

European Committee on Antimicrobial Susceptibility Testing

Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST

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Notes

1. In EUCAST quality control (QC) tables, both ranges and targets are listed. Repeat testing of EUCAST quality control strains should yield individual MIC and zone diameter values randomly distributed within the recommended ranges. If the number of tests is ≥ 10 , the mode MIC should be the target value and the mean zone diameter should be close to the target value.
2. For access to ISO standard documents, see http://www.eucast.org/documents/external_documents/.
3. EUCAST quality control strains for routine QC are used to monitor test performance. Control tests should be set up and checked daily, at least for antibiotic agents which are part of routine panels. For analysis of the QC test results, see [EUCAST Disk Diffusion Manual](#).
4. Specific β -lactamase-producing strains are recommended to check the inhibitor component of β -lactam- β -lactamase inhibitor combinations. This should be part of the routine QC. The active component is checked with a susceptible QC strain.
5. EUCAST quality control strains for extended QC are complementary to the EUCAST routine QC strains. These strains are recommended for detection of specific resistance mechanisms (ESBL, MRSA, VRE, HLGR and PBP mutations) and are used to check that routine susceptibility testing will result in the correct S, I and R categorisation. Extended QC should be performed with any change in the susceptibility testing system (with each new batch of disks or medium) and/or monthly.

Changes from previous version

Version 7.0 2017-01-01	Changes Cells containing a change or an addition from EUCAST QC Tables v. 6.1 are marked yellow.
ATCC 25922	<p>General</p> <ul style="list-style-type: none"> • Typo error on tigecycline MIC target value corrected <p>New QC ranges</p> <ul style="list-style-type: none"> • Ceftazidime-avibactam (MIC and zone diameter) • Fosfomycin (zone diameter) • Nitroxoline (zone diameter) <p>Revised QC ranges</p> <ul style="list-style-type: none"> • Cefixime (zone diameter) • Ciprofloxacin (zone diameter) <p>New comments</p> <ul style="list-style-type: none"> • Comments 8, 9, 12, 14, 15, 16 and 17 <p>Revised comments</p> <ul style="list-style-type: none"> • Comment 13
ATCC 27853	<p>New QC ranges</p> <ul style="list-style-type: none"> • Ceftazidime-avibactam (MIC and zone diameter) <p>New comments</p> <ul style="list-style-type: none"> • Comments 4, 5 and 8 <p>Revised comments</p> <ul style="list-style-type: none"> • Comment 9
ATCC 29213	<p>New QC ranges</p> <ul style="list-style-type: none"> • Telithromycin (MIC) <p>Revised comments</p> <ul style="list-style-type: none"> • Comment 6
ATCC 49619	<p>General</p> <ul style="list-style-type: none"> • Typo error on ceftobiprole MIC target value corrected
ATCC 49766	<p>Revised comments</p> <ul style="list-style-type: none"> • Comment 4
Control of the inhibitor component of inhibitor-combinations	<p>New QC ranges</p> <ul style="list-style-type: none"> • Ceftazidime-avibactam for <i>K. pneumoniae</i> ATCC 700603 (MIC and zone diameter) • Piperacillin-tazobactam for <i>K. pneumoniae</i> ATCC 700603 (MIC and zone diameter) <p>New comments</p> <ul style="list-style-type: none"> • Comment 8



