

CLOSTRIDIUM PERFRINGENS AGAR BASE
D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT
KANAMYCIN-POLYMYXIN B ANTIMICROBIC SUPPLEMENT

Medium base to be used with selective and enrichment supplements
for the enumeration of *C. perfringens* in foodstuffs.

TYPICAL FORMULAS**Clostridium Perfringens Agar Base (g/l)**

Tryptose	15
Beef Extract	5
Soy Peptone	5
Yeast Extract	5
Sodium Metabisulphite	1
Ferric Ammonium Citrate	1
Agar	13

D-Cycloserine Antimicrobial Supplement (vial contents for 500 ml of medium)

D-Cycloserine	200 mg
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Kanamycin-Polymyxin B Antimicrobial Supplement (vial contents for 500 ml of medium)

Kanamycin	6 mg
Polymyxin B Sulphate	15.000 IU

Directions

Suspend 22.5g of Clostridium Perfringens Agar Base in 500ml of cold distilled water, heat to boiling and autoclave at 121 °C for 15 minutes. For the preparation of Egg Yolk Free Tryptose Sulphite Cycloserine (TSC) Agar add the contents of one vial of D-Cycloserine Antimicrobial Supplement. (cat. N° 4240002) reconstituted with 5 ml of sterile distilled water. For the preparation of Shahidi Ferguson Perfringens (SFP) Agar add the contents of one vial of Kanamycin Polymyxin B Antimicrobial Supplement (code 4240005) reconstituted with 5 ml of sterile distilled water. Mix well and pour into sterile Petri dishes. Both the media may be prepared also by adding 50ml/litre of Egg Yolk Emulsion (code 42111601) to 450 ml of selective medium, cooled to 50°C.

Final pH 7.6 ± 0.2

Description

C. perfringens causes enteric disorders characterised by abdominal pain and diarrhoea generally without fever or vomiting. The illness is usually provoked by ingestion of inadequately cooked or reheated meats, such as meat pies, stews, gravies, etc, contaminated by soil or faeces. The food poisoning can be diagnosed using quantitative anaerobic cultures to test foods and faeces. The minimum infective dose is 10⁵ cells/g of food.

For the isolation and enumeration of *C. perfringens* the most widespread methods used in Europe and North America are those of Harmon at al. (Tryptose Sulphite Cycloserine -TSC Agar) and of Shahidi-Ferguson with Kanamycin and Polymyxin B (SFP Agar) with or without Egg Yolk Emulsion.

Technique

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned. ISO 6887 recommends the use of peptone salt (see Maximum Recovery Diluent cat. N° 401691) as general diluent for foods and animal foodstuffs.
2. Transfer by means of sterile pipettes 1ml of the test sample (if liquid) or 1ml of the initial suspension and 1ml of each decimal dilution, in duplicate, to the centre of empty Petri dishes.
3. Pour 15 – 20ml of Egg Yolk free TSC Agar into each dish and mix well with the inoculum.
4. When the medium has solidified add an over layer of 10ml of the same TSC Agar.
5. Allow to solidify and incubate in anaerobic jars or other suitable containers at 37°C for 20 hours. Longer incubation may result in excess blackening along the bottom rim of the plates.

6.Count the black colonies on the plates containing between 15 and 150 characteristic colonies. If parts of the plates are completely blackened count the colonies at the next higher dilution even if their number may be less than 15.

To confirm the presence of *C. perfringens* the following tests are recommended:

- reduction of nitrate to nitrite (+)
- motility test (-)
- gelatin liquefaction (+)

User quality assurance (37°C - 24 h - Anaer.)

Productivity control

With Antimicrobial Supplement: *C.perfringens* ATCC 13124: growth, black green colonies

Without Antimicrobial Supplement: *C.sporogenes* ATCC 19404: growth, black green colonies

Selectivity control

With Antimicrobial Supplement: *E.coli* ATCC 25922 : inhibited

Specificity control

Without Antimicrobial Supplement : *E.coli* ATCC 25922 : growth, white colonies

Storage

Dehydrated medium: 10-30°C

Selective supplements: 2-8°C

User prepared medium base in flasks: 1month at 2-8°C

References

- Haushild, A.H.W. & Hilaheimer, A. (1974). App. Microbiol. **27**, 78
- Harmon, S.M., Kautter, O.A. & Peeler, J.T. (1971). App. Microbiol., **22**,688
- Shehidi, SA. & Ferguson, AR. (1971). App. Microbiol., **21**, 500-606

Packaging

4013072 Clostridium Perfringens Agar Base,	500 g (11.1 l)
4240002 D-Cycloserine Antimicrobial Supplement,	10 vials, each for 500 ml of basal medium.
4240005 Kanamycin-Polymixin B Antim. Supp.	10 vials, each for 500 ml of basal medium.