BILE ESCULIN AGAR (7249)

Intended Use

Bile Esculin Agar is used for the selective isolation and differentiation of group D streptococci.

Product Summary and Explanation

Bile Esculin Agar is based on the formulation described by Swan and further evaluated by Facklam and Moody.[1,2] Rochaix first noted the value of esculin hydrolysis in the identification of enterococccci.[3] Meyer and Schonfeld added bile to the esculin medium and demonstrated 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while other streptococci could not.[4]

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.[5] The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,[1] and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.[2]

Bile Esculin Agar is in standard procedures for the microbiological examination of food products.[6-8]

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit Gram-positive bacteria other than enterococci. Beef Extract and Enzymatic Digest of Gelatin are the carbon and nitrogen sources used for general growth requirements in Bile Esculin Agar. Agar is the solidifying agent.

Formula / Liter

Beef Extract	11 g
Enzymatic Digest of Gelatin	34.5 g
Esculin	1 g
Oxbile	2 g
Ferric Ammonium Citrate	0.5 g
Agar	15 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 64 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to dark beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, opalescent, and grey-yellow.

Expected Cultural Response: Cultural response on Bile Esculin Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction Esculin Hydrolysis
Enterococcus faecalis ATCC® 19433	growth	+ (black colonies)
Enterococcus faecalis ATCC® 29212	growth	+ (black colonies)
Enterococcus faecalis ATCC® 33186	growth	+ (black colonies)
Escherichia coli ATCC® 25922	growth	-
Streptococcus pyogenes ATCC® 19615	inhibited	-

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results
Refer to appropriate references and procedures for results.

Storage
Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure
Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging
Bile Esculin Agar	**Code No.**	**7249A**	**500 g**
		7249B	**2 kg**
		7249C	**10 kg**

References

1. **Swan, A.** 1954.The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
2. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245.
3. **Rochaix, A.** 1924. Milieux a leculine pour le diagnostid differentieldes bacteries du grojps strepto-entero-pneumocoque. Comt. Rend. Soc. Biol. **90**:771-772.
4. **Meyer, K., and H. Schönfeld.** 1926. Über die Unterscheidung des Enterococcus vom Streptococus viridans und die Beziehunger beider zum Strptoccus lactis. Zentralb. Bakteriol Parasitenkd. Infektionskr. Hyg. Abt. I orig. **99**:402-416.
5. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
8. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information
Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.