EE BROTH ISO 21528-1

CAT Nº: 1362

For the detection and enumeration of Enterobacteriaceae by the MPN method

FORMULA IN g/l

Dehydrated Ox Bile	20.00	Glucose Monohydrate	5.00
Pancreatic Digest of Gelatin	10.00	Potassium Dihydrogen Phosphate	2.00
Disodium Hydrogen Phosphate	6.45	Brilliant Green	0.0135

Final pH 7.2 ± 0.2 at 25ºC

PREPARATION

Suspend 43.5 grams of the medium in one liter of distilled water. Dispense into appropriate containers and sterilize as soon as possible, either by using vapor flow during 10 minutes, or in autoclave at 121°C for 5 minutes. Cool immediately under tap water without contaminating the medium The prepared medium should be stored at 2-8°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and light green in color. If there are any physical changes, discard the medium.

USES

EE Broth is a medium recommended by the ISO normative 21528 for the detection and enumeration of Enterobacteriaceae by the MPN method (Most probable number method).

Pancreatic digest of gelatin and Glucose are the nitrogen and energy sources. Dehydrated ox bile and Brilliant green inhibit Gram positive bacteria and most Gram negative bacteria. Sodium phosphate and potassium phosphate act as a buffer system.

ISO 21528-1:2004 outlines a method, including preenrichment, for the detection of Enterobacteriaceae. It can be applied to products for human consumption and the feeding of animals, as well as environmental samples in the area of food production and food handling. This method is used when the microorganisms sought are expected to need resuscitation before enrichment, and when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.

For the enumeration of Enterobacteriaceae by the MPN method: inoculate 3 tubes of the sample in Buffered Peptone Water (Cat.1402), using the adequate dilutions to obtain the detection of the parameters required for the product for each dilution of the sample. Sub-cultivate in EE broth with Durham gas collecting tubes and incubate at 30 ± 1ºC (milk and lactic products), or 37 ± 1ºC (other food samples) for 24 hours. Sub-cultivate each tube in Violet Red Bile Agar w/Glucose (VRBG) (Cat.1092) and incubate at 30 ± 1ºC /milk and lactic products, or 37 ± 1ºC (other food samples) for 24 hours. Detection of Enterobacteriaceae will be confirmed with the presence of gases in EE Broth and red-purple colonies in VRBG Agar.

Determination must be carried out from the number of positive tubes of selected dilutions using an MPN table and calculation of the Enterobacteriaceae count per gram or millilitre of sample.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature to According to ISO 11133: 24 h/37°C (Productivity, Selectivity)

Microorganisms	Inoculum (cfu/ml)	Productivity Qualitative	Selectivity Qualitative
Escherichia coli ATCC 8739	$10\text{-}10^2$	≥ 10 colonies on VRBG	
Escherichia coli ATCC 25922	$10\text{-}10^2$	≥ 10 colonies on VRBG	
Salmonella thyphimurium ATCC 14028	$10\text{-}10^2$	≥ 10 colonies on VRBG	
Enterococcus faecalis ATCC 19433	$10^3\text{ -}10^4$		Total inhibition (0) on TSA
Enterococcus faecalis ATCC 29212	$10^3\text{ -}10^4$		Total inhibition (0) on TSA

BIBLIOGRAPHY

ISO 21528-1. 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

Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient identifiable information. London. December 1997.

The European Parliament and the Council of the European Union. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union. L226.

ISO

STORAGE

Once opened keep powdered medium closed to avoid hydration.