

Table 2A. Zone Diameter and Minimal Inhibitory Concentration (MIC) Interpretive Standards for *Enterobacteriaceae*

Testing Conditions	Minimal Quality Control (QC) Recommendations (See Tables 3A and 4A for acceptable QC ranges.)
<p>Medium: Disk diffusion: Mueller-Hinton agar (MHA) Broth dilution: cation-adjusted Mueller-Hinton broth (CAMHB) Agar dilution: MHA</p> <p>Inoculum: Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard</p> <p>Incubation: 35±2°C; ambient air; Disk diffusion: 16 to 18 hours Dilution methods: 16 to 20 hours</p>	<p><i>Escherichia coli</i> ATCC®* 25922 <i>Escherichia coli</i> ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</p>

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

Refer to Table 2A Supplemental Tables 1, 2, and 3 at the end of Table 2A for additional recommendations for testing conditions, reporting suggestions, and QC.

General Comments

- (1) For disk diffusion, **test a maximum of 12 disks on a 150-mm plate and 5 disks on a 100-mm plate (see M02 Section 9.2)**. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black, nonreflecting background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
- (2) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested.
- (3) The dosage regimens shown in the comment column below are those **required to achieve** plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with infectious disease practitioners, pharmacists, pharmacy and therapeutics committees, and infection control committees.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria nearest whole mm			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
PENICILLINS									
A	Ampicillin	10 µg	≥ 17	14–16	≤ 13	≤ 8	16	≥ 32	(4) Results of ampicillin testing can be used to predict results for amoxicillin. See comment (2).
B	Piperacillin	100 µg	≥ 21	18–20	≤ 17	≤ 16	32–64	≥ 128	
O	Mecillinam	10 µg	≥ 15	12–14	≤ 11	≤ 8	16	≥ 32	(5) For testing and reporting of <i>E. coli</i> urinary tract isolates only.
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS									
B	Amoxicillin-clavulanic acid	20/10 µg	≥ 18	14–17	≤ 13	≤ 8/4	16/8	≥ 32/16	
B	Ampicillin-sulbactam	10/10 µg	≥ 15	12–14	≤ 11	≤ 8/4	16/8	≥ 32/16	
B	Piperacillin-tazobactam	100/10 µg	≥ 21	18–20	≤ 17	≤ 16/4	32/4–64/4	≥ 128/4	
B	Ticarcillin-clavulanate	75/10 µg	≥ 20	15–19	≤ 14	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
<p>(6) WARNING: For <i>Salmonella</i> spp. and <i>Shigella</i> spp., first- and second-generation cephalosporins and cephamycins may appear active <i>in vitro</i>, but are not effective clinically and should not be reported as susceptible.</p> <p>(7) Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised interpretive criteria for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefazolin interpretive criteria were revised again in June 2010 and are listed below. Cefepime and cefuroxime (parenteral) were also evaluated; however, no change in interpretive criteria was required for the dosages indicated below. When using the current interpretive criteria, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current interpretive criteria, ESBL testing should be performed as described in Table 2A Supplemental Table 1.</p> <p>Note that interpretive criteria for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for <i>E. coli</i>, <i>Klebsiella</i>, or <i>Proteus</i> spp., ESBL testing should be performed (see Table 2A Supplemental Table 1). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.</p> <p>(8) <i>Enterobacter</i>, <i>Citrobacter</i>, and <i>Serratia</i> may develop resistance during prolonged therapy with third-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy. Testing of repeat isolates may be warranted.</p>									
A	Cefazolin	30 µg	≥ 23	20–22	≤ 19	≤ 2	4	≥ 8	(9) Interpretive criteria are based on a dosage regimen of 2 g every 8 h. See comment (7).
U	Cephalothin	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(10) Cephalothin interpretive criteria can be used only to predict results to the oral agents, cefadroxil, cefpodoxime, cephalexin, and loracarbef. Older data that suggest that cephalothin results could predict susceptibility to some other cephalosporins may still be correct, but there are no recent data to confirm this.

