



**EUCAST**

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# **Technical problems and controversies in antimicrobial susceptibility testing (AST)**

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# Standardised AST

- Results change with changed parameters.
  - It is crucial to adhere to the methodology to get reproducible and reliable results!
- Standardisation of:
  - Potency of antimicrobial agent/disk potency
  - Media
    - Type of media, supplements, pH, agar depth etc.
  - Inoculum
  - Incubation
  - Reading of results

# Troubleshooting in disk diffusion

- Quality control (QC)
  - QC results out of range
  - Evaluation of QC data over time
    - Mean value of  $> 1$  mm from target value ( $\geq 10$  tests)
    - Zone sizes changing over time
- Zone distributions for clinical isolates not in agreement with reference distributions.

# QC ranges and targets

Routine QC

EUCAST QC Tables v. 6.1, valid from 2016-03-01

## *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 <sup>1</sup>	Range <sup>2</sup>		Target <sup>1</sup>	Range <sup>3</sup>
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	2-8	-	-	-
Amoxicillin-clavulanic acid <sup>4,5</sup>	4	2-8	20-10	21	18-24 <sup>6</sup>
Ampicillin	4	2-8	10	18-19	15-22 <sup>6</sup>
Ampicillin-sulbactam <sup>5,7</sup>	2	1-4	10-10	21-22	19-24 <sup>6</sup>
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	25	23-27
Cefotaxime	0.06	0.03-0.125	5	28	25-31
Cefoxitin	4	2-8	30	26	23-29

