

# MANNITOL SALT AGAR with OXACILLIN

- For in vitro use only -

Cat No. PM31 | PM32 | PM33 6µg/mL | 2µg/mL | 4µg/mL

Our Mannitol Salt Agar with Oxacillin can be used as a primary screening medium for methicillin-resistant *Staphylococcus aureus* (MRSA).

The tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride was reported by Koch in 1942. Mannitol Salt Agar was first described by Chapman in 1945 and he demonstrated its ability to cultivate pathogenic staphylococci. Numerous researchers have since altered the salt concentration and added varying concentrations of antibiotics to devise a suitable, selective medium for isolating MRSA. The use of 6µg/mL of oxacillin was described by Van Enk and Thompson in 1991; 4µg/mL of oxacillin was described by Lally, Ederer, and Woolfrey in 1985; and 2µg/mL of oxacillin was described by Kampf et al. in 1997.

Pancreatic digest of casein, peptic digest of animal tissue, and beef extract are the nutritional sources that provide the bacterial cells with essential elements and growth factors. Mannitol can be utilized and fermented by pathogenic staphylococci to produce acid. Acid produced during fermentation is detected by phenol red, a pH indicator, which changes from red to yellow when a sufficient amount of acid is produced. The high sodium chloride level suppresses the growth of most bacteria, other than Staphylococcus species, and the higher salt concentration is beneficial for recovering resistant strains. The stability of oxacillin makes it the antibiotic of choice for screening for MRSA and gives the completed medium a longer shelf life than similar mediums using other penicillins.

The natural flora contained in some specimens can overgrow and obscure MRSA colonies when a non-selective medium is used; therefore the primary advantage of using Mannitol Salt Agar with Oxacillin is its increased sensitivity and recovery when only a low number of MRSA are present.

#### Formula per Litre of Medium

Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
D-Mannitol	10.0 g
Sodium Chloride	75.0 g
Beef Extract	1.0 g
Phenol Red	0.025 g
Agar	15.0 g
Oxacillin	_

 $pH 7.4 \pm 0.2$ 

#### **Recommended Procedure**

- 1. Allow medium to reach room temperature.
- 2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well isolated colonies. If the specimen is contained on a swab, roll the swab several times over a small area near the edge of the plate and proceed to streak the plate with a sterile loop starting where the swab was inoculated.
- 3. Incubate aerobically at 35°C.
- 4. Examine after 24 hours. If no typical MRSA colonies are observed re-incubate plates an additional 24 hours before discarding.

## **Interpretation of Results**

Although staphylococci are salt tolerant, they grow more slowly on high-salt media and

therefore a 48 hour incubation period is required for definitive results. MRSA ferment mannitol, producing acidic end-products which change the color of the indicator, phenol red, from red to yellow. Typically, MRSA colonies appear as small, yellow colonies surrounded by yellow halos.

Mannitol Salt Agar with Oxacillin is not completely selective for MRSA as some coagulase-negative staphylococci can ferment mannitol and are resistant to oxacillin.

If typical MRSA colonies are observed, rapid detection of the *mecA* gene is necessary to confirm the presence of MRSA. Additional biochemical, serological, and susceptibility tests may be required to fully evaluate MRSA isolates.

• The excellent selectivity of Mannitol Salt Agar permits the use of a heavy inoculum without danger of excessive overgrowth

#### **Quality Control**

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	<b>Expected Result</b>
Staphylococcus aureus ATCC 43300 (MRSA)	Yellow colonies with yellow halos
Staphylococcus aureus ATCC 25923	Inhibition

#### Storage and Shelf Life

Our Mannitol Salt Agar with Oxacillin should be stored away from direct light at 4 to 8°C with the medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 5 weeks from the date of manufacture.

## **Ordering Information**

Cat#	Description	Format
PM31	Mannitol Salt Agar w 6-µg/mL Oxacillin [Standard 15x100-mm plate]	10/pkg
PM32	Mannitol Salt Agar w 2-μg/mL Oxacillin [Standard 15x100-mm plate]	10/pkg
PM33	Mannitol Salt Agar w 4-μg/mL Oxacillin [Standard 15x100-mm plate]	10/pkg

#### References

- 1. Koch FE. Zentralbl Bakteriol Parasentenkd. 1942: 149:122.
- 2. Chapman GH. The significance of sodium chloride in studies of staphylococci. J Bacteriol 1945: 50:201.
- 3. Lally RT, Ederer MN, Woolfrey BF. Evaluation of mannitol salt agar with oxacillin as a screening medium for methicillin-resistant *Staphylococcus aureus*. J Clin Micro 1985; 22:501-4.
- Van Enk RA, Thompson KD. Use of a primary isolation medium for recovery of methicillin-resistant *Staphylococcus aureus*. J Clin Micro 1992; 30:504-505.
- 5. Kampf G, Weist K, Swindsinski S, Kegel M, Ruden H. Comparison of screening methods to identify methicillin-resistant *Staphylococcus aureus*. Eur J Micro Infect Dis 1997; 16:301-7.
- 6. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.

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