



Specification

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

Formula * in g/L

Casein peptone.....	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 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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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 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 (TSC Agar or bacteriological agar). After incubation à 44-46 °C for 24 ± 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 ± 1.0 **Incubation time:** 21 ± 3 h

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10⁴-10⁶ CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism

Clostridium perfringens ATCC® 10543

Clostridium perfringens ATCC® 13124

Bacillus subtilis ATCC® 6633

Clostridium perfringens ATCC® 13124

Growth

Productivity > 0.50

Productivity > 0.50

Inhibited

Productivity > 0.50

Remarks

Black colonies (Anaerobiosis)

Black colonies (Anaerobiosis)

-

Black colonies (Anaerobiosis)



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/ 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).