Tryptose Sulphite Agar

M2183I

Intended Use:

Recommended for the detection and enumeration of sulfite-reducing anaerobes. The composition and performance criteria of this media are as per the specifications laid down in ISO 6461-2:1986 (E).

Composition**

ISO 6461-2:1986-Tryptose- Sulphite Agar	
Ingredients	**g / L**
Tryptose	15.000
Soytone	5.000
Yeast extract	5.000
Sodium metabisulfite	1.000
Ammonium iron III citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.1

Iron Sulphite Agar Modified	**M2183I**
Ingredients	**g/L**
Tryptose	15.000
Soya peptone#	5.000
Yeast extract	5.000
Sodium metabisulfite	1.000
Iron III ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.1

**Formula adjusted, standardized to suit performance parameters, # Equivalent to Soytone

Directions

Suspend 42 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121±1°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense into sterile tubes or pour into sterile Petri plates.

Principle And Interpretation

Tryptose Sulphite Agar is recommended by ISO for the enumeration of sulphite reducing bacteria (1). Most *Clostridia* possess sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Peptone and soya peptone provides carbon, nitrogen compounds, vitamins, minerals and amino acids necessary for the growth of organism. Yeast extract serves as a rich reservoir of vitamins especially B-complex vitamins. Ferric citrate ammonium citrate and Disodium sulfite serves as are H2S indicators, wherein Clostridium perfringens reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Agar is the solidifying agent.

Enumeration with this medium can be performed using either tubes or plates. In case of Petri plates, Using a fresh sterile pipette, transfer to each dish of the first decimal dilution 10^{-1} of the test sample if the product is liquid, or of the first decimal dilution of the initial suspension 10^{-2} in the case of other products. Pour iron sulfite agar into each Petri dish. Carefully mix the inoculum with the medium by horizontal movements and allow the medium to solidify. After the medium has solidified, pour 5 to 10ml of the same medium into the dish as an overlay.

If tubes are used, inoculate a 1 ml volume from each dilution into each of two tubes of medium. Mix gently without forming bubbles, and leave the medium to solidify. After the medium has solidified, pour 2ml to 3ml of the same medium into each tube as an overlay. After solidification, incubate the medium at 36-38°C for 24-48 hours. If thermophilic bacteria are suspected, prepare a second set of Petri dishes. Incubate this set at 50°C ± 1°C. Black colonies, possibly surrounded by a black zone, are counted as sulfite-reducing bacteria.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.20% w/v aqueous solution at 25°C. pH : 7.6±0.1

pH
7.50-7.70

Cultural Response

Productivity : Cultural characteristics observed under anaerobic atmosphere, after an incubation at 37±1°C for 44±4h. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Specificity : Cultural characteristics observed after an incubation at 37±1°C for 44±4h.

Organism	Inoculum	Growth	Recovery	Characteristic reaction
Productivity				
Clostridium perfringens ATCC 13124 (00007)*	50-100	luxuriant	>=50%	black colonies
Clostridium perfringens ATCC 12916 (00080)*	50-100	luxuriant	>=50%	black colonies
Specificity				
Escherichia coli ATCC 25922 (00013)*	10^3-10^4	growth		no blackening
Escherichia coli ATCC 8739 (00012)*	10^3-10^4	growth		no blackening

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 (3,4).

Reference

1. Water quality- Detection and enumeration of the spores of sulfite-reducing anaerobes (Clostridia)- Part 2: Method by membrane filtration, ISO 6461-2:1986(E).
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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 :00/2024