Coagulase Mannitol HiVeg™ Agar Base

MV272

Intended Use:

With plasma it is recommended for isolation and differentiation of Staphylococci from specimens or for classifying pure cultures.

Composition**

Ingredients	g / L
HiVeg™ special infusion	5.000
HiVeg™ hydrolysate	10.500
Soya peptone	3.500
Sodium chloride	3.500
Mannitol	10.000
Bromo cresol purple	0.020
Agar	14.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 47.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118 - 121°C (12-15 lbs pressure respectively) for 15 minutes. Cool to 45 - 50°C. Add 7 - 15% v/v sterile, pre-tested, rabbit plasma the basal medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The genus *Staphylococcus* comprises 28 accepted or proposed species, 14 of which may be encountered in human clinical specimens. Staphylococci are generally found on the skin and mucous membranes of humans and other animals. Some of the pathogenic staphylococci in both humans and animals produce an enzyme called coagulase and detection of this enzyme is used in the laboratory to identify these organisms (1).

These media are used for the isolation of *Staphylococcus aureus* from clinical specimens and for differentiation of *S.aureus* from other species on the basis of coagulase production and mannitol fermentation. Chapman for the first time introduced a medium for selective isolation and differentiation of Staphylococci (2). Tellurite-glycine media were designed by Zebovitz et al (3) and Marwin (4) for selectively isolating coagulase-positive Staphylococcal species. Present medium is based on Esber and Faulconer formulation (5). Mutant or old cultures of *S.aureus* may be weak coagulase producers.

They should be freshly sub cultured and rechecked. Escherichia coli ferments mannitol and may be weakly coagulase positive. Coagulase production is dependent on the presence of a fermentable sugar like mannitol in this case. Coagulase Mannitol HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. It is also dependent on the presence of a protein factor in the HiVeg™ special infusion and blood plasma (6). When mannitol is fermented, the pH of the medium surrounding the coagulase positive colonies drops. This drop in pH is indicated by the change in colour of the bromocresol purple indicator, which turns yellow and exhibits yellow zones around the colonies. An opaque area of coagulated plasma forms around the colonies of coagulase positive organisms. *Staphylococcus epidermidis* is coagulase negative and mannitol non-fermenting species, which does not change the colour of the medium. Coagulase negative species may ferment mannitol and produce a yellow zone around the colonies but an opaque zone will not be formed.

Type of specimen

Please add specimens

Specimen Collection and Handling

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. Some mutant strains of *S.aureus* may show coagulase weak or negative reaction. The culture should be retested in case of doubt.
2. Some strains of *E.coli* may show mannitol fermentation and weak coagulase positive reaction.
3. Gram strain may help in distinguishing between species

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 light grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.45% Agar gel

Colour and Clarity of prepared medium

Purple coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added 7-15% v/v sterile pretested, rabbit plasma at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Coagulase production
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	negative reaction, purple colour	negative reaction, no opaque zone formation
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	positive reaction, yellow colour	positive reaction, colonies surrounded by opaque zone

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 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 (7,8).

Reference

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2. Chapman, 1944, J. Bacteriol., 48:113
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4. Marwin, 1958, Am. J. Clin. Pathol., 30:470.
5. Esber and Faulconer, 1959, Am. J. Clin. Pathol., 32:192.
6. Schaub and Merrit, 1960, Bull. Johns Hopkins Hosp., 106:25.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

8. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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