

Table 3G-2. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus*^a and *Staphylococcus lugdunensis*

Test	Detecting <i>mecA</i> -Mediated Resistance Using Cefoxitin ^b	Detecting <i>mecA</i> -Mediated Resistance Using Oxacillin	
Test method	Disk diffusion	Disk diffusion	Broth microdilution and agar dilution
Organism group	<i>Staphylococcus</i> spp. except: <i>S. aureus</i> (refer to Table 3G-1) <i>S. lugdunensis</i> (refer to Table 3G-1) <i>S. pseudintermedius</i> (not recommended) <i>S. schleiferi</i> (not recommended)	Testing is only indicated for the species listed below: <i>S. epidermidis</i> <i>S. pseudintermedius</i> <i>S. schleiferi</i>	<i>Staphylococcus</i> spp. except: <i>S. aureus</i> (refer to Table 3G-1) <i>S. lugdunensis</i> (refer to Table 3G-1)
Medium	MHA	MHA	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)
Antimicrobial concentration	30 µg cefoxitin disk	1-µg oxacillin disk	0.5 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard disk diffusion procedure	Standard broth microdilution procedure or standard agar dilution procedure
Incubation conditions	33 to 35°C; ambient air ^c		
Incubation length	24 hours (may be reported after 18 hours, if resistant)	16-18 hours	24 hours (may be reported after 18 hours, if resistant)
Results	≤ 24 mm = positive for <i>mecA</i> -mediated resistance ≥ 25 mm = negative for <i>mecA</i> -mediated resistance	≤ 17 mm = positive for <i>mecA</i> -mediated resistance ≥ 18 mm = negative for <i>mecA</i> -mediated resistance	≥ 1 µg/mL = positive for <i>mecA</i> -mediated resistance ≤ 0.5 µg/mL = negative 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		For <i>Staphylococcus</i> spp., excluding <i>S. aureus</i> , <i>S. lugdunensis</i> , <i>S. epidermidis</i> , <i>S. pseudintermedius</i> , and <i>S. schleiferi</i> , oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1-2 µg/mL may be <i>mecA</i> negative. Isolates from serious infections for which oxacillin MICs are 1-2 µg/mL may be tested for <i>mecA</i> or for PBP2a. Isolates that test <i>mecA</i> or PBP2a negative should be reported as methicillin (oxacillin) susceptible.
QC recommendations - routine ^e	<i>S. aureus</i> ATCC ^{®f} 25923 - <i>mecA</i> negative (zone 23-29 mm)	<i>S. aureus</i> ATCC [®] 25923 - <i>mecA</i> negative (zone 18-24 mm)	<i>S. aureus</i> ATCC [®] 29213 - <i>mecA</i> negative (MIC 0.12-0.5 µg/mL)
QC recommendations - lot/shipment ^g	N/A	<i>S. aureus</i> ATCC [®] 43300 - <i>mecA</i> positive (zone ≤ 24 mm)	<i>S. aureus</i> ATCC [®] 43300 - <i>mecA</i> positive (MIC ≥ 8 µg/mL)

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-2. (Continued)

Footnotes

- a. Including members of the *S. aureus* complex (see Table 2C, general comment [3]).
- b. Cefoxitin is tested as a surrogate for detecting *mecA*-mediated methicillin (oxacillin) resistance; however, recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*).¹
- 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 M02² and M07³)
 - 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
- f. ATCC® is a registered trademark of the American Type Culture Collection.
- g. QC Recommendations - lot/shipment
Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3G-2

- ¹ Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J Clin Microbiol.* 2020;59(1):e02290-20.
- ² CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.
- ³ CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.