Reinforced Clostridial Agar

Cat. 1087

For the cultivation and enumeration of Clostridium spp and other anaerobes

Practical information

Aplications	Categories
Non selective enumeration	Anaerobes
Detection	Clostridium

Industry: Food

Principles and uses

Reinforced Clostridial Agar is recommended for the cultivation and enumeration of anaerobes, particularly Clostridium, and other microorganisms in foods.

Hirsch and Grinstead formulated Reinforced Clostridial Medium (Cat. 1007) and found that this medium is superior to others in supporting growth and producing high cell counts of Clostridia. When incubated anaerobically, this medium grows various anaerobes and other bacteria. Barnes and Ingram also demostrated that it can be used to develop vegetative cells in assays of Clostridium perfringens.

Peptone and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Starch in the medium acts as a growth factor, probably functioning like a colloid protector and neutralizes toxic products that form during the development of the organisms. L-Cysteine hydrochloride is the reducing agent and Sodium acetate is the buffer. Bacteriological agar is the solidifying agent.

Since it is a non-selective enrichment medium, it allows the growth of various anaerobic microorganisms and facultative bacteria when incubated anaerobically.

Formula in g/L

Bacteriological agar	12,5	Beef extract	10
Cysteine hydrochloride	0,5	Dextrose	5
Peptone	10	Sodium acetate	3
Sodium chloride	5	Soluble starch	1
Yeast extract	3		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC and, if desired, add 0,02 g/l of Polymyxin B in a sterile filtered solution.

Instructions for use

Poured plate method:
- Deposit 1 ml of the initial suspension and/or diluted sample in an empty Petri dish.
- Add 12-15 ml of agar cooled to 44-47 ºC in each Petri dish and mix gently moving the plate.

- Allow the plates to solidify and incubate in an inverted position at a temperature of 35±2 ºC for 40-48 hours in anaerobiosis.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Cream	Clear amber, slightly opalescent	6,8±0,2

Microbiological test

Incubation conditions: (35±2 ºC, anaerobiosis / 40-48 h).

Microorganisms	Specification
Clostridium perfringens ATCC 12916	Good growth
Clostridium perfringens ATCC 13124	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Barnes, EMJE Despaul and M. Ingram 1963. The behavior of a food poisoning strain of Clostridium welchii in beef. J. Appl. Bacteriol 26:415.
MacFaddin JF. 1985 Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 660-668. Williams & Wilkins, Baltimore MD.