Routine quality control

***Escherichia coli* ATCC 25922**

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	2-8	-	-	-
Amoxicillin-clavulanic acid ^{4,5}	4	2-8	20-10	21	18-24 ⁶
Ampicillin	4	2-8	10	18-19	15-22 ⁶
Ampicillin-sulbactam ^{5,7}	2	1-4	10-10	21-22	19-24 ⁶
Aztreonam	0.125	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	17	14-20
Cefalexin	8	4-16	30	18	15-21
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37
Cefixime	0.5	0.25-1	5	23	20-26
Cefotaxime	0.06	0.03-0.125	5	28	25-31
Cefoxitin	4	2-8	30	26	23-29
Cefpodoxime	0.5	0.25-1	10	25-26	23-28
Ceftaroline	0.06	0.03-0.125	5	27	24-30
Ceftazidime	0.125-0.25	0.06-0.5	10	26	23-29
Ceftazidime-avibactam ^{8,9}	0.125-0.25	0.06-0.5	10-4	27	24-30
Ceftibuten	0.25	0.125-0.5	30	31	27-35
Ceftobiprole	0.06	0.03-0.125	5	28	25-31
Ceftolozane-tazobactam ^{10,11}	0.25	0.125-0.5	30-10	28	24-32
Ceftriaxone	0.06	0.03-0.125	30	32	29-35
Cefuroxime	4	2-8	30	23	20-26
Chloramphenicol	4	2-8	30	24	21-27
Ciprofloxacin	0.008	0.004-0.016	5	33	29-37
Colistin ¹²	0.5-1	0.25-2	-	-	-
Doripenem	0.03	0.016-0.06	10	31	27-35
Ertapenem	0.008	0.004-0.016	10	32-33	29-36
Fosfomycin ¹³	1	0.5-2	200 ¹⁴	30	26-34¹⁵
Gentamicin	0.5	0.25-1	10	22-23	19-26
Imipenem	0.125	0.06-0.25	10	29	26-32
Levofloxacin	0.016-0.03	0.008-0.06	5	33	29-37
Mecillinam ¹⁶	0.06-0.125	0.03-0.25	10	27	24-30
Meropenem	0.016-0.03	0.008-0.06	10	31-32	28-35
Moxifloxacin	0.016-0.03	0.008-0.06	5	31-32	28-35
Nalidixic acid	2	1-4	30	25	22-28
Netilmicin	-	≤0.5-1	10	21	18-24
Nitrofurantoin	8	4-16	100	20	17-23
Nitroxoline	Note ¹⁷	Note ¹⁷	30	21	18-24
Norfloxacin	0.06	0.03-0.125	10	31-32	28-35
Ofloxacin	0.03-0.06	0.016-0.125	5	31	29-33
Pefloxacin	-	-	5	29	26-32
Piperacillin	2	1-4	30	24	21-27
Piperacillin-tazobactam ^{10,11}	2	1-4	30-6	24	21-27
Ticarcillin	8	4-16	75	27	24-30
Ticarcillin-clavulanic acid ^{4,5}	8	4-16	75-10	27	24-30
Tigecycline ¹⁸	0.06-0.125	0.03-0.25	15	23-24	20-27
Tobramycin	0.5	0.25-1	10	22	18-26
Trimethoprim	1	0.5-2	5	24-25	21-28
Trimethoprim-sulfamethoxazole ¹⁹	≤0.5 ²	-	1.25-23.75	26	23-29

***Escherichia coli* ATCC 25922**

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

¹ Calculated by EUCAST.² From International Standards Organisation, ISO 20776-1: 2006 (with updates as in the latest CLSI M100 document), except ranges in bold/italics established by EUCAST. All ranges have been validated by EUCAST.³ From Clinical and Laboratory Standards Institute, M100-S26, 2016, except ranges in bold/italics established by EUCAST. All ranges have been validated by EUCAST.⁴ For MIC testing, the concentration of clavulanic acid is fixed at 2 mg/L.⁵ *E. coli* ATCC 35218 is used to check the inhibitor component (see Routine quality control for β -lactam- β -lactamase inhibitor combinations).⁶ Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agar.⁷ For MIC testing, the concentration of sulbactam is fixed at 4 mg/L.⁸ For MIC testing, the concentration of avibactam is fixed at 4 mg/L.⁹ *K. pneumoniae* ATCC 700603 is used to check the inhibitor component (see Routine quality control for β -lactam- β -lactamase inhibitor combinations).¹⁰ For MIC testing, the concentration of tazobactam is fixed at 4 mg/L.¹¹ Either *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603 can be used to check the inhibitor component (see Routine quality control for β -lactam- β -lactamase inhibitor combinations).¹² Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive). For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only on occasion be 2 or 8 mg/L.¹³ Agar dilution is the reference method for fosfomycin. Fosfomycin MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturer's instructions for commercial systems.¹⁴ Fosfomycin 200 g disks must contain 50 μ g glucose-6-phosphate.¹⁵ Ignore isolated colonies within the inhibition zone and read the outer zone edge (for reading examples see the EUCAST Reading Guide or Breakpoint Tables).¹⁶ Agar dilution is the reference method for mecillinam MIC determination.¹⁷ There is currently no MIC range for *E. coli* ATCC 25922 and nitroxoline.¹⁸ For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.¹⁹ Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

