

Specification

Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*, according to ISO Standards.

Presentation

	Packaging Details	Shelf Life	Storage
30 Membrane filtration plates 55 mm Plates for filtration purposes with: 9 ± 1 ml	1 box containing: 6 plastic bags with 5 plates of 55 mm/ bag.	6 months	2-25 °C

Composition

Composition (g/l):	
Enzymatic digest of casein.....	15.00
Soy Peptone.....	5.00
Yeast Extract.....	5.00
Sodium meta-bisulfite.....	1.00
Ferric ammonium citrate.....	1.00
Cycloserine.....	0.40
Agar.....	14.00

Description /Technique

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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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.

Technique:

Collect, dilute and prepare samples and volumes to be filtered as required according to specifications, directives, official standard regulations and/or expected results.

Filter the sample through a 0.45 mm pore membrane and apply it onto the surface of the agar.

Alternatively, a thin layer molten TSC agar or agar as an overlay on the membrane can be used.

Incubate the plates anaerobically at 44±1°C for 21±3h.

(Incubation times greater than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications,...)

After incubation, enumerate the colonies with a black iron sulfide precipitate.

Confirmation of characteristic colonies as *C.perfringens* is required, throughout further microbiological or biochemical tests.

Quality control

Physical/Chemical control

Color : yellow

pH: 7.6 ± 0.2 at 25°C

Microbiological control

Membrane Filtration /Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/10⁴-10⁶ CFU (selectivity)/ ≥10³ CFU (specificity).

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosis. Incubation at 44 ± 1 °C during 21 ± 3h.

Microorganism

Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237*Clostridium perfringens* ATCC® 10543, WDCM 00174*Bacillus subtilis* ATCC® 6633, WDCM 00003

A double layer with TSC agar favors the observation of the blackening of the SH2 (+) strains.

Growth

Good ≥ 50%. Black colonies

Good ≥ 50%. Black colonies

Inhibited

Sterility Control

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

Check at 7 days after incubation in same conditions.

Bibliography

- 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.
- 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.