

## Specification

Differential selective medium for the detection and enumeration of enterococci according to ISO Standard.

## Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90mm with: 21 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14 °C

## Composition

Composition (g/l):	
Tryptose.....	20.0
Yeast Extract.....	5.00
D-(+)-Glucose.....	2.00
Dipotassium phosphate.....	4.00
Sodium azide.....	0.40
TTC.....	0.10
Agar.....	10.0

## Description /Technique

### Description

Differential medium for enumeration and differentiation of enterococci in water samples based on the resistance to sodium azide and the ability of enterococci to reduce the TTC to formazan and so their colonies are red in colour.

Note: The color tone (light amber / pale pink) between batches can vary without modifying the characteristics of the medium.

### Technique

For the membrane filtration technique, take 100 mL of a well mixed water sample, and pass it through a sterile membrane filter. Then, wash with 30 mL of sterile water to rinse the funnel of the filtering system.

Transfer the membrane aseptically to the culture medium contained in a Petri dish, making sure that the filter surface faces upwards.

Close the lid and invert the plate. Incubate at 36°C for 48 hours.

The developed colonies that appear red or purple in colour must be considered as enterococci, since these bacteria reduce Triphenyltetrazolium-HCl to an insoluble formazan which is red in colour. The secondary or accompanying Gram negative bacteria are inhibited by sodium azide.

For food samples, from a decimal dilution bank of the sample, spread 0,1 mL of the dilutions onto the plated medium using a Drigalsky loop. Incubation and examination is then carried out in the same way as in the membrane filtration technique.

Note: the presence of enterococci must be confirmed with complementary biochemical tests (Catalase, Esculine, etc).

## Quality control

### Physical/Chemical control

Color : Light amber - pale pink      pH: 7.2 ± 0.1 at 25°C

### Microbiological control

Membrane Filtration /Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/10<sup>4</sup>-10<sup>6</sup> CFU (selectivity)/ ≥10<sup>3</sup> CFU (specificity).

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 36 ± 2 °C, reading at 44±4 h

### Microorganism

*Escherichia coli* ATCC® 25922, WDCM 00013

*Enterococcus faecalis* ATCC® 19433, WDCM 00009

*Enterococcus faecalis* ATCC® 29212, WDCM 00087

*Enterococcus faecium* ATCC® 6057, WDCM 00177

*Stph. aureus* ATCC® 25923, WDCM 00034

### Growth

Inhibited

Good (≥ 50%) Colonies Red-brow

Good (≥ 50%) Colonies Red-brow

Good (≥ 50%) Colonies Red-brow

Inhibited

### Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

## Bibliography

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- ISO 7899-2:2000 Standard. Water Quality. Detection and enumeration of enterococci by membrane filtration method.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- LACHICA, LV.F. and P.A. HARTMAN (1968) Two improved media for isolating and enumerating enterococci in certain frozen foods. J. appl. Bact. 31:151-156.
- SLANETZ, L.W. and BARTLEY, C.H. (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium. J. Bact. 74:591-596.
- UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.

## Storage

Storage conditions: 2-14°C

Alternatively the plates may also be stored at the range of 2 - 25°C, with a proper performance of the medium, but some precautions must be taken into account:

- In the range of 2 - 8 °C avoid direct contact with surfaces that can freeze product.
- In the range of 15 - 25 °C, dehydration control must be taking in account.