Reference: 401320ZA **Technical Data Sheet**

Product: **SPS Agar - Vial 200 ml**

Specification

Solid medium for the detection of *Clostridium perfringens* in food.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottles 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	8-25 ºC

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
Agar	15.00

Description /Technique

Description:

SPS Agar is a modification of the classic Wilson and Blair medium for the detection of clostridia. The current formulation exceeds that of Mossel and that of Angelotti *et al*., by achieving greater selectivity for *Cl. perfringens*, thanks to the addition of sulfadiazine and polymyxin.

On the other hand, the nutritional substrate, consisting of tryptone and yeast extract, is complemented by polysorbate and allows the recovery of the most delicate cells. Anaerobiosis conditions are greatly improved with the presence of thioglycollate, which allows the use of the medium in plates, regardless of the Miller-Pritchett tubes, used by Mossel and Wilson-Blair.

The differential system is made up of sodium sulphite and ferric citrate, which allow the detection of sulphite-reducing organisms, producing black colonies, due to precipitates of iron sulphide.

Recommended Technique:

The usual technique for using this medium is as follows: The material to be examined is crushed with a mixer or stomacher and a bank of decimal dilutions is made. From each of the dilutions, an aliquot is placed in Petri dishes and the molten medium, cooled to about 50 ° C, is poured over them. The plates are allowed to solidify and are incubated in an anaerobic system at 35 ± 2 ºC for 24-48 hours, although temperatures or incubation times may vary slightly depending on the regulations adopted by the laboratory. Detection is accelerated if the double layer method is applied with the same culture medium.

After incubation, the black colonies that appear in the different dilutions are listed and the results are expressed as “sulphite-reducing clostridia per unit of sample”.

Generally, 90% of the black colonies that occur can be attributed to *Clostridium perfringens*. However, and since the medium is not extremely selective, it should be verified that the black colonies are made up of Gram positive organisms, sporulated, immobile and incapable of reducing nitrates to nitrites.

Most clostridia are reducing sulphites and include *Cl. perfringens* and *Cl. botulinum* which together with *Cl. bifermentans* are the species most frequently related to food poisoning.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

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Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7.2 ± 0.2 at 25ºC

Microbiological control

Melting - pour plates - inoculation Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10^4-10^6 CFU (selectivity)

Double-layer inoculation

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosis. Incubation at 35 ± 2 ºC, reading after 24-48 hours

Microbiological control according to ISO 11133:2014/A1:2018.

Microorganism	Growth
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good - H_2S positive . Black colonies
Clostridium perfringens ATCC® 10543, WDCM 00174	Good - H_2S positive . Black colonies
Escherichia coli ATCC® 8739, WDCM 00012	Inhibited
Bacillus subtilis ATCC® 6633, WDCM 00003	Inhibited

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

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· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed., APHA. Washington.

· F.D.A. (1998) Bacteriological Analytical Manual. 8th ed. Rev. A., AOAC International. Gaithersburg. MD.

. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

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