BILE ESCULIN AGAR

- For in vitro use only - Catalogue No. PB65 & TB65

Our Bile Esculin Agar (BEA) is used as a differential medium for the isolation and presumptive identification of group D streptococci and enterococci from clinical specimens and food. This medium is also useful for differentiating *Klebsiella*, *Enterobacter*, and *Serratia* species from other *Enterobacteriaceae*.

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. During their comparative studies, Facklam and Moody demonstrated that Bile Esculin Agar was a reliable method of presumptively identifying group D streptococci and differentiating them from other streptococci. Lindell and Quinn as well as Edberg's group showed that Bile Esculin Agar could also be used for the rapid differentiation of Enterobacteriaceae based on esculin hydrolysis later showed it.

Gelatin peptone and beef extract provide the essential elements needed for growth. 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 iron-complex giving esculinase-positive colonies a brown-black halo. Selectivity is accomplished by the addition of bile (oxgall), which inhibits the growth of most gram-positive cocci other than enterococci and group D streptococci.

Formula per Litre of Medium

Gelatin Peptone	5.0 g
Beef Extract	3.0 g
Bile (Oxgall)	40.0 g
Esculin	1.0 g
Ferric Citrate	0.5 g
Agar	15.0 g

pH 6.6 ± 0.2

Recommended Procedure

Enterococci & Streptococci (Group D)

1. Allow medium to reach room temperature.
2. From a primary plate, pick suspect isolates of enterococci and perform a four-quadrant streak onto a Bile Esculin Agar plate. Alternatively, from a pure, overnight culture of streptococci or enterococci grown in Todd-Hewitt broth, add 2 drops onto the agar surface and perform a four-quadrant streak for maximum isolation. If inoculating a Bile Esculin Agar Slant follow the same procedure but streak the surface of the slant in a fish-tail motion.
3. Incubate aerobically at 35°C.
4. Examine after 18-24 hours for esculinase-positive colonies.
5. Hold up to 72 hours before reporting as negative.

Enterobacteriaceae

1. Allow medium to adjust to room temperature
2. Take a heavy inoculum from a confirmed gram-negative pure culture grown on heart infusion agar, Lysine Iron Agar, or Triple Sugar Iron Agar and perform a four-quadrant streak to maximize recovery of isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 18-24 hours.

Interpretation of Results

Characteristically, group D streptococci and enterococci grow in the presence of bile and hydrolyze esculin. On Bile Esculin 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.

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.

As mentioned, Bile Esculin Agar can be used as a rapid test for differentiating *Enterobacteriaceae*. Among the family, *Klebsiella, Enterobacte*r, and *Serratia* species are typically esculinase-positive and hydrolyze esculin, while most other *Enterobacteriaceae* are esculinase-negative.

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 enterococci and some strains of Lactococcus, Leuconostoc, Pediococcus, and Vagococcus, which have been isolated from human infections, may produce similar results on Bile Esculin Agar*

- *Bile Esculin Agar is a differential medium and not a primary plating medium; use Bile Esculin Azide Agar (Enterococcosel Agar) if a primary plating medium is desired*

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

- *Use a light inoculum when testing E. coli by touching the top of a colony using a bacteriological needle and inoculating the agar surface*

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
Streptococcus pyogenes ATCC 19615	Inhibition

Storage and Shelf Life

Our Bile Esculin 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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