PRODUCT SPECIFICATION SHEET

Bile Esculin Azide Agar (DM038)

Intended Use

Bile Esculin Azide Agar (DM038) is recommended for selective isolation and presumptive identification of faecal *Streptococci*.

Product Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg and Isenberg, Goldberg, and Sampson.[1, 2] This formula modifies Bile Esculin Agar by reducing the bile concentration and additional sodium azide is incorporated. The revised medium is more selective, but still provides rapid growth and efficient recovery of group D streptococci.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.[3] Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,[4] 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[3] for the isolation and identification of Group D Streptococci from food. Facklam and Moody[5, 6] 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.[7] The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld.[8] Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate.[9] The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix.[10] Bile Esculin Agar was also shown to aid differentiation of Enterobacteriaceae, Klebsiella, Enterobacter, Serratia from other Enterobacteriaceae genera[11] on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci.[12]

Sabbaj, Sutter, and Finegold evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces.[13] Bile Esculin Azide Agar selected for *S. bovis*, eliminated the requirement for special incubation temperatures, by displaying earlier distinctive reactions.

Principles of the Procedure

Bile Esculin Agar is highly nutritious. Peptic digest of animal tissue and beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and sodium azide is used to inhibit most of the other accompyning bacteria. Esculin 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. In slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	17.00
Peptic digest of animal tissue	3.00
Yeast extract	5.00
Oxgall	10.00
Sodium chloride	5.00
Esculin	1.00
Ferric ammonium citrate	0.50

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Sodium azide	0.15
Agar	15.00
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.
3. Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Directions

1. Suspend 56.65 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Dispense into appropriate containers. For slants dispense in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Mix well and pour into sterile petri plates.
6. Allow the tubed medium to solidify in slanted position.

Quality Control Specifications

Dehydrated Appearance	Light yellow to brownish yellow homogeneous free flowing powder
Prepared Medium	Amber coloured, clear to slightly opalescent solution with a bluish tinge forms in Petri plates
Reaction of 5.67% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
1.	*Enterococcus faecalis ATCC 29212*	50 -100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
2.	*Escherichia coli ATCC 25922*	>=10^3	Inhibited	0%	--
3.	*Staphylococcus aureus ATCC 25923*	50-100	good	40-50%	negative reaction
4.	*Proteus mirabilis ATCC 25933*	50-100	good	40-50%	negative reaction
5.	*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.

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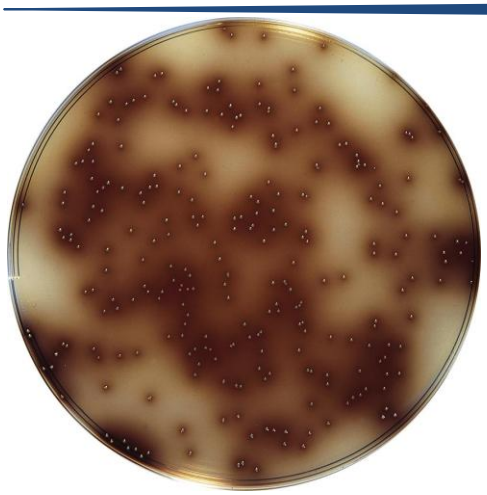

Enterococcus faecalis ATCC 29212

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
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 Azide Agar
Product Code : DM038
Available Pack sizes : 500gm

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References

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Further Information

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