Clostridial Agar

M497

Clostridial Agar is recommended for the selective isolation of pathogenic Clostridia from mixed flora.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Dextrose	6.000
Sodium chloride	2.500
Sodium thioglycollate	1.800
L-Cystine	0.250
Sodium formaldehyde sulphoxylate	1.000
Neomycin sulphate	0.150
Sodium azide	0.200
Agar	14.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.4 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118°C for 15 minutes. Mix well and pour into sterile Petri plates.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

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). The media is well supplemented to support luxuriant growth of *Clostridium* species.

Casein enzymic hydrolysate and papaic digest of soyabean meal provide the essential nutrients, mainly the nitrogen compounds. Dextrose serves as the carbon or fermentable carbohydrate source. L-cystine is an amino acid, which promotes the growth of Clostridia. Sodium thioglycollate and sodium formaldehyde sulphoxylate are the reducing agents that help to create low oxidation-reduction potential enabling the growth of Clostridia.

Accompanying enteric bacteria including *Proteus* , *Pseudomonas* and *Bacillus* species are inhibited by neomycin sulphate and sodium azide incorporated in the medium. 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 1.45% Agar gel

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

Reaction
Reaction of 4.64% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°Cfor 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50%
Clostridium tetani ATCC 10779	50-100	luxuriant	>=50%
Escherichia coli ATCC 25922	>=10^3	inhibited	0%
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. 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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