TM 038 – BILE ESCULIN AZIDE AGAR

INTENDED USE

For selective isolation and presumptive identification of faecal Streptococci.

PRODUCT SUMMARY AND EXPLANATION

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. The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld. Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate. The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix. Bile Esculin Agar was originally formulated by Swan for the isolation and identification of Group D Streptococci from food. Facklam and Moody 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 on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci. Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg. In this medium the bile concentration is reduced and additional sodium azide is incorporated.

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. To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum.

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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	17.000
Beef extract	5.000
Proteose peptone	3.000
Oxgall	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000

PRINCIPLE

Tryptone, proteose peptone and beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and sodium azide inhibits most of the other accompyning 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.

INSTRUCTION FOR USE

- Dissolve 56.65 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
pH (at 25°C) : 7.1±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Esculin hydrolysis	Incubation Temperature	Incubation Period
Enterococcus faecalis	29212	50-100	Luxuriant	>=70%	Positive reaction, blackening of medium around the colony	35-37°C	18-24 Hours
Escherichia coli	25922	50-100	Inhibited	0%	-	35-37°C	18-24 Hours
Staphylococcus aureus	25923	50-100	Good	40-50%	Negative reaction	35-37°C	18-24 Hours
Proteus mirabilis	25933	50-100	Good	40-50%	Negative reaction	35-37°C	18-24 Hours
Streptococcus pyogenes	19615	50-100	None-poor	0-10%	Negative reaction	35-37°C	18-24 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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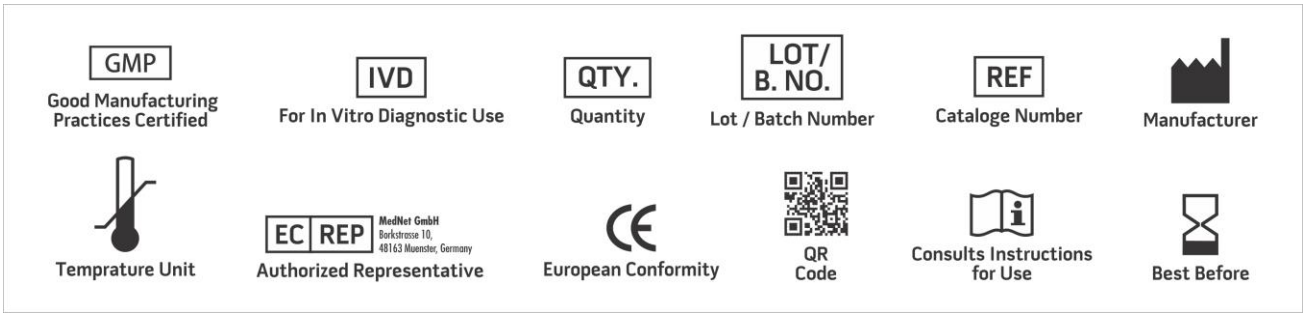

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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