Esculin Agar Procedure

I. Principle

Esculin agar is a non-selective, differential medium for demonstrating esculin hydrolysis by various microorganisms. This formulation is sometimes used to determine esculin hydrolysis without the selective bile component found in bile esculin agar (refer to gram-positive cocci differentiation charts). Hydrolysis of esculin yields esculetin and dextrose. In the presence of an iron salt, esculetin forms a brown-black complex that diffuses into the surrounding medium. Esculin agar has an iron salt, ferric citrate, already in the medium.

II. Specimen Information

Organisms to be tested using the esculin slant should be isolated in order to obtain a pure inoculum.

III. Reagents & Equipment

Esculin agar slant

- Store at 2 – 8°C until needed.
- Allow to reach room temperature prior to use.

Sterile inoculating loop

IV. Procedure

A. Using a sterile inoculating loop, inoculate esculin media with several isolated colonies.
B. Incubate tubes at 35 ± 2°C in ambient air with caps loosened for up to 48 h.

V. Interpretation

A. Positive: growth with blackening of the agar around colonies
B. Negative: growth without blackening of the agar around colonies

VI. Quality Control

Each new lot or shipment should be tested for performance using the following control organisms:
Enterococcus faecalis ATCC 29212 = Growth, blackening around colonies
Streptococcus pyogenes ATCC 19615 = Growth, partial or no blackening
Enter QC results into the computer.

VII. Limitations

Inoculum must be pure in order for results to be valid.

VIII. References

Package insert: BBL Prepared Media for Demonstration of Esculin Hydrolysis. 8807061, Revised: February 1999.

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Updates and Revisions: