PRODUCT SPECIFICATION SHEET

Reinforced Clostridial Broth (DM224)

Intended Use
Reinforced Clostridial Broth (DM224) is recommended for the cultivation and enumeration of *Clostridia* and other anaerobes.

Product Summary and Explanation
Hirsch and Grinstead formulated semisolid Reinforced Clostridial Medium and found that the medium outperformed other media in supporting growth of clostridia from small amount of inocula and produced higher viable cell counts.[1] Barnes and Ingram[2] used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens*. Reinforced Clostridial Broth is a nonselective enrichment medium and grows various anaerobic and facultative bacteria when incubated anaerobically.[3] This medium has been used to detect clostridia, bifidobacteria and other anaerobes in food products[4-7] and fecal samples.[8] Reinforced Clostridial Broth is listed in the *USP* as the recommended medium for the isolation of *Clostridium* sp. from nonsterile pharmaceutical products.[9] 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.[10]

Principles of the Procedure
Reinforced Clostridial Broth contains casein enzymic hydrolysate and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic byproducts. Cysteine hydrochloride is the reducing agent. Sodium acetate acts as a buffer. This medium can be made selective by addition of 15-20 mg polymyxin B per litre of media. The small amount of agar makes the medium semisolid.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Beef extract	10.00
Yeast extract	3.00
Dextrose	5.00
Sodium chloride	5.00
Sodium acetate	3.00
Starch, soluble	1.00
L-Cysteine hydrochloride	0.50
Agar	0.50
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing *C. botulinum* or *C. tetani* or their toxins.
4. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of these organisms and for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin.

Directions
1. Suspend 38.00 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 115°C, 10 psi pressure, for 15 minutes / validated cycle.
4. Mix well and dispense as desired.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder

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Prepared Medium	Light yellow coloured clear solution in tubes
Reaction of 3.8% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Not applicable

Expected Cultural Response: Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 24 - 48hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Bacteroides fragilis ATCC 23745*	50 -100	good-luxuriant
2.	*Bacteroides vulgatus ATCC 8482*	50 -100	good-luxuriant
3.	*Clostridium sporogenes ATCC 11437*	50 -100	good-luxuriant
4.	*Clostridium sporogenes ATCC 19404*	50 -100	good-luxuriant
5.	*Clostridium perfringens ATCC 13124*	50 -100	good-luxuriant

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer to appropriate references for standard test procedures.

Results
1. After appropriate incubation time and temperature, subculture each tube or bottle to two Columbia Agar plates.
2. Incubate under both aerobic and anaerobic conditions for 48 hours at 30-35°C to confirm the presence of anaerobic growth.
3. After incubation of these plates, if isolates grow anaerobically only (with or without endospores) and are catalase negative, this indicates the presence of *Clostridium* sp.
4. Perform other confirmatory biochemical testing as necessary.
5. Refer appropriate references and test procedures for interpretation of results.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Reinforced Clostridial Broth
Product Code : DM224
Available Pack sizes : 100gm / 500gm

References

1. Hirsch and Grinstead. 1954. J. Dairy Res. *21*:101.
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5. Roy. 2003. Media for the detection and enumeration of bifidobacteria in food products. *In* Corry, J.E.L. et al. (eds.), Handbook of culture media for food microbiology. Elsevier Science B.V. Amsterdam, The Netherlands.
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8. Hartemink and Rombouts. 1999. J. Microbiol. Methods. *36*:181.
9. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
10. Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

Further Information

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