



EUCAST

European Committee
on Antimicrobial
Susceptibility Testing

Quality control in the laboratory

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QC responsibility

- Manufacturers of AST materials:
 - To ensure that products have been appropriately manufactured and comply with published QC criteria.
- Laboratories/users:
 - To ensure that products are maintained properly (storage and handling).
 - To ensure that testing is performed according to the standardised methodology.

The final responsibility for AST results lies with the laboratory!

Internal routine quality control

- Control of materials and equipment
 - Agar plates
 - Antimicrobial disks
 - Incubators
- Control of the procedures
 - Inoculum and inoculation
 - Incubation time
 - Reading of results

Quality control strains

Organism	Number	Characteristics
<i>E. coli</i>	ATCC 25922	Susceptible, wild type
<i>P. aeruginosa</i>	ATCC 27853	Susceptible, wild type
<i>S. aureus</i>	ATCC 29213	Weak β -lactamase producer
<i>E. faecalis</i>	ATCC 29212	Susceptible, wild type
<i>S. pneumoniae</i>	ATCC 49619	Reduced susceptibility benzylpenicillin
<i>H. influenzae</i>	ATCC 49766	Susceptible, wild type
<i>Campylobacter jejuni</i>	ATCC 33560	Susceptible, wild type

Control of β -lactamase inhibitors

<i>E. coli</i>	ATCC 35218	TEM-1 β -lactamase
<i>K. pneumoniae</i>	ATCC 700603	SHV-18 ESBL
<i>K. pneumoniae</i>	ATCC BAA-2814	KPC-3, SHV-11 and TEM-1

QC of β -lactam- β -lactamase inhibitor combinations

Control of active component:

- Use a susceptible QC strain

Control of inhibitor:

- Use a β -lactamase-producing QC strain:
 - *E. coli* ATCC 35218: clavulanic acid, tazobactam
 - *K. pneu* ATCC 700603: avibactam, tazobactam
 - *K. pneu* ATCC BAA-2814: relebactam, vaborbactam
- Both tests should be part of the routine QC.

QC testing procedure

- Perform QC daily, or at least four times a week!
- Include antimicrobial agents which are part of routine panels.
- QC results should be read and evaluated before reporting AST results for clinical isolates.
- Evaluate results against criteria in EUCAST QC Tables.

QC ranges and targets

Escherichia coli ATCC 25922

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

Test according to EUCAST methodology for non-fastidious organisms (MH broth and agar). See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	2-8	-	-	-
Amoxicillin-clavulanic acid ^{3,4}	4	2-8	20-10	21	18-24 ⁵
Ampicillin	4	2-8	10	18-19	15-22 ⁵
Ampicillin-sulbactam ^{4,6}	2	1-4	10-10	21-22	19-24 ⁵
Azithromycin	-	-	15	17	14-20 ⁷

Range

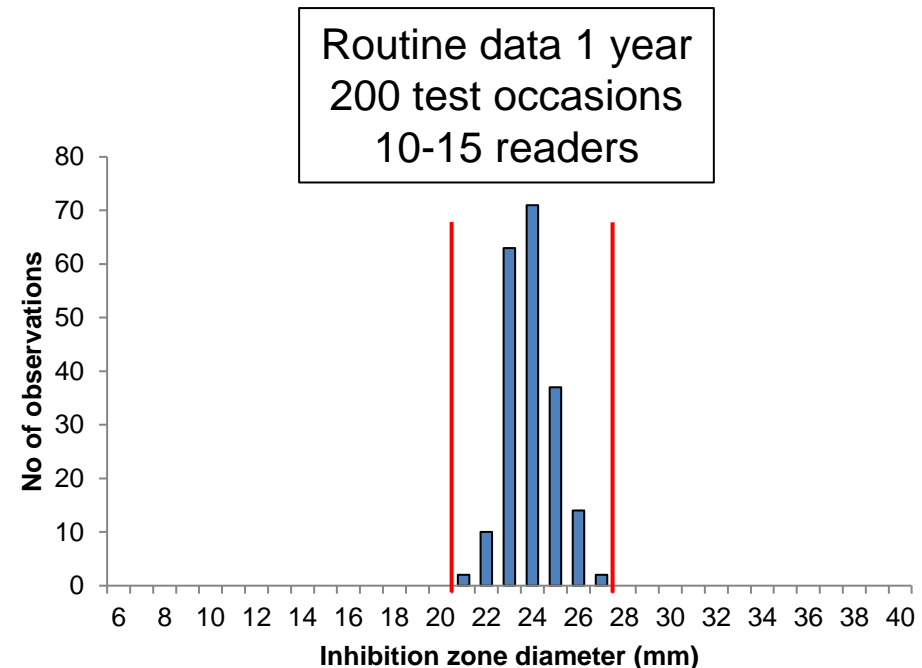
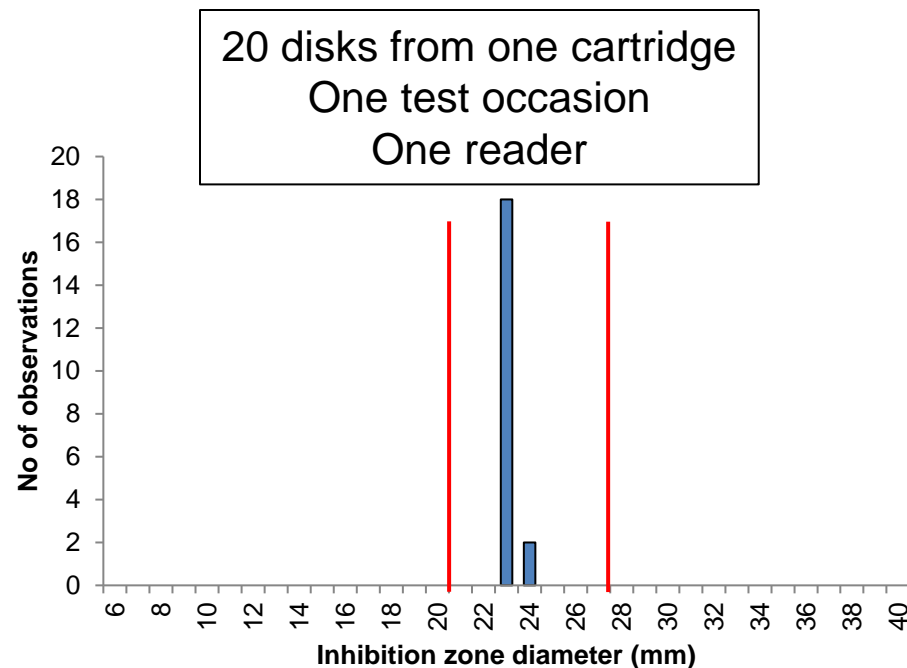
Set to allow for random variation

