Reinforced Clostridial Broth

M443

Reinforced Clostridial Broth 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
Starch, soluble	1.000
L-Cysteine hydrochloride	0.500
Sodium acetate	3.000
Agar	0.500
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes. Mix well and dispense as desired.

Principle And Interpretation

Reinforced Clostridial Broth is formulated by Hirsch and Grinsted (1). 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* (2). 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 (3). Other spore forming anaerobes, *Streptococci* and *Lactobacilli* also grow in this media. This is a nonselective enrichment media.

Casein enzymic hydrolysate, yeast extract, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia* . Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. This media can be made selective by addition of 15-20 mg Polymyxin B per litre of media (1).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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

Reaction
Reaction of 3.8% w/v aqueous solution at 25°C . pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 24 - 48hours.

Cultural Response

Organism	Inoculum (CFU)	Growth
Cultural Response		
Clostridium sporogenes ATCC 11437	50 -100	good - luxuriant

Clostridium sporogenes ATCC 19404	50 -100	good - luxuriant
Bacteroides vulgatus ATCC 8482	50 -100	good - luxuriant
Bacteroides fragilis ATCC 23745	50 -100	good - luxuriant
Clostridium perfringenes ATCC 13124	50 -100	good - luxuriant

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.Barnes and Ingram, 1956, J. Appl. Bact., 19:117.
3.Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

Revision : 2 / 2015