ENTEROCOCCOSEL AGAR
(BILE ESCULIN AZIDE AGAR)

- For in vitro use only - Catalogue No. PE53

Our Bile Esculin Azide Agar (BEA) is a selective and differential medium for the isolation and presumptive identification of group D streptococci and enterococci from clinical specimens.

Swan was the first to describe the formulation and use of a bile esculin medium, although Rochaix (1924) was the first to note the value of using esculin hydrolysis to identify enterococci. Our current formulation is based on the work of Isenberg, Goldberg, and Sampson, who modified bile esculin agar by adding of sodium azide and reducing the amount of bile. The resultant medium is more selective against gram-negative organisms, and allows clinical specimens to be streaked directly onto the medium.

BEA Agar contains numerous peptones and extracts which provide the organism with nitrogen, amino acids, and other trace elements. The inclusion of esculin allows for detection of esculin-hydrolysis by the bacterial enzyme, esculinase. Esculin hydrolysis liberates esculetin which in turn reacts with ferric ions (ferric citrate) in the medium to produce a black phenolic iron-complex giving esculinase-positive colonies a brown-black halo. Selectivity is accomplished by the addition of bile (oxgall) and sodium azide. Bile inhibits the growth of most gram-positive cocci other than enterococci and group D streptococci, while sodium azide inhibits gram-negative bacteria that may be contained in some clinical samples.

Formula per Litre of Medium

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Animal Tissue	3.0 g
Yeast Extract	5.0 g
Sodium Chloride	5.0 g
Oxgall	10.0 g
Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g
Sodium Azide	0.25 g
Agar	13.5 g

pH 7.1 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 18-24 hours for esculinase-positive colonies.

Interpretation of Results

Characteristically, group D streptococci and enterococci grow in the presence of bile and hydrolyze esculin. On Bile Esculin Azide Agar, typical group D streptococci and enterococci colonies appear as small transparent colonies with brown-black halos. If these colonies are observed then this is a presumptive positive for enterococci.

A negative result for enterococci on Bile Esculin Azide Agar would be no growth, or growth with no blackening of the medium.

To differentiate between enterococci and group D streptococci a PYR disk test (Dalynn DP95) and salt tolerance test (Dalynn TS27) can be performed. All enterococci are PYR positive and can grow in 6.5% NaCl (salt tolerance test) while group D streptococci are negative for both tests.

Further biochemical and/or serological tests should be performed on isolated colonies from pure culture to complete identification.

- *This is only a presumptive test for group D streptococci and enterococci and some strains of Lactococcus, Leuconostoc, Pediococcus, and Vagococcus, which have been isolated from human infections, may produce similar results on Bile Esculin Azide Agar*

- *The reduced concentration of bile allows some strains of staphylococci to grow, but can be differentiated from enterococci since they cannot hydrolyze esculin*

- *Approximately 3 % of viridans streptococci are esculinase-positive and can grow in the presence of bile*

- *Listeria monocytogenes can grow on the medium and exhibit blackening (esculinase-positive) although to a lesser extent than enterococci*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Enterococcus faecalis ATCC 29212	Growth with blackening of medium
Escherichia coli ATCC 25922	Inhibition
Streptococcus pyogenes ATCC 19615	Inhibition

Storage and Shelf Life

Our Bile Esculin Azide Agar should be stored at 4°C to 8°C and protected from light. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 11 weeks from the date of manufacture.

References

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5. MacFaddin JF. Media for isolation, cultivation, identification, maintenance of bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
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