Target

Mean values from repeated measurements should optimally be on target ± 1 mm (mode MIC on target)

Product accuracy vs. day-to-day variation

Example *E. coli* ATCC 25922 and piperacillin-tazobactam 30-6 µg

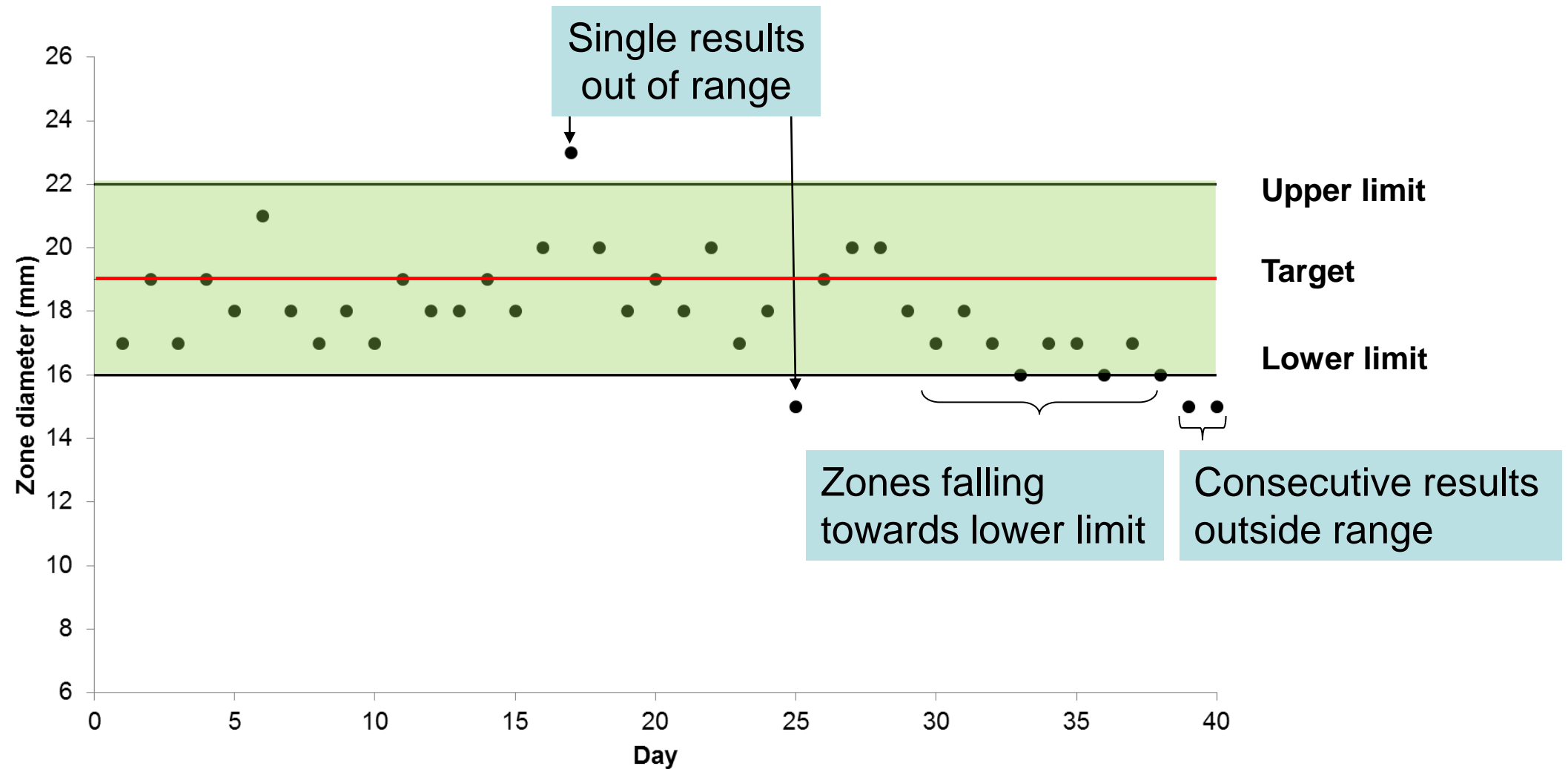


- Day-to-day variation due to small differences in:
 - Inoculum preparation and plate inoculation
 - Incubation time and temperature
 - Reading of results

