

CAT Nº: 1075

For the selective isolation of *Clostridium perfringens* from foods and other material

FORMULA IN g/l

Casein Peptone	15.00	Neomycin Sulfate	0.05
Yeast Extract	10.00	Polymyxin B Sulfate	0.02
Sodium Sulfite	1.00	Bacteriological Agar	13.50
Ferric Citrate	0.50		

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TSN AGAR (TRYPTONE SULFITE NEOMYCIN) is a selective medium that can be used in tubes or plates for the identification and enumeration of *Clostridium perfringens* in foods and other materials, especially from mixed contaminating flora.

The Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Ferric citrate and Sodium sulfite are H₂S indicators. *C. perfringens* reduces the sulfite to sulfide which reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Bacteriological agar is the solidifying agent. Polymyxin and Neomycin sulfates inhibit the growth of the majority of Enterobacteria and *Clostridium bifermentans*. The incubation at a temperature of 46°C allows specific and quantitative results.

Inoculate medium with sample and incubate at 46 ±1°C for 18-24 hours. Use an anaerobic jar for incubation in a H₂/CO₂ atmosphere if possible. For aerobic incubation in tubes, cover the tubes with a layer of sterile medium. Read within half an hour after taking the plates out of the jars and observe for black colonies which can lose their color by oxidation in air after this time period.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after anaerobic incubation at a temperature of 46 ± 1°C and observed after 18-24 hours.

Microorganisms	Growth	Colony Color
<i>Clostridium perfringens</i> ATCC 13124	Good	Black
<i>Clostridium sporogenes</i> ATCC 25781	Good	Black
<i>Escherichia coli</i> ATCC 25922	Inhibited	---
<i>Pseudomonas aeruginosa</i> ATCC 27853	inhibited	---

BIBLIOGRAPHY

Angelotti, Nall, Foter y Lewis. Applied Microbiol. 10: 193. 1962. Mossel. J.SCI. Agr. 10: 662. 1959. Mossel de Bruin Van Diepen, Vendrig y Zoutwelle J. Applied Bact, 19: 142. 1956.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

