CLSI Archived Methods

Method	Date and Edition of First Publication	M100 Edition in Which This Procedure Was Last Listed	Comments	Procedure Available on Page(s):
Modified Hodge test	January 2009, M100-S19	January 2017, M100, 27th ed.	No longer considered a reliable phenotypic method for carbapenemase detection; other methods included in M100, such as the CarbaNP test and the mCIM, are more reliable.	2-4
Test for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus* and *Staphylococcus lugdunensis*	January 2008, M100-S18	March 2023, M100-Ed33	Removed cefoxitin and oxacillin methods that applied standard disk diffusion or microdilution (broth or agar) procedures that are outlined in Table 2C.	5-6
Test for detecting methicillin (oxacillin) resistance in *Staphylococcus* spp. except *Staphylococcus aureus* and *Staphylococcus lugdunensis*	March 2021, M100-Ed31	March 2023, M100-Ed33	Removed cefoxitin and oxacillin methods that applied standard disk diffusion or microdilution (broth or agar) procedures that are outlined in Table 2C.	7-8

Abbreviation: mCIM, modified carbapenem inactivation method.

The Modified Hodge Test for Suspected Carbapenemase Production in Enterobacterales

Abbreviations

ATCC®	American Type Culture Collection
MHA	Mueller-Hinton agar
MHT	modified Hodge test
MIC	minimal inhibitory concentration
QC	quality control

NOTE: If using FORMER MIC breakpoints for carbapenems described in M100-S20 (January 2010), please refer to modifications in CLSI document M100.

<table>
<tr><th>Test</th><th>MHT</th></tr>
<tr><td>When to do this test:</td><td>For epidemiological or infection control purposes. NOTE: No change in the interpretation of carbapenem susceptibility test results is necessary for carbapenemase-positive isolates.</td></tr>
<tr><td>Test method</td><td>MHT</td></tr>
<tr><td>Medium</td><td>MHA</td></tr>
<tr><td>Antimicrobial concentration</td><td>10-µg ertapenem or meropenem disk</td></tr>
<tr><td>Inoculum</td><td>1. Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of Escherichia coli ATCC®[a] 25922 (the indicator organism) in broth or saline, and dilute 1:10 in saline or broth. Inoculate an MHA plate as for the routine disk diffusion procedure. Allow the plate to dry 3–10 minutes. Place the appropriate number of ertapenem or meropenem disks on the plate as noted below and shown in Figures 1 and 2.

2. Using a 10-µL loop or swab, pick 3–5 colonies of test or QC organism grown overnight on a blood agar plate and inoculate in a straight line out from the edge of the disk. The streak should be at least 20–25 mm in length. Test the number of isolates per plate as noted below and shown in Figures 1 and 2.

Capacity of small and large MHA plates (100-mm or 150-mm diameter, respectively):

<table>
<tr><th></th><th>Small</th><th>Large</th></tr>
<tr><td>Disks</td><td>1</td><td>1–4</td></tr>
<tr><td>Test isolates</td><td>1</td><td>1–6</td></tr>
<tr><td>QC isolates</td><td>2</td><td>2</td></tr>
</table>
</td></tr>
<tr><td>Incubation conditions</td><td>35°C ± 2°C; ambient air</td></tr>
<tr><td>Incubation length</td><td>16–20 hours</td></tr>
<tr><td>Results</td><td>Following incubation, examine the MHA plate for enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition (see Figures 1 and 2):

• Enhanced growth = positive for carbapenemase production

• No enhanced growth = negative for carbapenemase production

Some test isolates may produce substances that inhibit growth of E. coli ATCC® 25922. When this occurs, a clear area is seen around the streak (see Figure 3), and the MHT is uninterpretable for these isolates.

NOTE: Not all carbapenemase-producing isolates of Enterobacterales are MHT positive, and MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.</td></tr>
<tr><td>Additional testing and reporting</td><td>Report results of the MHT to infection control or those requesting epidemiological information.

No change in the interpretation of carbapenem susceptibility test results is necessary for MHT-positive isolates.</td></tr>
<tr><td>QC recommendations</td><td>Test positive and negative QC organisms each day of testing.

Klebsiella pneumoniae ATCC® BAA-1705™[b]—MHT positive

K. pneumoniae ATCC® BAA-1706™—MHT negative</td></tr>
</table>

NOTE 1: Test recommendations were largely derived following testing of US isolates of Enterobacterales and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting *K. pneumoniae* carbapenemase–type carbapenemases in these isolates.[1] The sensitivity and specificity of the test for detecting other carbapenemase production can vary.

NOTE 2: No data exist on the usefulness of the MHT for the detection of carbapenemase production in nonfermenting gram-negative bacilli.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC name.

Reference for the Modified Hodge Test

1 Anderson KF, Lonsway DR, Rasheed JK, et al. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. *J Clin Microbiol*. 2007;45(8):2723-2725.