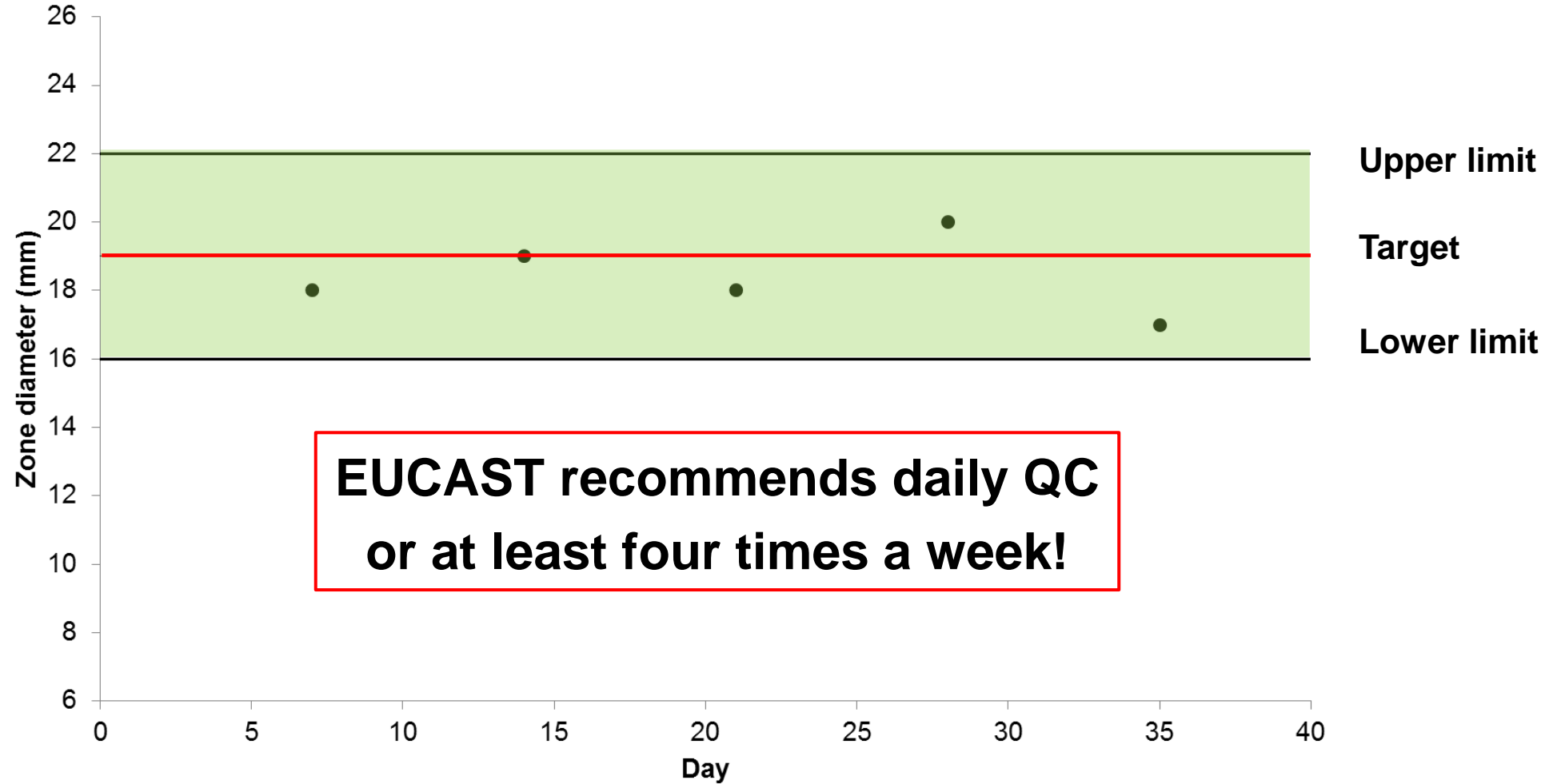
Evaluation of QC results

- Each day of QC:
 - Examine results of the last 20 tests.
- If two consecutive tests are outside range or if multiple disks are outside range on one day:
 - Investigate before reporting results for clinical isolates.
 - The tests may have to be repeated.

Monitoring Laboratory QC results

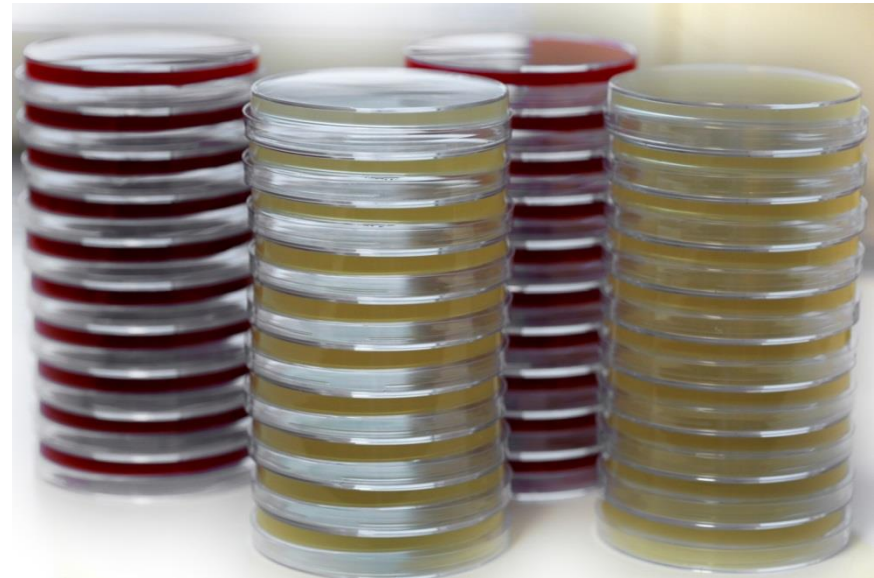


Daily vs. weekly QC



Potential sources of errors in disk diffusion

- Disks
- Media
- Not adhering to methodology
- Equipment
- QC strain



Antimicrobial disks

- Store according to manufacturers instructions.
- Store disks in use in sealed containers with an indicating desiccant and protected from light.
- Allow disks to reach room temperature before opening containers, to prevent condensation.
- Do not use disks beyond the expiry date.

Quality of disks and media

EUCAST evaluation of disk and media from different manufacturers, studies 2014-2020:

- Antimicrobial disks from 9 manufacturers

The quality of antimicrobial discs from nine manufacturers-EUCAST evaluations in 2014 and 2017. Åhman et al, CMI. 2019 Mar;25(3):346-352.

