INTENDED USE
BD Mannitol Salt Agar with Oxacillin is used for the isolation of methicillin resistant *Staphylococcus aureus* (MRSA) from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
Three different resistance mechanisms contribute to oxacillin (methicillin) resistance in *Staphylococcus aureus*. These are (1) the classic type, which involves production of a supplemental penicillin-binding protein (PBP) that is encoded by a chromosomal *meca* gene, (2) hyper β-lactamase production, and (3) production of modified PBPs, which lowers the organisms affinity for β-lactam antibiotics.¹
Strains that possess the *meca* gene (classic resistance) are either homogeneous or heterogeneous in their expression of resistance. With homogeneous expression, virtually all cells express resistance when tested by standard *in vitro* tests. With heteroresistant expression, some cells appear susceptible and others appear resistant. Often, only 1 in $10^4$ to 1 in $10^8$ cells in the test population expresses resistance. Heterogeneous expression occasionally results in MICs that appear to be borderline; i.e. oxacillin MICs of 4 to 8 µg/ml. Isolates that have classic resistance are usually resistant to other agents such as erythromycin, clindamycin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, a quinolone, or an aminoglycoside.¹
Resistance mediated by hyper β-lactamase production or the presence of modified PBPs also results in borderline resistance. Isolates that are resistant by either hyper β-lactamase production or the modified PBP mechanism usually do not have multiple-drug resistance.¹, ²

BD Mannitol Salt Agar with Oxacillin contains peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the detection of *Staphylococcus aureus* which is one of the mannitol positive *Staphylococcus* species. Due to the addition of oxacillin, all staphylococci that are sensitive to ≤ 5 mg per liter of medium are inhibited. This or comparable concentrations of this antimicrobial have been proposed for screening of MRSA by other authors and are used in Oxacillin Screen Agar which is, however, not an isolation medium.³⁶

REAGENTS
BD Mannitol Salt Agar with Oxacillin

<table>
<thead>
<tr>
<th>Formula* Per Liter Purified Water</th>
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</thead>
<tbody>
<tr>
<td><strong>Beef Extract</strong></td>
</tr>
<tr>
<td><strong>Pancreatic Digest of Casein</strong></td>
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<tr>
<td><strong>Peptic Digest of Animal Tissue</strong></td>
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<tr>
<td><strong>Sodium Chloride</strong></td>
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<tr>
<td><strong>D-Mannitol</strong></td>
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<tr>
<td><strong>Phenol Red</strong></td>
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<tr>
<td><strong>Oxacillin</strong></td>
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<tr>
<td><strong>Agar</strong></td>
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</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.
PRECAUTIONS
[IVD] For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE
On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL
Inoculate the plates with $10^2$ to $10^3$ cfu per plate of ATCC 33592, and with $10^4$ to $10^5$ cfu per plate of the remaining strains. Incubate for 24 hours at 32 to 35° C.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC™ 33592 (MRSA strain)</td>
<td>Growth; yellow colonies, medium yellow</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>No growth</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228</td>
<td>No growth</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Red</td>
</tr>
</tbody>
</table>

PROCEDURE
Materials Provided
BD Mannitol Salt Agar with Oxacillin (90 mm Stacker™ plates) Microbiologically controlled.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
This medium can be used for the isolation and detection of *S. aureus* resistant to methicillin/oxacillin (=MRSA) from all types of clinical specimens (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).

Test Procedure
Inoculate BD Mannitol Salt Agar with Oxacillin with the clinical specimen as soon as possible after it is received in the laboratory. Streak for isolation. Non-selective media, such as BD Columbia Agar with 5% Sheep Blood, selective media for Gram negatives, and, if required, Gram positive bacteria, including staphylococci sensitive to oxacillin, must be included in order to detect the whole range of pathogens involved in the infection. Incubate BD Mannitol Salt Agar with Oxacillin aerobically for not less than 24 hours, preferably between 32 and 35° C. Avoid temperature higher than 35° C and do not incubate in a carbon dioxide enriched atmosphere. If required, plates may be incubated for additional 18 to 24 hours.

Results
Inspect the plates for the presence of yellow colonies, surrounded by a yellow halo. Growth with these characteristics, indicates that the isolate is methicillin (oxacillin) resistant. Isolates that grow on this medium should be further tested for susceptibility against oxacillin with standard methods.1,4,6 Since mannitol fermentation alone is not indicative for *S. aureus* only, identification with coagulase testing or complete biochemical identification is necessary.
PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

**BD Mannitol Salt Agar with Oxacillin** is used for the isolation of methicillin resistant *Staphylococcus aureus* (MRSA) from clinical specimens.

**Performance Evaluation**

This medium was evaluated internally. Altogether, 26 staphylococci were tested which included 18 *Staphylococcus aureus*, 5 *S. epidermidis*, 2 *S. haemolyticus*, and 1 *S. hominis*.

Of the 18 *S. aureus* strains which consisted of 7 reference strains and 11 clinical isolates, 14 had been identified as MRSA strains earlier using standard methods. The remaining four strains were reference strains from the American Type Culture Collection, all labeled MRSA-negative.

Of the 14 MRSA strains, 12 produced significant growth of mannitol positive colonies on **BD Mannitol Salt Agar with Oxacillin** after 20 hours incubation at 35°C and were oxacillin resistant in the agar diffusion test (Mueller Hinton Agar with 1 µg Oxacillin disc). One clinical isolate and one reference strain (= ATCC 43300), both labeled MRSA positive, produced weak growth of pin-point, mannitol positive colonies growth on **BD Mannitol Salt Agar with Oxacillin**, and gave zone sizes of 15 and 11 mm with the 1 µg oxacillin disc, respectively. A zone size of 11 mm means “intermediate”, and 15 mm means “susceptible”.

The four MRSA-negative reference strains did not grow on **BD Mannitol Salt Agar with Oxacillin** after 20 hours of incubation. They were susceptible in the oxacillin disc test (zone sizes ≥ 22 mm).

The five *S. epidermidis* strains (clinical isolates, all labeled methicillin resistant) did not grow after 20 hours of incubation on **BD Mannitol Salt Agar with Oxacillin**. All of them were resistant in the oxacillin disc test.

The two *S. haemolyticus* strains (clinical isolates) grew on **BD Mannitol Salt Agar with Oxacillin** after 20 hours and were resistant in the Oxacillin disc test. Both were mannitol positive.

The *S. hominis* strain produced growth on **BD Mannitol Salt Agar with Oxacillin** after 20 hours and was resistant in the oxacillin disc test. The strain was mannitol negative.

In summary, **BD Mannitol Salt Agar with Oxacillin** was comparable to the standard diffusion test method as described in the NCCLS standard M2-A7 for *S. aureus*.

**Limitations of the Procedure**

This medium can be used for the isolation of MRSA from clinical specimens. It must not be used for the isolation of methicillin/oxacillin resistant coagulase-negative staphylococci. Coagulase testing or a complete biochemical identification of the isolates from this medium must be performed since species other than *S. aureus* may be mannitol positive and resistant to oxacillin.

It is recommended that strains producing weak growth of mannitol positive colonies on this medium after 18 to 25 hours of incubation are further investigated, using currently recommended methods, e.g., growth on Oxacillin Screen Agar or molecular tests for the presence of *mecA* gene.

**REFERENCES**


**PACKAGING/AVAILABILITY**

**BD Mannitol Salt Agar with Oxacillin**

Cat. No. 257021 Prepared Plated Media, cpu 20

**FURTHER INFORMATION**

For further information please contact your local BD representative.

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