Staphylococcus Medium 110

Intended Use

Staphylococcus Medium 110, also known as Stone Gelatin Agar,[1] is used for isolating and differentiating staphylococci based on mannitol fermentation, pigment formation and gelatinase activity.

Summary and Explanation

Stone[2] described a culture medium on which food-poisoning staphylococci gave a positive gelatinase test. Chapman, Lieb and Curcio[3] later reported that pathogenic staphylococcal strains typically ferment mannitol, form pigment and produce gelatinase. Chapman[4] suggested adding 7.5% NaCl to Phenol Red Mannitol Agar to make a selective isolation medium for staphylococci using a high salt content. Further studies by Chapman[5] led to the development of Staphylococcus Medium 110. This medium is recommended for selectively isolating pathogenic staphylococci from foods.

Principles of the Procedure

Staphylococcus Medium 110 contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Sodium chloride, in high concentration, inhibits most bacteria other than staphylococci. Lactose and D-mannitol are the carbohydrates. Gelatin is included for testing liquefaction. Agar is the solidifying agent.

Pathogenic staphylococci (coagulase-positive staphylococci) typically resist the high salt concentration and form colonies with a yellow-orange pigment. These organisms typically ferment mannitol and produce acid, and liquefy gelatin, producing zones of clearing around the colonies.

Formula

Difco™ Staphylococcus Medium 110

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	2.5	g
Gelatin	30.0	g
Lactose	2.0	g
D-Mannitol	10.0	g
Sodium Chloride	75.0	g
Dipotassium Phosphate	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Staphylococcus Medium 110

Dehydrated Appearance:	Very light beige to beige, free-flowing, homogeneous.
Solution:	14.9% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with heavy precipitate.
Prepared Appearance:	Light amber, slightly opalescent to opalescent.
Reaction of 14.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Staphylococcus Medium 110

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. To observe mannitol fermentation, remove a colony from the medium and add a drop of 0.04% bromthymol blue to the area from which the colony was removed. Observe for the formation of a yellow color (positive reaction).

To observe the gelatinase reaction, flood the plate with 5 mL of saturated ammonium sulfate solution and incubate at 35 ± 2°C for 10 minutes. Observe for a zone of clearing around the colonies (positive reaction).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	PIGMENT*	GELATINASE	MANNITOL
Escherichia coli	25922	10^2-3×10^2	Marked to complete inhibition	–	N/A	N/A
Staphylococcus aureus	25923	10^2-3×10^2	Good	+	+	+
Staphylococcus epidermidis	12228	10^2-3×10^2	Good	–	+	–

**Pigment is seen as a yellow to orange color.*

Directions for Preparation from Dehydrated Product

1. Suspend 149 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes.
4. Evenly disperse the precipitate when dispensing.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for procedures concerning selection and enumeration of staphylococci.

Expected Results

Growth of pathogenic staphylococci produces colonies with yellow-orange pigment.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Medium 110 as tiny colonies with mannitol fermentation. Differentiate these organisms from staphylococci with the Gram stain and catalase test.
2. Suspected staphylococci must be subcultured to Nutrient Broth, Blood Agar, BHI Broth, or Tryptose Phosphate Broth for coagulase testing as the high salt content of Staphylococcus Medium 110 may interfere with results.
3. Pigment production is not a reliable criterion for differentiation of staphylococcal species.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Stone. 1935. Proc. Soc. Exp. Biol. Med. *33*:185.
3. Chapman, Lieb and Curcio. 1937. Food Res. *2*:349.
4. Chapman. 1945. J. Bacteriol. *50*:201.
5. Chapman. 1946. J. Bacteriol. *51*:409.

Availability

Difco™ Staphylococcus Medium 110

Cat. No. 229730 Dehydrated – 500 g

Japan

Cat. No. 251358 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*