions

Suspend 43,5 g in 1000 ml of cold distilled water, heat to dissolve completely. Dispense 100 ml portions in 250 ml flasks (or 10 ml in tubes) and autoclave at 121 °C for 5 minutes. Cool rapidly in running water. This medium is heat sensitive.

Final pH 7.2 ± 0.2

### Description

Enterobacteriaceae (EE) Broth Mossel is a liquid medium for the enumeration or detection of *Enterobacteriaceae* in foods by means of MPN Technique and Presence Absence Test. The MPN method is described above; for the Presence/Absence Test, refer to technical sheet of Violet Red Bile Glucose Agar. Prepared according to the original formulation of Mossel et al. and the specifications given by ISO 7402 and ISO 8523 (Buffered Brilliant Green Bile Glucose Broth), the medium contains glucose and thus supports the growth of all *Enterobacteriaceae*, including lactose not fermenting strains. Because it contains brilliant green and bile salts, Mossel's medium is far more selective than Lactose Broth.

### Technique

The technique reported in ISO 7402 (MPN) and here summarised is recommended when the number of *Enterobacteriaceae* is expected to be in the range 1 to 100 per ml or per g of the test sample.

1. Take three tube of double- strength medium. Transfer to each of these tubes 10ml of the test sample if the product is liquid or 10 ml of the initial suspension in the case of other products.
2. Take three tube of single- strength medium Transfer to these each of three-tube 1ml of the test sample if the product is liquid or 1ml of the initial suspension in the case of other products.
3. Take three more tube of single- strength medium. Transfer to these each of three tube 1ml of the first decimal dilution ( $10^{-1}$ ) if the product is liquid or 1ml of first decimal dilution of the initial suspension ( $10^{-2}$ ) in the case of other products.
4. Incubate these nine tubes at 37°C for 24 h.
5. Streak a loopful from each of the nine incubated cultures on Violet Red Bile Glucose Agar plates and incubate at 37°C for 24 h
6. Observe for the presence of pink to red violet colonies, oxidase negative. The tubes from which the subculture is derived shall be regarded as being positive.
7. Using the MPN table determine from the number of positive tubes in the different dilutions, the most probable index.

The technique recommended by ISO 8523 include also a non selective pre-enrichment in Buffered Peptone Water incubated at 35 or 37°C for 16 to 20 hours followed by a subculture of 1ml to 10ml of EE Broth. After 18-24 h of incubation at 35 to 37°C streak a plate of Violet Red Bile Glucose Agar.

### User quality assurance (37°C - 24 h)

Productivity and selectivity control

*E.coli* ATCC 25922+ *E.faecalis* ATCC 29212: purple red colonies after subculture on VRBG

*S.typhimurium* ATCC 14028+ *E.faecalis* ATCC 29212: purple red colonies after subculture on VRBG

### Storage

Dehydrated medium: 10-30°C

User prepared tubes: 1 month at 2-8°C

**References**

- ISO 7402: 1993 - Microbiology- general guidance for the enumeration of *Enterobacteriaceae* without resuscitation - MPN technique and colony count technique.1993-09-15
- ISO 8523: 1991 - Microbiology- general guidance for the detection of *Enterobacteriaceae* with pre-enrichment 1991-10-01
- Mossel, D.A.A., Visser, M. & Connelissen A. M.R. (1963) J. Appl. Bact., **26**, 444

**Packaging**

4014661	Enterobacteriaceae (EE) Broth Mossel,	100 g (2.3 L)
4014662	Enterobacteriaceae (EE) Broth Mossel,	500g (11.5 L)