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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.

REF: BS.1/BE01.100.0100	100 Gram	REF: BS.1/BE01.250.0250	250 Gram
REF: BS.1/BE01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE [1]

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*. 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 (9). 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

Item	Formula per liter of medium
- Tryptone	17.00 gm
- Beef extract	5.000 gm
- Proteose peptone	3.000 gm
- Ox gall	10.00 gm
- Esculin	1.000 gm
- Ferric ammonium citrate	0.500 gm
- Sodium chloride	5.000 gm
- Sodium azide	0.150 gm
- Agar	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 [2]

BioScien 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 ***BioScien*** 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 samples; Clinical 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

Organism	Growth	Esculine Hydrolysis
Enterococcus faecalis ATCC 29212	Good	positive reaction, blackening of medium around the colony
Esch erichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Inhibited	-
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 ***BioScien*** technical support.

REFERENCES

1.Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
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4. Salfinger Y., andTortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Swan, 1954, J. Clin. Pathol., 7:160.
6. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
7. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
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SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		