



### Specification

Solid medium for the isolation and differentiation of *Clostridium perfringens*, according to ISO standards and other regulations.

### Formula \* in g/L

Tryptone .....	15.00
Soy peptone .....	5.00
Yeast extract .....	5.00
Sodium disulphite .....	1.00
Ammonium iron(III) citrate .....	1.00
Agar .....	18.00

Final pH 7.6 ±0.2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

### Directions

Suspend 45 g of powder in 1L of distilled water and let soak minutes. Bring to a boil and distribute volumes of 500 ml in suitable containers. Sterilize the autoclave for 10 minutes at 121 ° C. Cool to 60 ° C and add 1 vial of Selective Supplement D-Cycloserine (Ref. DSHB3021) to 500 ml. Mix well and distribute on plates. If you wish yolk, while adding the antibiotic, sterile egg yolk (Ref .BA1019) at 80 ml/l.

### Description

The medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine. Cycloserine has been found more selective for *Clostridium perfringens*, and reduces the production of diffuse blackening. *Clostridium perfringens* is more resistant to cycloserine than to sulfadiazine, polymyxin and neomycin, hence reducing the dosage. The presence of sodium meta-bisulfite and ferric ammonium citrate allow three differential characteristics of this anaerobic species to be verified with just one assay. These characteristics are sulfite reduction, growth at 46°C and cycloserine resistance.

Cycloserine does not tolerate temperatures above 100°C and its stability in a solution is variable. Therefore, it is advisable to prepare the exact number of plates that are going to be used.

A solution of cycloserine in phosphate buffer at pH 8,0 may be prepared (Di potassium phosphate 16,73 g/L and mono-potassium phosphate 0,52 g/L) and if it is maintained refrigerated, can be used for approx. 5 days. This product , store at (-20±5 ) ° C can be use within 4 weeks of preparation. If stored frozen at (-20 ± 5) ° C could extend the expiration to 4 weeks or 12 months if stored at (-70 ± 10) ° C.

This lyophilized product, has a much higher expiration indicated on the manufacturer's label.

### Necessary supplements

D-Cycloserine Selective Supplement (Ref. DSHB3021)

Vial contents:

Necessary amount for 250 mL of complete medium.

D-Cycloserine 200,00 mg

Distilled water (Solvent)

### Technique

The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis. After incubation at 44-46 °C for 24 ±3h, proceed to enumerate the black colonies that appear in the plate.

Proceed according to standards or standardized methods.

### Quality control

**Incubation temperature:** 44°C ±1,0

**Incubation time:** 21 ± 3 h

**Inoculum:** Practical range 100 ±20 CFU. Min. 50 CFU (productivity)/10<sup>3</sup>-10<sup>6</sup> CFU (selectivity), according to ISO 11133:2014/Amd 1:2018 .

### Microorganism

*Clostridium perfringens* ATCC® 10543

*Clostridium perfringens* ATCC® 13124

*Bacillus subtilis* ATCC® 6633

### Growth

Productivity > 0.50

Productivity > 0.50

Inhibited

### Remarks

Black colonies (Anaerobiosis)

Black colonies (Anaerobiosis)

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**References**

- ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DIN Standard 10165. Referenz Verfahren für Bestimmung von Clostridium perfringens. Fleisch und Fleischerzeugnissen.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. American Public Health Association. Washington.
- DIRECTIVA 2015/1787/UE de la Comisión por la que se modifica la Directiva 98/ 83/CE relativa a la calidad de las aguas destinadas al consumo humano (DO L260 de 7.10.2015 pg 6 y ss)
- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International Inc. Gaithersburg. MD.
- ISO 7937 (2004) Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for Enumeration of C. perfringens. Colony-count technique.
- ISO Norma 6461-2 (1986) Water Quality.- Detection and enumeration of the spores of sulfite-reducing anaerobes (Clostridia).- Part 2: Method by Membrane Filtration.
- ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 14189 (2013) Water quality. Enumeration of Clostridium perfringens — Method using membrane filtration
- SMITH, L.D. (1981) Clostridial Anaerobic Infections, in Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections. 6th ed. APHA. Washington.
- UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.- Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.

**Storage**

Keep tightly closed, away from light, in a dry place (4-30 °C).