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria nearest whole mm			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)									
B	Cefepime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(11) Interpretive criteria are based on a dosage regimen of 1 g every 8 h or 2 g every 12 h. See comment (7).
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	23–25 20–22	≤ 22 ≤ 19	≤ 1 ≤ 1	2 2	≥ 4 ≥ 4	(12) Interpretive criteria are based on a dosage regimen of 1 g every 24 h for ceftriaxone and 1 g every 8 h for cefotaxime. See comment (7).
B	Cefotetan	30 µg	≥ 16	13–15	≤ 12	≤ 16	32	≥ 64	
B	Cefoxitin	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(13) The interpretive criteria for cefoxitin are based on a dosage regimen of at least 8 g per day (eg, 2 g every 6 h).
B	Cefuroxime (parenteral)	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(14) Interpretive criteria are based on a dosage regimen of 1.5 g every 8 h. See comment (7).
C	Ceftazidime	30 µg	≥ 21	18–20	≤ 17	≤ 4	8	≥ 16	(15) Interpretive criteria are based on a dosage regimen of 1 g every 8 h. See comment (7).
O	Cefamandole	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	See comment (7).
O	Cefmetazole	30 µg	≥ 16	13–15	≤ 12	≤ 16	32	≥ 64	(16) Insufficient new data exist to reevaluate interpretive criteria listed here.
O	Cefonicid	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	See comment (7).
O	Cefoperazone	75 µg	≥ 21	16–20	≤ 15	≤ 16	32	≥ 64	See comment (7).
O	Ceftizoxime	30 µg	≥ 25	22–24	≤ 21	≤ 1	2	≥ 4	(17) Interpretive criteria are based on a dosage regimen of 1 g every 12 h. See comment (7).
O	Moxalactam	30 µg	≥ 23	15–22	≤ 14	≤ 8	16–32	≥ 64	See comment (7).
CEPHEMS (ORAL)									
B	Cefuroxime (oral)	30 µg	≥ 23	15–22	≤ 14	≤ 4	8–16	≥ 32	
O	Loracarbef	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(18) Because certain strains of <i>Citrobacter</i> , <i>Providencia</i> , and <i>Enterobacter</i> spp. have been reported to give false-susceptible results when tested by disk diffusion with cefdinir and loracarbef, strains of these genera should not be tested by disk diffusion with these agents .
O	Cefaclor	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	See comment (18).
O	Cefdinir	5 µg	≥ 20	17–19	≤ 16	≤ 1	2	≥ 4	
O	Cefixime	5 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	(19) For disk diffusion, not applicable for testing <i>Morganella</i> spp.
O	Cefpodoxime	10 µg	≥ 21	18–20	≤ 17	≤ 2	4	≥ 8	See comment (19).
O	Cefprozil	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	(20) Because certain strains of <i>Providencia</i> spp. have been reported to give false-susceptible results when tested by disk diffusion with cefprozil, strains of this genus should not be tested by disk diffusion with this agent .
Inv.	Cefetamet	10 µg	≥ 18	15–17	≤ 14	≤ 4	8	≥ 16	See comment (19).
Inv.	Ceftibuten	30 µg	≥ 21	18–20	≤ 17	≤ 8	16	≥ 32	(21) For testing and reporting of urine isolates only.

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria nearest whole mm			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
MONOBACTAMS									
C	Aztreonam	30 µg	≥21	18–20	≤17	≤4	8	≥16	(22) Interpretive criteria are based on a dosage regimen of 1 g every 8 h. See comment (7).
CARBAPENEMS									
<p>(23) Following evaluation of PK-PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase producing strains, revised interpretive criteria for carbapenems were first published in June 2010 (M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens, as has been reported in the literature.¹⁻⁴ Consultation with an infectious diseases practitioner is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges.</p> <p>Until laboratories can implement the current interpretive criteria, the MHT should be performed as described in the updated Table 2A Supplemental Table 3. After implementation of the current interpretive criteria, the MHT does not need to be performed other than for epidemiological or infection control purposes (refer to Table 2A Supplemental Table 2).</p> <p>The following information is provided as background on carbapenemases in <i>Enterobacteriaceae</i> that are largely responsible for MICs and zone diameters in the new intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints:</p> <ul style="list-style-type: none"> The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the new intermediate (I) range is uncertain due to lack of controlled clinical studies. Imipenem MICs for <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Morganella morganii</i> tend to be higher (eg, MICs in the new intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated MICs by mechanisms other than production of carbapenemases. 									
B	Doripenem	10 µg	≥23	20–22	≤19	≤1	2	≥4	(24) Interpretive criteria are based on a dosage regimen of 500 mg every 8 h.
B	Ertapenem	10 µg	≥22	19–21	≤18	≤0.5	1	≥2	(25) Interpretive criteria are based on a dosage regimen of 1 g every 24 h.
B	Imipenem	10 µg	≥23	20–22	≤19	≤1	2	≥4	(26) Interpretive criteria are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.
B	Meropenem	10 µg	≥23	20–22	≤19	≤1	2	≥4	(27) Interpretive criteria are based on a dosage regimen of 1 g every 8 h.
AMINOGLYCOSIDES									
(28) WARNING: For <i>Salmonella</i> spp. and <i>Shigella</i> spp., aminoglycosides may appear active <i>in vitro</i> but are not effective clinically and should not be reported as susceptible.									
A	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
A	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
B	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Kanamycin	30 µg	≥18	14–17	≤13	≤16	32	≥64	
O	Netilmicin	30 µg	≥15	13–14	≤12	≤8	16	≥32	
O	Streptomycin	10 µg	≥15	12–14	≤11	–	–	–	(29) There are no MIC interpretive standards.
TETRACYCLINES									
(30) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
C	Tetracycline	30 µg	≥15	12–14	≤11	≤4	8	≥16	
O	Doxycycline	30 µg	≥14	11–13	≤10	≤4	8	≥16	
O	Minocycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	

