

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Oxacillin Screen Agar (originally named MRSA Screen Agar) was developed for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA).

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Preparation of inocula
Suspend several well-isolated colonies of the test organism from an 18- to 24-h plate culture into a tube of **BD BBL™ Trypticase™** Soy Broth and adjust the turbidity to a 0.5 McFarland turbidity standard.
 - b. Spot inoculate 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 swab over an approximately 1 inch (2.54 cm) area. Include a **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plate as a nonselective growth control.
 - c. Incubate plates at 30 – 35 °C (ambient atmosphere).
2. Examine plates at 24 h of incubation, as indicated below.
3. Expected Results

Organisms	ATCC®	Recovery
* <i>Staphylococcus aureus</i>	29213	No growth at 24 h of incubation (susceptible)
* <i>Staphylococcus aureus</i>	43300	Growth at 24 h of incubation (resistant)
<i>Staphylococcus aureus</i>	43387	No growth at 24 h of incubation (susceptible)
<i>Staphylococcus aureus</i>	25923	No growth at 24 h of incubation (susceptible)
<i>Staphylococcus epidermidis</i>	12228	No growth at 24 h of incubation (susceptible)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2 .
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination. (Note: The test for methicillin/oxacillin-resistant *S. aureus* is incubated at 30 – 35 °C.)

PRODUCT INFORMATION

IV INTENDED USE

Oxacillin Screen Agar (originally named MRSA Screen Agar) was originally developed for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA). These strains are resistant to penicillinase-resistant penicillins (PRPs), such as methicillin, oxacillin and nafcillin. 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.

V SUMMARY AND EXPLANATION

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 oxacillin 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 β -lactamase production, and (3) production of modified PBPs, which lowers the organism's affinity for β -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*, 7th ed., p. 1566.⁴

Strains that possess the *mec* 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 express resistance. Heterogeneous expression occasionally results in MICs that appear to be borderline; i.e. oxacillin MICs of 4 – 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 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.³ Additionally, **these isolates are unlikely to grow on the agar screen plate.**³⁻⁶

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

VI PRINCIPLES OF THE PROCEDURE

Mueller Hinton Agar is a medium that has been standardized for the disc diffusion procedure for antimicrobial susceptibility testing of aerobic bacteria.⁷ Sodium chloride is added to improve the growth of the PRP-resistant sub-populations.

VII REAGENTS

Oxacillin Screen Agar

Approximate Formula* Per Liter Purified Water

Beef Extract	2.0 g
Acid Hydrolysate of Casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Sodium Chloride	40.0 g
Oxacillin	6.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This medium is not intended for use with specimens or mixed cultures. The organism to be tested must first be in pure culture and presumptively identified as a *Staphylococcus aureus*.

IX PROCEDURE

Material Provided: Oxacillin Screen Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

1. Prepare the inoculum by selecting several well-isolated colonies of the *S. aureus* test isolate from an 18- to 24-h plate culture and suspending them in a tube of suitable broth medium, such as **Trypticase™** Soy Broth. Adjust the turbidity to a 0.5 McFarland turbidity standard, or use the **BD BBL™** Prompt™ inoculation system.
2. Spot inoculate with 10 µL of the test suspension using a micropipette.
3. Alternately, 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.
4. Include a **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) 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 BBL Oxacillin Screen Agar plate.**
6. Incubate plates at 30 – 35 °C for a full 24 h. **Do not exceed 35 °C.**

User Quality Control: See “Quality Control Procedures.”

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Following incubation, observe plates for growth. Any growth, even one colony, indicates 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* β-lactam antimicrobial agents, including β-lactam/β-lactamase inhibitor combinations and cephalosporins.

NOTE: Supplemental tables to CLSI Document M2-A9, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. For information on current publications, call BD Technical Services at (800) 638-8663. The complete standard and supplements can be ordered from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. Telephone: (610) 688-0100.

XI 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.

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 heterogeneous resistant population, is improved with the larger inoculum obtained by a using a micropipette and inoculating the plate with 10 µL.¹⁰

Any isolate that grows on this medium should be tested quantitatively by broth or agar dilution to confirm oxacillin resistance and also resistance to other antimicrobial agents that are characteristic of MRSA, such as chloramphenicol, clindamycin, erythromycin, gentamicin and tetracycline.

The use of Oxacillin Screen Agar for the detection of methicillin/oxacillin resistant coagulase-negative staphylococci is not recommended.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{11–16}

XII PERFORMANCE CHARACTERISTICS

In a field trial at a large metropolitan hospital, 152 *S. aureus* isolates were tested on **BD BBL™** MRSA Screen Agar (now Oxacillin Screen Agar) in comparison with a reference agar dilution procedure for methicillin susceptibility.¹⁰ 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%.

XIII AVAILABILITY

Cat. No.	Description
221952	BD BBL™ Oxacillin Screen Agar (Mueller Hinton Agar with 6 µg/mL Oxacillin and 4% NaCl)

XIV REFERENCES

1. Chain, E., H.W. Florey, and M.A. Jennings. 1949. Acquired resistance of microorganisms 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.F. Hindler, and J.H. Jorgensen. 2003. Special phenotypic methods for detecting antibacterial resistance, p. 1178-1195. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
4. Swenson, J.M., J.A. Hindler, and L.R. Peterson. 1995. Special tests for detecting antibacterial resistance, p. 1356-1367. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
5. 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.
6. 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.
7. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests; 9th ed. Clinical and Laboratory Standards Institute, Wayne, Pa.
8. Clinical and Laboratory Standards Institute. 2006. Disk diffusion supplemental tables: M100-S16 (M2). Clinical and Laboratory Standards Institute, Wayne, Pa.
9. Clinical and Laboratory Standards Institute. 2006. MIC testing supplemental tables: M100-S16 (M7). Clinical and Laboratory Standards Institute, Wayne, Pa.
10. Data on file, BD Diagnostic Systems.
11. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Tenover (ed.). 2003. *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
12. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11th ed. Mosby, Inc., St. Louis.
13. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
14. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
15. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
16. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.



Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA



Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland

ATCC is a trademark of the American Type Culture Collection.

Prompt is a trademark of 3M.

BD, BD Logo, and all other trademarks are property of Becton, Dickinson and Company. © 2015 BD