PRODUCT SPECIFICATION SHEET

Staphylococcus Agar No. 110 (Gelatin Mannitol Salt Agar) (DM249)

Intended Use

Staphylococcus Agar No 110/Gelatin Mannitol Salt Agar is use for for isolating and differentiating *Staphylococci* based on mannitol fermentation, pigment formation and gelatinase activity.

Product Summary and Explanation

Staphylococcus Medium No.110 is a selective medium for isolation and differentiation of pathogenic staphylococci (Chapman[1,2]) on a basis of salt tolerance, pigmentation, mannitol fermentation, and gelatin liquefaction. Pathogenic staphylococci (coagulase-positive) are able to grow on the high-salt mannitol medium to form orange colonies which give positive reactions for acid production and gelatine liquefaction. Stone[3]suggested that gelatinase activity was indicative of food poisoning strains but Chapman et al.[4] reported that typical food poisoning staphylococci should also produce an orange pigment, be haemolytic, be coagulase-positive, and ferment mannitol. Smuckler & Appleman[6] made Staphylococcus Medium No.110 selective, for the determination of coagulase-positive staphylococci in meat pies containing large numbers of Bacillus species, by the addition of sodium azide 0.75mM (4.875 grams per litre). Chapman[5] showed that incubation at 35°C produced deeper pigmentation and no interference with the Stone reaction or with acid production from mannitol - both of the latter being about as intense as at 35°C. Staphylococcus Agar No. 110 is recommended by APHA[7] and AOAC[8]. The medium can be used with Egg Yolk Emulsion(MS038) to study the egg yolk reactions.[9] 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 also known as Stone Gelatin Agar[10] 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 .

Principles of the Procedure

Casein enzymic 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.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Yeast extract	2.50
Gelatin	30.00
Lactose	2.00
D-Mannitol	10.00
Sodium chloride	75.00
Dipotassium phosphate	5.00
Agar	15.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 149.5 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.

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3. Autoclave at 121°C , 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 14.9% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel and 3.0% gelatin gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Pigment Production	Gelatinase production
1.	*Staphylococcus aureus ATCC 25923*	50 -100	Good-luxuriant	>=50%	Positive Reaction	Positive	Positive Reaction
2.	*Staphylococcus epidermidis ATCC 12228*	50 -100	Good-luxuriant	>=50%	Variable Reaction	Negative	Positive Reaction
3.	*Enterococcus faecalis ATCC29212*	50-100	None-poor	<=10%	Slight reaction	Negative	Variable Reaction
4.	*Escherichia coli ATCC25922*	>=10^3	Good-luxuriant	0%	----	----	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Mannitol fermentation is indicated by a color change of bromthymol blue or bromcresol purple (0.04% in 50:50 ethanol: distilled water) after placing a drop of the dye onto the agar surface where colonies have been removed.
2. Gelatin hydrolysis is determined by flooding the plate with 5 mL of a saturated aqueous solution of ammonium sulfate and incubating plates at 35°C for 10 minutes.

Results

Growth of pathogenic staphylococci produce yellow to orange pigmented colonies. Perform additional biochemical tests on suspected colonies for complete identification of staphylococci.
Gelatin hydrolysis is indicated by a clear zone around the colonies after flooding the medium with ammonium sulfate.

Storage

Store the sealed bottle containing the dehydrated medium at10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Agar # 110 as tiny colonies with mannitol fermentation.
2. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

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Packaging
Product Name : Staphylococcus Agar No. 110
Product Code : DM249
Available Pack sizes : 100gm / 500gm

References

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3. Stone R. V. (1935) Proc. Soc. Exper. Biol. & Med. 33. 185±187.
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5. Chapman G. H. (1947) J. Bact. 53. 365-366.
6. Smuckler S. A. and Appleman M. D. (1964) Appl. Microbiol. 12. 335-339.
7. Speck M. L., (Eds.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C.
8. Association of Official Analytical Chemists (AOAC), Bacteriological Analytical Manual, 5th Ed., 1978, AOAC International, Gaithersburg, Md.
9. Carter C. H., 1960, J. Bacteriol., 79:753.
10. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

Further Information

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