Table 2A
Enterobacteriaceae
M02 and M07

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria nearest whole mm			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
FLUOROQUINOLONES									
(31) NOTE: Reevaluation of fluoroquinolones is ongoing. See comment (2).									
B	Ciprofloxacin	5 µg	≥21	16–20	≤15	≤1	2	≥4	(32) For testing and reporting against <i>Enterobacteriaceae</i> other than <i>S. typhi</i> and extraintestinal <i>Salmonella</i> spp.
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
B	Ciprofloxacin	5 µg	≥31	21–30	≤20	≤0.06	0.12–0.5	≥1	(33) For reporting against <i>S. typhi</i> and extraintestinal <i>Salmonella</i> spp. only. (34) Because of limited clinical experience in the treatment of infections caused by <i>S. typhi</i> and extraintestinal <i>Salmonella</i> spp. with ciprofloxacin MICs or zone diameters in the intermediate range, clinicians may wish to use maximal oral or parenteral dosage regimens. See comment (36).
U	Lomefloxacin or ofloxacin	10 µg	≥22	19–21	≤18	≤2	4	≥8	
U	Norfloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
U	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	
O	Enoxacin	10 µg	≥18	15–17	≤14	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥18	15–17	≤14	≤2	4	≥8	
O	Gemifloxacin	5 µg	≥20	16–19	≤15	≤0.25	0.5	≥1	(35) FDA-approved for <i>Klebsiella pneumoniae</i> .
O	Grepafloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
Inv.	Fleroxacin	5 µg	≥19	16–18	≤15	≤2	4	≥8	
QUINOLONES									
O	Cinoxacin	100 µg	≥19	15–18	≤14	≤16	32	≥64	See comment (21).
O	Nalidixic acid	30 µg	≥19	14–18	≤13	≤16	–	≥32	(36) In addition to testing urine isolates, nalidixic acid may be used to test for reduced fluoroquinolone susceptibility in isolates from patients with extraintestinal <i>Salmonella</i> infections. Strains of <i>Salmonella</i> that test resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintestinal salmonellosis. However, nalidixic acid may not detect all mechanisms of fluoroquinolone resistance. Therefore, <i>Salmonella</i> strains may also be tested with ciprofloxacin and reported using the <i>Salmonella</i> spp. interpretive criteria above. See comments (32) and (33). See comments (21) and (31).
FOLATE PATHWAY INHIBITORS									
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	See comment (2).
U	Sulfonamides	250 or 300 µg	≥17	13–16	≤12	≤256	–	≥512	(37) Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	11–15	≤10	≤8	–	≥16	

Table 2A. (Continued)

PHENICOLS									
C	Chloramphenicol	30 µg	≥18	13–17	≤12	≤8	16	≥32	(38) Not routinely reported on isolates from the urinary tract.
FOSFOMYCINS									
O	Fosfomycin	200 µg	≥16	13–15	≤12	≤64	128	≥256	(39) For testing and reporting of <i>E. coli</i> urinary tract isolates only. (40) The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate. (41) The only approved MIC method for testing is agar dilution using agar media supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution MIC testing should not be performed.
NITROFURANS									
U	Nitrofurantoin	300 µg	≥17	15–16	≤14	≤32	64	≥128	

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; FDA, US Food and Drug Administration; MHA, Mueller-Hinton agar; MHT, modified Hodge test; MIC, minimal inhibitory concentration; PK-PD, pharmacokinetic-pharmacodynamic; QC, quality control.

