

Reinforced Clostridial Medium EP/USP

Cat. 1007

For the cultivation and enumeration of Clostridium and other anaerobes.

Practical information

Applications	Categories
Non selective enumeration	Anaerobes
Detection	Clostridium

Industry: Pharmaceutical/Veterinary

Regulations: USP / European Pharmacopoeia

Principles and uses

Reinforced Clostridial Medium is a semisolid medium. It is recommended for the cultivation and enumeration of anaerobes, particularly Clostridium and other microorganisms, in foods and clinical specimens.

It was formulated by Hirsch and Grinstead in 1954. Their work demonstrated that the medium outperformed other media in supporting the growth of Clostridium from small inoculum and produced higher viable cell counts.

Peptone and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. yeast extract is the 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.

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

The European Pharmacopoeia, USP, recommends the Reinforced Clostridial Medium in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" for the testing of Clostridia in products.

Formula in g/L

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

Preparation

Suspend 38 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°C for 15 minutes. Cool to 45-50 °C and, if desired, add 0,02 g/l of polymyxin B in a sterile filtered solution.

Instructions for use

Test of specified microorganisms (Clostridia) according to European Pharmacopoeia:

- Used without blood.
- Inoculate and incubate, under anaerobic conditions, the Reinforced Clostridial Medium at a temperature of 30-35 °C for 48 hours.
- Make subcultures on Columbia Agar (Cat. 1104).
- Add 20 mg / liter of gentamicin if necessary
- Incubate and incubate, under anaerobic conditions, at 30 - 35°C for 48 hours
- Colonies grown in anaerobiosis that are negative catalases indicate presence of clostridia. This result must be confirmed by biochemical tests.

Quality control

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

Microbiological test

According to European Pharmacopoeia; Clostridium sporogenes:
Incubation conditions: (30-35 °C, under anaerobic conditions / 48-72 h).
Inoculation conditions: (<=100 CFU).

Rest of strains:
Incubation conditions: (30-35 °C, under anaerobic conditions / 48 h).

Microrganisms	Specification
Clostridium perfringens ATCC 13124	Good growth
Clostridium sporogenes ATCC 19404	Good growth
Clostridium sporogenes ATCC 25781	Good growth
Clostridium difficile ATCC 9689	Good growth

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

Andrews, W.H. (ed) 1995. Microbial methods p. 1-119. In Official methods of analysis of AOAC International. 16th ed.
European Pharmacopoeia. 9.0.