



Bile Esculin Azide Broth, Modified

M1799

Bile Esculin Azide Broth, Modified is used to differentiate between Enterococci and Group D Streptococci.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Casein enzymic hydrolysate | 17.000 |
| Peptic digest of animal tissue | 3.000 |
| Yeast extract | 5.000 |
| Oxgall | 10.000 |
| Sodium chloride | 5.000 |
| Esculin | 1.000 |
| Ferric ammonium citrate | 0.500 |
| Sodium azide | 0.250 |
| Sodium citrate | 1.000 |
| Final pH (at 25°C) | 7.1±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.75 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Caution: 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

Bile Esculin Azide Broth, Modified is similar to the medium developed by Isenberg et. al. (1) but with agar omitted.

Casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract serve as a source of carbon, nitrogen and essential nutrients. Sodium azide inhibits growth of gram-negative organisms and permits the cultivation of Enterococci and group D Streptococci. Oxgall inhibits gram-positive bacteria other than Enterococci. Sodium citrate acts as a buffering agent. Esculin is hydrolysed by Enterococci and group D streptococci to esculin which reacts with ferric ammonium citrate to form dark brown or black complex (2). Colonies suspected of being Enterococci can be emulsified in 2ml of broth (Bile esculin azide broth, modified) and incubated at 35- 37°C. The combination of esculin and bile in presence of sodium azide permits the selection and differentiation of Enterococci by esculin hydrolysis (Blackening of medium) within 2hours (1), when heavy inoculum is used.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Amber coloured, clear to slightly opalescent solution with a bluish tinge.

Reaction

Reaction of 4.275% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

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

Cultural Response

| Organism | Inoculum (CFU) | Growth | Esculin Hydrolysis |
|----------|-------------------|--------|-----------------------|
|----------|-------------------|--------|-----------------------|

Cultural Response

| | | | |
|---|--------|-----------|--|
| <i>Enterococcus faecalis</i> ATCC 50-100 29212 | | luxuriant | positive reaction, blackening of medium |
| <i>Escherichia coli</i> ATCC 25922 | 50-100 | none-poor | negative reaction |
| <i>Staphylococcus aureus</i> ATCC 25923 | 50-100 | good | negative reaction |
| <i>Streptococcus pyogenes</i> ATCC 19615 | 50-100 | none-poor | negative reaction |

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, Goldberg and Sampson, 1970, Appl. Microbiol. 20:433.
- 2.MacFaddin, 2000. Biochemical test for identification of medical bacteria, 3rd ed. Lippincott William & Wilkins, Baltimore, Md.

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