

# TMKH 025 - REINFORCED CLOSTRIDIAL BROTH (as per USP/BP/JP/EP)

#### **INTENDED USE**

For isolation, cultivation and enumeration of *Clostridia* species, highly nutritive for *Clostridium sporogenes* and other anaerobes

#### PRODUCT SUMMARY AND EXPLANATION

Reinforced Medium for Clostridia was formulated by Hirsch and Grinsted and is in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP. 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*. 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. Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media.

### **COMPOSITION**

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

#### **PRINCIPLE**

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.

#### **INSTRUCTION FOR USE**

Label the ready to use bottle. Inoculate the sample and Incubate at specified temperature and time.

## **QUALITY CONTROL SPECIFICATIONS**

Appearance of Prepared media : Light yellow coloured solution.

Sterility test : Passes the release criteria.

# INTERPRETATION

Cultural characteristics observed after incubation.













Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Clostridium sporogenes	11437	50 – 100	Good-luxuriant	30-35°C	24-48 Hours
Clostridium sporogenes	19404	50 – 100	Good-luxuriant	30-35°C	24-48 Hours
Bacteroides vulgatus	8482	50 - 100	Good-luxuriant	30-35°C	24-48 Hours
Bacteroides fragilis	23745	50 – 100	Good-luxuriant	30-35°C	24-48 Hours
Clostridium sporogenes	13124	50 – 100	Good-luxuriant	30-35°C	24-48 Hours

## **PACKAGING:**

In pack size of 100 ml X 25 bottles.

## **STORAGE**

On receipt, store bottles in the dark at 10–25 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Bottled media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation

#### **DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## **REFERENCES**

- 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. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India
- 7. Barnes and Ingram, 1956, J. Appl. Bact., 19:117
- 8. Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.







































**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only Revision: 08 Nov., 2019







