

Specification

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

Presentation

20 Tubes
Tube 16 x 113 mm
with: 10 ± 0,2 ml

Packaging Details

1 box with 20 tubes, 16x113 mm glass tubes, ink
labelled and metal-Non injectable cap.

Shelf Life

12 months

Storage

8-25°C

Composition

Composition (g/L):

Casein peptone..... 15.0
Yeast extract..... 10.0
Sodium sulfite..... 0.50
Polymixin (B) sulfate..... 0.01
Sodium sulfadiazine..... 0.12
Ferric citrate..... 0.50
Agar..... 15.0

Description /TechniqueDescription

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 overheating.

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

Incubate the tube in anaerobically conditions at 44±1°C 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 25°C

Microbiological control

Inoculate by stabbing

Anaerobiosis. Incubation at $35 \pm 2.5^\circ\text{C}$, reading after 24-48 hours

Microorganism**Growth**

Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237 Good - H₂S positive . Black colonies

Clostridium perfringens ATCC® 10543, WDCM 00174 Good - H₂S positive . Black colonies

Escherichia coli ATCC® 8739, WDCM 00012 Inhibited

Sterility Control

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

Check at 7 days after incubation in same conditions

Bibliography

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