

Mannitol Salt Agar Plate

MPH118

Intended Use

Recommended for selection and subculture of *Staphylococcus aureus* in accordance with the harmonized method of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Peptone #	5.000
Tryptone ##	5.000
HM Peptone B ###	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
pH after sterilization (at 25°C)	7.4±0.2

Peptic digest of animal tissue ## Pancreatic digest of casein ### Equivalent to Beef extract

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e. *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (1,4,5,6,7). It is also used in the performance of microbial limit tests for the selective isolation of *Staphylococcus*. The formulation is in accordance with the harmonization of USP/EP/BP/JP/IP (8-12).

The medium contains HM peptone B, tryptone and peptone which makes it very nutritious as they provide carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. Mannitol is the fermentable carbohydrate fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Presumptive coagulase-positive yellow colonies of *S.aureus* should be confirmed by performing the coagulase test [tube or slide (1)]. A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth)(M002) (13). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (14).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8-12).

Enrichment : Sample is initially enriched in Tryptone Soya Broth (MH011) and incubated at 30-35°C for 18-24 hours.

Selection and subculture : The enriched culture is then subcultured on Mannitol Salt Agar (MPH118) and incubated at 30-35°C for 18-72 hours.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is a selective medium, some strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol.
2. Certain other bacteria are also mannitol fermenting other than *Staphylococcus*, therefore further biochemical testing is required for identification.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
5. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile Mannitol Salt Agar in 90mm disposable plate with smooth surface and absence of black particles/cracks/bubbles

Colour of medium

Red coloured medium

Quantity of medium

27ml of medium in disposable plate

pH

7.20-7.60

Sterility Check

Passes release criteria

Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BPJP/IP, after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu (at 30-35°C for ≤ 18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating ≤ 100 cfu (at 30-35°C for 18-72 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating ≥ 100 cfu (at 30-35°C for ≥ 72 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Growth Promoting + Indicative <i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50 -100	luxuriant	≥ 50 %	yellow/white colonies surrounded by yellow zone

Inhibitory

Escherichia coli ATCC 8739 (00012*) $\geq 10^3$ inhibited 0 %

Additional Microbiological testing

Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*) 50 -100 luxuriant ≥ 50 % yellow/white colonies surrounded by yellow zone

Staphylococcus epidermidis ATCC 12228 (00036*) 50 -100 fair - good 30 -40 % red

Staphylococcus epidermidis ATCC 14990 (00132*) 50 -100 fair - good 30 -40 % red

Proteus mirabilis ATCC 12453 50 -100 none-poor 0 -10 % yellow

Escherichia coli ATCC 25922 (00013*) $\geq 10^3$ inhibited 0%

Klebsiella aerogenes ATCC 13048 (00175*) $\geq 10^3$ inhibited 0%

Key: (#) Formerly known as *Enterobacter aerogenes*, (*) Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (13,14).

Reference

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