



Reinforced Clostridium Medium Base

M1915

For mesophilic anaerobic spore count in milk and cheese by MPN method

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	3.000
Beef extract	10.000
Sodium acetate	5.000
Cysteine hydrochloride	0.500
Soluble starch	1.000
Agar	1.000
Final pH (at 25°C)	5.6±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.50 grams in 1000 ml distilled water. Adjust the pH to 5.5-5.7 with 20% aqueous lactic acid solution. Heat if necessary to dissolve the medium completely. Add 28 ml of 72% Sodium lactate. Mix well and dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Reinforced Clostridial Medium Base is formulated by Wehr & Frank (1). The Mesophilic sporeforming anaerobes belonging to the genus *Clostridia* of food concern are Gram-positive, catalase negative, rods of varying sizes.

The medium 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.

Tryptone, yeast extract, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia*. The small amount of agar helps in maintaining low redox potential for stabilizing the medium.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.05% w/v aqueous solution at 25°C. pH : 5.6±0.1

pH

5.50-5.70

Cultural Response

Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 40 - 48 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)
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Cultural Response

Clostridium perfringens good - luxuriant50-100
ATCC 13124
Clostridium tyrobutyricum good - luxuriant50-100
ATCC 25755
Clostridium butyricum good - luxuriant50-100
ATCC 13732

Storage and Shelf Life

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Standard Methods for the Examination of Dairy products, Wehr & Frank, 2004, (item 8.100).
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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