REINFORCED CLOSTRIDAL MEDIUM EUROPEAN PHARMACOPOEIA, USP

CAT Nº: 1007 For the cultivation and enumeration of *Clostridium* and other anaerobes

FORMULA IN g/l

Beef Extract	10.00	Sodium Acetate	3.00
Peptone	10.00	Soluble Starch	1.00
Glucose Monohydrate	5.00	L-Cysteine Hydrochloride	0.50
Sodium Chloride	5.00	Bacteriological Agar	0.50
Yeast Extract	3.00		

Final pH 6.8 ± 0.2 at 25ºC

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 45-50ºC and, if desired, add 0.02 g/l of Polymyxin B in a sterile filtered solution. The prepared medium should be store at 2-8°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

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.

European Pharmacopoeia recommends in Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified micro-organisms" the following preparations for the sample:

Take two equal portions corresponding to no less than 1 gram or 1 ml of the product to be examined. Heat one portion at 80ºC for 10 minutes and cool rapidly. Do not heat the other portion. Transfer 10 ml of each of the mixed portions to two containers, containing 100ml of reinforced medium for clostridia. Incubate under anaerobic conditions at 30-35ºC for 48 hours. After incubation, make subcultures from each tube on Columbia Agar (Cat. 1104) and incubate under anaerobic conditions at 30-35ºC for 48-72 hours.

Interpretation: The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This is confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30-35ºC under anaerobic conditions and observed after 48 hours

Microorganisms	Growth
Clostridium bifementans ATCC 19299	Good
Clostridium difficile NCTC 11024	Good
Clostridium perfringens ATCC 13124	Good
Clostridium perfringens ATCC 10543	Good
Clostridium sporogenes ATCC 19404	Good

BIBLIOGRAPHY

Andrews, W.H. (ed) 1995. Microbial methods p. 1-119. In Official methods of analysis of AOAC International. 16th ed.
European Pharmacopoeia. 7.0

EP

STORAGE

Once opened keep powdered medium closed to avoid hydration.