

## 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 Prepared Plates 55 mm Plates for filtration purposes with: $9 \pm 1$ ml	1 box containing: 5 plastic bags with 6 plates of 55 mm/ bag.	6 months	2-25°C

## Composition

Composition (g/l):

Tryptose.....	15.00
Soy Peptone.....	5.00
Yeast Extract.....	5.00
Sodium meta-bisulfite.....	1.00
Ferric ammonium citrate.....	1.00
Cycloserine.....	0.40
Agar.....	14.0

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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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

Cover the membrane with a second layer of room temperature melted agar.

Incubate the plates anaerobically at  $44 \pm 1^\circ\text{C}$  for  $21 \pm 3$ h.

(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:  $7.6 \pm 0.2$  at 25°C

### Microbiological control

Membrane Filtration /Practical range  $100 \pm 20$  CFU; Min. 50 CFU (Productivity)./ $10^4$ - $10^6$  CFU for Selectivity.  
Microbiological control according to ISO 11133:2014/ Adm 1:2018.

Anaerobiosis. Incubation at  $44 \pm 1^\circ\text{C}$  during  $21 \pm 3$ h.

### Microorganism

<i>Clostridium perfringens</i> ATCC® 13124, WDCM 00007, NCTC® 8237	Good $\geq 50\%$ . Black colonies
<i>Clostridium perfringens</i> ATCC® 10543, WDCM 00174	Good $\geq 50\%$ . Black colonies
<i>Bacillus subtilis</i> ATCC® 6633, WDCM 00003	Inhibited

### Growth

### Sterility Control

Incubation 48 hours at 30-35°C and 48 hours 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. 4<sup>th</sup> 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. 8<sup>th</sup> 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. 6<sup>th</sup> 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.