



## Medium 14. Reinforced medium for Clostridia

MM443

Medium 14. Reinforced Medium for Clostridia is used for the enrichment of Clostridia from pharmaceutical products in accordance with Indian pharmacopoeia, 2010.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	10.000
Beef extract	10.000
Yeast extract	3.000
Dextrose monohydrate	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Soluble starch	1.000
Cysteine hydrochloride	0.500
Agar	0.500
pH after sterilization ( at 25°C)	6.8±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 37.54 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

### Principle And Interpretation

Reinforced Medium for Clostridia was formulated by Hirsch and Grinsted (1) and is in accordance with Indian pharmacopoeia (2) the microbial limit testing by harmonized methodology of USP/EP/BP/JP (3,4,5,6). It is recommended for sterility checking of non-sterile products, nutritional and dietary supplements. 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* (7,8). 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 (8). Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media. These are enriched but non-selective media.

Peptone, yeast extract and beef extract provide all the necessary nutrients for the growth of clostridia. Dextrose monohydrate is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. Cysteine hydrochloride acts as reducing agent. Small amount of soluble starch removes toxic metabolites from the medium. Sodium acetate also acts as a good buffering agent. Small quantity of agar keeps the medium semi solid and helps in maintaining anaerobic conditions.

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Light yellow coloured clear solution in tubes.

#### pH

6.60-7.00

#### Growth Promotion Test

Growth promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP/IP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for <=48 hours

#### Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

**Cultural Response**

MM443: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30-35°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
<b>Growth Promoting</b>				
<i>Clostridium sporogenes</i> ATCC 11437	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
<i>Clostridium sporogenes</i> ATCC 19404	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
<i>Bacteroides vulgatus</i> ATCC 8482	50 -100	good - luxuriant	30 -35 °C	<=24 hrs
<b>Additional Microbiological testing</b>				
<i>Bacteroides fragilis</i> ATCC 23745	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
<i>Clostridium sporogenes</i> ATCC 13124	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs

**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.Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India
- 3.The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
- 4.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
- 5.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
- 6.Japanese Pharmacopoeia, 2008.
- 7.Barnes and Ingram, 1956, J. Appl. Bact., 19:117.
- 8.Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

Revision : 1 / 2011

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