Mannitol Salt Broth, 2 mL
(6545)

Intended Use

Ampouled Mannitol Salt Broth, 2 mL is used for the isolation of staphylococci by the membrane filtration method.

Product Summary and Explanation

Mannitol Salt Broth, 2ml was developed to perform similarly to Mannitol Salt Agar in applications where isolation of the staphylococci by the membrane filtration method is used.

Chapman formulated Mannitol Salt Agar to isolate staphylococci by inhibiting growth of most other bacteria by the effects of a high salt concentration.[4] Chapman added 7.5% Sodium Chloride to Phenol Red Mannitol Agar, and noted pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium. Mannitol Salt Agar is highly selective, and specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth.[5] Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics, and microbial limit tests.[1,2,3,5,6]

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Broth. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic staphylococci ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and will form red colonies.

Medium Composition: Per Liter

Enzymatic Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	5 g
Beef Extract	1 g
D-Mannitol	10 g
Sodium Chloride	75 g
Phenol Red	0.025 g

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Physical Characteristics

Appearance of medium:	Clear to slightly hazy, red
pH at 25ºC:	7.4 ± 0.2

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.
2. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
3. Using the same gentle twisting motion, secure the Neogen Filter onto the funnel adapter.

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter (if using a manifold, open only one valve at a time.)
3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: This step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the rubber stopper to release any remaining vacuum pressure, and then re-secure into the stopper.
5. Add the Mannitol Salt Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: The media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a Petri dish for sample incubation.
8. Remove the filter from the funnel adapter, and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubate at 35 ± 2ºC. Read and record results after 40 – 48 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response:

Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampoulized Mannitol Salt Broth and the filtration housing removed. Plates were incubated aerobically at 35 ± 2ºC and examined for growth at 40 – 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated Media	NA	No Growth
Staphylococcus aureus	10-300	≥ 85% recovery; light yellow colonies
Staphylococcus aureus	10-300	≥ 85% recovery; yellow colonies
Staphylococcus epidermidis	10-300	≥ 85% recovery; pink/colorless colonies
Escherichia coli	300-1000	Inhibited
Escherichia coli	300-1000	Inhibited
Proteus mirabilis	300-1000	Suppressed to Inhibited

Results

Staphylococci will grow on this medium, while the growth of most other bacteria will be inhibited. Coagulase-positive staphylococci will produce growth of yellow colonies and may have a yellow halo around the colony. Coagulase-negative staphylococci will produce small colorless to pink colonies with no color change to the medium.

Storage

Store Ampouled Mannitol Salt Broth, 2 mL at 2 - 8 ºC.

Expiration

Refer to expiration date printed on the front of the box container.

Limitations of the Procedure

1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium

Packaging

Mannitol Salt Broth, 2 mL	**Code No.**	**6545**	**Box of 50**
Neogen Filter "White"	**Code No.**	**6550**	**Box of 50**
Neogen Filter "Black"	**Code No.**	**6555**	**Box of 50**

References

1. **United States Pharmacopeial Convention.** 2007. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.
2. **Directorate for the Quality of Medicines of the Council of Europe (EDQM).** 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
3. **Japanese Pharmacopoeia.** 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.
4. **Chapman, G. H.** The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
5. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1995. Microbiology methods for cosmetics, p. 23.01-23.12. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

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