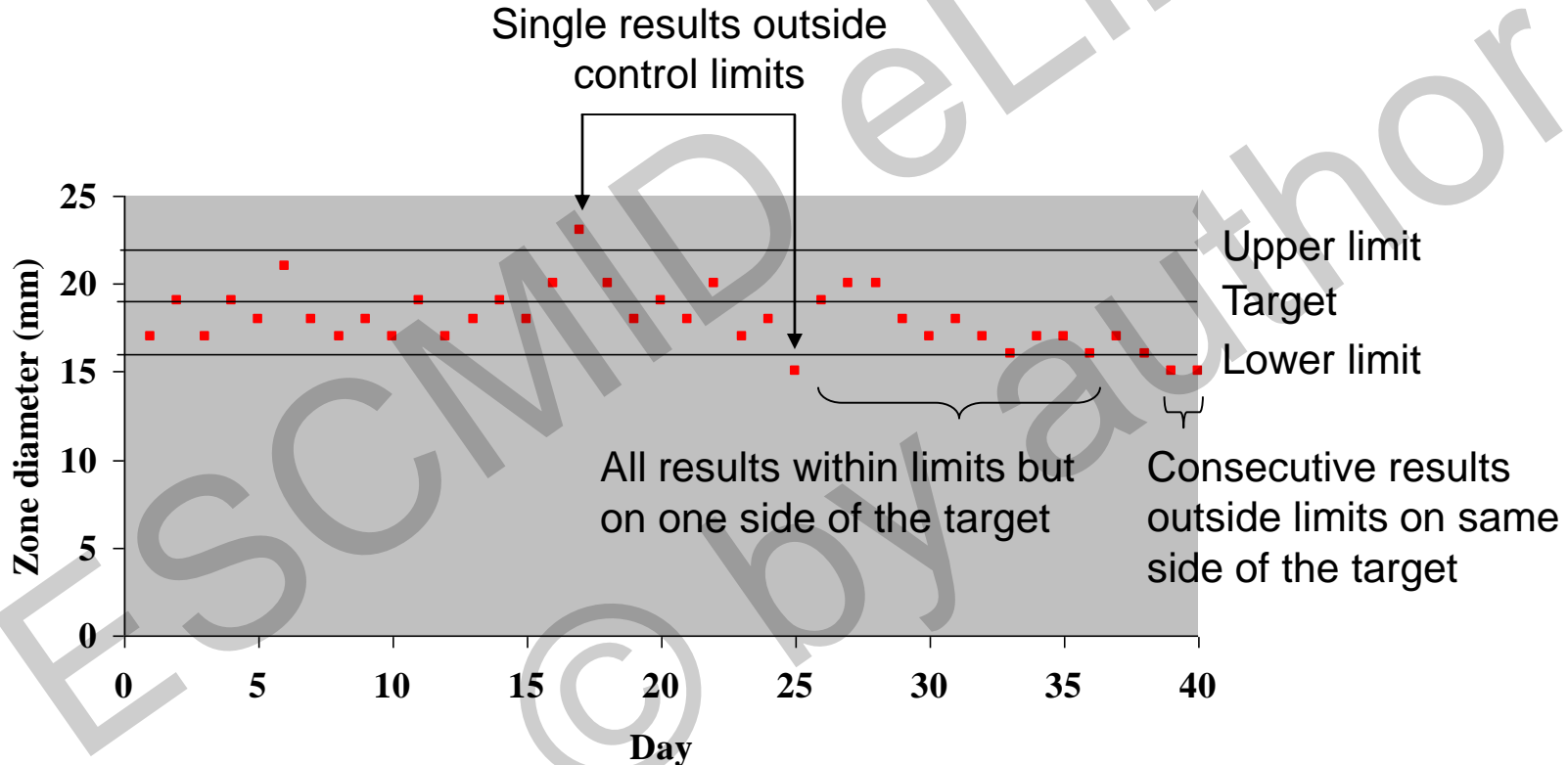
### Range

Used to allow occasional variation

### Target

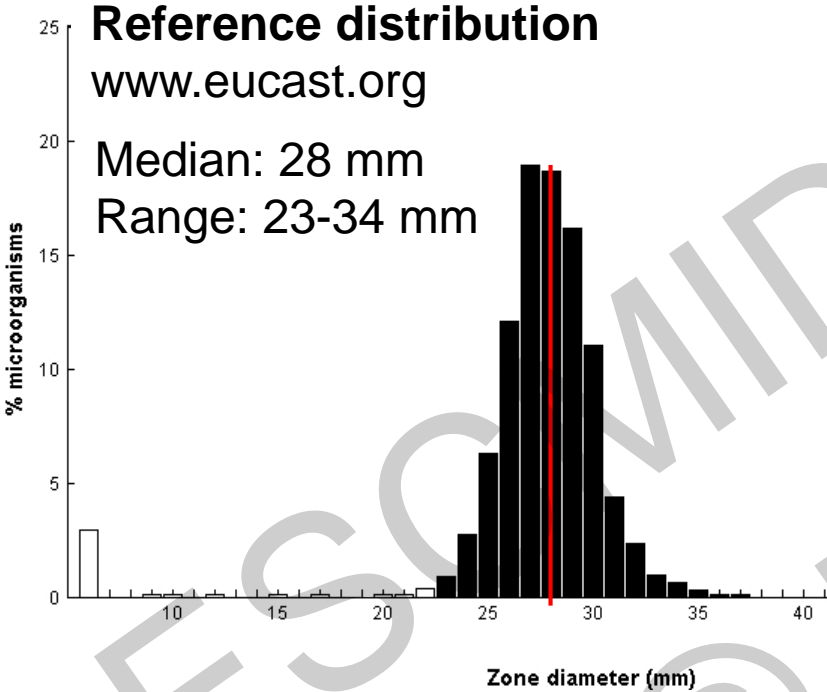
Mean values from repeated measurements should optimally be on target ± 1 mm (mode MIC on target)

# Monitoring of QC results over time

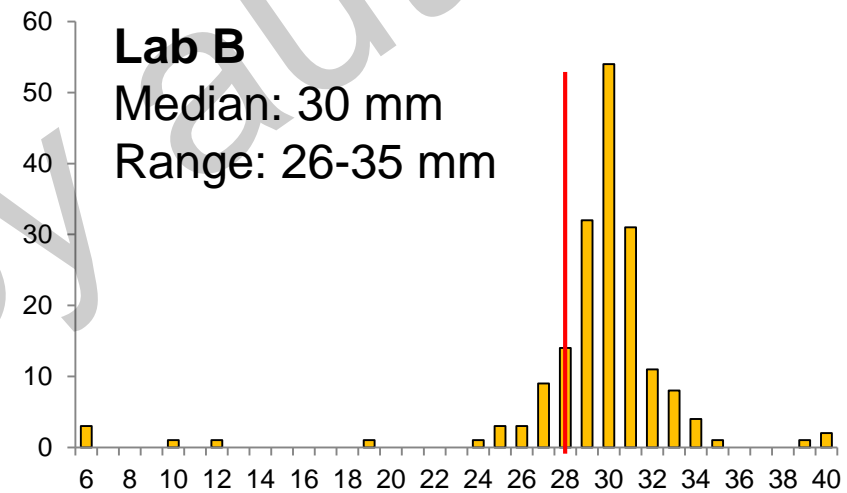
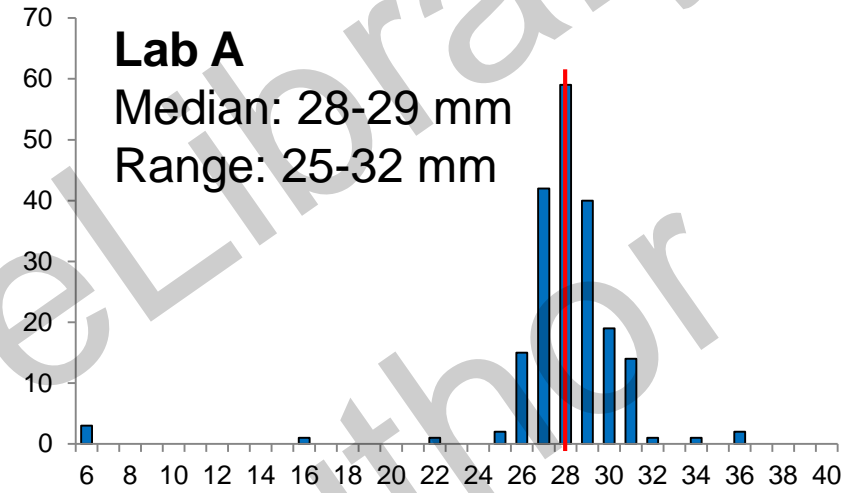


