

Technical Information

Reinforced Clostridial Broth (without Agar)

Product Code: DM 1443A

Application: Reinforced Clostridial Broth w/o Agar is recommended for the cultivation and enumeration of Clostridia when a semisolid medium is preferred.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Beef extract	10.000
Yeast extract	3.000
Dextrose	5.000
Sodium chloride	5.000
Sodium acetate	3.000
Soluble starch	1.000
L-Cysteine hydrochloride	0.500
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Hirsch and Grinsted ⁽¹⁾ formulated Reinforced Clostridial Broth w/o Agar. The media can be used to start growth from small inocula 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 can also grow in these media. These are enriched but nonselective media.

Casein enzymic hydrolysate, yeast extracts, beef extract, 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. These media can be made selective by addition of 15-20 mg Polymyxin B per litre of media ⁽¹⁾.

Methodology

Suspend 37.5 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense as desired. Sterilize by autoclaving 115°C for 15 minutes (10 lbs pressure)

Quality Control

Physical Appearance

Light yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured slightly opalescent solution

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH range 6.60-7.00



Dehydrated Culture Media
Bases / Media Supplements

Cultural Response/Characteristics

DM 1443A: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth
<i>Bacteroides fragilis</i> ATCC 23745	50-100	good - luxuriant
<i>Bacteroides vulgatus</i> ATCC 8482	50-100	good – luxuriant
<i>Clostridium butyricum</i> ATCC 13732	50-100	good – luxuriant
<i>Clostridium perfringens</i> ATCC 12924	50-100	good - luxuriant

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

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.

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