***Pseudomonas aeruginosa* ATCC 27853**

(NCTC 12903, CIP 76.110, DSM 1117, CCUG 17619, CECT 108)

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amikacin	2	1-4	30	22	18-26
Aztreonam	4	2-8	30	26	23-29
Cefepime	1-2	0.5-4	30	28	25-31
Ceftazidime	2	1-4	10	24	21-27
Ceftazidime-avibactam ^{4,5}	1-2	0.5-4	10-4	24	21-27
Ceftolozane-tazobactam ^{6,7}	0.5	0.25-1	30-10	28	25-31
Ciprofloxacin	0.5	0.25-1	5	29	25-33
Colistin ⁸	1-2	0.5-4	-	-	-
Doripenem	0.25	0.125-0.5	10	31-32	28-35
Fosfomycin ⁹	4	2-8	-	-	-
Gentamicin	1	0.5-2	10	20	17-23
Imipenem	2	1-4	10	24	20-28
Levofloxacin	1-2	0.5-4	5	22-23	19-26
Meropenem	0.5	0.25-1	10	30	27-33
Netilmicin	2	0.5-8	10	18	15-21
Piperacillin	2-4	1-8	-	-	-
Piperacillin-tazobactam ^{6,7}	2-4	1-8	30-6	26	23-29
Ticarcillin	16	8-32	-	-	-
Ticarcillin-clavulanic acid ^{10,11}	16	8-32	75-10	24	20-28
Tobramycin	0.5	0.25-1	10	23	20-26

¹ Calculated by EUCAST.² From International Standards Organisation, ISO 20776-1: 2006 (with updates as in the latest CLSI M100 document). All ranges have been validated by EUCAST.³ From Clinical and Laboratory Standards Institute, M100-S26, 2016, except ranges in bold/italics established by EUCAST. All ranges have been validated by EUCAST.⁴ For MIC testing, the concentration of avibactam is fixed at 4 mg/L.⁵ *K. pneumoniae* ATCC 700603 is used to check the inhibitor component (see Routine quality control for β-lactam-β-lactamase inhibitor combinations).⁶ For MIC testing, the concentration of tazobactam is fixed at 4 mg/L.⁷ Either *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603 can be used to check the inhibitor component (see Routine quality control for β-lactam-β-lactamase inhibitor combinations).⁸ Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive). For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only on occasion be 2 or 8 mg/L.⁹ Agar dilution is the reference method for fosfomycin. Fosfomycin MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturer's instructions for commercial systems.¹⁰ *E. coli* ATCC 35218 is used to check the inhibitor component (see Routine quality control for β-lactam-β-lactamase inhibitor combinations).¹¹ For MIC testing, the concentration of clavulanic acid is fixed at 2 mg/L.

***Staphylococcus aureus* ATCC 29213**

(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

β-lactamase-producing strain (weak)

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amikacin	2	1-4	30	21	18-24
Ampicillin	-	-	2	18	15-21
Azithromycin	1	0.5-2	-	-	-
Benzylpenicillin	0.5-1	0.25-2	1 unit	15	12-18
Cefoxitin	2	1-4	30	27	24-30
Ceftaroline	0.25	0.125-0.5	5	27	24-30
Ceftobiprole	0.25-0.5	0.125-1	5	25	22-28
Chloramphenicol	4-8	2-16	30	24	20-28
Ciprofloxacin	0.25	0.125-0.5	5	24	21-27
Clarithromycin	0.25	0.125-0.5	-	-	-
Clindamycin	0.125	0.06-0.25	2	26	23-29
Dalbavancin ⁴	0.06	0.03-0.125	-	-	-
Daptomycin ⁵	0.25-0.5	0.125-1	-	-	-
Doxycycline	0.25	0.125-0.5	-	-	-
Erythromycin	0.5	0.25-1	15	26	23-29
Fosfomycin ⁶	1-2	0.5-4	-	-	-
Fusidic acid	0.125	0.06-0.25	10	29	26-32
Gentamicin	0.25-0.5	0.125-1	10	22	19-25
Levofloxacin	0.125-0.25	0.06-0.5	5	26	23-29
Linezolid	2	1-4	10	24	21-27
Minocycline	0.125-0.25	0.06-0.5	30	26	23-29
Moxifloxacin	0.03-0.06	0.016-0.125	5	28	25-31
Mupirocin	0.125	0.06-0.25	200	34	31-37
Netilmicin	≤0.25 ²	-	10	23	20-26
Nitrofurantoin	16	8-32	100	20	17-23
Norfloxacin	1	0.5-2	10	21	18-24
Oflloxacin	0.25-0.5	0.125-1	5	24	21-27
Oritavancin ⁴	0.03-0.06	0.016-0.125	-	-	-
Quinupristin-dalfopristin	0.5	0.25-1	15	24	21-27
Rifampicin	0.008	0.004-0.016	5	33	30-36
Tedizolid	0.5	0.25-1	-	-	-
Teicoplanin	0.5	0.25-1	-	-	-
Telavancin ⁴	0.06	0.03-0.125	-	-	-
Telithromycin	0.125	0.06-0.25	15	IP	IP
Tetracycline	0.25-0.5	0.125-1	30	27	23-31
Tigecycline ⁷	0.06-0.125	0.03-0.25	15	22	19-25
Tobramycin	0.25-0.5	0.125-1	10	23	20-26
Trimethoprim	2	1-4	5	25	22-28
Trimethoprim-sulfamethoxazole ⁸	≤0.5 ²	-	1.25-23.75	29	26-32
Vancomycin	1	0.5-2	-	-	-

