



## Reinforced Medium for Clostridia

MH443

Reinforced Medium for Clostridia is used for the enrichment of Clostridia from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (Medium 14).

### Composition\*\*

Ingredients	Gms / Litre
Peptone	10.000
Beef extract	10.000
Yeast extract	3.000
Glucose monohydrate	5.000
Sodium chloride	5.000
Soluble starch	1.000
Cysteine hydrochloride	0.500
Sodium acetate	3.000
Agar	0.500

If necessary adjust the pH so that after sterilization it is  $6.8 \pm 0.2$

\*pH can also be measured after sterilization at 25°C

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

### Directions

Suspend 37.54 grams (the equivalent weight of dehydrated medium per litre) 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). This media is prepared in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (2,3,4,5,8). 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* (6, 7). 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 (7). 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. Glucose 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  $\leq 48$  hours

#### Growth promoting properties

Please refer disclaimer Overleaf.

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

MH443: 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	<=48 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 Grinsted, 1954, J. Dairy Res., 21:101.
- 2.The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
- 5.Japanese Pharmacopoeia, 2008.
- 6.Barnes and Ingram, 1956, J. Appl. Bact., 19:11
- 7.Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
- 8.Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India

Revision : 2 / 2015

### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.