Differential Reinforced Clostridial Broth Base

M549I

Differential Reinforced Clostridial Broth Base is used for the cultivation of Clostridia from water.

Composition**

Ingredients	Gms / Litre
Tryptose	10.000
Meat extract	10.000
Yeast extract	1.500
Sodium acetate, hydrated	5.000
Starch	1.000
Glucose	1.000
L-Cysteine hydrochloride	0.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Just before use add 0.5 ml filter sterilized solution prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate, to 25 ml of single strength medium or 0.4 ml and 2 ml to 10 ml and 50 ml of double strength medium respectively. Mix well.

Principle And Interpretation

Differential Reinforced Clostridial Agar was originally described by Hirsch and Grinstead (1) to initiate the growth from small inoculum and get a higher Clostridial count. Later, Barnes and Ingram (2) used the medium to develop vegetative cells in assays of *Clostridium perfringens* . This medium is developed for the isolation of sulphite-reducing Clostridia from food and for their enumeration in water by multiple tube method. Differential Reinforced Clostridial Broth is used to determine the count of sulphite reducing bacteria by MPN technique (3).

Tryptose, meat extract, yeast extract, starch, and sodium acetate provide essential nutrients for bacterial metabolism. Glucose is the fermentable carbohydrate and serves as carbon and energy source. L-cysteine hydrochloride acts as reducing agent. Sodium sulphite and ferric citrate are added as indicators. Sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black coloured medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured, clear solution without any precipitate

Reaction
Reaction of 2.9% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed in an anaerobic atmosphere, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate after an incubation at 30-35°C within 1 week.

Cultural Response

Organism	Inoculum (CFU)	Growth	H2S production

Clostridium perfringens ATCC 13124	50-100	good to luxuriant	positive reaction, blackening of medium
Clostridium sporogenes ATCC 11437	50-100	good to luxuriant	positive reaction, blackening of medium

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 A. and Grinstead E., 1954, J. Dairy Res. 21:101
2. Barnes E. M. and Ingram M., 1956, J. Appl. Bacteriol., 19(1):117.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone

Revision : 2 / 2015