***Staphylococcus aureus* ATCC 29213**

(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

 β -lactamase-producing strain (weak)¹ Calculated by EUCAST.² From International Standards Organisation, ISO 20776-1: 2006 (with updates as in the latest CLSI M100 document). All ranges have been validated by EUCAST.³ Established and validated by EUCAST.⁴ MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.⁵ Daptomycin MICs must be determined in the presence of Ca²⁺ (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.⁶ Agar dilution is the reference method for fosfomycin. Fosfomycin MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturer's instructions for commercial systems.⁷ For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.⁸ Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

IP = In Preparation

***Enterococcus faecalis* ATCC 29212**

(NCTC 12697, CIP 103214, DSM 2570, CCUG 9997, CECT 795)

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Ampicillin	1	0.5-2	2	18	15-21
Ciprofloxacin	0.5-1	0.25-2	5	22	19-25
Gentamicin	8	4-16	30 ⁴	15	12-18
Imipenem	1	0.5-2	10	27	24-30
Levofloxacin	0.5-1	0.25-2	5	22	19-25
Linezolid	2	1-4	10	22	19-25
Nitrofurantoin	8	4-16	100	21	18-24
Norfloxacin	4	2-8	10	19	16-22
Quinupristin-dalfopristin	4	2-8	15	14	11-17
Streptomycin	Note ⁵	Note ⁵	300 ⁶	17	14-20 ⁷
Teicoplanin	0.5	0.25-1	30	18	15-21
Tigecycline ⁸	0.06	0.03-0.125	15	23	20-26
Trimethoprim	0.25	0.125-0.5	5	28	24-32
Trimethoprim-sulfamethoxazole ⁹	≤0.5 ²	-	1.25-23.75	30	26-34
Vancomycin	2	1-4	5	13	10-16

¹ Calculated by EUCAST.² From International Standards Organisation, ISO 20776-1: 2006 (with updates as in the latest CLSI M100 document). All ranges have been validated by EUCAST.³ Established and validated by EUCAST.⁴ Screening disk for high-level aminoglycoside-resistance in enterococci.⁵ There is currently no MIC range for *E. faecalis* ATCC 29212 and streptomycin.⁶ Screening disk for high-level streptomycin-resistance in enterococci.⁷ From Clinical and Laboratory Standards Institute, M100-S26, 2016.⁸ For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.⁹ Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

Streptococcus pneumoniae* ATCC 49619

(NCTC 12977, CIP 104340, DSM 11967, CCUG 33638)

Strain with reduced susceptibility to benzylpenicillin

* Zone edges for *S. pneumoniae* on MH-F are often accompanied by α-haemolysis. Read inhibition of growth and not inhibition of haemolysis. Tilt the plate to easier differentiate between haemolysis and growth. There is usually growth in the whole area of α-haemolysis but on some MH-F media, there is additional α-haemolysis without growth.

