Reference: 064-TA2115 **Scharlau Microbiology - Technical Data Sheet**

Product: # SPS Agar with paraffin

Specification

Solid medium for the detection of *Clostridium perfringens*.

Presentation

	Packaging Details	Shelf Life	Storage
20 Tubes Tube 16 x 113 mm with: 10 ± 0,2 ml	16x113 mm glass tubes, ink labelled. Metalic-Non injectable cap. - 20 tubes per box	12 months	8-25ºC

Composition

Composition (g/l):

Sodium sulfite	0.50
Polymixin (B) sulfate	0.01
Sodium sulfadiazine	0.12
Casein peptone	15.00
Yeast extract	10.00
Ferric citrate	0.50
Sodium thioglycolate	0.10
Polysorbate 80	0.05
Agar	15.00
Paraffin	1 ml

Description /Technique

Description

SPS Agar (Sulfite Polymyxin Sulfadiazine) is a modification of the original Wilson & Blair medium for the detection of clostridia. The present medium betters the formulation of Mossel and also the later modification of Angelotti et al.. It achieves a higher selectivity for C. perfringens with the addition of Sulfadiazine and Polymyxin.

The nutritional substrates constituted by the tryptone and the yeast extract are complemented by the polysorbate, which also allows the recovery of the most delicate cells. The anaerobic conditions are improved by the thioglycolate, which permits the use of the medium on the plates.

The differential system consists of sodium sulfite and ferric citrate which allows the detection of sulfite reducing organisms, which form black colonies due to ferrous sulfide precipitate.

Technique

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and / or expected results.

Melt the medium contained in the tubes in a water bath or in a microwave oven, avoiding overhating.

Add the sample directly into tube after making a dilution bank, or stab needle.

Incubate the tube in anaerobically conditions at 44±1ºC for 24-48h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications,...)

after incubation, enumerate all the colonies that have appeared into the agar, with a black precipitate.

Each laboratory must evaluate the results according to their specifications.

Presumptive isolation of Clostridium sp must be confirmed by further microbiological and biochemical tests.

Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by the inverse dilution factor if streaked a diluted sample. Report results as Colony Forming Unit (CFU's) per ml or g along with incubation time and temperature.

Since the medium is not extremely selective, it is advisable to verify black colonies by checking that they are Gram positive sporulated non-motile organisms incapable of reducing nitrates to nitrites.

Most clostridia are sulfite reducers. Among them are C. perfringens and C. botulinum which along with C. bifermentans are the species most frequently involved in food poisoning.

Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7 ± 0.2 at 25ºC

Microbiological control

Anaerobiosis. Incubation at 35 ± 2.5ºC, reading after 24-48 hours

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Microorganism	Growth
Clostridium perfringens ATCC® 13124	Good - H_2S positive . Black colonies
Clostridium perfringens ATCC® 10543	Good - H_2S positive . Black colonies
Bacillus subtilis ATCC® 6633	Inhibited

Sterility Control

Incubation 48 hours at 30-35ºC and 48 hours at 20-25ºC: NO GROWTH

Check at 7 days after incubation in same conditions

Bibliography

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· MOSSEL, D.A.A. (1959) Enumeration of sulfite-reducing bacteria occurring in foods. J. Sci. Food Agric. 19:662.