

Technical Data

Reinforced Clostridial Broth

Intended Use:

Recommended for cultivation and enumeration of Clostridia and other anaerobes.

Composition**

Ingredients	g / L
Tryptone	10.000
HM peptone B #	10.000
Yeast extract	3.000
Dextrose (Glucose)	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 # Equivalent to Beef extract

Directions

Suspend 38.00 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes. Mix well and dispense as desired.

Principle And Interpretation

Reinforced Clostridial Broth 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 *Clostridia*. Barnes and Ingram used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens* (2). 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 (3). Other spore forming anaerobes, Streptococci and Lactobacilli also grow in this media. This is a nonselective enrichment media. Tryptone, yeast extract, HM peptone B, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia*. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. This media can be made selective by addition of 15-20 mg Polymyxin B per litre of media (1).

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,6). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further subculturing is required for confirmatory tests.

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

M443

Appearance

Cream to yellow homogeneous free flowing powder Colour and Clarity of prepared medium

Light yellow coloured clear solution 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

Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 40 - 48hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Bacteroides fragilis</i> ATCC 23745	50-100	good - luxuriant	>=50%
<i>^Phocaeicola vulgatus</i> ATCC 8482	50-100	good - luxuriant	>=50%
<i>Clostridium butyricum</i> ATCC 13732	50-100	good - luxuriant	>=50%
Clostridium perfringens ATCC 13124 (00007*)	50-100	good - luxuriant	>=50%

Key: (*) Corresponding WDCM numbers

^ Formerly known as *Bacteroides vulgatus*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. 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 (7,8).

Reference

- 1. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.
- 2. Barnes and Ingram, 1956, J. Appl. Bact., 19:117.

3. Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

6. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

8. 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.

Revision : 03/2024

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 HiMediaTM 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. HiMediaTM 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.