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD, McFarland 0.5, 5% CO₂, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amoxicillin	0.06	0.03-0.125	-	-	-
Ampicillin	0.125	0.06-0.25	2	28	25-31
Azithromycin	0.125	0.06-0.25	-	-	-
Benzylpenicillin	0.5	0.25-1	1 unit	19	16-22
Cefaclor	2	1-4	30	28	25-31
Cefepime	0.06-0.125	0.03-0.25	30	34	31-37
Cefotaxime	0.06	0.03-0.125	5	31	28-34
Cefpodoxime	0.06	0.03-0.125	10	32	29-35
Ceftaroline	0.016	0.008-0.03	-	-	-
Ceftobiprole	0.008-0.016	0.004-0.03	-	-	-
Ceftriaxone	0.06	0.03-0.125	30	35	32-38
Cefuroxime	0.5	0.25-1	30	31	28-34
Chloramphenicol	4	2-8	30	27	24-30
Ciprofloxacin	-	-	5	25	22-28
Clarithromycin	0.06	0.03-0.125	-	-	-
Clindamycin	0.06	0.03-0.125	2	25	22-28
Dalbavancin ⁴	0.016	0.008-0.03	-	-	-
Daptomycin ⁵	0.125-0.25	0.06-0.5	-	-	-
Doripenem	0.06	0.03-0.125	10	34	31-37
Doxycycline	0.03-0.06	0.016-0.125	-	-	-
Ertapenem	0.06-0.125	0.03-0.25	10	31	28-34
Erythromycin	0.06	0.03-0.125	15	29	26-32
Imipenem	0.06	0.03-0.125	10	38	34-42
Levofloxacin	1	0.5-2	5	24	21-27
Linezolid	0.5-1	0.25-2	10	26	23-29
Meropenem	0.125	0.06-0.25	10	34	30-38
Minocycline	-	-	30	28	25-31
Moxifloxacin	0.125	0.06-0.25	5	27	24-30
Nitrofurantoin	8	4-16	100	28	25-31
Norfloxacin	4	2-8	10	21	18-24
Oflloxacin	2	1-4	5	21	18-24
Oritavancin ⁴	0.002	0.001-0.004	-	-	-
Oxacillin ⁶	-	-	1	11	8-14 ⁶
Rifampicin	0.03	0.016-0.06	5	29	26-32
Tedizolid	0.25	0.125-0.5	-	-	-
Teicoplanin	-	-	30	21	18-24
Telithromycin	0.008-0.016	0.004-0.03	15	30	27-33
Tetracycline	0.125-0.25	0.06-0.5	30	31	28-34
Tigecycline ⁷	0.03-0.06	0.016-0.125	15	27	24-30
Trimethoprim-sulfamethoxazole ⁸	0.25-0.5	0.125-1	1.25-23.75	22	18-26
Vancomycin	0.25	0.125-0.5	5	20	17-23

Streptococcus pneumoniae* ATCC 49619

(NCTC 12977, CIP 104340, DSM 11967, CCUG 33638)

Strain with reduced susceptibility to benzylpenicillin

¹ Calculated by EUCAST.² From International Standards Organisation, ISO 20776-1: 2006 (with updates as in the latest CLSI M100 document). All ranges have been validated by EUCAST.³ Established and validated by EUCAST.⁴ MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.⁵ Daptomycin MICs must be determined in the presence of Ca²⁺ (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.⁶ *S. aureus* ATCC 29213 can be used for quality control of oxacillin 1 µg with target 22 mm and range 19-25 mm (according to disk diffusion methodology for *S. aureus*).⁷ For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.⁸ Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

***Haemophilus influenzae* ATCC 49766**

(NCTC 12975, CIP 103570, DSM 11970, CCUG 29539)

