Reinforced Clostridial HiVeg™ Broth

MV443

Reinforced Clostridial HiVeg Broth is used for cultivation and enumeration of *Clostridium* species and other anaerobes.

Composition**

Ingredients	Gms / Litre
HiVeg hydrolysate	10.000
HiVeg 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 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Reinforced Clostridial HiVeg Broth is specially developed using HiVeg hydrolysate and HiVeg extract to avoid BSE/TSE risks associated with animal origin peptones and extracts. It is the modification of Reinforced Clostridial Broth which 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 *Clostridium* species. This medium like the conventional medium can be used for diluting an inoculum of vegetative cells of *Clostridium perfringens* as suggested by Barnes and Ingram (2). It can be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese, for enumeration of *Clostridium* species in tube dilution counts. Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media. This is enriched but non-selective medium. HiVeg hydrolysate, yeast extract, HiVeg extract and starch, provide all the necessary nutrients for the growth of *Clostridium* species. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. Cystine hydrochloride is the reducing agent whereas sodium acetate acts as buffer. This medium 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
Yellow coloured solution with slight precipitate 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
Growth promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for 48 hours

Cultural Response

Organism	Inoculum (CFU)	Growth

Cultural Response

Clostridium sporogenes ATCC 11437	50 -100	good - luxuriant
Clostridium sporogenes ATCC 19404	50 -100	good - luxuriant
Clostridium sporogenes NBRC 14293	50 -100	good - luxuriant
Bacteroides fragilis ATCC 23745	50 -100	good - luxuriant
Clostridium sporogenes ATCC 13124	50 -100	good - luxuriant
Bacteroides vulgatus ATCC 8482	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, Grinsted. J Dairy Res. 1954;21.
2.Barnes, Ingram. J Appl Bact. 1956;19.

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