Reference: 101141AA **Technical Data Sheet**

Product: **TSC Agar + Egg Yolk**

Specification

Medium recommended for isolation, cultivation and enumeration of *Clostridium perfringens* according to Iso standars.

Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm Plates with: 21 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14 ºC

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
Egg yolk emulsion	50.00 ml
Agar	18.00

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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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 44±1°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.
Inoculate the sample and alternatively, a thin layer molten TSC agar or agar as an overlay on the membrane can be used.
Incubate the plates anaerobically at 44±1ºC 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 sulfida precipitate.(H2S+)
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 25ºC

Microbiological control

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

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 ºC during 21 ± 3h.

Microorganism	Growth
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good ≥ 50%. Black colonies
Clostridium perfringens ATCC® 10543, WDCM 00174	Good ≥ 50%. Black colonies
Bacillus subtilis ATCC® 6633, WDCM 00003	Inhibited

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

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: 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 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.
. 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

Storage conditions: 2-14ºC
Alternatively the plates may also be stored at the range of 2 - 25ºC, with a proper performance of the medium, but some precautions must be taken into account:
-In the range of 2 - 8 ºC avoid direct contact with surfaces that can freeze product.
-In the range of 15 - 25 ºC, dehydration control must be taking in account.