

# BILE ESCULIN TEST

## SUMMARY AND EXPLANATION

The ability of organisms to hydrolyze esculin was first observed by Harrison and Van der Leck<sup>1</sup> in 1909. In 1924, Rochaix<sup>2</sup> introduced esculin hydrolysis for identification of group D streptococci (which includes the organism now named *Enterococcus faecalis* [formerly *Streptococcus faecalis*]). This group of bacteria have the ability to grow in the presence of bile, an emulsifying agent produced in the liver. Smith<sup>3</sup> later employed the use of the esculin hydrolysis test for differentiation of anaerobic bacteria.

## PRINCIPLE

Incorporation of bile salts into the culture medium selects for those organisms capable of growing in the intestine, where they normally encounter bile. The bile selects against organisms that cannot grow in the intestine. The beef extract and peptone in this medium supply the nutritional requirements for growth of microorganisms. When an organism enzymatically hydrolyzes the esculin present in the medium, two products are produced, esculetin and glucose (Fig. 6-8). Black ferric salts of esculetin precipitate out in the presence of ferric ammonium citrate. This results in a brownish-black color being formed in the medium. Other bacteria capable of growing in the presence of bile, but incapable of hydrolyzing esculin do not turn the medium black.

## PROCEDURE

1. Inoculate a slant tube of bile esculin agar with fresh colonies of *Enterococcus faecalis*. Inoculate a separate tube with *Staphylococcus epidermidis*. Inoculate the surface of the agar slant using a multiple S pattern.
2. Incubate the medium under aerobic or anaerobic conditions at 35-37°C for 24-48 hours.
3. Observe for a brownish-black color development.

## INTERPRETATION OF THE TEST (Fig. 6-9, p. 47-48)

Positive Test (esculin hydrolysis) – brownish-black color development, esculin hydrolysis

Negative Test – no color change

## LIMITATIONS

Iron sulfide may be produced and make the medium difficult to interpret. When iron sulfide is produced, a sooty black color is produced on the medium as compared with the brownish-black color produced by the hydrolysis of esculin.

## BIBLIOGRAPHY

1. Harrison, R.C. and J. Van der Leck. 1909. Bakt. Abt. 222:549.
2. Rochaix, A. 1924. Cr. Soc. Biol. Pans, 90:771.
3. Smith, L. 1975. The Pathogenic Anaerobic Bacteria. 2nd ed. Chas. C. Thomas, Springfield, IL.