PRODUCT SPECIFICATION SHEET

Reinforced Medium for Clostridia (DM224U)

Intended Use

Reinforced Medium for Clostridia (DM224U) is recommended for the cultivation and enumeration of *Clostridia* from pharmaceutical products using the microbial limit testing in compliance with USP.

Product Summary and Explanation

Clostridia are a highly polyphyletic class of firmicutes including *Clostridium* and other similar genera and are obligate anaerobes, oxygen being toxic to them. Hirsch and Grinstead formulated semisolid Reinforced Medium for Clostridia 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 Ingramused the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens*.[2,3] This media is prepared as per United States Pharmacopeia[4] and in accordance with the microbial limit testing by harmonized methodology of USP/BP/EP/JP/IP.[4,5,6,7,8] It is recommended for sterility checking of non-sterile products, nutritional and dietary supplements. 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.[9] These are enriched but non-selective media and also allows growth of other spore forming anaerobes, Streptococci and Lactobacilli.

Principles of the Procedure

Reinforced Medium for Clostridia contains peptone, yeast extract and beef extract as sources of carbon, nitrogen, vitamins and minerals which provide all the necessary nutrients for the growth of clostridia. Glucose monohydrate is a fermentable carbohydrate in the medium. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Cysteine hydrochloride is the reducing agent. Small amount of soluble starch removes toxic metabolites from the medium. Sodium acetate also acts as a good buffering agent. The small amount of agar makes the medium semisolid and helps in maintaining anaerobic conditions.

Formula / Liter

Ingredients	Gms / Liter
Peptone	10.00
Beef extract	10.00
Yeast extract	3.00
Glucose monohydrate	5.00
Sodium chloride	5.00
Sodium acetate	3.00
Starch, soluble	1.00
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.

PRODUCT SPECIFICATION SHEET

Directions

1. Suspend 37.54 grams of the medium in one liter of purified/distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear solution in tubes
Reaction of % Solution	Not applicable
Gel Strength	Not applicable

Growth Promotion Test

Growth promotion was carried out in accordance with the harmonized method of USP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for <=48 hours.

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

Expected Cultural Response: Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30-35°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Incubation Temperature	Incubation period
	Growth Promoting				
1.	*Clostridium sporogenes ATCC 11437*	50 -100	good-luxuriant	30 -35 °C	<=48 hrs
2.	*Clostridium sporogenes ATCC 19404*	50 -100	good-luxuriant	30 -35 °C	<=48 hrs
3.	*Bacteroides vulgatus ATCC 8482*	50 -100	good-luxuriant	30 -35 °C	<=48 hrs
	Additional Microbiological testing				
4.	*Bacteroides fragilis ATCC 23745*	50 -100	good-luxuriant	30 -35 °C	24 -48 hrs
5.	*Clostridium perfringens ATCC 13124*	50 -100	good-luxuriant	30 -35 °C	24 -48 hrs

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

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.

PRODUCT SPECIFICATION SHEET

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 Medium for Clostridia

Product Code : DM224U

Available Pack sizes : 100gm / 500gm

References

1. Hirsch and Grinstead. 1954. J. Dairy Res. *21*:101.
2. Barnes E. M. and Ingram J. E., 1956. J. Appl. Bacteriol. 19:117.
3. Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
4. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
5. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
6. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
7. Japanese Pharmacopoeia, 2008.
8. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India
9. 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.

Further Information

For further information please contact your local MICROMASTER Representative.

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	Checked By	Approved By
01.01.2018	01.01.2018	01.01.2018
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