- Mueller-Hinton dehydrated media from 21 brands

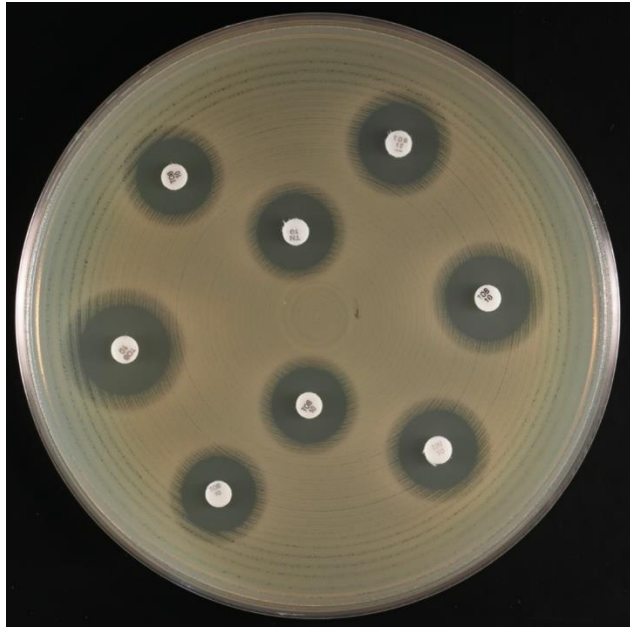
EUCAST evaluation of 21 brands of Mueller-Hinton dehydrated media for disc diffusion testing. Åhman et al. CMI. 2020 Oct;26(10):1412.e1-1412.e5.

- Mueller-Hinton pre-poured plates from 10 brands

Evaluation of 10 brands of pre-poured Mueller-Hinton agar plates for EUCAST disc diffusion testing. Åhman et al. CMI. 2022 May 31;S1198-743X(22)00289-0

Disks from 9 manufacturers

- Disks tested in triplicate against several QC strains.
- Disks from all manufacturer placed on the same agar plate.



Tobramycin 10 µg
E. coli ATCC 25922



Benzylpenicillin 1 unit
S. pneumoniae ATCC 49619

Data analysis

- Results evaluated against targets and ranges in EUCAST QC Tables.

Mean value within ± 1 mm of the target value

Mean value >1 mm but within ± 2 mm of the target value

Mean value >2 mm from target value but still within the QC range

Mean value out of the QC range

H = High (above target)

L = Low (below target)

Disks from 9 manufacturers

Antimicrobial disk	Oxoid	Mast	BD	SirScan	Bio-analyse	Bio-Rad	Liofilchem	Abtek	HiMedia
Benzylopenicillin 1 unit			L	H	H				NA
Amoxicillin-clavulanic acid 20-10 µg						H		L	H
Piperacillin-tazobactam 30-6 µg				H				L	NA
Oxacillin 1 µg			L	L	L		L		H
Mecillinam 10 µg				H	H			L	H
Cefotaxime 5 µg				L				NA	NA
Cefoxitin 30 µg						H	H	NA	L
Ceftazidime 10 µg								L	L
Meropenem 10 µg	H	H		H	H	H	H	L	H
Ciprofloxacin 5 µg		L	L			L		L	H
Norfloxacin 10 µg				L				L	H
Pefloxacin 5 µg			L	NA			L	NA	H
Gentamicin 10 µg			H	NA				L	H
Tobramycin 10 µg						NA	H		H
Erythromycin 15 µg			L	L	L		L	L	H
Tetracycline 30 µg		L	L	L	L		L	L	

Disks from 9 manufacturers

Disk manufacturer	Readings within range	Readings outside range
Oxoid	100%	0%
Mast	100%	0%
BD	99%	1%
SirScan	94%	6%
Bio-analyse	93%	7%
Bio-Rad	93%	7%
Liofilchem	90%	10%
Abtek	89%	11%
HiMedia	67%	33%

21 brands of dehydrated Mueller-Hinton media

Total rating	MH agar brand	Percent zones on QC target ± 1 mm	Percent zones outside QC range	Agents outside range
-4	Bio-Rad MH Agar	86	0	
-10	Biolife MH Agar II	81	1.1	TS
-10	Oxoid MH Agar	78	1.1	TS
-11	Sigma MH Agar 2	81	0	
-12	BD BBL MH II Agar	73	0	
-12	Hardy Diagnostics MH Agar	71	0	
-13	BD Difco MH Agar	70	3.3	AM
-14	Alpha Biosciences MH Agar	71	3.3	FQ
-17	E&O Labs MH Agar	82	8.9	CA, FQ, AM, TS
-18	Sigma MH Agar	57	3.3	CS
-20	HiMedia MH Agar	56	0	
-21	bioMérieux MHE Agar	64	3.3	TS
-22	Acumedia MH Agar	63	3.3	AM
-24	Remel MH Agar	64	6.7	AM, TS
-25	Lab M MH Agar	69	6.7	AM, TS
-25	Merck MH Agar acc. to CLSI	66	6.7	AM, TS
-27	Mast MH Agar	59	8.9	CA, FQ, TS
-31	Sifin MH Agar	60	6.7	AM, TS
-32	HiMedia MH Agar No. 2	50	6.7	CA, AM
-40	Biolab MH II Agar	52	10	PC, MA, TE, TS
-55	Merck MH Agar	44	23	CS, CA, FQ, AM, TE

Abbreviations

PC - Penicillins; CS - Cephalosporins; CA - Carbapenems; FQ - Flouroquinolones; AM - Aminoglycosides; MA - Macrolides; TE - Tetracyclines; TS - Trimethoprim-sulfamethoxazole

10 brands of pre-poured Mueller-Hinton plates

Total rating	MH agar brand	Percent zones on QC target ± 1 mm	Percent zones outside QC range	Agents outside range
-11	BD BBL MH II Agar	70	0.0	
-14	bioMérieux MHE Agar	66	2.2	TS
-17	Hardy Diagnostics MH Agar	60	0.0	
-22	Oxoid MH Agar	56	3.3	TS
-23	E&O Laboratories MH Agar	58	5.6	CA, TE
-25	Bio-Rad MH Agar	48	1.1	AM
-30	Biolife MH Agar II	50	7.8	AM, TS
-40	Liofilchem MH II Agar	50	8.9	AM, TS
-54	HiMedia MH Agar No. 2	34	18	PC, CS, CA, FQ,
-61	HiMedia MH Agar	27	20	PC, CS, CA, FQ, AM, TS

The quality of pre-poured MH plates were generally poorer than for in-house prepared plates of the same brand in our previous study!

