Bile Esculin Agar

Intended Use
Bile Esculin Agar is a differential medium used for isolation and presumptive identification of group D Streptococci / Enterococci from food samples.

Summary
Swan formulated Bile Esculin Agar for the isolation and identification of group D Streptococci from foods. Originally, Bile Esculin Test was used for the identification of Enterococci. However, since group D Streptococci share the test with Enterococci, it is advisable that other tests such as salt tolerance be performed while identifying Enterococci. Meyer and Schonfeld showed that majority of Enterococci were able to grow in esculin and split it, while other Streptococci could not. This medium is used to differentiate Enterococci and *Streptococcus bovis* from other Streptococci.

Principle
Oxgall inhibits gram-positive bacteria other than group D Streptococci / Enterococci. Ferric citrate is an indicator of esculin hydrolysis and resulting esculetin formation. Enterococci / group D Streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with ferric citrate producing brownish black complex. This medium is also shown to aid differentiation of genus *Klebsiella-Enterobacter-Serratia* from other *Enterobacteriaceae* on the basis of esculin hydrolysis.

Formula*

Ingredients	**g/L**
Pancreatic Digest of Gelatin	5.0
Beef Extract	3.0
Oxgall	40.0
Ferric Citrate	0.5
Esculin	1.0
Agar	15.0
Final pH (at 25°C)	6.6 ± 0.2

*Adjusted to suit performance parameters

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

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

Quality Control

Dehydrated Appearance: Brownish yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Yellow to amber coloured, clear to slightly opalescent gel with 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 42 to 48 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 42 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤ 100 cfu of appropriate microorganism.
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 ≥ 48 hours.

Organisms (ATCC)	Growth	Esculin Hydrolysis
Enterococcus faecalis (29212)	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.

Interpretation of Results

1. Blackening of the medium around the colonies indicates a positive result for esculin hydrolysis.
2. For slants, if more than half of the slant is blackened within 26-48 hours, the test is positive; if less than half of it is blackened or no blackening occurs within 24-48 hours, the test is negative.

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. Use a light inoculum. A heavy inoculum may cause difficulty in Interpretation of Results and decrease the ability of the bile to inhibit growth of other gram-positive organisms that may hydrolyze esculin.
2. A few Streptococci may grow on the medium in the presence of bile without hydrolysis of esculin.
3. Growth without blackening of the medium is a negative result.
4. Gram- negative rods may grow on this medium and hydrolyze esculin.
5. Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* if present (isolated from human infections) may give a positive bile-esculin reaction.
6. Occasionally, strains of *Streptococcus viridans* blacken the medium or show weakly positive reactions.
7. This medium is not recommended for primary isolation of specimens.

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 A, 1954, J.Clin. Path; 7:160.
2. Downes and Ito (ed.) 2001, Compedium Of Methods for The Microbiological Examination of Foods, 4th edition, APHA Washington DC.
3. Greenberg AE; Clesceri LS and Eaton AD (Eds), 1998, Std Methods for The Examination of Water and Wastewater, 20th edition, APHA, Washington, DC.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020120100	Dehydrated Culture Media	100 g
201020120500	Dehydrated Culture Media	500 g