ESCULIN HYDROLYSIS AGAR

INTENDED USE
Remel Esculin Hydrolysis Agar is a solid medium recommended for use in qualitative procedures for the detection of esculin hydrolysis by microorganisms.

SUMMARY AND EXPLANATION
The ability of organisms to hydrolyze esculin was first observed by Harrison and Van der Leck in 1909.[1] In 1924, Rochaix introduced esculin hydrolysis for identification of group D streptococci.[2] Smith later used the esculin hydrolysis test for differentiation of anaerobic bacteria.[3] In 1974, Dowell et al. reported the usefulness of the esculin hydrolysis test in the differentiation of *Bacteroides* species.[4]

PRINCIPLE
Casein peptone, yeast extract, and beef heart infusion supply nitrogenous compounds, amino acids, vitamins, and trace minerals necessary for the growth of microorganisms. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Vitamin K and hemin are growth factors which enhance the growth of anaerobic bacteria. Organisms that hydrolyze esculin in the medium, form black ferric salts in the presence of ferric ammonium citrate. This results in the formation of a brownish-black color in the medium. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	13.0 g	Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5.0 g	Vitamin K	10.0 mg
Yeast Extract	5.0 g	Hemin	5.0 mg
Beef Heart Infusion	2.0 g	Agar	15.0 g
Esculin	1.0 g	Demineralized Water	1000.0 ml

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate Esculin Hydrolysis Agar with 2 or 3 colonies from an 18-24 hour culture of the test isolate and streak for isolation. If a tubed medium is being inoculated, streak the surface of the agar slant.
2. Incubate the medium in ambient or anaerobic air at 33-37°C for 24-48 hours.
3. Examine for the development of a brownish-black color.

INTERPRETATION OF THE TEST
Positive Test - Brownish-black color development
Negative Test - No color change

QUALITY CONTROL
All lot numbers of Esculin Hydrolysis Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Bacteroides fragilis ATCC® 25285	Anaerobic, up to 48 h @ 33-37°C	Growth, blackening of medium
Prevotella melaninogenica ATCC® 25845	Anaerobic, up to 48 h @ 33-37°C	Growth, no color change in medium

LIMITATIONS
1. Organisms which produce iron sulfide may make the medium difficult to interpret, because iron sulfide produces a sooty black color as compared with the brownish-black color produced by the hydrolysis of esculin.[4]

BIBLIOGRAPHY

1. Harnson, R.C. and J. Van der Leck. 1909. Bakt. Abt. 222:549.
2. Rochaix, A. 1924. Cr. Soc. Biol. 90:771-772.
3. Smith, L. 1975. The Pathogenic Anaerobic Bacteria. 2nd ed. Chas. C. Thomas, Springfield, IL.
4. Dowell, V.R. and T.M. Hawkins. 1974. Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual. U.S. Dept. of H.H.S. CDC, Atlanta, GA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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