Condalab

Reference: 4661 **Technical Data Sheet**

Product: # TRYPTOSE SULFITE CYCLOSERINE (TSC) AGAR BASE

Specification

Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*, according to ISO Standards.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottle 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml Non injectable cap.	12 months	8-25°C

Composition

Composition (g/l):

Tryptose	15.0
Peptone from soymeal	5.00
Yeast extract	5.00
Sodium disulfite	1.00
Ammonium iron III citrate	1.00
Agar	18.0

Description /Technique

Description

The medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine. Cycloserine has been found more selective for Clostridium perfringens, and reduces the production of diffuse blackening. Clostridium perfringens is more resistant to cycloserine than to sulfadiazine, polymyxin and neomycin, hence reducing the dosage. The presence of sodium meta-bisulfite and ferric ammonium citrate allow three differential characteristics of this anaerobic species to be verified with just one assay. These characteristics are sulfite reduction, growth at 46°C and cycloserine resistance.

Cycloserine does not tolerate temperatures above 100°C and its stability in a solution is variable. Therefore, it is advisable to prepare the exact number of plates that are going to be used.

A solution of cycloserine in phosphate buffer at pH 8,0 may be prepared (Di potassium phosphate 16,73 g/L and mono-potassium phosphate 0,52 g/L) and if it is maintained refrigerated, can be used for approx. 5 days.

Technique

To use, the contents of the bottle should be poured into plates. The melting of the culture medium should be carried out according to the manufacturer's instructions, either in a water bath (100°C) or microwave oven. Add the Cycloserine at a concentration of 400 mg / L, before pouring the culture medium on the plates or tubes.

Never apply direct heat to melt a medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source. Before melting any medium loosen the screwcap of the container to avoid breaking the container. The medium should be melted only once and used. Media with agar should not be melted repeatedly as their characteristics change with each remelting. Overheating should be avoided as much as prolonged heating, especially with regard to media with an acidic or alkaline pH. Once melted pour the plates using aseptic techniques.

To inoculate, follow standard laboratory methods or the applicable norms. Spiral plate method, streak plating, econometric methods, dilution banks, spread plating etc...

The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis. After incubation at 44±1°C for 23±1h, proceed to enumerate the black colonies that appear in the plate.

Note: An alternative method would be the use of TSC Base Medium + Selective Supplement MUP (25 mg) / 200 ml medium. This reagent allows the identification of *Cl perfringens* by their Fluorescence.

Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7.6 ± 0.2 at 25°C

Microbiological control

Before addition of Cycloserine; Quality control according to ISO 11133:2014/ Adm 1 : 2018.
Melt Medium - Prepare Plates - Spiral Spreading: Practical range 100±20 CFU; Min. 50 CFU (Productivity)
Anaerobiosis. Incubation at 44 ± 1 °C during 21 ± 3h.

Microorganism	Growth
Clostridium perfringens ATCC® 10543, WDCM 00174	Good - black colonies
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good - black colonies
Bacillus subtilis ATCC® 6633, WDCM 00003	Inhibited

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH
Check at 7 days after incubation in same conditions

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