Abbreviations

PC - Penicillins; CS - Cephalosporins; CA - Carbapenems; FQ - Flouroquinolones; AM - Aminoglycosides; MA - Macrolides; TE - Tetracyclines; TS - Trimethoprim-sulfamethoxazole

Adhering to methodology

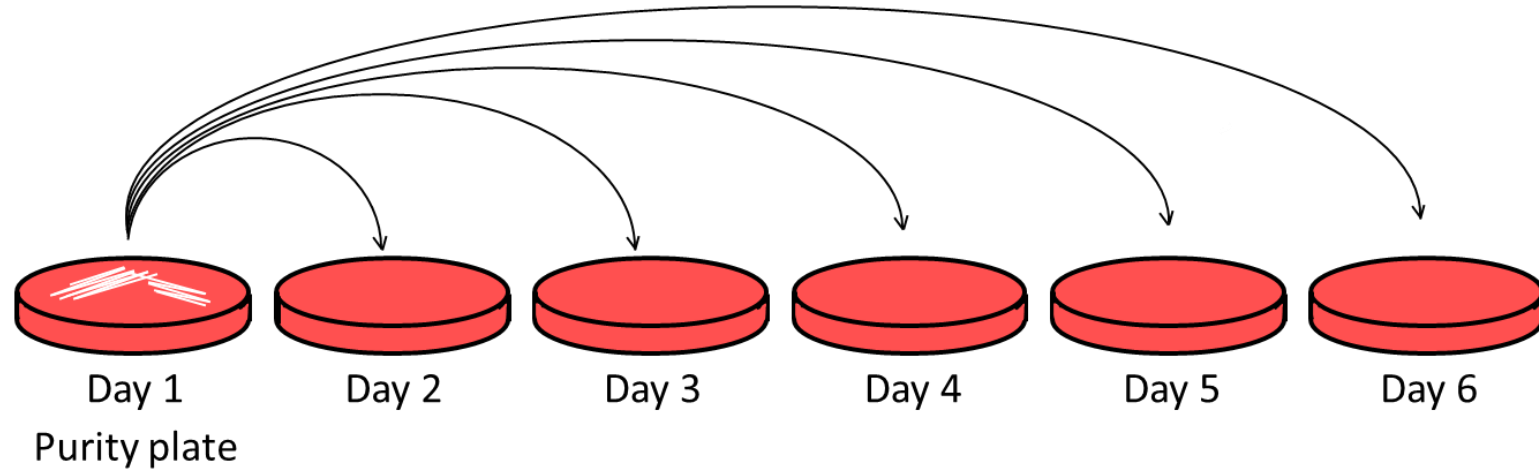
- 15-15-15 minutes rule
- Inoculum density
- Incubation temperature
- Incubation atmosphere
- Incubation time (16-20 h)
- Reading instructions



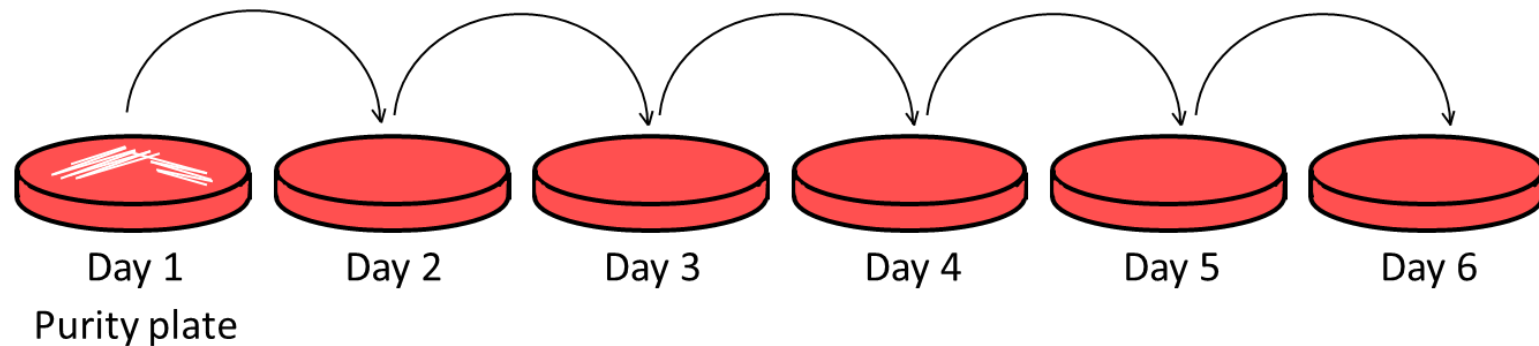
Modifications to the methodology will affect the end result and may affect the susceptibility categorisation!

Subculturing of QC strains

Non-fastidious QC strains



Fastidious QC strains



Comparison with reference distributions

- MIC and zone diameter distributions are available in the EUCAST database (www.eucast.org).
- Compare the median for the wild-type distribution of clinical isolates with the median in the reference distribution.
- May detect systematic deviations not detected by regular QC testing.

