BILE ESCULIN AZIDE AGAR (7133)

Intended Use

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

Product Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg and Isenberg, Goldberg, and Sampson.[1,2] This formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The revised medium is more selective, but still provides rapid growth and efficient recovery of group D streptococci.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described group D streptococci, in the genus *Enterococcus*.[3] 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, and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.[4] 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.[5]

Sabbaj, Sutter, and Finegold evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces.[6] Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside 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 enterococci, while Sodium Azide inhibits Gram-negative bacteria. Enzymatic Digest of Casein and Yeast Enriched Meat Peptone are the carbon, nitrogen, and vitamin sources used for general growth requirements in Bile Esculin Agar. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate acts as a preservative. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	25 g
Yeast Enriched Meat Peptone	9.5 g
Oxbile	1 g
Sodium Chloride	5 g
Sodium Citrate	1 g
Ferric Ammonium Citrate	0.5 g
Esculin	1 g
Sodium Azide	0.25 g
Agar	14 g

Final pH: 7.1 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 56 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.

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

Expected Cultural Response: Cultural response on Bile Esculin Azide Agar at 35± 2°C after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions
Enterococcus faecalis ATCC® 29212	10 - 300	Poor	Blackening of medium
Escherichia coli ATCC® 25922	10^3	Inhibited	---
Streptococcus pyogenes ATCC® 19615	10^3	Partial to complete inhibition	Colorless colonies

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 the container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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 Azide Agar	**Code No.**	**7133A**	**500 g**
		7133B	**2 kg**
		7133C	**10 kg**

References

1. **Isenberg, H. D.** 1970. Clin. Lab. Forum.
2. **Isenberg, H. D., D. Goldberg, and J. Sampson. 1970.** Laboratory studies with a selective enterococcus medium. Appl. Microbiol. **20**:433.
3. **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.
4. **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.
5. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20:**245.
6. **Sabbaj, J., V. L. Sutter, and S. M. Finegold.** 1971. Comparison of selective media for isolation of presumptive group D streptococci from human feces. Appl. Microbiol. **22**:1008.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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