Esculin Mannitol Agar

M2072

Intended use

Recommended as a selective and differential media for the isolation of Staphylococci and *Enterococcus* based on mannitol fermentation and esculin hydrolysis.

Composition**

Ingredients	Gms / Litre
Peptone	23.000
Mannitol	10.000
Sodium chloride	5.000
Corn starch	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Phenol red	0.025
Nalidixic acid	0.015
Colistin sulphate	0.010
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.55 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.

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen and also considered potent pathogen from the point of view of food hygiene The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (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 (2). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4).

Peptone supplies nitrogeneous and carbonaceous compounds, long chain amino acids, other essential growth factors and trace nutrients to the growing bacteria. Sodium chloride maintains osmotic balance Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator. Corn starch helps in neutralizing the toxic compounds. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. Esculin hydrolysis is shown by *Enterococcus* species. Nalidixic acid and Colistin sulphate helps in inhibiting gram negative bacteria. *S.aureus* ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S.aureus* should be confirmed by performing the coagulase test [tube or slide](1). *Enterococcus* species hydrolyse esculin and hence black precipitate is observed around the colonies.This medium helps in smultaneous differentiation of *Enterococcus* and *Staphylococcus* based on mannitol fermentation and esculin hydrolysis.

Type of specimen

Food and dairy samples ; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(8)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. 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 guidleines should be followed while handling specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations :

Due to nutritional variations, some 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 pink homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.55% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Esculin hydrolysis
Staphylococcus aureus ATCC 6538 (00032)*	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	
Staphylococcus aureus ATCC 25923 (00034)*	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	
Enterococcus faecalis ATCC 29212 (00087)*	50-100	luxuriant	>=50%		positive reaction,blackening of medium around the colony
Escherichia coli ATCC 25922 (00013)*	>=10^3	inhibited	0%		
Enterobacter aerogenes ATCC 13048 (00175)*	>=10^3	inhibited	0%		

Key:- *(*) Corresponding WDCM numbers*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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. Use before expiry date on the label.
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 (9,10).

Reference

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