Iron Sulphite Agar

M868

Intended Use:

Recommended for detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Sodium sulphite	0.500
Iron (III) citrate	0.500
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America (7). It was shown by Beerens (2) that 0.1% sulphite concentration in the original formula was inhibitory to some strains of *Clostridium sporogenes*. This observation was later confirmed by Mossel et al (5), who consequently showed that 0.05% sulphite concentration was not inhibitory to the organisms. Most clostridia have 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.

Tryptone provides nitrogen and other nutrients necessary to support bacterial growth. Sulphite-reducing bacteria usually produce black colonies as a result of the reduction of sulphite to sulphide, which reacts with the iron (III) salt.

For the detection of organisms causing sulphide spoilage, two methods can be followed:

a) Deep-Shake Culture Method: Dispense the medium in 10 ml amounts in tubes. Inoculate the sample when the medium is at about 50°C. Allow to set and incubate at 55°C for 24-48 hours.
Typical thermophilic species -*Desulfotomaculum nigrificans*, produces distinct black spherical colonies in the depth of the medium.

b) Attenborough and Scarr (1) Method: In this method, diluted samples of sugar or any other food are filtered through membrane filters.

These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them. The medium is allowed to set and then incubated at 55-56°C for 24-48 hours. After incubation, the number of black colonies on the membrane filter is counted. Confirmation tests are further carried out to identify the organism growing in the medium. This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples. The blackening reaction is only presumptive evidence of clostridial growth. Confirmation test must be carried out for identification. There are many gram-negative bacteria that are able to reduce sulfite with iron sulfide production in this medium, but in these cases the enzymes are extra cellular and the entire medium becomes dark, rendering their enumeration impossible.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).
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.This medium is general purpose medium and may not support the growth of fastidious organisms.
2.Further biochemical tests must be carried out for confirmation.

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 2.6% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed under anaerobic conditions, after an incubation at 55-56°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
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
Escherichia coli ATCC 25922 (00013*)	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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Attenborough J. and Scarr M., 1957, J. Appl. Bacteriol., 20: 460.
2.Beerens H., 1958, DSIR, Proc. 2nd Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.
3.3. 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.
5.Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S., 1959, J. Path. Bacteriol., 78:290.
6.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.
7.Tanner F. W., 1944, “The Microbiology of Foods”, 2nd Ed., Garrard Press, Illinois, P. 1127.