Table 2A Supplemental Table 1. Screening and Confirmatory Tests for ESBLs in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis* for Use With Table 2A

NOTE: Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised interpretive criteria for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in Table 2A. Cefepime and cefuroxime (parenteral) were also evaluated; however, no change in interpretive criteria was required with the dosages included in Table 2A. When using the **current** interpretive criteria, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant). **However**, ESBL testing may still be useful for epidemiological or infection control purposes. **For laboratories that have not implemented the current interpretive criteria, ESBL testing should be performed as described in this table.**

Test	Initial Screen Test		Phenotypic Confirmatory Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB	MHA	CAMHB
Antimicrobial concentration	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime 10 µg or Ceftazidime 30 µg or Aztreonam 30 µg or Cefotaxime 30 µg or Ceftriaxone 30 µg</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime 10 µg or Ceftazidime 30 µg or Cefotaxime 30 µg</p> <p>(The use of more than one antimicrobial agent for screening improves the sensitivity of detection.)</p>	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime 4 µg/mL or Ceftazidime 1 µg/mL or Aztreonam 1 µg/mL or Cefotaxime 1 µg/mL or Ceftriaxone 1 µg/mL</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime 1 µg/mL or Ceftazidime 1 µg/mL or Cefotaxime 1 µg/mL</p> <p>(The use of more than one antimicrobial agent for screening improves the sensitivity of detection.)</p>	<p>Ceftazidime 30 µg Ceftazidime-clavulanic acid^a 30/10 µg</p> <p>and</p> <p>Cefotaxime 30 µg Cefotaxime-clavulanic acid 30/10 µg</p> <p>(Confirmatory testing requires use of both cefotaxime and ceftazidime, alone and in combination with clavulanic acid.)</p>	<p>Ceftazidime 0.25–128 µg/mL Ceftazidime-clavulanic acid 0.25/4–128/4 µg/mL</p> <p>and</p> <p>Cefotaxime 0.25–64 µg/mL Cefotaxime-clavulanic acid 0.25/4–64/4 µg/mL</p> <p>(Confirmatory testing requires use of both cefotaxime and ceftazidime, alone and in combination with clavulanic acid.)</p>
Inoculum	Standard disk diffusion recommendations	Standard broth dilution recommendations	Standard disk diffusion recommendations	Standard broth dilution recommendations
Incubation conditions	35±2°C; ambient air	35±2°C; ambient air	35±2°C; ambient air	35±2°C; ambient air
Incubation length	16–18 hours	16–20 hours	16–18 hours	16–20 hours
Results	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime zone ≤17 mm Ceftazidime zone ≤22 mm Aztreonam zone ≤27 mm Cefotaxime zone ≤27 mm Ceftriaxone zone ≤25 mm</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime zone ≤22 mm Ceftazidime zone ≤22 mm Cefotaxime zone ≤27 mm</p> <p>Zones above may indicate ESBL production.</p>	<p>Growth at or above the screening concentrations may indicate ESBL production (ie, for <i>E. coli</i>, <i>K. pneumoniae</i>, and <i>K. oxytoca</i>, MIC ≥8 µg/mL for cefpodoxime or MIC ≥2 µg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for <i>P. mirabilis</i>, MIC ≥2 µg/mL for cefpodoxime, ceftazidime, or cefotaxime).</p>	<p>A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanic acid zone = 21).</p>	<p>A ≥3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 µg/mL; ceftazidime-clavulanic acid MIC = 1 µg/mL).</p>

Table 2A Supplemental Table 1. (Continued)

