Reinforced Medium for Clostridia

M443B

Reinforced Medium for Clostridia is used for the enrichment of Clostridia from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of BP.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Beef extract	10.000
Yeast extract	3.000
Glucose monohydrate	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Soluble starch	1.000
Cysteine hydrochloride	0.500
Agar	0.500
pH after sterilization (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.54 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

Reinforced Medium for Clostridia was formulated by Hirsch and Grinsted (1). This media is prepared as described in BP (3) and is in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (2,3,4,5,8). It is recommended for sterility checking of non-sterile products, nutritional and dietary supplements. It can be used to initiate growth from small inocula and to obtain the highest viable count of clostridia. Barnes and Ingram used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens* (6, 7). It can be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese, for enumeration of Clostridia in tube dilution counts or for preparation of plates for isolation (7). Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media. These are enriched but non-selective media.

Peptone, yeast extract and beef extract provide all the necessary nutrients for the growth of clostridia. Glucose monohydrate is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. Cysteine hydrochloride acts as reducing agent. Small amount of soluble starch removes toxic metabolites from the medium. Sodium acetate also acts as a good buffering agent. Small quantity of agar keeps the medium semi solid and helps in maintaining anaerobic conditions.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution in tubes.

pH
6.60-7.00

Growth Promotion Test
Growth promotion was carried out in accordance with the harmonized method of BP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for <=48 hours

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

Cultural Response

M443B: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30-35°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Growth Promoting				
Clostridium sporogenes ATCC 11437	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 19404	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Bacteroides vulgatus ATCC 8482	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Additional Microbiological testing				
Bacteroides fragilis ATCC 23745	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
Clostridium sporogenes ATCC 13124	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs

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 and Grinsted, 1954, J. Dairy Res., 21:101.
2.The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
4.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5.Japanese Pharmacopoeia, 2008.
6.Barnes and Ingram, 1956, J. Appl. Bact., 19:117.
7.Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

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