



Reinforced Clostridial Agar

M154

Reinforced Clostridial Agar is used for the cultivation and enumeration of *Clostridia* and other anaerobes.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Beef extract	10.000
Yeast extract	3.000
Dextrose	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Starch, soluble	1.000
L-Cysteine hydrochloride	0.500
Agar	13.500
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.00 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. Mix well and dispense as desired.

Principle And Interpretation

Hirsch and Grinstead formulated Semisolid Reinforced Clostridial Medium and found that clostridia from small amount of samples could be grown well on this medium with higher viable cell counts (1). This medium can also be used for growing anaerobic and facultative bacteria (2). Barnes et al used a solid (agar) version of the medium (2) to develop vegetative cells in assays of *Clostridium perfringens*. Reinforced Clostridial Medium was used in the enumeration of clostridia from food (3).

Reinforced Clostridial Agar contains casein enzymic hydrolysate and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic byproducts. Cysteine hydrochloride is the reducing agent. Sodium acetate acts as a buffer. This medium can be made selective by addition of 15-20 mg polymyxin B per litre of media (3).

Material to be examined is homogenized in a stomacher, and dilutions are prepared. For enumeration, pour plate technique is employed. Incubate anaerobically. If tubes are used, they are covered with sealing Anaerobic Agar immediately after the Reinforced Clostridium Medium has solidified.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M154: Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 40 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Bacteroides fragilis</i> ATCC 23745	50-100	good - luxuriant	>=50%
<i>Bacteroides vulgatus</i> ATCC 8482	50-100	good - luxuriant	>=50%
<i>Clostridium butyricum</i> ATCC 13732	50-100	good - luxuriant	>=50%
<i>Clostridium perfringens</i> ATCC 13124	50-100	good - luxuriant	>=50%

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. Hirsch A. and Grinstead C., 1954, J. Dairy Res. 21:101.
2. Barnes E. M., Despaul J. E. and Ingram M., 1963. J. Appl. Bacteriol. 26:415.
3. Barnes E. M. and Ingram J. E., 1956. J. Appl. Bacteriol. 19:117.

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