Slanetz and Bartley Medium

M612I

Intended use

Recommended for detection and enumeration of faecal Streptococci from water samples by membrane filtration technique. The composition and performance criteria of this medium are as per the specifications laid down in ISO/ DIS 7899 -2: 2000 (E) and APHA.

Composition**

ISO 7899-2:2000 (E), APHA	
Ingredients	**g / L**
Tryptose	20.000
Yeast extract	5.000
Glucose	2.000
Dipotassium hydrogen phosphate	4.000
Sodium azide	0.400
TTC Solution (1%)	10.00ml
Agar	8-18
Final pH (at 25°C)	7.2±0.1

Slanetz and Bartley Medium	**M612I**
Ingredients	**g/ L**
Tryptose	20.000
Yeast extract	5.000
Dextrose	2.000
Dipotassium hydrogen phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	15.000
Final pH (at 25°C)	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Slanetz and Bartley Medium was originally devised by Slanetz and Bartley (1) for the detection and enumeration of Enterococci by membrane filtration technique. It can be also used as a direct plating medium (2,3). M612I differs from M612 in the type of buffering system used. This medium composition is as per specifications laid in ISO (4), APHA (5).

Tryptose and yeast extract serves as a source of essential nutrients along with B-complex vitamins and nitrogenous nutrients. The medium is highly selective for Enterococci. Sodium azide has inhibitory effect on gram-negative organisms. Triphenyl Tetrazolium Chloride is reduced to the insoluble formazan inside the bacterial cell forming dark red-coloured colonies. When the medium is incubated at higher temperature (44-45°C), all red or maroon colonies can be considered as presumptive Enterococci (6,7).

The Department of Health (8) has recommended this medium to be used for enumeration of Enterococci in water supplies. Water is filtered through a membrane filter which is then placed on the surface of the Slanetz and Bartley Medium plates and incubated at 35°C for 4 hours and then at 44-45°C for 44-48 hours. Red or maroon colonies are counted as Enterococci. The preliminary incubation at 35°C helps for the recovery of stressed organisms. Not all the species reduce TTC, hence pale colonies also should be considered. Food samples are homogenized and so diluted with physiological saline to give 15-150 colonies on each petri plate. Homogenates or dilutions are spread on agar surface and incubated at 35°C for 48 hours. Pink or dark red colonies with a narrow whitish border are counted (9).

Type of specimen

Water samples

Specimen Collection and Handling:

ISO 7899-2:2000:

Preparation of test sample: Prepare tenfold dilutions of water samples

Choice of technique:

a) Pour plate method
b) Spread plate method
c) Membrane filtration method

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 biochemical testing is required for identification of species.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.65% w/v aqueous solution at 25°C. pH : 7.2±0.1

pH

7.10 -7.30

Cultural Response

Cultural characteristics observed after an incubation at 44-45°C for 44-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecalis ATCC 19433 (00009*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecalis WDCM 00176	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecium ATCC 6057 (00177*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecium WDCM 00178	50-100	good-luxuriant	>=50%	red or maroon
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034)*	>=10^4	inhibited	0%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 (10,11).

Reference

1. Slanetz L. W. and Bartley C.H., 1957, J. Bact., 74:591.
2. Burkwall M.K. and Hartman P.A., 1964, Appl. Microbiol., 12:18.
3. Nordic Committee on Food Analysis, 1968, Leaflet 68.
4. ISO 7899-2:2000, Water Quality - Detection and enumeration of intestinal enterococci - Part 2 :Membrane filtration method.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Mead G.C., 1966, Proc. Soc. Wat. Treat. Exam., 15:207.
7. Taylor E.W. and Burman N.P., 1964, J. Appl. Bact., 27:294.
8. Department of Health and Social Security, 1982, Report 71, HMSO, London.
9. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
11. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 07/2024