Test	Initial Screen Test		Phenotypic Confirmatory Test		
	Test Method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Reporting			<p>For all confirmed ESBL-producing strains: If laboratories do not use current cephalosporin and aztreonam interpretive criteria, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam.</p> <p>If laboratories use current cephalosporin and aztreonam interpretive criteria, then test interpretations for these agents do not need to be changed.</p>		
QC recommendations	<p>When testing ESBL-screening antimicrobial agents, <i>K. pneumoniae</i> ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competency, or test evaluation). Either strain, <i>K. pneumoniae</i> ATCC® 700603 or <i>E. coli</i> ATCC® 25922, may then be used for routine QC (eg, weekly or daily).</p> <p><i>E. coli</i> ATCC® 25922 (see acceptable QC ranges in Table 3A)</p> <p><i>K. pneumoniae</i> ATCC® 700603: Cefpodoxime zone 9–16 mm Ceftazidime zone 10–18 mm Aztreonam zone 9–17 mm Cefotaxime zone 17–25 mm Ceftriaxone zone 16–24 mm</p>	<p>When testing ESBL-screening antimicrobial agents, <i>K. pneumoniae</i> ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competency, or test evaluation). Either strain, <i>K. pneumoniae</i> ATCC® 700603 or <i>E. coli</i> ATCC® 25922, may then be used for routine QC (eg, weekly or daily).</p> <p><i>E. coli</i> ATCC® 25922 = No growth (also see acceptable QC ranges listed in Table 4A).</p> <p><i>K. pneumoniae</i> ATCC® 700603 = Growth: Cefpodoxime MIC ≥ 8 µg/mL Ceftazidime MIC ≥ 2 µg/mL Aztreonam MIC ≥ 2 µg/mL Cefotaxime MIC ≥ 2 µg/mL Ceftriaxone MIC ≥ 2 µg/mL</p>	<p>When performing the ESBL confirmatory tests, <i>K. pneumoniae</i> ATCC® 700603 and <i>E. coli</i> ATCC® 25922 should be used for routine QC (eg, weekly or daily).</p> <p>Acceptable QC: <i>E. coli</i> ATCC® 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanic acid vs the zone diameter when tested alone.</p> <p><i>K. pneumoniae</i> ATCC® 700603: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanic acid vs ceftazidime alone; ≥ 3-mm increase in zone diameter of cefotaxime-clavulanic acid vs cefotaxime alone.</p>	<p>When performing the ESBL confirmatory tests, <i>K. pneumoniae</i> ATCC® 700603 and <i>E. coli</i> ATCC® 25922 should be tested routinely (eg, weekly or daily).</p> <p>Acceptable QC: <i>E. coli</i> ATCC® 25922: < 3 twofold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanic acid vs the MIC of the agent when tested alone.</p> <p><i>K. pneumoniae</i> ATCC® 700603: ≥ 3 twofold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanic acid vs the MIC of the agent when tested alone.</p>	

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK-PD, pharmacokinetic-pharmacodynamic; QC, quality control.

Footnotes

- a. Preparation of ceftazidime-clavulanic acid (30 µg/10 µg) and cefotaxime-clavulanic acid (30 µg/10 µg) disks: Using a stock solution of clavulanic acid at 1000 µg/mL (either freshly prepared or taken from small aliquots that have been frozen at -70°C), add 10 µL of clavulanic acid to ceftazidime (30 µg) and cefotaxime (30 µg) disks. Use a micropipette to apply the 10 µL of stock solution to the ceftazidime and cefotaxime disks within one hour before they are applied to the plates, allowing about 30 minutes for the clavulanic acid to absorb and the disks to be dry enough for application. Use disks immediately after preparation or discard; do not store.

Table 2A Supplemental Table 2. Confirmatory Test for Suspected Carbapenemase Production in *Enterobacteriaceae* for Use With Table 2A

Until laboratories can implement the revised carbapenem interpretive criteria (now considered current), the MHT should be performed as described in Table 2A Supplemental Table 3. If using current interpretive criteria, the MHT does not need to be performed other than for epidemiological or infection control purposes (refer to Table 2A Supplemental Table 2) and no change in the interpretation of carbapenem susceptibility test results is required for MHT-positive isolates.

When to do this test:	Institutional infection control procedures or epidemiological investigations may require identification of carbapenemase-producing <i>Enterobacteriaceae</i> . Carbapenemase-producing isolates usually test intermediate or resistant to one or more carbapenems using the current interpretive criteria as listed in Table 2A (Note: Ertapenem nonsusceptibility is the most sensitive indicator of carbapenemase production), and test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). Therefore, for infection control or epidemiological investigations , testing could be limited to isolates with these characteristics.												
Test method	MHT												
Medium	MHA												
Antimicrobial concentration	Ertapenem disk 10 µg or Meropenem disk 10 µg												
Inoculum	(1) Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of <i>E. coli</i> ATCC® 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 to 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 to 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 to 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 border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th></th> <th>Small</th> <th>Large</th> </tr> </thead> <tbody> <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> </tbody> </table>		Small	Large	Disks	1	1–4	Test isolates	1	1–6	QC isolates	2	2
	Small	Large											
Disks	1	1–4											
Test isolates	1	1–6											
QC isolates	2	2											
Incubation conditions	35 ± 2°C; ambient air												
Incubation length	16 to 20 hours												

