

CM 21498 – REINFORCED CLOSTRIDIAL BROTH

INTENDED USE

For cultivation and enumeration of Clostridia and other anaerobes.

PRODUCT SUMMARY AND EXPLANATION

Reinforced Clostridial Broth is formulated by Hirsch and Grinstead. 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
Agar	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 38.0 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 10 psi pressure (115°C) for 15 minutes.
- Mix well and dispense as desired.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Light yellow coloured clear solution in tubes.
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 sporogenes	11437	50-100	Good-luxuriant	35-37°C	24-48 Hours
Clostridium sporogenes	19404	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 perfringens	13124	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.

