BILE ESCULIN AGAR

INTENDED USE
Remel Bile Esculin Agar is a solid medium recommended for use in qualitative procedures for presumptive identification of group D streptococci and enterococci.

SUMMARY AND EXPLANATION
Rochaix first demonstrated the value of esculin hydrolysis for identification of enterococci.[1] Meyer et al. found that 61 of 62 strains of enterococci hydrolyzed esculin in a medium containing bile.[2] Swan determined that positive results obtained on esculin agar containing 40% bile correlated well with serologically confirmed group D streptococci.[3] Using Swan's formula, Facklam and Moody tested over 700 strains of streptococci and enterococci representing all known serological groups and found all strains to be bile-resistant and esculin-positive.[4]

PRINCIPLE
Group D streptococci and enterococci hydrolyze esculin in the presence of bile to form esculetin and dextrose. Esculetin reacts with ferric ions supplied by ferric ammonium citrate to form brown-black colonies on Bile Esculin Agar. Oxgall in a concentration of 4% (equivalent to 40% bile) inhibits most strains of streptococci and enterococci other than group D.

REAGENTS (CLASSICAL FORMULA)*

Oxgall (40% Bile)	40.0	g	Esculin	1.0	g
Gelatin Peptone	5.0	g	Ferric Ammonium Citrate	0.5	g
Beef Extract	3.0	g	Agar	15.0	g
			Demineralized Water	1000.0	ml

pH 6.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate Bile Esculin Agar with 2-3 colonies from a pure culture of the test isolate. Alternatively, inoculate with 2 drops of a pure, 24 hour Todd-Hewitt Broth culture.
2. Incubate aerobically at 33-37°C for up to 72 hours.
3. Examine plate for blackening of the medium around and under the growth.

INTERPRETATION OF THE TEST
Positive Test - Dark-brown to black color around colonies and diffusing into the medium
Negative Test - No blackening in the medium

QUALITY CONTROL
All lot numbers of Bile Esculin Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[5] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Enterococcus faecalis* ATCC® 29212	Aerobic, 18-24 h @ 33-37°C	Growth, blackening around colonies
Streptococcus gallolyticus ATCC® 9809	Aerobic, 18-24 h @ 33-37°C	Growth, blackening around colonies
**Streptococcus pyogenes* ATCC® 19615	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)

*CLSI recommended organism

LIMITATIONS
1. Organisms other than streptococci and enterococci can grow on this medium and hydrolyze esculin. A Gram stain and additional biochemical/serological testing are required for definitive identification. Consult appropriate references for further instructions.[6]

BIBLIOGRAPHY

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2. Meyer, K. and H. Schonfeld. 1926. Zentralbl. Bakteriol. Abt. I. Orig. 99:402-419.
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5. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.
6. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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