Table 2A Supplemental Table 2. (Continued)

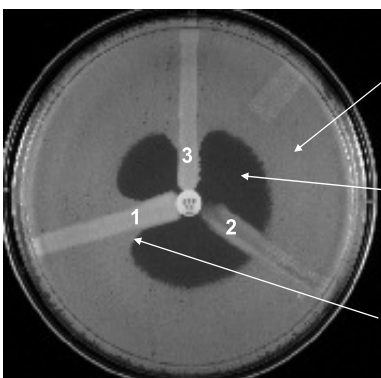
<p>Results</p>	<p>Following incubation, examine the MHA plate for enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition (see Figures 1 and 2).</p> <p>Enhanced growth = positive for carbapenemase production.</p> <p>No enhanced growth = negative for carbapenemase production.</p> <p>Some test isolates may produce substances that will inhibit growth of <i>E. coli</i> ATCC® 25922. When this occurs, a clear area will be seen around the streak (see Figure 3), and the MHT is uninterpretable for these isolates.</p> <p>For isolates with positive MHTs, perform MIC tests before reporting any carbapenem results, since carbapenem MIC interpretations are based solely on the MIC and should not be changed regardless of the MHT result.</p> <p>NOTE: Not all carbapenemase-producing isolates of <i>Enterobacteriaceae</i> are MHT positive and MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.</p>
<p>Reporting</p>	<p>Report results of the MHT to infection control or those requesting epidemiological information.</p> <p>No change in the interpretation of carbapenems susceptibility test results is required for MHT-positive isolates.</p>
<p>QC recommendations</p>	<p>Test positive and negative QC organisms each day of testing.</p> <p><i>K. pneumoniae</i> ATCC® BAA-1705—MHT positive</p> <p><i>K. pneumoniae</i> ATCC® BAA-1706—MHT negative</p>

Abbreviations: ATCC, American Type Culture Collection; KPC, *Klebsiella pneumoniae* carbapenemase; MHA, Mueller-Hinton agar; MHT, modified Hodge test; MIC, minimal inhibitory concentration; QC, quality control.

Table 2A Supplemental Table 2. (Continued)

NOTES:

1. Test recommendations were largely derived following testing of US isolates of *Enterobacteriaceae*, and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting low-level metallo- β -lactamase production are not known.
2. No data exist on the usefulness of these tests for the detection of carbapenemase production in nonfermenting gram-negative bacilli.



E. coli ATCC® 25922

Inhibition of *E. coli* ATCC® 25922
by ertapenem

Enhanced growth of *E. coli* ATCC®
25922. Carbapenemase produced by *K.
pneumoniae* ATCC® BAA-1705
inactivated ertapenem that diffused into
the media. Thus, there is no longer
sufficient ertapenem here to inhibit *E.
coli* ATCC® 25922 and an indentation of
the zone is noted.

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.

Table 2A Supplemental Table 2. (Continued)

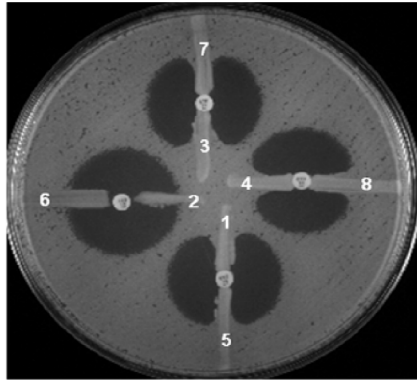


Figure 2. The MHT Performed on a Large MHA Plate With Ertapenem. (1) *K. pneumoniae* ATCC® BAA-1705, positive result; (2) *K. pneumoniae* ATCC® BAA-1706, negative result; (3–8) clinical isolates; (6) negative result; (3, 4, 5, 7, 8) positive result.