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β -NAD, McFarland 0.5, 5% CO₂, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Amoxicillin-clavulanic acid ^{3,4}	0.25	0.125-0.5	2-1	20	17-23
Amoxicillin	0.25	0.125-0.5	-	-	-
Ampicillin	0.125	0.06-0.25	2	22	19-25
Ampicillin-sulbactam ⁵	0.125	0.06-0.25	-	-	-
Azithromycin	1	0.5-2	-	-	-
Benzylpenicillin	-	-	1 unit	18	15-21
Cefepime	0.06	0.03-0.125	30	33	30-36
Cefixime	0.03	0.016-0.06	5	32	29-35
Cefotaxime	0.008	0.004-0.016	5	33	29-37
Cefpodoxime	0.06	0.03-0.125	10	33	30-36
Ceftaroline	0.008	0.004-0.016	-	-	-
Ceftibuten	0.03	0.016-0.06	30	34	31-37
Ceftriaxone	0.004	0.002-0.008	30	38	34-42
Cefuroxime	0.5	0.25-1 ⁶	30	30	26-34
Chloramphenicol	0.5	0.25-1	30	34	31-37
Ciprofloxacin	0.008	0.004-0.016	5	36	32-40
Clarithromycin	8	4-16	-	-	-
Doripenem	0.125	0.06-0.25 ⁶	10	29	26-32
Doxycycline	0.5	0.25-1	-	-	-
Ertapenem	0.03	0.016-0.06 ⁶	10	30	27-33
Erythromycin	4	2-8	15	13	10-16
Imipenem	0.5	0.25-1 ⁶	10	27	24-30
Levofloxacin	0.016	0.008-0.03	5	35	31-39
Meropenem	0.06	0.03-0.125 ⁶	10	31	27-35
Minocycline	0.25	0.125-0.5	30	29	26-32
Moxifloxacin	0.016	0.008-0.03	5	33	30-36
Nalidixic acid	-	-	30	30	27-33
Oflloxacin	0.03	0.016-0.06	5	34	31-37
Rifampicin	0.5	0.25-1	5	24	21-27
Roxithromycin	8	4-16	-	-	-
Telithromycin	2	1-4	15	17	14-20
Tetracycline	0.5	0.25-1	30	31	28-34
Trimethoprim-sulfamethoxazole ⁷	0.03	0.016-0.06	1.25-23.75	31	27-35

¹ Calculated by EUCAST.² Established and validated by EUCAST.³ For MIC testing, the concentration of clavulanic acid is fixed at 2 mg/L.⁴ *E. coli* ATCC 35218 (MIC) and *S. aureus* ATCC 29213 (disk diffusion) are used to check the inhibitor component (see Routine quality control for β -lactam- β -lactamase inhibitor combinations).⁵ For MIC testing, the concentration of sulbactam is fixed at 4 mg/L.⁶ From Clinical and Laboratory Standards Institute, M100-S26, 2016, and validated by EUCAST.⁷ Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

***Campylobacter jejuni* ATCC 33560**

(NCTC 11351, CIP 702, DSM 4688, CCUG 11284)

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β -NAD, McFarland 0.5, microaerobic environment, 41 \pm 1°C, 24h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. The MH-F plates should be dried prior to inoculation to reduce swarming (at 20-25°C over night or at 35°C, with the lid removed, for 15 min).

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target	Range		Target ¹	Range ²
Ciprofloxacin	IP	IP	5	38	34-42
Erythromycin	IP	IP	15	31	27-35
Tetracycline	IP	IP	30	34	30-38

¹ Calculated by EUCAST.² Established and validated by EUCAST.

IP = In Preparation

Control of the inhibitor component of β -lactam- β -lactamase inhibitor combinations

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Escherichia coli ATCC 35218

(NCTC 11954, CIP 102181, DSM 5923, CCUG 30600, CECT 943)

TEM-1 β -lactamase-producing strain (non-ESBL)

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Amoxicillin-clavulanic acid ³	8-16	4-32	20-10	19-20	17-22 ⁴
Ampicillin-sulbactam ⁵	32-64	16-128	10-10	16	13-19 ⁴
Ceftolozane-tazobactam ^{6,7}	0.125	0.06-0.25	30-10	28	25-31
Piperacillin-tazobactam ^{6,7}	1	0.5-2	30-6	24	21-27
Ticarcillin-clavulanic acid ³	16	8-32	75-10	23	21-25

Klebsiella pneumoniae ATCC 700603

(NCTC 13368, CCUG 45421, CECT 7787)

SHV-18 ESBL producer

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Ceftazidime-avibactam ⁸	0.5-1	0.25-2	10-4	21	18-24
Ceftolozane-tazobactam ^{6,7}	1	0.5-2	30-10	21	17-25
Piperacillin-tazobactam ^{6,7}	16	8-32	30-6	17	14-20

Staphylococcus aureus ATCC 29213

(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

β -lactamase-producing strain (weak)

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Amoxicillin-clavulanic acid ³	Note ⁹	Note ⁹	2-1	22	19-25

¹ Calculated by EUCAST.

