

Specification

A sterile selective supplement used for isolation and presumptive identification of *Clostridium perfringens*, according to ISO Standards and other regulations.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 °C

Composition

Compositon (g/vial)	
D-Cycloserine.....	0.100

NOTE : Each vial is sufficient to supplement 250 ml of medium Base: TSC Agar Base.

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....6 ml

Description /Technique

Description:

D-cycloserine selective supplement is added to TSC Agar in order to obtain a final selective medium which has the advantage to simplify the counting of plates with high numbers of colonies because smaller colonies of *C.perfringens* are formed. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction made by *Clostridium perfringens* spp. that produce black colonies in TSC agar.

Technique:

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

Reconstitute the vial with 6ml of sterile diluent in aseptic conditions and add it to 250 ml of melted Agar base cooled to 50°C.

Do not overheat once supplemented.

Pour the complete medium into Petri dishes (or tubes) and, once solidified on a flat surface, spread the plates either by streaking by spiral method or dilution banc.

Incubate the plates in anaerobic atmosphere at $35 \pm 2^\circ\text{C}$ for 20-24h. To obtain a more selective medium, incubated at $44^\circ\text{C} \pm 1$.

Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications.

After incubation, count all the colonies that have appeared onto the surface of the agar.

C.perfringens grows in black colonies, due to the iron sulfide precipitation.

Presumptive isolation of *Clostridium perfringens* must be confirmed by further microbiological and biochemical tests.

Quality control**Physical/Chemical control**

Color : White-Gray

Microbiological control

Add 1 vial to 225/ 250 ml of medium base. DO NOT HEAT once supplemented

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

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

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

Microorganism

Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237

Clostridium perfringens ATCC® 10543, WDCM 00174

Bacillus subtilis ATCC® 6633, WDCM 00003

Growth

Good - black colonies

Good - black colonies

Inhibited

Sterility control

Add 5ml of the sample to 100 ml of TSB and to 100 ml Thioglycollate.

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

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