

### 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	23x60 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

Reconstitute the original freeze-dried vial  
by adding

Sterile Distilled Water.....6 ml

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

### 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 ± 2°C for 20-24h. To obtain a more selective medium, incubated at 44° C ± 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  $44 \pm 1$  °C during  $21 \pm 3$ 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 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

**Bibliography**

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