Differential Reinforced Clostridial Broth Base, Granulated

GM549

Differential Reinforced Clostridial Medium, granulated is used for the cultivation of *Clostridia* from water.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Beef extract	10.000
Yeast extract	1.500
Starch	1.000
Sodium acetate, hydrated	5.000
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 freshly prepared sulphite reducing solution as below.Mix well and dispense as desired.

Preparation of Sulphite Reducing Solution:Sepaarte solutions of 4% w/v sodium sulphite and 7% w/v ferric citrate should be prepared and filter sterilized. Equal volumes of both solutions should be mixed separately for adding into single strength or double strength media prepared.

Single Strength: For 25 ml, add 0.5 ml sterile sulphite reducing solution.

Double Strength: For 10 ml, add 0.4 ml sterile sulphite reducing solution,
For 50 ml add 2.0 ml sterile sulphite reducing solution.

Principle And Interpretation

Hirsch and Grinstead (1) originally described Differential Reinforced Clostridial Medium 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).

Peptic digest of animal tissue, beef extract, yeast extract, starch, 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 coloured granular medium

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	H_2S production
Cultural Response			
Clostridium perfringens ATCC 13124	50-100	good - luxuriant	positive reaction, blackening of medium
Clostridium sporogenes ATCC 11437	50-100	good - 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

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