

# BILE ESCULIN AZIDE AGAR

## INTENDED USE

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

## SUMMARY AND EXPLANATION

Rochaix first demonstrated the value of esculin hydrolysis in the identification of enterococci.<sup>1</sup> Meyer et al. found that 61 of 62 strains of enterococci hydrolyzed esculin in a medium containing bile.<sup>2</sup> Swan determined that positive results obtained on esculin agar containing 40% bile correlated well with serologically confirmed group D streptococci.<sup>3</sup> 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.<sup>4</sup> Isenberg et al. modified the formulation by reducing the concentration of bile and adding the selective agent, sodium azide.<sup>5</sup> The lower concentration of bile is less inhibitory to non-group D streptococci.<sup>5</sup>

## PRINCIPLE

Group D streptococci and enterococci hydrolyze esculin in the presence of bile to form esculetin and dextrose. Esculetin reacts with the ferric ions supplied by ferric ammonium citrate to form brown-black colonies. Sodium azide and 1% Oxgall (equivalent to 10% bile) are selective agents added to inhibit gram-negative bacilli and most gram-positive bacteria other than group D streptococci and enterococci.

## REAGENTS (CLASSICAL FORMULA)\*

Casein Peptone.....	17.0 g	Sodium Citrate.....	1.0 g
Oxgall (10% Bile) .....	10.0 g	Esculin.....	1.0 g
Yeast Extract.....	5.0 g	Ferric Ammonium Citrate .....	0.5 g
Sodium Chloride.....	5.0 g	Sodium Azide .....	0.25 g
Meat Peptone.....	3.0 g	Agar.....	15.0 g
		Deminerlized Water.....	1000.0 ml

pH 7.1 ± 0.2 @ 25°C

\*Adjusted as required to meet performance standards.

## PRECAUTIONS

This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

## PREPARATION OF DEHYDRATED CULTURE MEDIUM

1. Suspend 58 g of medium in 1000 ml of demineralized water.
2. Heat to boiling with agitation to completely dissolve.
3. Sterilize by autoclaving at 121°C for 15 minutes or following established laboratory procedures.
4. Dispense into appropriate containers.

## PROCEDURE

1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, testing, and interpretation.

## QUALITY CONTROL

Each lot number of Bile Esculin Azide Agar has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

## CONTROL

*Enterococcus faecalis* ATCC® 29212  
*Streptococcus gallolyticus* ATCC® 9809  
*Escherichia coli* ATCC® 25922  
*Streptococcus pyogenes* ATCC® 19615

## INCUBATION

Aerobic, 24-48 h @ 33-37°C  
Aerobic, 24-48 h @ 33-37°C  
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## RESULTS

Growth, blackening around colonies  
Growth, blackening around colonies  
Inhibition (partial to complete)  
Inhibition (partial to complete)

## LIMITATIONS

1. Organisms other than streptococci and enterococci can grow on this medium and hydrolyze esculin. Additional biochemical and serological testing is required for definitive identification. Consult appropriate references for further instructions.<sup>7</sup>

## BIBLIOGRAPHY

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2. Meyer, K. and H. Schonfeld. 1926. Zentralbl. Bakteriol. Abt. I. Orig. 99:402-419.
3. Swan, A. 1954. J. Clin. Pathol. 7:160-163.
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6. Facklam, R.R. 1972. Appl. Microbiol. 23:1131-1139.
7. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

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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