SPS Agar, Modified

M898

Intended Use:

Recommended for selective isolation and enumeration of *Clostridium perfringens* from foodstuffs.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	10.000
Ferric citrate	0.500
Sodium sulphite	0.500
Sodium thioglycollate	0.100
Polysorbate 80 (Tween 80)	0.050
Sulphadiazine	0.120
Polymyxin B sulphate	0.010
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.28 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 50°C and pour in sterile Petri plates containing inoculum. Allow to solidify and if desired, pour the cover layer using about 5 ml sterile medium. Incubate anaerobically.

Principle And Interpretation

SPS (Sulphite Polymyxin Sulphadiazine) Agar was developed by Angelotti et al (2) based on the Wilson and Blair medium and the medium described by Mossel et al (3,8) for selective isolation and enumeration of *Clostridium perfringens* from foods. The medium of Mossel et al included the use of Miller-Prickett tubes. The modified SPS Agar however obviates the inclusion of Miller-Prickett tubes.

Tryptone and yeast extract supplies nitrogenous compounds, vitamin B complex and other essential growth nutrients to the growing *Clostridium perfringens.* This organism reduces sulphite to sulphide which reacts with iron of ferric citrate to form a black precipitate of iron sulphide and hence the colonies are black (7). Polysorbate 80 monooleate supplies fatty acids for the organisms. Polymyxin and Sulphadiazine inhibit a wide variety of gram-positive and gram-negative bacteria (9). Few organisms found in food other than *Clostridium perfringens* also form black colonies on this medium. Some strains of *Clostridium perfringens* fail to grow on this medium.

Type of specimen

Food and dairy samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,9,10).

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. Some strains of *Clostridium perfringens* fail to grow on this medium.

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 beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared Medium

Medium amber coloured slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.13% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours under anaerobic conditions.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium perfringens ATCC 13124 (00007*)	50-100	good-luxuriant	>=50%	black
Clostridium sporogenes ATCC 11437	50-100	fair-good	30-40%	black
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	none-poor	<=10%	white
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated 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 (4,5).

Reference

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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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9. Salfinger Y., and Tortorello M., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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