

## CM 21501 – REINFORCED CLOSTRIDIAL BROTH W/O AGAR

### INTENDED USE

For cultivation and enumeration of Clostridia.

### PRODUCT SUMMARY AND EXPLANATION

Reinforced Clostridial Broth is formulated by Hirsch and Grinsted. 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 this media. This is a nonselective enrichment media.

### COMPOSITION

| Ingredients                | Gms / Ltr |
|----------------------------|-----------|
| Casein enzymic hydrolysate | 10.000    |
| Beef extract               | 10.000    |
| Yeast extract              | 3.000     |
| Dextrose                   | 5.000     |
| Sodium chloride            | 5.000     |
| Sodium acetate             | 3.000     |
| Starch, soluble            | 1.000     |
| L-Cysteine hydrochloride   | 0.500     |

### PRINCIPLE

This medium consists of Casein enzymic hydrolysate, yeast extract, beef extract, starch, L-cysteine and sodium acetate which 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.

### INSTRUCTION FOR USE

Dissolve 37.5 grams in 1000 ml distilled water.

Heat if necessary to dissolve the medium completely. Dispense as desired.

Sterilize by autoclaving at 10 psi pressure (115°C) for 15 minutes.

### QUALITY CONTROL SPECIFICATIONS

|                               |                                                          |
|-------------------------------|----------------------------------------------------------|
| Appearance of Powder          | : Light yellow coloured homogeneous free flowing powder. |
| Appearance of prepared medium | : Medium amber coloured slightly opalescent solution.    |
| pH (at 25°C)                  | : 6.8 ± 0.2                                              |

### INTERPRETATION

Cultural characteristics observed in an anaerobic atmosphere after incubation.



| Microorganism            | ATCC  | Inoculum (CFU/ml) | Growth         | Incubation Temperature | Incubation Period |
|--------------------------|-------|-------------------|----------------|------------------------|-------------------|
| Clostridium butyricum    | 13732 | 50-100            | Good-luxuriant | 35-37°C                | 24-48 Hours       |
| Bacteroides vulgatus     | 8482  | 50-100            | Good-luxuriant | 35-37°C                | 24-48 Hours       |
| Bacteroides fragilis     | 23745 | 50-100            | Good-luxuriant | 35-37°C                | 24-48 Hours       |
| Clostridium perfringenes | 12924 | 50-100            | Good-luxuriant | 35-37°C                | 24-48 Hours       |

#### PACKAGING:

In pack size of 100 gm and 500 gm bottles.

#### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

#### DISPOSAL

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

#### REFERENCES

1. Hirsch and Grinstead, 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.

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|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| <br>Good Manufacturing Practices Certified | <br>Best Before        | <br>Quantity                       | <br>Catalogue Number | <br>Manufacturer |
| <br>Temperature Unit                       | <br>Lot / Batch Number | <br>Consults Instructions for Use | <br>QR Code          |                                                                                                       |

