PRODUCT SPECIFICATION SHEET

Bile Esculin Agar Base (DM460)

Intended Use
Bile Esculin Agar Base (DM460) is recommended for differential isolation and presumptive identification of group D *Streptococci* in food and pharmaceutical products.

Product Summary and Explanation
Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*. [1] Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping, [2] and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Bile Esculin Agar was originally formulated by Swan [2] for the isolation and identification of Group D Streptococci from food. Facklam and Moody [3, 4] further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci. Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci. [5] The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld. [6] Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate. [7] The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix. [8] Bile Esculin Agar was also shown to aid differentiation of Bile Esculin Agar Base with added supplements is recommended for selective isolation and presumptive identification of group D streptococci from food and pharmaceutical products.

Principles of the Procedure
Bile Esculin Agar Base is highly nutritious and contains peptic digest of animal tissue and beef extract which serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall is used to inhibit most of the other accompyning bacteria. Esculin when added as a supplement in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Beef extract	3.00
Oxgall	40.00
Ferric citrate	0.50
Agar	15.00
Final pH: 6.6 ± 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 63.5 grams in one liter distilled water.
2. Heat to boiling to dissolve the medium completely.

3. Add rehydrated contents of 1 vial of Esculin (MS073).
4. Mix and dispense into tubes or flasks as desired.
5. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
6. Allow the tubed medium to solidify in slanted position.

Quality Control Specifications

Dehydrated Appearance	Cream to brownish yellow homogeneous free flowing powder
Prepared Medium	Amber coloured, clear to slightly opalescent solution with a bluish tinge forms in Petri plates or in tubes as slants.
Reaction of 6.35% Solution	pH : 6.6 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Esculin (MS073) in an increased atmosphere of Carbon dioxide, after an incubation at 35-37°C for 18-24 hours .

Sr. No.	Organisms	Results to be achieved			
		Inoculum	Growth	Recovery	Esculin Hydrolysis
1.	*Enterococcus faecalis ATCC 29212*	50-100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
2.	*Proteus mirabilis ATCC 25933*	50-100	good-luxuriant	>=50%	negative reaction
3.	*Streptococcus pyogenes ATCC 19615*	50-100	none-poor	<=10%	negative reaction

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

Test Procedure

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results

1. Any blackening around the colony of the plated medium indicates a positive result; if no blackening occurs, the test is negative.
2. For slants, if more than half of the slant is blackened within 24-48 hours, the test is positive; if less than half is blackened or no blackening occurs within 24-48 hours, the test is negative.

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

PRODUCT SPECIFICATION SHEET

1. Consult appropriate texts for detailed information and recommended procedures.
2. Strains of *Lactococcus, Leuconostoc* and *Pediococcus* that give a positive bile-esculin reaction have been isolated from human infections.
3. Occasional strains of viridans streptococci blacken the medium or display weakly positive reactions.

Packaging
Product Name : Bile Esculin Agar Base
Product Code : DM460
Available Pack sizes : 500gm

References

1. Schleifer, K. H., and R. Kilpper-Balz. 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. 10:1-19.
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4. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
5. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4 th Ed., J. B. Lippinccott Company
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7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.

Further Information

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PRODUCT SPECIFICATION SHEET

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