



Reinforced Clostridial Broth (without Agar)

M443A

Reinforced Clostridial Broth w/o Agar is recommended for the cultivation of Clostridia.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Meat extract B#	10.000
Yeast extract	3.000
Dextrose	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Soluble starch	1.000
L-Cysteine hydrochloride	0.500
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 37.5 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. Sterilize by autoclaving 115°C (10 lbs pressure) for 15 minutes.

Principle And Interpretation

Reinforced Clostridial Broth w/o Agar 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 these media. These are enriched but nonselective media.

Casein enzymic hydrolysate, yeast extract, meat extract B, 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. These media can be made selective by addition of 15-20 mg Polymyxin B per litre of media (1).

Quality Control

Appearance

Light yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured slightly opalescent solution

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M443A: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth
Cultural Response <i>Bacteroides fragilis</i> ATCC 23745	50-100	good - luxuriant

<i>Bacteroides vulgatus</i> ATCC 50-100 8482	good - luxuriant
<i>Clostridium butyricum</i> ATCC 50-100 13732	good - luxuriant
<i>Clostridium perfringens</i> ATCC 12924	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 Grinstead, 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.

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