Iron Sulphite Agar Modified

M1852I

Intended Use:

Recommended for the enumeration of sulfite – reducing bacteria growing under anaerobic conditions. The composition and performance criteria of this medium are as per the specifications laid down in ISO 15213:2003

Composition**

Ingredients	Gms / Litre
Casitose ▲	15.000
Soya peptone	5.000
Yeast extract	5.000
Disodium disulfite	1.000
Ferric ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters
▲ - Equivalent to Enzymatic digest of casein

Directions

Suspend 42 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°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

Iron Sulphite Agar, Modified is recommended by ISO for the enumeration of sulphite reducing bacteria.(3). 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.

Casitose and soya peptone provides carbon, nitrogen ompounds, 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 H_2S 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 tubes distribute 20-25 ml of the medium in tubes and inoculate 1 ml of test sample or 1 ml of serial dilutions of 10-1 and 10-2 in molten state. Allow to solidify, and pour 2-3 ml of the same medium in each tube to overlay. In case of Petri plates, transfer 1 ml of test sample or initial dilution. Further dilution can be carried out and 1 ml of each dilution (10-1 and 10-2) is transferred to an empty Petri plate. Cool the medium to 44-47°C and pour 15-20 ml of the medium to the Petri plate containing the inoculum. Mix the inoculum and allow the medium to solidify. Overlay the medium with 5-10 ml of the same medium.

After solidification , incubate the medium at 36-38°C for 24-48 hours. If thermophilic bacteria are suspected, a second of tubes is incubated at 49-51°C for 24-48 hours. After incubation, black coloured colonies, possibly surrounded by a black zone are counted as sulphite reducing bacteria.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

For isolated microorganisms, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3). 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. Further biochemical and serological testing is required for complete identification.

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 4.2% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed under anaerobic conditions, after an incubation at 36-38°C for 24-48 hours.

Organism	Inoculum	Growth	Recovery	Colour of colony
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	black
Clostridium butyricum ATCC 13732	50-100	luxuriant	>=50%	black
*Clostridium sporogenes ATCC 19404 (00008)**	50-100	luxuriant	>=50%	black
*Clostridium perfringens ATCC 13124 (00007)**	50-100	luxuriant	>=50%	black
*Clostridium perfringens ATCC 12916 (00080)**	50-100	luxuriant	>=50%	black
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
*Escherichia coli ATCC 25922 (00013)**	50-100	good	40-50%	no blackening
*Escherichia coli ATCC 8739 (00012)**	50-100	good	40-50%	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 20-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of sulphite reducing bacteria growing under anaerobic conditions, ISO 15213.

Revision : 03 / 2019