# Reinforced Clostridium Medium Base

M1915

## Intended Use:

Recommended for mesophilic anaerobic spore count in milk and cheese by MPN method

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Tryptone | 10.000 |
| Yeast extract | 3.000 |
| HM peptone B # | 10.000 |
| Sodium acetate | 5.000 |
| Cysteine hydrochloride | 0.500 |
| Soluble starch | 1.000 |
| Agar | 1.000 |
| Final pH ( at 25°C) | 5.6±0.1 |

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

## Directions

Suspend 30.50 grams in 1000 ml purified / distilled water.Adjust the pH to 5.5-5.7 with 20% aqueous lactic acid solution.Heat if necessary to dissolve the medium completely.Add 28 ml of 72% Sodium lactate. Mix well and dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

## Principle And Interpretation

Reinforced Clostridial Medium Base is formulated by Wehr & Frank (7). The Mesophilic sporeforming anaerobes belonging to the genus Clostridia of food concern are Gram-positive, catalase negative, rods of varying sizes.

The medium 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 (5). 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 provides carbon, nitrogen, long chain amino acids, vitamins and other necessary nutrients for the growth of Clostridia. The small amount of agar helps in maintaining low redox potential for stabilizing the medium.

## 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 (1,6,8). 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

**Appearance**

Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 3.05% w/v aqueous solution at 25°C. pH : 5.6±0.1

**pH**

5.50-5.70

**Cultural Response**

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

| Organism | Growth | Inoculum (CFU) |
|---|---|---|
| *Clostridium perfringens* *ATCC 13124* (00007*) | good - luxuriant | 50-100 |
| *Clostridium tyrobutyricum* *ATCC 25755* | good - luxuriant | 50-100 |
| *Clostridium butyricum* *ATCC 13732* | good - luxuriant | 50-100 |

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 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 (3,4).

## Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Barnes and Ingram, 1956, J. Appl. Bact., 19:117.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
6. 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.
7. Standard Methods for the Examination of Dairy products,Wehr & Frank, 2004, (item 8.100).
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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