

**Specification**

Solid medium used for the biochemical confirmation of *Clostridium perfringens*, according to ISO 7937 standard.

**Formula \* in g/L**

Casein peptone.....	15,00
Yeast Extract.....	10,00
Lactose.....	10,00
Gelatin.....	120,00
Phenol red.....	0,05

Final pH 7,50 ±0,2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

**Directions**

Dissolve 155 g of powder in 1 L distilled water, heating if necessary. Adjust pH to 7.5 ± 0.2 (add 10-15 ml of sodium carbonate solution at 10%). Dispense in tubes in suitable volumes and sterilize at 121°C for 15 minutes. If not used the same day, store in the refrigerator. Just prior to use heat in a boiling water bath or flowing steam for 15 minutes, then cool rapidly to the incubation temperature. Discard unused medium 1 month after preparation. Note: Before preparing the medium, shake the container vigorously until the powder is homogeneous. The different granulometry of gelatin, can cause disaggregation of the medium in transport.

**Description**

This medium with the Nitrate Motility Medium (Art. No. 03-612) are used in the confirmation technique for *Clostridium perfringens* according to the 7937:2004 ISO Standard.

**Technique**

Inoculate each selected colony from the Tryptose-Sulfite-Cycloserine Agar into the Lactose Gélatine Medium and incubate under anaerobic conditions for 24 hours à 37°C. Examine the tubes of Lactose Gélatine Medium for the presence of gas and a yellow colour due to acid formation indicating fermentation of lactose. Chill the tubes for 1 hour à 5 ± 3°C and check for Gélatine liquefaction. If the medium has solidified, re-incubate for an additional 24 hours and again check for Gélatine liquefaction.

**Interpretation**

Non-motile bacteria that produce black colonies in Tryptose Sulfite Cycloserine Agar liquefy Gélatine in 48 hours are considered to be *Clostridium perfringens*.

Cultures that show a faint reaction for nitrite should be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.

**Quality control**

**Incubation temperature:** 35°C ±2,0                      **Incubation time:** 24-48 h

**Inoculum:** Pure cultures using and inoculating needle

Microorganism	Growth	Remarks
<i>Staphylococcus aureus</i> ATCC® 25923	Poor	-
<i>Escherichia coli</i> ATCC® 25922	Good	-
<i>Clostridium perfringens</i> ATCC® 10543	Good	L (+) Gas (+) Gelatinase (+)
<i>Clostridium perfringens</i> ATCC® 13124	Good	L (+) Gas (+) Gelatinase (+)
<i>Clostridium sporogenes</i> ATCC® 11437	Good	L (+) Gas (D) Gelatinase (+)

**References**

- ISO 7937 Standard (2004) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony count technique.
- ISO 11133:2014/ Adm 1:2018/ Adm 2:2020/ Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

**Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).