PRODUCT SPECIFICATION SHEET

Enterobacteria Enrichment Broth, Mossel (DM616U)

Intended Use

Enterobacteria Enrichment Broth, Mossel (DM616U) is recommended for selective enrichment of *Enterobacteriaceae* from pharmaceutical products using the microbial limit testing in compliance with USP.

Product Summary and Explanation

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. The *Enterobacteriaceae* family consists of *Salmonella*, *Shigella* and other enteric pathogens. These organisms find entry into the foodsystem through faecally contaminated water. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers. Recovery relies on proper resuscitation of damaged cells.[1] The resuscitation procedure is recommended for dried foods,[2] animal feeds[3] and semi-preserved foods.[4] Mossel et al[5] formulated EE Broth, Mossel, which is recommended as an enrichment medium for bile tolerant gram-negative bacteria in the biological examination of foods, animal feed stuffs.[5,6] This medium is prepared as per United States Pharmacopeia[7] and is in accordance with the harmonized method of USP/BP/EP/JP/IP.[7,8,9,10,11]

Principles of the Procedure

Enterobacteria Enrichment Broth, Mossel contains pancreatic digest of gelatin and glucose monohydrate which provides nitrogen, vitamins and amino acids for the growth of members of *Enterobacteriaceae*. Brilliant green and ox bile, purified are selective agents which inhibit the growth of gram-positive bacteria. Phosphates form the buffering system of the medium and neutralizes acids produced by lactose fermenters that otherwise would adversely affect the growth of the organism. Lactose is replaced by glucose in this medium as lactose negative, anaerogenic lactose-positive or late lactose fermenting *Enterobacteriaceae* are often missed by the standard Coli-aerogenes test. The cells damaged while drying or low pH is resuscitated in well-aerated Soybean Casein Digest Broth (DM277U) for 2 hours at 25°C prior to enrichment in EE Broth.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatin	10.00
Glucose monohydrate	5.00
Dehydrated ox-bile	20.00
Disodium hydrogen phosphate, dihydrate	8.00
Potassium dihydrogen phosphate	3.00
Brilliant green	0.015
Final pH: 7.2 ± 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.

Directions

1. Suspend 42.93 grams of the medium in one liter of purified/distilled water.
2. Dispense 120 ml amounts in 250 ml flasks or 9 ml amounts in tubes.
3. Stopper with cotton plugs or loose fitting caps.
4. Heat in free flowing steam or boiling water for 30 minutes.
5. Avoid overheating of the medium. DO NOT AUTOCLAVE.

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Quality Control Specifications

Dehydrated Appearance	Light yellow to greenish yellow homogeneous free flowing powder
Prepared Medium	Emerald green coloured, clear solution without any precipitate
Reaction of 4.29% Solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP. Cultural response was observed after an incubation at 30-35°C for specified time.

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 <=100 cfu (at 30-35°C for <=24 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >= 100cfu (at 30-35°C for >= 48 hours).

Expected Cultural Response: Cultural response was observed after an incubation at 30-35°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Acid	Incubation Temperature /period
	Growth Promoting				
1.	*Escherichia coli ATCC 8739*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C <=24 hrs
2.	*Pseudomonas aeruginosa ATCC 9027*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C <=24 hrs
	Inhibitory				
3.	*Staphylococcus aureus ATCC 6538*	>=10^3	inhibited	--	30 -35 °C >=48 hrs
	Additional Microbiological Testing				
4.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
5.	*Escherichia coli NCTC 9002*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
6.	*Pseudomonas aeruginosa ATCC 27853*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
7.	*Enterobacter aerogenes ATCC 14028*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
8.	*Proteus mirabilis ATCC 25933*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
9.	*Salmonella Enteritidis ATCC 13076*	50 -100	good-luxuriant	positive reaction, yellow colour	30 -35 °C 24 -48 hrs
10.	*Shigella boydii ATCC 12030*	50 -100	good-luxuriant	negative reaction	30 -35 °C

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				no colour change	24 -48 hrs
11.	*Staphylococcus aureus* *A TCC 25923*	>=10^3	inhibited	--	30 -35 °C >=48 hrs

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

Test Procedure

1. EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (DM287U). A loopful of the enriched sample from EE Broth is sub-cultured onto Violet Red Bile Glucose Agar (DM287U) after an initial incubation at 30-35°C for 24 hours.
2. Typical pink colonies from DM287U are sub-cultured for biochemical confirmation by oxidase and fermentation reactions.[7] Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (DM616U)
3. Refer to appropriate references for standard test procedures.

Results

1. A positive reaction results in acid production which causes the colour of EE Broth Mossel to become yellow.
2. A negative reaction results in no colour change and the medium remains green.

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. Some strains may grow poorly or fail to grow on this medium.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Enterobacteria Enrichment Broth, Mossel
Product Code : DM616U
Available Pack sizes : 100gm / 500gm

References

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10. Japanese Pharmacopoeia, 2008.
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Further Information

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DM616UPSS,QAD/FR/024,Rev.00/01.01.2018

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