# Comparison with reference distributions

Example *E. coli* and cefotaxime 5 µg



Median of wild-type distribution should be at median for reference  $\pm 1$  mm.



# Sources of errors in disk diffusion

- Disks
  - Incorrect disk potency
  - Incorrect handling and storage
  - Disk quality
- Testing procedures
  - Inoculation
  - Incubation
  - Reading of zones
- Media
  - Quality of agar base
  - Supplements
  - pH, agar depth
  - Excess humidity
- QC strains

# Variability in disk quality

Warning on  
www.eucast.org

1 = 1st study, 2 = Follow-up study

Antimicrobial disk	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Benzylpenicillin 1 unit					L				H	H			NA	NA	H	H		
Amoxicillin-clav. 30 µg	H	H*					L						H	H		L		
Piperacillin-tazo. 36 µg							L	L	H				NA	NA				
Oxacillin 1 µg			L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg							NA		L				NA	NA				
Cefoxitin 30 µg	H*	H*	H	H*			NA	L					L*	L*		L		
Ceftazidime 10 µg							L	L					L	H				
Meropenem 10 µg	H		H*				L	L			H		H					
Ciprofloxacin 5 µg	L				L		L	L					H	H*		L	L	
Norfloxacin 10 µg							L		L				H*	H				
Pefloxacin 5 µg			L	L	L		NA	NA	NA				H					
Gentamicin 10 µg					H		L		NA				H	H				
Tobramycin 10 µg	NA	NA	H										H*	H*				
Erythromycin 15 µg			L		L		L		L				H	H	L*	L		
Tetracycline 30 µg			L	L*	L*		L		L*					L	L		L	

Mean value within  $\pm 1$  mm of the target value

Mean value  $>1$  mm but within  $\pm 2$  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

Disk included in first study, but not supplied for second study

NA = Not Available

H = High, mean value  $>1$  mm above target

L = Low, mean value  $>1$  mm below target

\* One or more readings out of QC range



# Variability in disk quality - conclusions

- Antimicrobial activity differs significantly between disks from different manufacturers.
  - Second follow-up study planned for 2017.
- Disk manufacturers:
  - Ensure that the activity of each disk comply with the target values in the EUCAST QC Tables!
- Laboratories:
  - Perform internal quality control!

# Excess humidity of agar plates - example with MH-F



If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min.

# Reading of zones

- See EUCAST Disk Diffusion Manual and pictures in EUCAST Reading Guide.
- Pictures for important exceptions are also available in EUCAST Breakpoint Table.
  - *E. coli* and fosfomycin
  - *S. maltophilia* and trimethoprim-sulfamethoxazole
  - *S. aureus* and benzylpenicillin
  - Enterococci and vancomycin

# Harmonisation of reading

- Reading exercises where all laboratory staff read zones from the same plate.
- Read more:
  - [www.eucast.org](http://www.eucast.org), AST of bacteria, Disk diffusion implementation
  - Matuschek E *et al.* Clin Microbiol Infect. 2014 Apr;20(4):O255-66

# Reading exercise at Clinical Microbiology, Växjö

## *Staphylococcus aureus*, clinical isolate 1st reading occasion

Lab tech	Cefoxitin	Erythromycin	Clindamycin
1	26	27	26
2	26	28	28
3	26	29	28
4	29	28	28
5	26	27	27
6	26	28	29
7	26	29	29
8	26	27	27
9	25	27	26
10	25	25	26
11	28	27	26
<b>Mean</b>	<b>26</b>	<b>27</b>	<b>27</b>
<b>SD</b>	<b>1.2</b>	<b>1.1</b>	<b>1.2</b>

