

m Staphylococcus Broth

Intended Use

m Staphylococcus Broth is used for isolating staphylococci by the membrane filtration technique.

Summary and Explanation

Staphylococci, along with other bacteria, are indicators of recreational water quality.¹ Indicators of health risk include normal skin flora that are likely to be shed, such as *Pseudomonas*, *Streptococcus* and *Staphylococcus*.² These organisms account for a large percentage of swimming pool-associated illness.¹

The coagulase-positive species, *Staphylococcus aureus*, is well documented as a human opportunistic pathogen.³ Coagulase-negative *Staphylococcus* spp. are a major component of the normal microflora of humans.³ Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds.³

Chapman⁴ added 7.5% NaCl to Phenol Red Mannitol Agar to achieve a selective medium for staphylococci. While studying this medium formulation, Chapman⁵ developed Staphylococcus Medium 110. m Staphylococcus Broth is patterned after the formula of Staphylococcus Medium 110.

m Staphylococcus Broth, with the addition of sodium azide, is used in a multiple-tube procedure to monitor swimming pool water for the presence of *S. aureus*.¹

Principles of the Procedure

Peptone provides the nitrogen, amino acids and minerals in m Staphylococcus Broth. Yeast extract is the vitamin source in this formula. Lactose and mannitol are the carbohydrates for bacterial growth. Dipotassium phosphate is the buffering agent. The high concentration of sodium chloride permits this medium to be selective for staphylococci.

Formula

Difco™ m Staphylococcus Broth

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	10.0 g
Yeast Extract	2.5 g
Lactose	2.0 g
Mannitol	10.0 g
Dipotassium Phosphate	5.0 g
Sodium Chloride	75.0 g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 104 g of the powder in 1 L of purified water.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

NOTE: For field studies where autoclaving is not practical, boil the medium for 5 minutes.

4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Follow the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.¹
2. Use 2.0-2.5 mL of medium to saturate the paper pads on which the inoculated membrane is placed.
3. Incubate at 35 ± 2°C for 40-48 hours.

Expected Results

Observe membranes for growth and pigment production. Test for mannitol fermentation by adding a drop of bromthymol blue to the site from which a colony is removed; a yellow color indicates mannitol fermentation.

User Quality Control

Identity Specifications

Difco™ m Staphylococcus Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	10.4% solution, soluble in purified water upon warming. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 10.4% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ m Staphylococcus Broth

Prepare the medium per label directions. Use the membrane filtration technique with the test organisms. Inoculate and incubate at 35 ± 2°C under humid conditions for 40-48 hours. Observe the membranes for recovery and pigment production. Detect mannitol fermentation by adding a drop of bromthymol blue to the site where a colony was removed. A yellow color indicates a positive result for mannitol fermentation.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	MANNITOL FERMENTATION	PIGMENT PRODUCTION
<i>Escherichia coli</i>	25922	20-200	Inhibition	N/A	–
<i>Staphylococcus aureus</i>	25923	20-200	Good	+	+
<i>Staphylococcus epidermidis</i>	12228	20-200	Good	–	–

Limitation of the Procedure

Confirm positive isolates using biochemical reactions.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Seyfried, Tobin, Brown and Ness. 1985. Am. J. Public Health 75:1071.
3. Kloos and Bannerman. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Chapman. 1945. J. Bacteriol. 50:201.
5. Chapman. 1946. J. Bacteriol. 51:409.

Availability

Difco™ m Staphylococcus Broth

SMWW

Cat. No. 264920 Dehydrated – 500 g