

Clostridium perfringens Agar Base

Basal medium for detection of *C. perfringens* from clinical specimens and other materials according to ISO 7937 and ISO 14189.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	15.0
Enzymatic Digest of Soya	5.0
Yeast Extract	5.0
Sodium Disulfite Anhydrous	1.0
Iron(III) Ammonium Citrate	1.0
Agar	15.0
Final pH 7.6 ± 0.2 at 25°C	

DESCRIPTION

Clostridium perfringens Agar Base is a basal medium used either on its own or with selective agents for the presumptive identification of *Clostridium perfringens* from clinical specimens, food, water and environmental samples.

D-Cycloserine can be added to this culture medium to comply with the specifications given by ISO 7937, ISO 14189 and APHA.

When supplemented with polymyxin B and kanamycin, the medium conforms to the formulation developed by Shahidi and Ferguson.

If used without any supplement added, this medium is known as Iron Sulfite Agar and recommended by ISO 15213 for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.

PRINCIPLE

Enzymatic digest of casein and enzymatic digest of soya provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a rich source of vitamins of B-group. Ferric ammonium citrate and sodium metabisulfite are H₂S indicators. Agar is the solidifying agent. Clostridia reduce sulfite to sulfide which reacts with iron to form a black iron sulfide precipitate.

One of the following selective supplements can be added to the medium:

- *Clostridium perfringens* (T.S.C.) Supplement (ref. 81011), containing D-Cycloserine as inhibitor of accompanying flora;
- Kanamycin/Polymyxin B Supplement (ref. 81031);
- D-Cycloserine 4-MUP Supplement (ref. 81098), which contains in addition to D-Cycloserine, 4-Methyl-umbelliferyl-phosphate (MUP) to detect acid phosphatase by its fluorescence under UV light.

PREPARATION

Suspend 42.0 g of powder in 1 liter of distilled or deionized water (*). Heat to boiling until completely dissolved. Autoclave at 121°C for 15 minutes. Cool to 45-50°C. Aseptically, add rehydrated content of 2 vials (10 ml) of:

- *Clostridium perfringens* (T.S.C.) Supplement for TSC (Tryptose Sulfite Cycloserine Agar) Agar or
- Kanamycin/Polymyxin B Supplement for SFP (Shahidi-Ferguson) Agar or
- D-Cycloserine 4-MUP Supplement for TSC Agar with MUP.

Mix well and pour in Petri dishes.

(*) If desired, 100 ml Egg Yolk Emulsion (ref. 80219) can also be added after sterilization to detect lecithinase activity (not indicated in ISO 7937 or ISO 14189 either). Take this into account for calculating the final volume of 1.01 liters. For either TSC Agar or SFP Agar used as an overlay, the egg yolk emulsion is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

TECHNIQUE

Inoculate the medium by streak/spread plating, pour-plate method or using the membrane filtration technique. Incubate plates anaerobically at 37 ± 1°C (food examination) or 44 ± 1°C (water analysis) for 18-24 hours.

INTERPRETATION OF RESULTS

Count all black colonies on the plates. For confirming presumptive colonies of *Clostridium perfringens* the following tests are recommended: reduction nitrate to nitrite (+), motility test (-), gelatine liquefaction (+).

On TSC Agar with MUP, fluorescence is detected with an UV lamp: light blue fluorescing black colonies indicate *C. perfringens*.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 14189: 2011. Water quality – Enumeration of *Clostridium perfringens* – Method using membrane filtration.
3. ISO 7937:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Clostridium perfringens* - Colony-count technique.
4. ISO 15213:2003. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
5. Rapporti ISTISAN 07/5 ISSA 005B Rev.00. Determinazione di *Clostridium perfringens* (solo su acque provenienti o contaminate da acque superficiali).
6. Downes F.P., and K. Ito (2001) Compendium of methods for the microbiological examination of foods. 4thed. American Public Health Association, Washington, D.C.
7. Haushild, A.H.W., and A. Hilsheimer (1974) Evaluation and modifications of media for enumeration of *C. perfringens*. App. Microbiol. 27:78.
8. Harmon, S.M., O.A. Kautler and J.T. Peeler (1971) Improved medium for enumeration of *Clostridium perfringens*. App. Microbiol. 22:688.
9. Shahidi, SA. and AR Ferguson (1971) App. Microbiol. 21:500-606.

PRODUCT SPECIFICATIONS

NAME

Clostridium perfringens Agar Base

PRESENTATION

Dehydrated medium

STORAGE

10-30°C

PACKAGING

Ref.	Content	Packaging
610207	500 g	500 g of powder in plastic bottle
620207	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM

7.6 ± 0.2

USE

Clostridium perfringens Agar Base is a medium used with supplements for the selective isolation and differentiation of *C. perfringens* from clinical specimens, food, water and environmental samples according to ISO 7937, ISO 14189 and APHA

TECHNIQUE

Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM

Dehydrated medium

Appearance: free-flowing, homogeneous

Colour: beige

Prepared medium

Appearance: clear (opaque if egg yolk emulsion has been added)

Colour: amber

SHELF LIFE








4 years

QUALITY CONTROL

- Control of general characteristics, label and print
- Microbiological control
 Complete medium: TSC Agar
 Inoculum for productivity: 50-100 CFU
 Inoculum for selectivity: 10⁴-10⁶ CFU
 Incubation Conditions: 20 ± 2 h at 37 ± 1°C ^(a) and/or 18-24 at 44 ± 1°C ^(b), in anaerobiosis

Microorganism		Growth	Colony Appearance
<i>Clostridium perfringens</i> ^{a,b}	WDCM 00007	Good	Black colonies
<i>Escherichia coli</i> ^a	WDCM 00012	Inhibited	---
<i>Bacillus subtilis</i> ^b	WDCM 00003	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by	 Fragile, handle with care
REF Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult instructions for use	 Do not reuse

Distribué par :

Z.A de Gesvrine - 4 rue Képler - B.P.4125
 44241 La Chapelle-sur-Erdre Cedex - France
 t. : +33 (0)2 40 93 53 53 | f. : +33 (0)2 40 93 41 00
 commercial@humeau.com

