HiCrome Clostridial Agar Base

M2026

For selective isolation and presumptive identification of *Clostridium* species

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	10.000
Dextrose	1.000
Sodium chloride	5.000
Sodium thioglycollate	0.500
Chromogenic mixture	3.310
Agar	13.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 47.81 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of one vial of Perfringens supplement II (FD012). Mix well and pour into sterile Petri plates.

Principle And Interpretation

One of the major species of anaerobic bacteria to cause disease in humans is *Clostridium* . *Clostridium* species cause tetanus and gas gangrene that ultimately leads to tissue damage. Another *Clostridium* species produces the lethal botulinum toxin, the causative agent of botulism (1). Clostridial Agar formulated by Vera is recommended for the selective isolation of pathogenic Clostridia form mixed flora (2). HiCrome is the modification for chromogenic differentiation.

Tryptone and yeast extract provide the essential nutrients, mainly the nitrogen compounds. Dextrose serves as the carbon or fermentable carbohydrate source. Sodium thioglycollate is the reducing agents that help to create low oxidation-reduction potential enabling the growth of Clostridia.Also the media is well supplemented to support luxuriant growth of Clostridium species.The selective supplements inhibits other enteric bacteria.

The ideal method of inoculation of Clostridial Agar is direct inoculation of sterile, cooled medium with the specimen (in tubes). Alternatively agar plates of the medium can also be inoculated by streaking.

Quality Control

Appearance
Cream to beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel

Colour and Clarity of prepared medium
Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.78% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48hours(under anaerobic condition).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium perfringens NCTC 8237	50-100	luxuriant	>=50%	Pale yellowish green
Clostridium perfringens ATCC 13124	50-100	luxuriant	>=50%	Pale yellowish green
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50%	Pale green-bluish green
Clostridium sporogenes ATCC 19404	50-100	luxuriant	>=50%	Pale green-bluish green
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life

Store dehydrated powder and the prepared medium at 2-8°C in tightly closed container. Use before expiry date on the label.

Reference

1. Alcamo E. I., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. Vera, 1962, Presented Pa. Soc. Med. Tech., York, Pa.

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