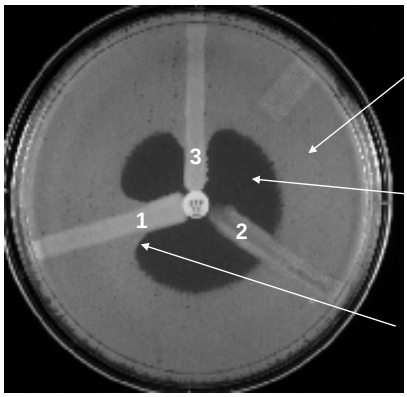

Figure 1. The MHT Performed on a Small MHA Plate.
(1) *K. pneumoniae* ATCC® BAA-1705™, positive result;
(2) *K. pneumoniae* ATCC® BAA-1706™, negative result;
and (3) a clinical isolate, positive result.

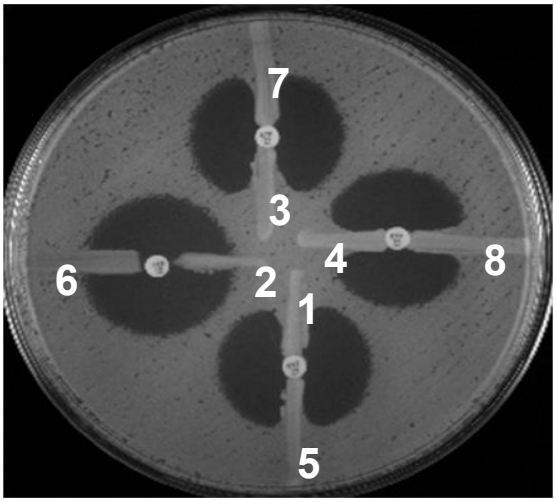

Figure 2. MHT Performed on a Large MHA Plate With Ertapenem. (1) *K. pneumoniae* ATCC® BAA-1705™, positive result; (2) *K. pneumoniae* ATCC® BAA-1706™, negative result; (3–8) clinical isolates; (6) negative result; (3, 4, 5, 7, 8) positive result.

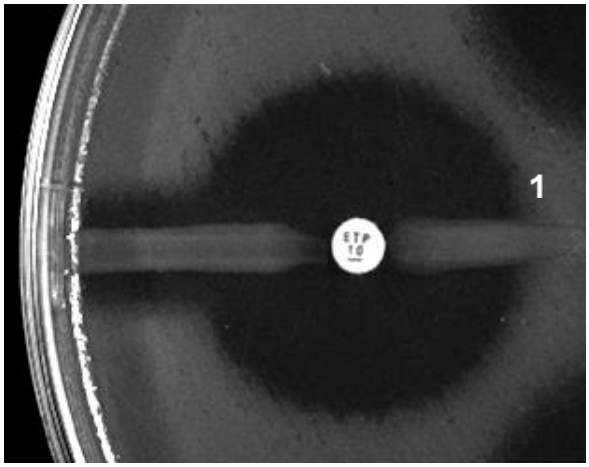

Figure 3. Example of an Indeterminate Result. (1) A clinical isolate with an indeterminate result; and (2) a clinical isolate with a negative result.

Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]		Detecting *mecA*-Mediated Resistance Using Oxacillin	Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar for *S. aureus* Only
Test method	Disk diffusion	Broth microdilution	Broth microdilution and agar dilution	Agar dilution for *S. aureus*
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 *mecA*-mediated resistance ≥ 22 mm = negative for *mecA*-mediated resistance	≥ 8 µg/mL = positive for *mecA*-mediated resistance ≤ 4 µg/mL = negative for *mecA*-mediated resistance	≥4 µg/mL = positive for *mecA*-mediated resistance ≤ 2 µg/mL = negative for *mecA*-mediated resistance	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = positive for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-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]	*S. aureus* ATCC®[g] 25923 – *mecA* negative (zone 23-29 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 1-4 µg/mL)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)	*S. aureus* ATCC®[c] 29213 – susceptible (≤ 1 colony; with each test day)
QC recommendations - lot/shipment[h]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* 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.

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 ß-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 M02[1] and M07[2])

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 Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus* and *Staphylococcus lugdunensis*

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.

Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]	Detecting *mecA*-Mediated Resistance Using Oxacillin	
Test method	Disk diffusion	Disk diffusion	Broth microdilution and agar dilution
Organism group	*Staphylococcus* spp. except: *S. aureus* (refer to previous table) *S. lugdunensis* (refer to previous table) *S. pseudintermedius* (not recommended) *S. schleiferi* (not recommended)	Testing is only indicated for the species listed below: *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	*Staphylococcus* spp. except: *S. aureus* (refer to previous table) *S. lugdunensis* (refer to previous table)
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 *mecA*-mediated resistance ≥ 25 mm = negative for *mecA*-mediated resistance	≤ 17 mm = positive for *mecA*-mediated resistance ≥ 18 mm = negative for *mecA*-mediated resistance	≥ 1 µg/mL = positive for *mecA*-mediated resistance ≤ 0.5 µg/mL = negative for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other ß-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]		
			For *Staphylococcus* spp., excluding *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*, oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 1–2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible.
QC recommendations - routine[e]	*S. aureus* ATCC®[f] 25923 – *mecA* negative (zone 23–29 mm)	*S. aureus* ATCC® 25923 – *mecA* negative (zone 18–24 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)
QC recommendations - lot/shipment[g]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 24 mm)	*S. aureus* ATCC® 43300 – *mecA* 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.

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 ß-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 M02[2] and M07[3])
- 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 Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus* and *Staphylococcus lugdunensis*

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.