MIC and zone distributions and ECOFFs

[Organization](#)

[Consultations](#)

[EUCAST News](#)

[New definitions of S, I and R](#)

[Clinical breakpoints and dosing](#)

[Rapid AST in blood cultures](#)

[Expert rules and expected phenotypes](#)

[Resistance mechanisms](#)

[Guidance documents](#)

[SOP](#)

[MIC and zone distributions and ECOFFs](#)

[New and revised ECOFFs](#)

[AST of bacteria](#)



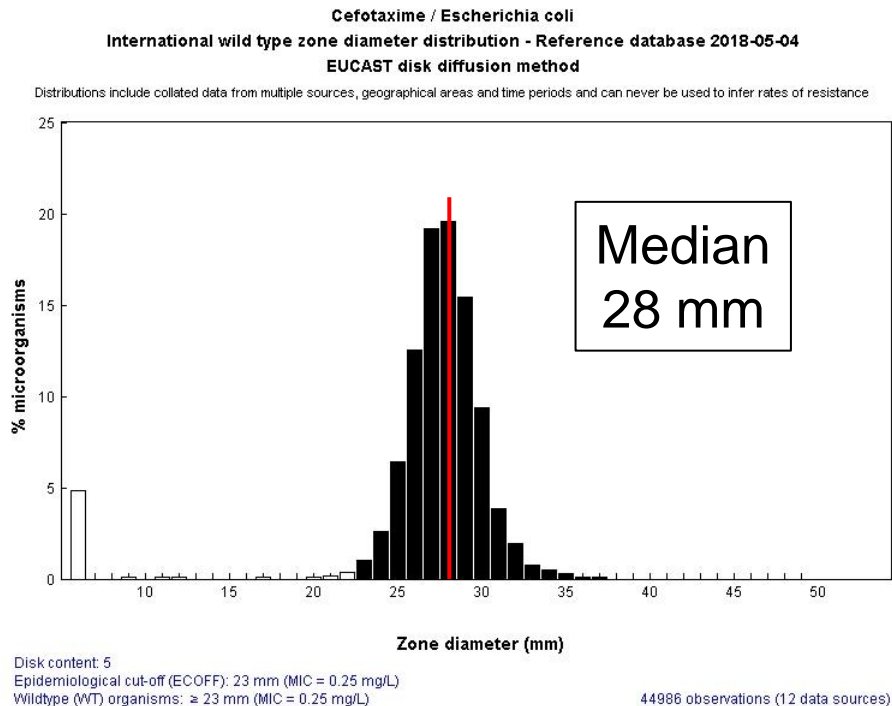
MIC and zone diameter distributions and ECOFFs

1. [Distributions and ECOFFs](#)
2. [MIC and zone diameter correlations](#)

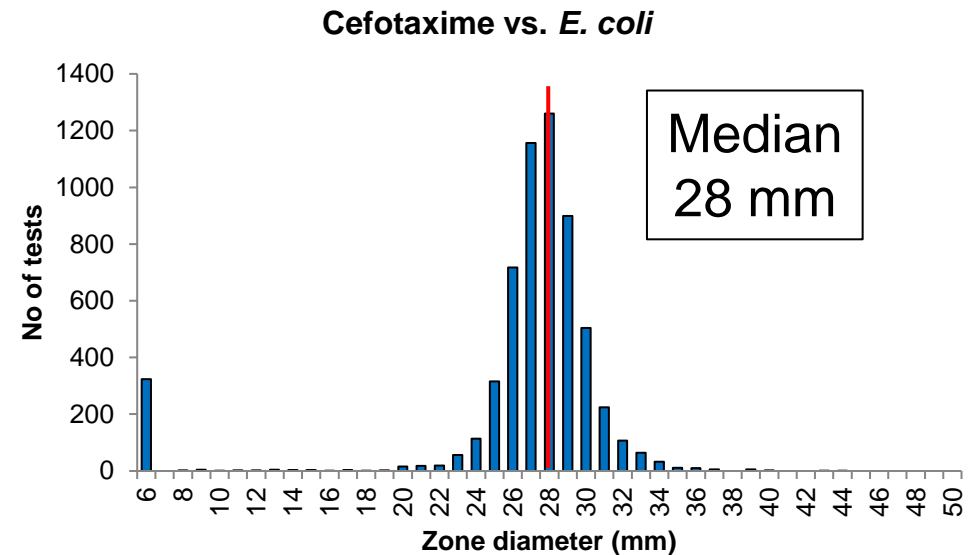
The EUCAST software, originally created in 2003, for displaying distributions of MIC-values (generated with methods calibrated to broth microdilution or agar dilution) and inhibition zone diameters (generated with EUCAST disk diffusion methodology) was re-programmed during 2020 and re-launched on 24 November, 2020. Each graph is shown in two versions where one is constructed by adding all approved distributions and the other by adding weighted distributions. The later is generated through converting numbers to per cent before adding individual distributions. This prevents large distributions from dominating or even

