Staphylococcus HiVeg™ Agar No. 110

MV521

Intended Use:

Recommended for selective isolation and differentiation of pathogenic Staphylococci.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	10.000
Yeast extract	2.500
Gelatin	30.000
Lactose	2.000
D-Mannitol	10.000
Sodium chloride	75.000
Dipotassium hydrogen phosphate	5.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 149.5 grams in 1000 ml of purified / distilled water. Mix thoroughly. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Resuspend the precipitate by gentle agitation to avoid bubbles and pour the plates while the medium is hot. Alternatively, cool the medium to 45 - 50°C and add blood or egg yolk if desired. This medium may also be used without sterilization; it should be boiled for 5 minutes and used at once.

Principle And Interpretation

Staphylococci are widespread in nature though they are mainly found living on the skin, skin glands and mucous membrane of mammals and birds. These organisms are also associated with staphylococcal food poisoning. Staphylococcus Agar No. 110 (1,2,3) also known as Stone Gelatin Agar (4) is used for the selective isolation of pathogenic Staphylococci on the basis of pigment production, mannitol fermentation and gelatin liquefaction. These properties are few of the characteristics of pathogenic Staphylococci (5, 6).

Staphylococcus Agar No. 110 is recommended by APHA (7) and AOAC (8). The medium can be used with Egg Yolk Emulsion (FD045) to study the egg yolk reactions (9).

Staphylococcus HiVeg™ Agar No. 110 is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg™ hydrolysate and yeast extract serve as sources of carbon, nitrogen and other essential nutrients and growth factors including vitamins. D-Mannitol is the fermentable carbohydrate with lactose being an additional source of carbon. Sodium chloride maintains the osmotic equilibrium while phosphate buffers the medium. Gelatin serves as the substrate for gelatin liquefaction.

Mannitol fermentation can be visualized as yellow colouration by addition of a few drops of bromothymol blue to the areas of the plates where colonies have been removed. Gelatin liquefaction can be seen when the plates are flooded with a saturated aqueous solution of ammonium sulphate. On incubation at 35-37°C for 10 minutes, clear zone are observed.

Enterococcus faecalis may grow on this medium as small colonies with slight mannitol fermentation (1).

Type of specimen

Food samples; Water samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(11) 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. Ammonium sulfate solution for gelatin liquefication test should be over-saturated and be warmed up to temprature of medium before use.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel and 3.0% gelatin gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 14.95% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 48 hours. (Mannitol fermentation - on addition of BTB; Gelatinase production : flooding plate with standard aqueous solution of ammonium sulphate)

Organism	Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Pigment Production	Gelatinase production
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant	>=50%	positive reaction	positive	positive reaction
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	good-luxuriant	>=50%	variable reaction	negative	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	slight reaction	negative	variable reaction
Escherichia coli ATCC 25922 (00013*)	>=10^4	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 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 (12,13).

Reference

1. Carter C. H., 1960, J. Bacteriol., 79:753.
2. Chapman G. H., 1946, J. Bacteriol., 51:409.
3. Chapman G. H., 1947, J. Bacteriol., 53:504.
4. Stone R. V., 1935. Proc. Soc. Exper. Biol. and Med. 33:185-187.
5. Chapman G. H., 1952, J. Bacteriol., 63:147.
6. Chapman G. H., Lieb C. W. and Cumco L. G., 1937, Food Research 2., 349-367
7. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

8. Association of Official Analytical Chemists (AOAC), Bacteriological Analytical Manual, 5th Ed., 1978, AOAC International, Gaithersburg, Md.
9. Smucker S. A. and Appleman. M. D., 1964, Appl. Microbiol., 12(4):355.
10. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
11. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
12. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
13. 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.

Revision : 04/2024