



Esculin Azide Broth

M749

Esculin Azide Broth is used for selective cultivation and identification of Streptococci.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Yeast extract	5.000
Bile salts	10.000
Sodium citrate	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.250
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.75 grams in 1000 ml distilled water. Heat, if necessary to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Principle And Interpretation

Esculin Azide Broth is prepared as per the modification of original formula of Isenberg (1). Enterococci are able to hydrolyze esculin whereas other streptococci are not able to do so. Swan (2) used an esculin medium containing 40% bile salts and reported that a positive reaction on bile esculin medium could be correlated with a serological group D precipitin reaction. Further studies by Facklam and Moody presumptively identified group D Streptococci and found that the bile esculin test provided a reliable means of identifying group D Streptococci and differentiating them from non-group D Streptococci (3). The present formulation is a modification of Bile Esculin Agar formulated by Isenberg et al in which bile concentration was 40 g/l. In Esculin Azide Broth, the concentration of bile was reduced to 10 g/l and also additional sodium azide was added (1).

The broth is selective due to presence of bile salts and sodium azide and provides rapid growth of Streptococci. Peptic digest of animal tissue and yeast extract provide nitrogenous nutrients to the organisms. Bile salts inhibit other gram-positive bacteria while sodium azide inhibits gram-negative bacteria. Streptococci hydrolyze esculin to esculetin and dextrose. Esculetin and ferric ammonium citrate forms dark brown to black complex, imparting dark brown colour to the broth.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Amber coloured, clear solution having slight purplish tinge

Reaction

Reaction of 3.78% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M749: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis
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<i>Enterococcus faecalis</i> ATCC 50-100 29212		good to luxuriant	positive reaction, blackening of medium
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	
<i>Streptococcus bovis</i> ATCC 27960	50-100	good to luxuriant	positive reaction, blackening of medium
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	poor	negative reaction, no change
<i>Proteus mirabilis</i> ATCC 25933	50-100	poor to fair	negative reaction, no change

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Isenberg, 1970, Clin. Lab. Forum. 2. Swan A., 1954, J. Clin. Pathol., 7:1603. Facklam R. R. and Moody M. D., 1970, Appl. Microbiol., 20:2451. Isenberg, 1970, Clin. Lab. Forum.
2. Swan A., 1954, J. Clin. Pathol., 7:160
3. Facklam R. R. and Moody M. D., 1970, Appl. Microbiol., 20:245

Revision : 2 / 2015

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