Comparison with reference distributions for clinical isolates

Reference distribution



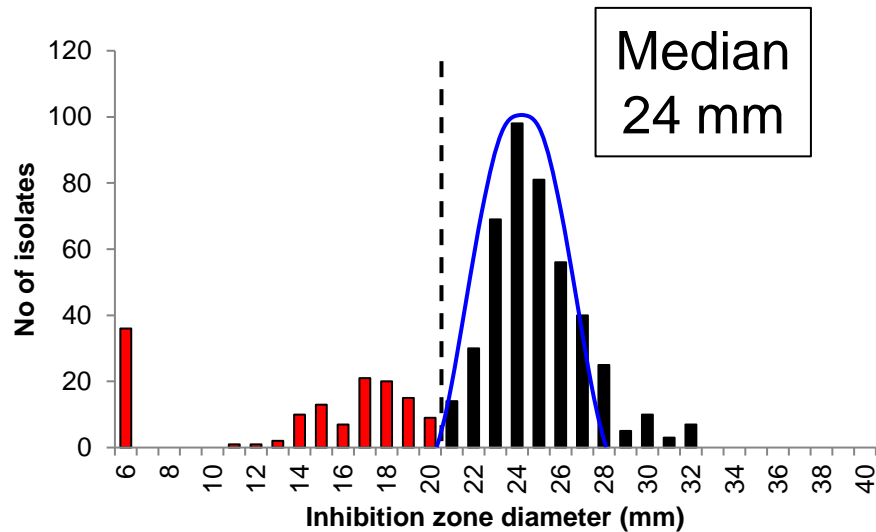
Routine distribution



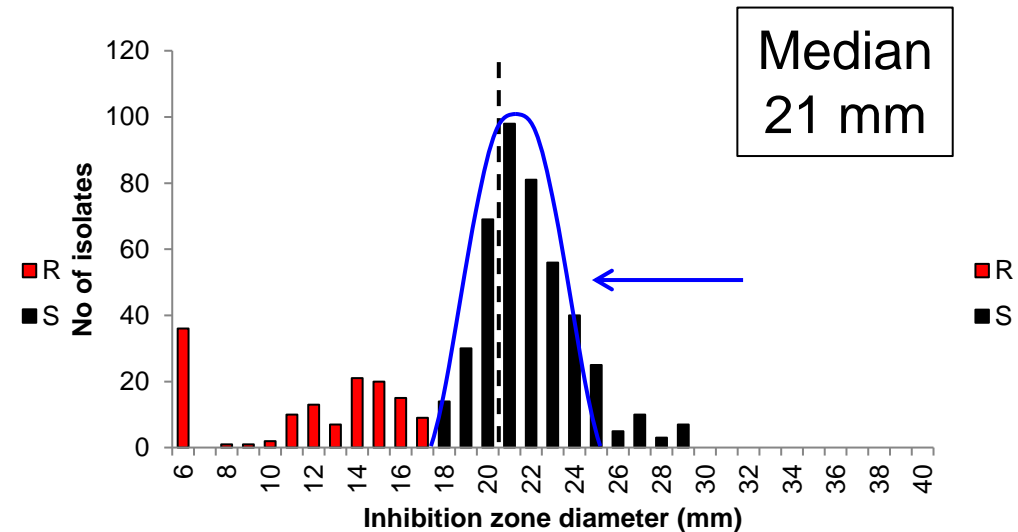
Compare the median values for wild-type isolates!

Example – too small zones

Reference distribution



Routine distribution



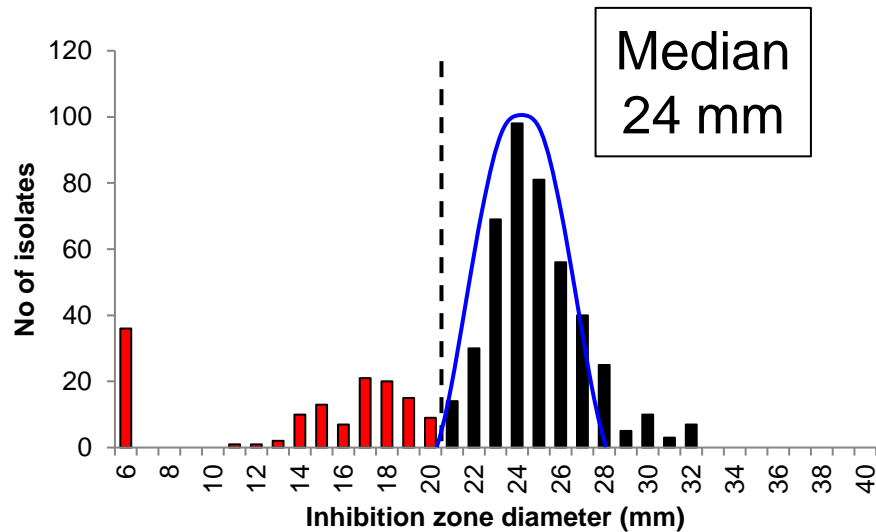
Risk of reporting isolates as false resistant!

Potential errors:

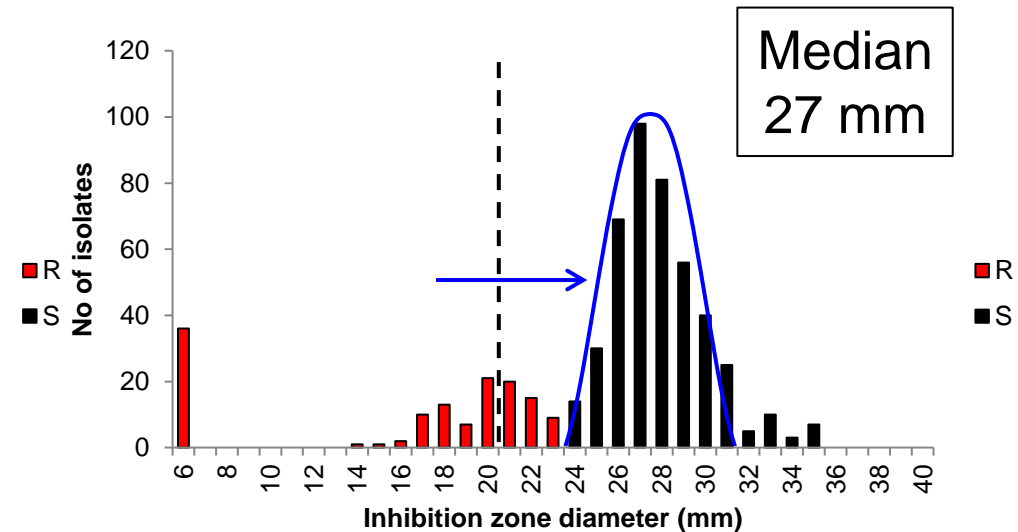
- Disk lost potency
- Over-incubation
- Over-inoculation
- Humid agar plates

Example – too large zones

Reference distribution



Routine distribution



Risk of reporting isolates as false susceptible!

