BD™ Oxacillin Screen Agar

INTENDED USE

BD Oxacillin Screen Agar (originally named MRSA Screen Agar) was developed for the detection of methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA, ORSA). Since the method to detect MRSA uses the same inoculum as the Bauer-Kirby antimicrobial disc susceptibility test procedure, the oxacillin screen test may be conveniently performed on isolates at the same time as routine susceptibility testing.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Resistance to penicillin in *S. aureus* was observed soon after the introduction of penicillin in the late 1940s.[1] By the late 1960s, methicillin/oxacillin resistant strains of *S. aureus* began to be isolated in the United States.[2]

Three different resistance mechanisms contribute to oxacilllin resistance in *S. 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 ß-lactamase production, and (3) production of modified PBPs, which lowers the affinity of organisms for ß-lactam antibiotics.[3]

Characteristics that might help differentiate the three types of oxacillin (methicillin) resistance can be found outlined in the *Manual of Clinical Microbiology,* 9th ed., p. 1181.[3]

Strains that possess the *mec* gene (classic resistance) are resistant to penicillinase-resistant penicillins (PRPs), such as methicillin, oxacillin and nafcillin, and 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.[3]

Resistance mediated by hyper ß-lactamase production or the presence of modified PBPs also results in borderline resistance. Isolates that are resistant by either hyper ß-lactamase production or the modified PBP mechanism usually do not have multiple-drug resistance.[3] Additionally, **these isolates are unlikely to grow on the agar screen plate.**[3-5]

The methicillin-resistant population grows more slowly, prefers a lower temperature of incubation and a high salt concentration.

BD Oxacillin Screen Agar consists of Mueller Hinton Agar which is a medium that has been standardized for the disc diffusion procedure for antimicrobial susceptibility testing of aerobic bacteria.[6] Sodium chloride is added to improve the growth of the PRP-resistant sub-populations. Oxacillin is preferred for the detection of PRP resistance since it is more stable and results are more reliable (see Table 2C [M100 (M2)] and Table 2C in CLSI document M100 [M7]). [7,8]

REAGENTS

BD Oxacillin Screen Agar

Formula* Per Liter Purified Water

Beef Extract	2.0 g	Oxacillin	0.006
Acid Hydrolysate of Casein	17.5	Agar	17.0
Starch	1.5	pH 7.3 ± 0.2	
Sodium Chloride	40.0		

*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 representative samples with the cultures listed below. Suspend several well-isolated colonies of the test organism from an 18- to 24-h plate culture into a tube of **BD Trypticase™ Soy Broth** and adjust the turbidity to a 0.5 McFarland turbidity standard. Spot inoculate **BD Oxacillin Screen Agar** with 10 µl of test suspension using a micropipette. Alternatively, saturate a cotton swab with the test suspension and gently press out excess fluid against the inner wall of the tube. Streak plate by drawing the swab over an approximately 2.5 cm area. Include a **BD Trypticase Soy Agar II with 5% Sheep Blood (TSA II)** or **BD Columbia Agar with 5% Sheep Blood** plate as a nonselective growth reference.

Incubate plates at 30 - 35°C aerobically. Do not exceed 35° C. Incubate plates for a full 24 h.

Strains	**Growth Results**
Staphylococcus aureus ATCC™ 29213	No growth at 24 h of incubation (susceptible)
Staphylococcus aureus ATCC 43300	Growth at 24 h of incubation (resistant)
Uninoculated	Light amber, clear to very slightly opaque

PROCEDURE

Materials Provided

BD Oxacillin Screen Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is not intended to be used for the isolation of MRSA/ORSA from clinical specimens. Instead, the medium is inoculated with pure cultures of isolates presumptively identified as a *Staphylococcus aureus* (see **Test Procedure** and **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

1. Presumptively identify the isolate as a *Staphylococcus aureus*, e.g., by slide or tube coagulase testing (or by a complete biochemical identification).
2. Prepare the inoculum by suspending several well-isolated colonies of the *S. aureus* test isolate from an 18- to 24-h plate culture into a tube of suitable broth medium, such as **Trypticase** Soy Broth and adjust the turbidity to a 0.5 McFarland turbidity standard.
3. Spot inoculate **BD Oxacillin Screen Agar** with 10 µl of the test suspension using a micropipette.
4. Alternatively, saturate swab with the test suspension and gently press out excess fluid against the inner wall of the tube. Streak plate by drawing swab over an approximately 1 inch (2.54 cm) area.
   Include a **BD Trypticase Soy Agar II with 5% Sheep Blood (TSA II)** or **BD Columbia Agar with 5% Sheep Blood** plate as a nonselective growth control.

