Technical Data Sheet

Tryptose cycloserine agar (TCA) base

Art. **85054.0500**

DEHYDRATED MEDIUM

Intended use

Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*.

Formula * - Composition in g/L

Tryptose..... 15.00
Soya peptone..... 5.00
Yeast extract..... 5.00
Sodium pyruvate..... 0.5
Ferric ammonium citrate..... 1.00
Agar.....19.00

Final pH 7.6 ±0.2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Instructions for preparation

Suspend 45.5 g of powder in 1l of purified water. Heat to boiling and distribute into suitable containers, but not more than 250 ml in each one. Sterilize in the autoclave at 121°C for 10 minutes. Let it cool to 60°C and add 1 flask of D-Cycloserine Selective Supplement (Art. 928330NL) to every 250 ml of medium. Mix well and pour it into containers. If it is desired to include egg yolk, then add Egg Yolk Sterile Emulsion (Art. 351430ZF) in a concentration of 80 ml/l, simultaneously to the antibiotic.

Principle of the method and general information

Tryptone Cycloserine Agar (TCA) is used for incubating the membranes through which the water samples with suspected presence of *Clostridium perfringens* have filtered.
This medium is a modification of Agar Tryptose Sulfite Cycloserine (TSC Agar), proposed by Watkins and Sartory (2014), which was deleted sodium metabisulphite (necessary for the production of sulfur) and added sodium pyruvate to improve recovery of stressed cells.
Cyclosporin solution is very unstable. A cycloserine solution in phosphate buffer at pH 8 (di-potassium phosphate 16.73 g / L mono-potassium phosphate and 0.52 g / L) can be prepared and it may be used for 5-7 days only if kept refrigerated. Furthermore, it should be noted that the cycloserine not tolerate temperatures above 100 ° C, so it is advisable to prepare the complete medium for the right amount of plates that are to be used immediately.

Instruction for use

The membranes used for filtration of the water sample (appropriately diluted to obtain counts 10-100 CFU / 100 mL) was deposited on the surface of the culture medium avoiding the formation of bubbles and incubated in anaerobiosis at 44 ° C for 21 ± 3 hours.
All the colonies grown in these conditions on the TCA are considered presumptively as Clostridium perfringens. After counting, the membrane is transferred from the plate TCA to a pad or filter-paper impregnated with Reagent for Alkaline Phosphatase and left there for 10 minutes. All colonies that are colored purple during this period are considered confirmed as Cl. perfringens.

Quality control

Incubation temperature: 44°C ±1,0 **Incubation time:** 21 ± 3 h

Inoculum: Practical range 10-30 CFU (Productivity) / 10^4-10^6 CFU (Selectivity). MF Methods. Anaerobiosis. Double layer.

Microorganism	Growth	Remarks
Clostridium perfringens ATCC® 10543	Productivity > 0.50	White - cream colonies
Clostridium perfringens ATCC® 13124	Productivity > 0.50	White - cream colonies
Bacillus subtilis ATCC® 6633	Inhibited	-

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Storage conditions

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

Ordering information

85054.0500 Tryptose cycloserine agar (TCA) base Bulk of 500 g.
Note: For supplements see the section - Instructions for preparation.