

Bile Esculin Azide Agar (NCM0166)

Intended Use

Bile Esculin Azide Agar is used for the selective isolation and differentiation of group D streptococci in a laboratory setting. Bile Esculin Azide Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg and Isenberg, Goldberg, and Sampson. 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*. 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. 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.

Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Typical Formulation

Enzymatic Digest of Casein	25.0 g/L
Yeast Enriched Meat Peptone	9.5 g/L
Oxbile	1.0 g/L
Sodium Chloride	5.0 g/L
Sodium Citrate	1.0 g/L
Ferric Ammonium Citrate	0.5 g/L
Esculin	1.0 g/L
Sodium Azide	0.25 g/L
Agar	14.0 g/L

Final pH: 7.1 ± 0.2 at 25°C

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

Precaution

Refer to SDS

Preparation

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.
4. Cool to 45-50°C.

Quality Control Specifications

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

Prepared Appearance: Prepared medium is grey to green-yellow and trace 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
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Poor	Blackening of medium
<i>Escherichia coli</i> ATCC® 25922	10 ³	Completely Inhibited	---
<i>Streptococcus pyogenes</i> 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.

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.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

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.