



Clostridial Agar

M497

Intended use

Recommended for the selective isolation of pathogenic Clostridia from mixed flora.

Composition**

Ingredients	g / L
Tryptone	17.000
Soya peptone	3.000
Dextrose	6.000
Sodium chloride	2.500
Sodium thioglycollate	1.800
L-Cystine	0.250
Sodium formaldehyde sulphonylate	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 purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118°C for 15 minutes. Cool to 45-50°C. 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 from mixed flora (2). The media is well supplemented to support luxuriant growth of *Clostridium* species.

Tryptone and soya peptone provide the nitrogenous and carbonaceous compounds, long chain amino acids and other 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 sulphonylate 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.

Type of specimen

Clinical samples - faeces, wounds, Food and dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitation:

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

3. Further biochemical test must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	≥50%
<i>Clostridium sporogenes</i> ATCC 11437	50-100	luxuriant	≥50%
<i>Clostridium tetani</i> ATCC 10779	50-100	luxuriant	≥50%
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%

* - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

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5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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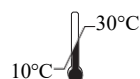
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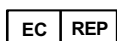
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Storage temperature



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