> 1 mm above mean

> 1 mm below mean



# Reading exercise at Clinical Microbiology, Växjö

## *Staphylococcus aureus*, clinical isolate After analysis and discussion

Lab tech	Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
1	27	24	25	26	18	28
2	27	25	26	28	18	29
3	27	25	26	27	17	27
4	27	25	26	27	16	27
5	27	25	26	27	19	28
6	28	24	24	26	17	25
7	27	26	27	29	19	29
8	27	24	26	28	18	28
9	27	25	23	26	18	27
10	28	25	26	27	18	30
11	27	24	24	25	17	27
<b>Mean</b>	<b>27</b>	<b>25</b>	<b>25</b>	<b>27</b>	<b>18</b>	<b>28</b>
<b>SD</b>	<b>0.4</b>	<b>0.6</b>	<b>1.2</b>	<b>1.1</b>	<b>0.9</b>	<b>1.3</b>

> 1 mm above mean

> 1 mm below mean

# EUCAST disk diffusion antimicrobial susceptibility testing method

## Preparation of inoculum



0:03 / 2:33



Warnings!

Documents

Videos from EUCAST

Translations

representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has several subcommittees - [see page Subcommittees](#).

Most antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST. Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated

### EUCAST videos (English)

Gunnar Kahlmeter • 1/5 videos



1 Preparation of inoculum (english)

Gunnar Kahlmeter

2



2 Inoculation english

Gunnar Kahlmeter

3



3 Application of disks and incubation english

Gunnar Kahlmeter

4



4 Reading of zones english

Gunnar Kahlmeter

5



5 Guidance on breakpoint table english

Gunnar Kahlmeter

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MIC distributions and ECOFFs

Zone distributions and ECOFFs

AST of bacteria

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10 May 2016

## The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2012), Rafael Canton (2012 - 2016) and Christian Giske (2016 - ). Its scientific secretary is Derek Brown (1997 - 2016) and John Turnidge (2016 - ). Its webmaster is Gunnar Kahlmeter (2001 - ). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

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### EUCAST News



27 Mar 2017

**EUCAST instruction videos in Czech**

15 Mar 2017

**China joins EUCAST**

13 Mar 2017

**Breakpoint table v 7.1 published**

13 Mar 2017

**Coagulase negative staphylococci - MIC vs. zone diameters uploaded**

09 Mar 2017

**Consultation (9 March - 14 May) on MIC distributions and ECOFFs**

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# EUCAST warnings

1. Problems with piperacillin-tazobactam gradient tests from two manufacturers
2. Wide variation in disk quality in 16 disks from nine manufacturers
3. Problems with colistin gradient tests from two manufacturers

# AST of colistin

## Reference methodology

- Broth microdilution (BMD): ISO 20776-1
  - Sulphate salts
  - Standard polystyrene trays
  - No additives or pre-treatment of plates
- See EUCAST Guidance Documents  
[www.eucast.org/guidance\\_documents/](http://www.eucast.org/guidance_documents/)

# EUCAST evaluation of colistin MIC methods

- 75 Gram-negative bacteria with varying colistin MICs (0.25-128 mg/L)
  - *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp.
- BMD (ISO 20776-1 and EUCAST/CLSI recommendations)
  - Frozen panels as references
  - Commercial freeze-dried panels
    - Sensititre, MICRONAUT-S, MICRONAUT MIC Strip
- Gradient tests
  - Etest (Oxoid, BBL and MHE Mueller-Hinton agar)
  - MIC Test Strip (Oxoid and BBL Mueller-Hinton agar)

# Results colistin MIC methods

- Correlation with reference MICs was good for all BMD methods.
- Gradient tests generally underestimated colistin MICs resulting in false susceptibility (very major errors).
  - Problems probably related to poor diffusion of colistin in agar.
- The poor performance of gradient tests could not be detected with QC strains.





# EUCAST recommendations for colistin MIC determination

- Use BMD, but perform extended quality control:
  - Susceptible strain: *E. coli* ATCC 25922 and/or *P. aeruginosa* ATCC 27853
  - Colistin resistant (*mcr-1* positive) *E. coli* NCTC 13846
- Currently available gradient tests underestimate colistin MIC values (and resistance) and should be avoided.
  - Even if QC results are within range.

For more information, see poster 161, Saturday 15.30-16.30.

# Thanks!

- Jenny Åhman, Gunnar Kahlmeter, Catherine Webster, Cecilia Alexandersson and Amra Kulic, EUCAST Development Laboratory.
- All staff at Clinical Microbiology, Växjö and Karlskrona, Sweden.
- EUCAST Network Laboratories contributing with data and/or isolates.





# EUCAST

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- Check the EUCAST website regularly for updates on methodology, QC ranges and breakpoints.

[www.eucast.org](http://www.eucast.org)

- For questions and comments, please contact [erika.matuschek@escmid.org](mailto:erika.matuschek@escmid.org) or the EUCAST secretariat (see website).