SPS Agar

M632

Sulphite-Polymyxin-Sulphadiazine Agar is used for the detection of *Clostridium perfringens* in foods.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Yeast extract	10.000
Sodium sulphite	0.500
Polymyxin B sulphate	0.010
Sulphadiazine	0.120
Ferric citrate	0.500
Agar	13.900
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.03 grams in 1000 ml distilled water. Heat, to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

SPS (Sulphite Polymyxin Sulphadiazine) Agar was developed by Angelotti et al (1) based on the Wilson and Blair Medium and the medium described by Mossel et al (2, 3) for selective isolation and enumeration of *C. 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.

Casein enzymic hydrolysate and yeast extract supply nitrogenous compounds, vitamin B complex and other essential growth nutrients to the growing *C. 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 appear black (4). Polymyxin B and sulphadiazine inhibit a wide variety of gram-positive and gram-negative bacteria (5). Few organisms found in food other than *C. perfringens* also form black colonies on this medium.

Prepare serial dilutions of the samples to be tested and inoculate onto SPS Agar using the pour plate technique. If desired, pour cover layers using about 5 ml medium. Incubate the plates anaerobically. Enumerate the black colonies. Presumptive black *C. perfringens* colonies should be confirmed by standard biochemical tests.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.39% Agar gel

Colour and Clarity of Prepared Medium
Medium amber coloured slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.0% 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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%	black
Clostridium sporogenes ATCC 11437	50-100	fair-good	30-40%	black
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	50-100	none-poor	<=10%	white

Storage and Shelf Life

Store dehydrated medium and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Angelotti R., Han H. E., Foter M. J. and Lewis K. H., 1962, Appl. Microbiol., 10:193.
2.Mossel D. A. A., De Bruit A. S., Van Dipen H. M. J., Vendring C. M. A. and Zoutewelle G., 1956, J. Appl. Microbiol., 19:142.
3.Mossel R. S., 1959, J. Sci. Food Agric., 19:662.
4.Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

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