² From Clinical and Laboratory Standards Institute, M100-S26, 2016, except ranges in bold/italics established by EUCAST. All ranges have been validated by EUCAST.

³ For MIC testing, the concentration of clavulanic acid is fixed at 2 mg/L.

⁴ Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agar.

⁵ For MIC testing, the concentration of sulbactam is fixed at 4 mg/L.

⁶ For MIC testing, the concentration of tazobactam is fixed at 4 mg/L.

⁷ Either *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603 can be used to check the inhibitor component.

⁸ For MIC testing, the concentration of avibactam is fixed at 4 mg/L.

⁹ For MIC testing, *E. coli* ATCC 35218 is used to check the inhibitor component.



Extended quality control for detection of resistance mechanisms with disk diffusion

Quality control strains for detection of resistance mechanisms with disk diffusion on Mueller-Hinton agar

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

ESBL production in Enterobacteriaceae

Klebsiella pneumoniae ATCC 700603

(NCTC 13368, CCUG 45421, CECT 7787)

SHV-18 ESBL-producer

Antimicrobial agent	Disk content (µg)	Target susceptibility ¹	Range ² (mm)	Comments
Aztreonam	30	R	9-17	
Cefotaxime	5	I or R	12-18	
Cefpodoxime	10	R	9-16	
Ceftazidime	10	I or R	6-12	
Ceftriaxone	30	I or R	16-22	

Methicillin resistance in *Staphylococcus aureus*

Staphylococcus aureus NCTC 12493

(CCUG 67181)

Methicillin resistant (MRSA), *mecA* positive

Antimicrobial agent	Disk content (µg)	Target susceptibility ¹	Range ² (mm)	Comments
Cefoxitin	30	R	14-20	

vanB-mediated glycopeptide resistance in enterococci

Enterococcus faecalis ATCC 51299

(NCTC 13379 ,CIP 104676, DSM 12956, CCUG 34289)

vanB-positive strain

Antimicrobial agent	Disk content (µg)	Target susceptibility ¹	Range ² (mm)	Comments
Teicoplanin	30	S	16-20	
Vancomycin	5	R	6-12	Examine zone edge with transmitted light (plate held up to light). Inhibition zones with fuzzy zone edges are interpreted as resistant, even if the zone diameter is above the susceptible breakpoint (for reading examples see the EUCAST Reading Guide or Breakpoint Tables).

High-level aminoglycoside resistance in enterococci

Enterococcus faecalis ATCC 51299

(NCTC 13379 ,CIP 104676, DSM 12956, CCUG 34289)

High-level gentamicin and streptomycin resistant

Antimicrobial agent	Disk content (µg)	Target susceptibility ¹	Range ² (mm)	Comments
Gentamicin	30	R	6	
Streptomycin	300	R	6	

¹ Targets comply with EUCAST clinical breakpoints and are set to ensure that resistance mechanisms are correctly detected. Interpretation according to EUCAST clinical breakpoints: S=Susceptible, I=Intermediate, R=Resistant.

² From Clinical and Laboratory Standards Institute, M100-S26, 2016, except ranges in bold/italics established by EUCAST. All ranges have been validated by EUCAST.

Quality control strains for detection of resistance mechanisms with disk diffusion on Mueller-Hinton fastidious (MH-F) agar

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β -NAD, McFarland 0.5, 5% CO₂, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.

Reduced susceptibility to β -lactam agents due to PBP mutations in *Haemophilus influenzae*

Haemophilus influenzae ATCC 49247

(NCTC 12699, CIP 104604, DSM 9999, CCUG 26214)

Antimicrobial agent	Disk content (µg)	Target susceptibility ¹	Range ² (mm)	Comments
				Inhibition zone diameters are particularly affected by variation in medium, inoculum and incubation conditions. Inhibition zones with growth of small colonies up to the disk are interpreted as no zone.
Ampicillin	2	R	6-12	
Benzylpenicillin	1 unit	R	6-9	

¹ Targets comply with EUCAST clinical breakpoints and are set to ensure that resistance mechanisms are correctly detected.
Interpretation according to EUCAST clinical breakpoints: S=Susceptible, I=Intermediate, R=Resistant.

² Established and validated by repeated testing by EUCAST.