

Bile Esculin Agar

Intended Use

Bile Esculin Agar is used to differentiate enterococci and the *Streptococcus bovis* group from other streptococci.^{1,2}

Summary and Explanation

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.³ The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.⁴ Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.⁵

Principles of the Procedure

Enterococci and certain streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt to form a dark brown or black complex.⁶ Ferric citrate is incorporated into the medium as an indicator of esculin hydrolysis and resulting esculetin formation. Oxgall is used to inhibit gram-positive bacteria other than enterococci.

Formula

BBL™ Bile Esculin Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	5.0	g
Beef Extract	3.0	g
Oxgall	20.0	g
Ferric Citrate	0.5	g
Esculin	1.0	g
Agar	14.0	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 43.5 g of the powder in 1 liter of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium with two or three colonies and incubate overnight at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Any blackening of the plated medium indicates a positive result; if no blackening occurs, the test is negative.

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.

Limitations of the Procedure

1. Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* that give a positive bile-esculin reaction have been isolated from human infections.^{1,2}
2. Occasional strains of viridans streptococci blacken the medium or display weakly positive reactions.²

User Quality Control

Identity Specifications

BBL™ Bile Esculin Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a moderate amount of very small dark particles.
Solution:	4.35% solution, soluble in purified water upon boiling. Solution is dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Prepared Appearance:	Dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Reaction of 4.35% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Bile Esculin Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
<i>Enterococcus faecalis</i>	29212	10 ³ -10 ⁴	Good	Blackening
<i>Streptococcus pyogenes</i>	19615	10 ⁴ -10 ⁵	Partial to complete inhibition	No blackening

References

1. Ruoff, Wiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Facklam, Sahm and Teixeira. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Rochaix. 1924. Compt. Rend. Soc. Biol. 90:771.
4. Meyer and Schonfeld. 1926. Zentralbl. Bakteriell. Parasitenk. Infektionskr. Hyg. Abt. Orig. 99:402.
5. Swan. 1954. J. Clin. Pathol. 7:160.
6. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.

Availability

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COMP

Cat. No.	299068	Dehydrated – 500 g
	221838	Prepared Plates – Pkg. of 10*
	221409	Prepared Slants – Pkg. of 10*
	221410	Prepared Slants – Ctn. of 100*

*Store at 2-8°C.