Bile Esculin

Intended Use:
Differential media is used for isolating and presumptively identifying group D streptococci. It is also helpful in the differentiation of *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp.

Principle:
Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin (derived from the horse chestnut tree) to esculetin and dextrose. Esculin is involved in Vitamin P activity. The esculetin reacts with ferric citrate in the media to form a dark brown or black complex. Bile inhibits growth of non-group D esculin hydrolyzing streptococci, most anaerobes, and most facultative anaerobes.

Test Procedure:
1. Select 2-3 well isolated colonies or a loop-full of pure culture broth and streak the surface of the slant with an S motion.
2. Incubate for 18-24 hours at 35°C in an aerobic atmosphere (cap should be loose).
3. Re-incubate up to 48 hours if testing streptococci or enterococci.
    - Incubate only 18-24 hours if testing Enterbacteriaceae.

Results:
- A positive result is indicated by a dark brown or black color that diffuses into half or more of the medium.
- Blackening of less than half of the medium after 48 hours is a negative result.

Limitations:
- Some group D streptococci, such as *S. mutans*, may display weakly positive result. While they hydrolyze esculin they usually do not grow well in the presence of bile.
- The bile esculin test cannot be used alone but must be used in combination with other tests to presumptively identify Enterococcus.