



Staphylococcus Agar No.110 (Gelatin Mannitol Salt Agar)

M521

Staphylococcus Agar No.110 is used as a selective medium for the isolation and testing of pathogenic Staphylococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	2.500
Gelatin	30.000
Lactose	2.000
D-Mannitol	10.000
Sodium chloride	75.000
Dipotassium 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 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 (2, 3, 1) 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).

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.

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

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

M521: 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
<i>Staphylococcus aureus</i> ATCC 25923	50-100	good-luxuriant	≥50%	positive reaction	positive	positive reaction
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant	≥50%	variable reaction	negative	positive reaction
<i>Enterococcus faecalis</i> ATCC 29212	50-100	none-poor	≤10%	slight reaction	negative	variable reaction
<i>Escherichia coli</i> ATCC 25922	≥10 ³	inhibited	0%			

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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