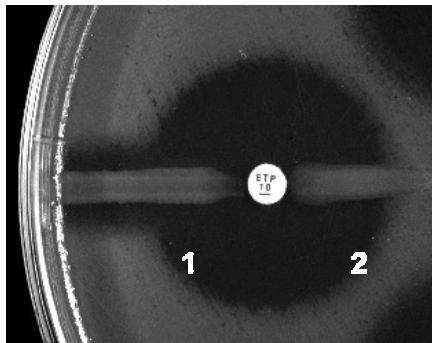


Figure 3. An Example of an Indeterminate Result. (1) A clinical isolate with an indeterminate result; and (2) a clinical isolate with a negative result.

Table 2A Supplemental Table 2
Confirmatory Test for Suspected
Carbapenemase Production
in *Enterobacteriaceae*

Table 2A Supplemental Table 3. Screening and Confirmatory Tests for Suspected Carbapenemase Production in *Enterobacteriaceae* When Using “Old” Interpretive Criteria for Carbapenems (for Use With Table 2A in M100-S20 [January 2010])

Until the current interpretive criteria for carbapenems are implemented, the screen and confirmatory tests should be performed and reported using the new instructions for a positive MHT described below. It is not necessary to test an isolate for a carbapenemase by the MHT when all of the carbapenems that are reported by a laboratory test either intermediate or resistant (ie, intermediate or resistant results should be reported as tested). However, if the isolate tests intermediate or resistant, the MHT may be performed for epidemiological purposes to determine if a carbapenemase is present.

Test	Initial Screen Test		Phenotypic Confirmatory Test												
When to do this test	The following applies ONLY when using interpretive criteria for carbapenems described in M100-S20 (January 2010).		Positive screening test and resistance to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone).												
Test method	Disk diffusion	Broth microdilution	MHT												
Medium	MHA	CAMHB	MHA												
Antimicrobial concentration	Ertapenem 10 µg or Meropenem 10 µg (NOTE: The imipenem disk test performs poorly as a screen for carbapenemases.)	Ertapenem 1 µg/mL or Imipenem 1 µg/mL or Meropenem 1 µg/mL	Ertapenem disk 10 µg or Meropenem disk 10 µg												
Inoculum	Standard disk diffusion recommendations	Standard broth dilution recommendations	(1) Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of <i>E. coli</i> ATCC® 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 to 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 to 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 to 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 style="margin-left: auto; margin-right: auto;"> <tr> <td></td> <td>Small</td> <td>Large</td> </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>		Small	Large	Disks	1	1–4	Test isolates	1	1–6	QC isolates	2	2
	Small	Large													
Disks	1	1–4													
Test isolates	1	1–6													
QC isolates	2	2													
Incubation conditions	35±2°C; ambient air	35±2°C; ambient air	35±2°C; ambient air												
Incubation length	16–18 hours	16–20 hours	16–20 hours												

Table 2A Supplemental Table 3. (Continued)

Test	Initial Screen Test		Phenotypic Confirmatory Test
Results	Ertapenem 19–21 mm Meropenem 16–21 mm The zone diameters of inhibition listed above may indicate carbapenemase production, despite the fact that they are in the old susceptible interpretive categories. For confirmation, perform the MHT. (NOTE: The imipenem disk test performs poorly as a screen for carbapenemases.)	Ertapenem 2 µg/mL Imipenem 2–4 µg/mL Meropenem 2–4 µg/mL MICs listed above may indicate carbapenemase production, despite the fact that they are in the old susceptible interpretive categories in M100-S20 (January 2010). For confirmation, perform the MHT.	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 will inhibit growth of <i>E. coli</i> ATCC® 25922. When this occurs, a clear area will be seen around the streak (see Figure 3) and the MHT is uninterpretable for these isolates. For isolates positive with the ertapenem or meropenem disk screen AND positive with the MHT, perform the MIC test before reporting any carbapenem results.
Reporting			The following applies ONLY when using interpretive criteria for carbapenems described in M100-S20 (January 2010). For isolates that are MHT positive and have an ertapenem MIC of 2–4 µg/mL, imipenem MIC of 2–8 µg/mL, or meropenem MIC of 2–8 µg/mL, report all carbapenems as resistant. If the MHT is negative, interpret the carbapenem MICs using CLSI interpretive criteria as listed in Table 2A in M100-S20 (January 2010). NOTE: Not all carbapenemase-producing isolates of <i>Enterobacteriaceae</i> are MHT positive and MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.
QC recommendations	<i>E. coli</i> ATCC® 25922 (see acceptable QC ranges in Table 3A).	<i>E. coli</i> ATCC® 25922 (see acceptable QC ranges in Table 4A).	Test positive and negative QC organisms each day of testing. <i>K. pneumoniae</i> ATCC® BAA-1705—MHT positive <i>K. pneumoniae</i> ATCC® BAA-1706—MHT negative

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; KPC, *Klebsiella pneumoniae* carbapenemase; MHA, Mueller-Hinton agar; MHT, modified Hodge test; MIC, minimal inhibitory concentration; QC, quality control.

