Bile Esculin Azide Agar

Intended Use
Bile Esculin Azide Agar is used for selective isolation and presumptive identification of fecal Streptococci.

Summary
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. The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld. Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate. The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix. Bile Esculin Agar was originally formulated by Swan for the isolation and identification of Group D Streptococci from food. Facklam and Moody further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non-group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae*, *Klebsiella*, *Enterobacter*, *Serratia* from other *Enterobacteriaceae* genera on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci.

Principle
Bile Esculin Azide Agar is a medium rich in casein enzymic hydrolysate, proteose peptone and Cara beef extract. Sodium azide acts as an inhibitor for gram-negative organisms. Gall powder is used to inhibit gram positive bacteria other than Enterococci. Hydrolysis of esculin helps to detect Group D Streptococci. 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 this medium the bile concentration is reduced and additional sodium azide is incorporated. *Leuconostoc, Pediococcus, Lactococcus species* causing human infections give a positive bile esculin test.

Formula*

Ingredients	**g/L**
Casein Enzymic Hydrolysate	17.0
Proteose Peptone	3.0
Cara Beef Extract #	5.0
Gall Powder ##	10.0
Sodium Chloride	5.0
Esculin	1.0
Ferric Ammonium Citrate	0.5
Sodium Azide	0.15
Agar	15.0
Final pH (at 25°C)	7.1 ± 0.2

*Adjusted to suit performance parameters.
Equivalent to Beef Extract
Equivalent to intended performance of Oxgall.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Food and dairy samples; Water samples; Pharmaceutical samples

Specimen Collection and Handling

Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 56.65 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Warning: Sodium azide has a tendency to form explosive metalazides with plumbing materials and it is advisable to flush off disposables with water.

Quality Control

Dehydrated Appearance: Cream to yellow coloured, homogenous free flowing powder.
Prepared Appearance: Medium amber to amber coloured, clear to slightly opalescent gel with a bluish tinge forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18-24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed is within the specified temperature and time, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18-24 hours.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating >100 cfu of the appropriate microorganism at 30°C-35°C for ≥ 24 hours.

Organisms (ATCC)	**Growth**	**Esculin Hydrolysis**
Enterococcus faecalis (29212)	Good	+
Proteus mirabilis (25933)	Complete inhibition	-
Staphylococcus aureus subsp. *aureus* (25923)	Good	-
Streptococcus pyogenes Strain Bruno (19615)	Partial inhibition	-

Key: + Blackening of the medium
- No change

Note: For Good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
For inhibition no growth of test microorganism should occur.
Inoculum for Good growth is 10 - 100 cfu and that for inhibition is greater than 100 cfu.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:
1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. *Streptococcus viridans* sometimes exhibits a weak positive reaction.
2. Further biochemical tests must be carried out for confirmation.

Warranty

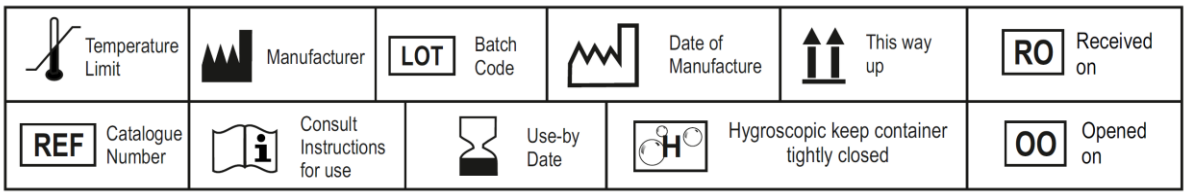
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Swan, 1954, J. Clin. Pathol., 7:160.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020140100	Dehydrated Culture Media	100 g
201020140500	Dehydrated Culture Media	500 g



Revision: 0725/VER-03

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.