HARMONIZED HIMEDIA®

Technical Data

Reinforced Medium for Clostridia

MH443

Intended use

Recommended for the enrichment of Clostridia from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM Peptone B#	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±0.2
*pH can also be measured after sterilization at 25°C
**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

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 into tubes or flasks as desired. 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 (2,3,4,5,6). 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 HM peptone B carbonaceous and nitrogenous substances, long chain amino acids, vitamins and other 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.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (10,2,3,8,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some *Clostridium* species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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
Growth Promoting				
Clostridium sporogenes ATCC 11437	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Bacteroides vulgatus ATCC 8482	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Additional Microbiological testing				
Bacteroides fragilis ATCC 23745	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
Clostridium perfringens ATCC 13124 (00007*)	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on thelabel. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.
2. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
3. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
4. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
5. The Japanese Pharmacopoeia, 17th edition, 2016, The Ministry of Health, Labour and welfare.
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7. Barnes and Ingram, 1956, J. Appl. Bact., 19:11.
8. Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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