



Reinforced Medium for Clostridia

LQ130CC

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	g L
HM Pepone B #	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
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters # Equivalent to Beef Extract

Directions

Label the ready to use LQ130CC bottle. Inoculate the sample and incubate at specified temperature and time.

Principle And Interpretation

Reinforced Medium for Clostridia was formulated by Hirsch and Grinsted (1) and is in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (2-6). 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 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.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (2-6).

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. Some *Clostridium* species may show poor growth due to nutritional variations.

2. Further isolation and biochemical tests must be carried out for complete identification.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Sterile clear to slightly opalescent Reinforced Medium for Clostridia in glass bottle.

Colour

Light yellow coloured medium.

Quantity of medium

200 ml of medium in glass bottle

pH

6.60- 7.00

Sterility Check

Passes release criteria.

Cultural Response

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

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 ≤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).

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Growth Promoting				
<i>Clostridium sporogenes</i> ATCC 11437	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
<i>Clostridium sporogenes</i> ATCC 19404 (00008*)	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
<i>§ Phocaeicola vulgatus</i> ATCC 8482	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs

Key : (*) Corresponding WDCM numbers § - Formerly known as *Bacteroides vulgatus*

Storage and Shelf Life

On receipt, store between 15-30°C. Use before expiry date on the label. 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, 2022, The United States Pharmacopoeial Convention. Rockville, MD.
3. European Pharmacopoeia, 2022, European Dept. for the quality of Medicines.
4. British Pharmacopoeia, 2022, The Stationery office British Pharmacopoeia.
5. Japanese Pharmacopoeia, 2016.
6. Indian Pharmacopoeia, 2022 Ministry of Health and Family Welfare, Govt. of India.

7. Barnes and Ingram, 1956, J. Appl. Bact., 19:11. Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. 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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