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**Bile Esculin Azide Agar**

Bile Esculin Azide Agar is a selective medium used for isolation and presumptive identification of faecal Streptococci.

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| REF: V/BE01.100 100 Gram  REF: V/BE01.500 500 Gram | REF: V/BE01.250 250 Gram |

**CLINICAL SIGNIFICANCE**

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non-Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of Enterobacteriaceae, Klebsiella, Enterobacter, Serratia from other Enterobacteriaceae genera (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5). Bile Esculin Azide Agar is a modification of Bile Esculin Agar (6, 8) as per Isenberg (10). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

**METHOD PRINCIPLE**

Tryptone, proteose peptone and Beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, Leuconostoc, Pediococcus, Lactococcus species causing human infections give a positive bile esculin test (11). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium. Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation. All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (11). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

**MEDIA COMPOSITION**

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| Item | Formula per liter of medium |
| - Tryptone  - Beef extract  - Proteose peptone  - Ox gall  - Esculin  - Ferric ammonium citrate  - Sodium chloride  - Sodium azide  - Agar | 17.00 gm  5.000 gm  3.000 gm  10.00 gm  1.000 gm  0.500 gm  5.000 gm  0.150 gm  15.00 gm |

***Final pH 7.1 ± 0.2 at 25°C***

**PRECAUTIONS AND WARNINGS**

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses,).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
* Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or   
special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Bile Esculin Azide agar material safety data sheet.

**STORAGE AND STABILITY**

**Lab.Vie**. Bile Esculin Azide Agar are stable until expiration date stated on label when properly stored between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

**MEDIA PREPARATION**

Suspend 56.65 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

**Deterioration**

The color of **Lab.Vie**. Bile Esculin Azide Agar is cream to yellow coloured, homogeneous, free flowing powder. If there are any physical changes, discard the medium.

The prepared medium is Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates, media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

**SPECIMEN COLLECTION AND PRESERVATION**

Food and dairy samples; Water samples

**EQUIPMENT REQUIRED NOT PROVIDED**

* Sterile loops
* Sterile petri-dishes
* Incubator

**PERFORMANCE CHARACTERISTICS**

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

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| Organism | Growth | Esculine Hydrolysis |
| Enterococcus faecalis ATCC 29212 | Good -luxuriant | positive reaction, blackening of medium around the colony |
| Esch erichia coli ATCC 25922 | Inhibited | - |
| Staphylococcus aureus ATCC 25923 | Good | negative reaction |
| Proteus mirabilis ATCC 25933 | Good | negative reaction |
| Streptococcus pyogenes ATCC 19615 | None- poor | negative reaction |

**QUALITY CONTROL**

# To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact **Lab.Vie**.technical support.

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| Number of <n> test in the pack |  | For in-vitro diagnostic use | **IVD** |
| Caution |  | Batch Code/Lot number | **LOT** |
| Do not use if package is damaged |  | Catalogue Number | **REF** |
| Consult Instruction for use |  | Temperature Limitation |  |
|  |  | Expiration Date |  |
|  |  | Manufactured by |  |