Potential errors:

- Agar depth too low
- Incorrect reading
- Agar problems
- Disk problems

Gradient tests

- No EUCAST recommendations for gradient tests.
- Manufacturers are responsible for calibrating the product against reference methodology (broth microdilution).
- Users should follow the manufacturers instructions for storage and testing:
 - Media, inoculum, incubation, species



bioMérieux Etest

BIOMÉRIEUX

Etest[®]

IVD

TABLE 1: Summary of ETEST[®] Performance, Interpretive Criteria and Quality Control Ranges

15211L - 2018/12

ANTIBIOTIC (1) MIC µg/mL	CODE (2)	PERFORMANCE (3)	N	% EA (4)	INTERPRETIVE CRITERIA (5) MIC µg/mL	S ≤	S-DD (8)	I	R ≥	QUALITY CONTROL (6)	MIC µg/mL				
Amikacin 0.016 - 256	AK	Aerobes	553	95	Enterobacteriaceae	16	-	32	64	<i>S. aureus</i>	ATCC [®] 29213 TM	1 - 4			
					<i>P. aeruginosa</i>	16	-	32	64	<i>E. coli</i>	ATCC [®] 25922 TM	0.5 - 4			
					Acinetobacter	16	-	32	64	<i>P. aeruginosa</i>	ATCC [®] 27853 TM	1 - 4			
					Staphylococci	16	-	32	64						
Amoxicillin 0.016 - 256	AC	<i>S. pneumoniae</i>	200	98	<i>S. pneumoniae</i>	Non meningitis	2	-	4	8	<i>S. pneumoniae</i>	ATCC [®] 49619 TM	0.032 - 0.125		
Amoxicillin*/ Clavulanic acid (2/1) 0.016 - 256*	XL	Aerobes	440	99	Enterobacteriaceae	8	-	16	32	<i>E. coli</i>	ATCC [®] 25922 TM	2 - 8			
					Staphylococci	Not available (7)	-	-	-	<i>E. coli</i>	ATCC [®] 35218 TM	4 - 16			
					<i>H. influenzae</i>	211	99	<i>H. influenzae</i>	4	-	-	8	<i>H. influenzae</i>	ATCC [®] 49247 TM	2 - 16
					<i>S. pneumoniae</i>	223	98	<i>S. pneumoniae</i>	2	-	4	8	<i>S. pneumoniae</i>	ATCC [®] 49619 TM	0.032 - 0.125
Anaerobes	230	97	Anaerobes	4	-	8	16	<i>B. fragilis</i>	ATCC [®] 25285 TM	0.25 - 1					
Ampicillin 0.016 - 256	AM	Gram-negative aerobes	385	98	Enterobacteriaceae	8	-	16	32	<i>S. aureus</i>	ATCC [®] 29213 TM	0.25 - 1			
					Gram-positive aerobes	160	96	Staphylococci	Not available (7)	-	-	-	<i>E. faecalis</i>	ATCC [®] 29212 TM	0.5 - 2
					Enterococci	8	-	-	16	<i>E. coli</i>	ATCC [®] 25922 TM	2 - 8			
					<i>H. influenzae</i>	438	95	<i>H. influenzae</i>	1	-	2	4	<i>H. influenzae</i>	ATCC [®] 49247 TM	2 - 8

Liofilchem MIC Test Strip



© Liofilchem® - Table no.1 MTS™ Interpretative Criteria and Quality Control - Rev.33 / 19.07.2022

The present MTS™ Interpretative Criteria and Quality Control table might be out of date.

Check on-line for the latest update: https://www.liofilchem.com/images/brochure/mic_test_strip_patent/tabella_interpretazione.pdf

- The present MTS™ Interpretative Criteria and Quality Control table does not replace the official documents by CLSI, EUCAST and FDA.
- The present MTS™ Interpretative Criteria and Quality Control table has been produced in part under ECDC service contracts and made available by EUCAST at no cost to the user and can be accessed on the EUCAST website www.eucast.org. EUCAST recommendations are frequently updated and the latest versions are available at www.eucast.org.

Clinical

Antibiotic	CODE	CLSI			EUCAST			QUALITY CONTROL		MIC µg/mL	
		INTERPRETATIVE CRITERIA ¹	S≤	I	R≥	INTERPRETATIVE CRITERIA ²	S≤	R>			
AMIKACIN 0.016 - 256 µg/mL	AK	<i>Enterobacterales</i>	16	32	64	<i>Enterobacterales</i>	8	8	<i>S. aureus</i>	ATCC® 29213	1-4
		<i>P. aeruginosa</i>	16	32	64	<i>Pseudomonas</i> spp.	16	16	<i>E. faecalis</i>	ATCC® 29212	64-256
		<i>Acinetobacter</i> spp.	16	32	64	<i>Acinetobacter</i> spp.	8	8	<i>E. coli</i>	ATCC® 25922	0.5-4
		Other Non-Enterobacterales	16	32	64	<i>S. aureus</i>	(16)	(16)	<i>P. aeruginosa</i>	ATCC® 27853	1-4
		EDA ⁴				Coagulase-negative staphylococci	(16)	(16)			
		<i>S. aureus</i>	16	32	64	PK/PD (Non-species related) breakpoints	1	1			
AMOXICILLIN 0.016 - 256 µg/mL	AML	<i>S. pneumoniae</i> (nonmeningitis)	2	4	8	<i>Enterobacterales</i>	8	8	<i>K. pneumoniae</i>	ATCC® 700603	> 128
						<i>Enterococcus</i> spp.	4	8	<i>E. coli</i>	ATCC® 25922	2-8
						<i>S. pneumoniae</i> (iv, meningitis)	0.5	0.5	<i>S. pneumoniae</i>	ATCC® 49619	0.03-0.12
						<i>S. pneumoniae</i> (oral)	0.5	1	<i>H. influenzae</i>	ATCC® 49766	0.125-0.5
						Viridans group streptococci	0.5	2			
						<i>H. influenzae</i> (iv)	2	2			
						<i>H. influenzae</i> (oral)	0.001	2			
						<i>N. meningitidis</i>	0.12	1			
						<i>H. pylori</i>	0.12	0.12			
						<i>P. multocida</i>	1	1			
						<i>K. kingae</i>	0.125	0.125			
						PK/PD (Non-species related) breakpoints	2	8			

QC of gradient tests

- Use EUCAST QC strains and criteria only
 - Do not use criteria provided by the manufacturer (package inserts)
- Susceptible QC strains will only control the lower end of the concentration range.
- Add specific QC strains to control the inhibitor component.

Contact us for support!



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