

Bile Esculin Azide Agar, Granulated®

GM493I

Intended Use:

Recommended for isolation and presumptive identification of faecal Streptococci. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7899-2:2000 and ISO 11333:2014, Amd.2 : 2020.

Composition**

ISO 7899-2:2000 Specification - Bile Esculin Azide Agar

Ingredients	g / L
Tryptone	17,0 g
Peptone	3,0 g
Yeast extract	5,0 g
Ox-bile, dehydrated	10,0 g
Sodium chloride (NaCl)	5,0 g
Aesculin	1,0 g
Ammonium iron(III) citrate	0,5 g
Sodium azide (NaN ₃)	0,15 g
Agar	8-18.000
Final pH (at 25°C)	7.1±0.1

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Ingredients	g / L
Tryptone	17.000
Peptone	3.000
Yeast extract	5.000
Bile #	10.000
Sodium chloride	5.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivqlent to Oxgall

Directions

Suspend 56.65 grams in 1000 ml purified / 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.

Principle And Interpretation

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 (5) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (6,7) 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 (8) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (9).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar (5,6) as per Isenberg (10). In this medium the bile concentration is reduced and additional sodium azide is incorporated. Bile Esculin Azide Agar, recommended by the ISO Committee (11) is a modification of Bile Esculin Azide Agar (M493), in the type of carbon sources used.

Tryptone, Peptone and yeast 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 (12). 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 (M612I).

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 $44 \pm 0.5^\circ\text{C}$ 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.

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection and processing as per guidelines (13).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variations certain strains may show poor growth.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to brownish yellow coloured granular medium

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Amber coloured, clear to slightly opalescent solution with a bluish tinge forms in Petri plates.

Reaction

Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC 50-100 29212 (00087*)		luxuriant	$\geq 50\%$	positive reaction, blackening of medium around the colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	$\geq 10^4$	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
<i>Proteus mirabilis</i> ATCC 25933	50-100	good	40-50%	negative reaction
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	none-poor	$\leq 10\%$	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store 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 prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in 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.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (14,15).

Reference

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