Also known as
EE Mossel

Specification
Liquid culture medium used for the enrichment of enterobacteria, according to the ISO standards and Pharmacopeial Harmonized Methods.

Formula * in g/L
Enzymatic digest of animal tissues (Gelatin peptone)..... 10.000
Dextrose 5.000
Ox bile 20.000
Disodium phosphate (anhy.) 6.450
Potassium dihydrogenphosphate 2.000
Brilliant green 0.015

Final pH 7.2 ±0.2 at 25 °C
* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 43.46 g of powder in 1 l of distilled water and heat until dissolved. DO NOT AUTOCLAVE. Heat at 105 °C for 5 minutes and cool down immediately.

Description
As the name suggests, this medium is for the enrichment of Enterobacteria, and is a modification by Mossel (1963) of the classic Brilliant Green Bile Broth. Substitution of lactose by glucose makes it more suitable for enteric bacteria detection, including both gas or non-gas-producers, in food and other samples.

Technique
The most common technique is as follows: The sample to be studied is added to sterile broth in a proportion of 10 %. After thorough homogenization, the mixture is incubated for a period of 24-48 hours à 30-35 °C.
After incubation, subcultures are performed on a solid media appropriate for the selective isolation of enterobacteria.
For this step, Violet Red Bile Glucose Agar is recommended, although MacConkey, VRBLA, deoxycholate or Vert brilliant based media can also be used.
Presumptive colonies isolated on this media, can be verified following the usual methodology.
Note: Temperatures or culture media may vary according to normatives adopted by the laboratory.
In the post-incubation reading, an excess of microbiological growth causes a sharp drop in pH which causes the medium to turn from green to yellowish.

Quality control
Incubation temperature: 30-35 °C / 37 ºC **Incubation time:** 24-48 h / 24 h

Inoculum: Practical range 50 - 100 CFU (productivity)/ 10^4-10^6 CFU (selectivity), according to ISO 11133:2014/Amd 1:2018 and Ph. Eur. (ATCC® 6538 / ATCC® 8739/ ATCC® 9027 temperature 30 - 35ºC).

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 6538	Inhibited	Recovery in TSA (18-24h)
Pseudomonas aeruginosa ATCC® 9027	Good	Recovery in VRBG (18-24h) >10 CFU
Escherichia coli ATCC® 8739	Good	Recovery in VRBG (18-24h)>10 CFU
Escherichia coli ATCC® 25922	Good	Recovery in VRBG (18-24h)>10 CFU
Salmonella typhimurium ATCC® 14028	Good	Recovery in VRBG (18-24h)>10 CFU
Enterococcus faecalis ATCC® 19433	Inhibited	Recovery in TSA (18-24h)
Escherichia coli ATCC® 11775	Good	Recovery in VRBG (18-24h)>10 CFU

References
· EUROPEAN PHARMACOPOEIA 11.0 (2023) 11th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
· ISO 21528-1:2004 Standard. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MOSSEL, VISSER & CORNELISSEN (1963) The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of salmonellae J. Appl. Bact. 26:444-452.
· PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos. S.A. Madrid.
· USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).