

Table 3G-1. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*^a and *Staphylococcus lugdunensis*

Test	Detecting <i>mecA</i> -Mediated Resistance Using Cefoxitin ^b		Detecting <i>mecA</i> -Mediated Resistance Using Oxacillin	Detecting <i>mecA</i> -mediated Resistance Using Oxacillin Salt Agar for <i>S. aureus</i> Only
Test method	Disk diffusion	Broth microdilution	Broth microdilution and agar dilution	Agar dilution for <i>S. aureus</i>
Medium	MHA	CAMHB	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)	MHA with 4% NaCl
Antimicrobial concentration	30-µg cefoxitin disk	4 µg/mL cefoxitin	2 µg/mL oxacillin	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure	Standard broth microdilution procedure or standard agar dilution procedure	Colony suspension to obtain 0.5 McFarland turbidity Using a 1-µL loop that was dipped in the suspension, spot an area 10-15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
Incubation conditions	33 to 35° C; ambient air ^c			
Incubation length	16-18 hours	16-20 hours	24 hours (may be reported after 18 hours, if resistant)	24 hours; read with transmitted light
Results	≤ 21 mm = positive for <i>mecA</i> -mediated resistance ≥ 22 mm = negative for <i>mecA</i> -mediated resistance	≥ 8 µg/mL = positive for <i>mecA</i> -mediated resistance ≤ 4 µg/mL = negative for <i>mecA</i> -mediated resistance	≥ 4 µg/mL = positive for <i>mecA</i> -mediated resistance ≤ 2 µg/mL = negative for <i>mecA</i> -mediated resistance	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = positive for <i>mecA</i> -mediated resistance
Additional testing and reporting	Isolates that test positive for <i>mecA</i> -mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported. ^d			
QC recommendations - routine ^{e,f}	<i>S. aureus</i> ATCC [®] 25923 - <i>mecA</i> negative (zone 23-29 mm)	<i>S. aureus</i> ATCC [®] 29213 - <i>mecA</i> negative (MIC 1-4 µg/mL)	<i>S. aureus</i> ATCC [®] 29213 - <i>mecA</i> negative (MIC 0.12-0.5 µg/mL)	<i>S. aureus</i> ATCC ^{®c} 29213 - susceptible (≤ 1 colony; with each test day)
QC recommendations - lot/shipment ^h	N/A	<i>S. aureus</i> ATCC [®] 43300 - <i>mecA</i> positive (MIC ≥ 8 µg/mL)	<i>S. aureus</i> ATCC [®] 43300 - <i>mecA</i> positive (MIC ≥ 8 µg/mL)	<i>S. aureus</i> ATCC [®] 43300 - <i>mecA</i> positive (>1 colony)

Abbreviations: ATCC[®], American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-1. (Continued)

Footnotes

- a. Including members of the *S. aureus* complex (see Table 2C, comment [3]).
- b. Cefoxitin is used as a surrogate test for detecting *mecA*-mediated methicillin (oxacillin) resistance.
- c. Testing at temperatures above 35°C may not detect MRS.
- d. Testing of other β -lactam agents, except ceftaroline, is not advised.
- e. QC recommendations - routine
Test negative (susceptible) QC strain:
 - With each new lot/shipment of testing materials
 - Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02¹ and M07²)
- f. Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met
- g. ATCC® is a registered trademark of the American Type Culture Collection.
- h. QC Recommendations - lot/shipment
Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3G-1

- ¹ CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.
- ² CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.