



## **Specification**

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

## Formula \* in g/L

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 15 minutes at 121 ° C. Cool to 45-50° 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 .FLWR3035) 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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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 monopotassium phosphate 0,52 g/L) and if it is maintained refrigerated, can be used for approx. 5 days. This product, store at (-20 $\pm$ 5) ° C can be use within 4 weeks of preparation. If stored frozen at (-20  $\pm$  5) ° C could extend the expiration to 4 weeks or 12 months if stored at (-70  $\pm$  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

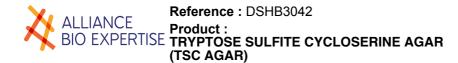
The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis (TSC Agar or bacteriológical agar). After incubation à 44-46 °C for  $24 \pm 3$  h, proceed to enumerate the black colonies that appear in the plate. Proceed according to standards or standardized methods.

#### Quality control

# Incubation temperature:44 °C $\pm$ 1.0Incubation time:21 $\pm$ 3 hInoculum:Practical range 100 $\pm$ 20 CFU. min. 50 CFU (productivity)/ 10<sup>4</sup>-10<sup>6</sup> CFU (selectivity), according to ISO

11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Clostridium perfringens ATCC <sup>®</sup> 10543	Productivity > 0.50	Black colonies (Anaerobiosis)
Clostridium perfringens ATCC <sup>®</sup> 13124	Productivity > 0.50	Black colonies (Anaerobiosis)
<i>Bacillus subtilis</i> ATCC <sup>®</sup> 6633	Inhibited	-
Clostridium perfringens ATCC <sup>®</sup> 13124	Productivity > 0.50	Black colonies (Anaerobiosis)





## References

- · ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DIN Standard 10165. Referenz Verfahren fur 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 Adminstrations) (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/ Adm 1:2018. 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.

## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).