Table 2A Supplemental Table 3
 Screening and Confirmatory Tests
 for Suspected Carbapenemase
 Production in *Enterobacteriaceae*
 Using “Old” Interpretive Criteria

Table 2A Supplemental Table 3. (Continued)

NOTES:

1. *Proteus* spp., *Providencia* spp., and *Morganella* spp. may have elevated MICs to imipenem by mechanisms other than production of carbapenemases; thus, the usefulness of the imipenem MIC screen test for the detection of carbapenemases in these three genera is not established. Also, the imipenem disk test performs poorly as a screen for carbapenemases for all *Enterobacteriaceae*.
2. The screening and confirmatory test recommendations were largely derived following testing of US isolates of *Enterobacteriaceae*, and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting low-level metallo- β -lactamase production are not known.
3. No data exist on the usefulness of these tests for the detection of carbapenemase production in nonfermenting gram-negative bacilli.

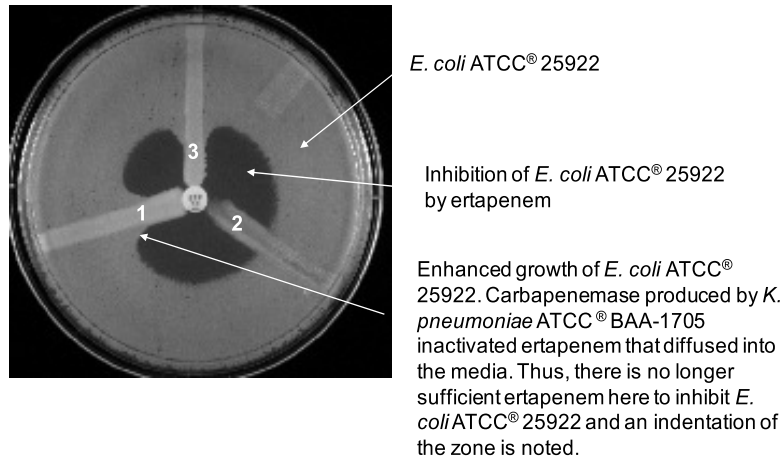


Figure 1. The MHT Performed on a Small MHA Plate.
(1) *K. pneumoniae* ATCC® BAA-1705, positive result;
(2) *K. pneumoniae* ATCC® BAA-1706, negative result;
and (3) a clinical isolate, positive result.

Table 2A Supplemental Table 3. (Continued)

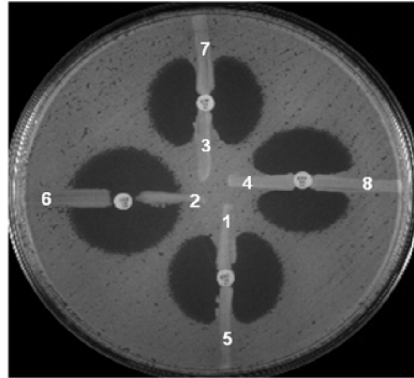


Figure 2. The MHT Performed on a Large MHA Plate With Ertapenem. (1) *K. pneumoniae* ATCC® BAA-1705, positive result; (2) *K. pneumoniae* ATCC® BAA-1706, negative result; (3–8) clinical isolates; (6) negative result; (3, 4, 5, 7, 8) positive result.

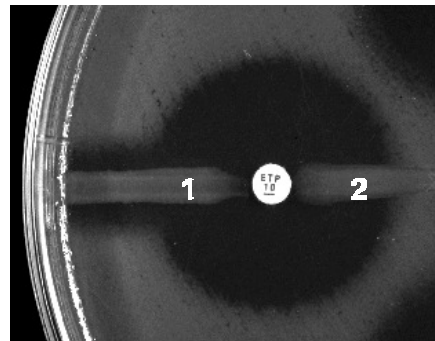


Figure 3. An Example of an Indeterminate Result. (1) A clinical isolate with an indeterminate result; and (2) a clinical isolate with a negative result.

Table 2A Supplemental Table 3. (Continued)

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