5. The test and control plates may be divided into several wedge-shaped sectors by marking the bottom of the plate. Several isolates may be tested on each plate. However, use and incubate each plate only once. DO NOT REUSE AND REINCUBATE a **BD Oxacillin Screen Agar**.
6. Incubate plates at 30-35°C for a **full 24 h**. **Do not exceed 35°C**.

Results
Following incubation, observe plates for growth. Note that plates of this medium must be carefully inspected. Also very small colonies, even one colony, indicate that the isolate is methicillin (oxacillin) resistant. No growth indicates that the organism is susceptible to PRPs (methicillin, nafcillin and oxacillin). Isolates that grow on Oxacillin Screen Agar should be reported as resistant to all ß-lactam antimicrobial agents, including ß-lactam/ß-lactamase inhibitor combinations and cephalosporins.

Note: Informational supplements to CLSI Document M2, or revised versions, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. The complete standard and informational supplements can be ordered from the Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA. Telephone: ++1-610-688-1100. **www.clsi.org**

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Oxacillin Screen Agar is a standard medium for the detection of methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA, ORSA).[3,6-8]
In-house studies have shown that there is a difference in inoculum size between inoculating with 10 µl of the test suspension using a micropipette and inoculating the plate with a swab. The likelihood of the emergence of the resistant subpopulation is greater in a large population of bacterial cells. Detection of resistance, especially with the heterogeneously resistant population, is improved with the larger inoculum obtained by a using a micropipette and inoculating the plate with 10µl.[9]
Any isolate that grows on this medium should be tested quantitatively by broth or agar dilution or by molecular methods (determination of the *mec*A gene) to confirm oxacillin resistance and also resistance to other antimicrobial agents that are characteristic of MRSA, such as chloramphenicol, clindamycin, erythromycin, gentamicin and tetracycline.

Performance Results [9]
In a field trial at a large metropolitan hospital, 152 *S. aureus* isolates were tested on **BD Oxacillin Screen Agar** (formerly MRSA Screen Agar) in comparison with a reference agar dilution procedure for methicillin susceptibility. A total of 121 isolates were found susceptible by both methods. There were 30 isolates found resistant (MRSA) by both methods. The one remaining isolate grew on the MRSA Screen Agar but was methicillin susceptible. Thus, the sensitivity of the test was 100 and specificity was 99.2%.

Limitations of the Procedure
Occasionally *S. aureus* isolates with borderline resistant MICs may not grow within 24 h. It is recommended that any equivocal results demonstrated on the screening plate be confirmed with a standard MIC test.
This medium must not be used for isolation of MRSA directly from clinical specimens.
The use of **BD Oxacillin Screen Agar** for the detection of methicilin/oxacillin resistant coagulase-negative staphylococci is not recommended.

REFERENCES

1. Chain, E., H.W. Florey, and M.A. Jennings. 1949. Acquired resistance of micro-organisms to penicillin, p. 1111-1136. *In* H.W. Florey, E. Chain, N.G. Heatley, M.A. Jennings, A.G. Sanders, E.P. Abraham, and M.E. Florey (ed.), Antibiotics, vol. II. Oxford University Press, London.

2. Barrett, F.F., R.F. McGehee, Jr., and M. Finland. 1968. Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital. Bacteriologic and epidemiologic observations. N. Engl. J. Med. *279*:444-448.
3. Swenson, J.M., J.B. Patel, and J.H. Jorgensen. 2007. Special phenotypic methods for detecting antibacterial resistance. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. L. Landry, and M.A. Pfaller (ed.). Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C. USA.
4. Leitch, C., and S. Boonlayangoor. 1994. Test to detect oxacillin (methicillin)-resistant staphylococci with an oxacillin screen plate, p. 5.5.1-5.5.7. *In* H.D. Isenberg (ed.), Clinical microbiology procedures manual, vol. 1 (suppl.1). American Society for Microbiology, Washington, D.C. USA.
5. Haberberger, R.L., A. J. Kallen, T.J. Driscoll, and M.R. Wallace. 1998. Oxacillin-resistant phenotypes of *Staphylococcus aureus*. Lab. Med. 29: 302-305.
6. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Approved standard: M7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
7. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Disk diffusion supplemental tables: M100 (M2). CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
8. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). MIC testing supplemental tables: M100 (M7). CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
9. Data on file. BD Diagnostic Systems. Sparks, MD. USA

PACKAGING/AVAILABILITY

BD Oxacillin Screen Agar
Cat. No. 254570 Ready-to-use Plated Media, cpu 10

FURTHER INFORMATION

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