



Quality problems in antimicrobial susceptibility testing

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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:
 - Media
 - Type of media, supplements, pH, agar depth etc.
 - Disk potency
 - Inoculum
 - Incubation
 - Reading of results

Internal routine quality control

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

QC testing procedure

- Follow the same testing procedure as for clinical isolates.
- Test 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)

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 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

Range

Set to allow for random variation

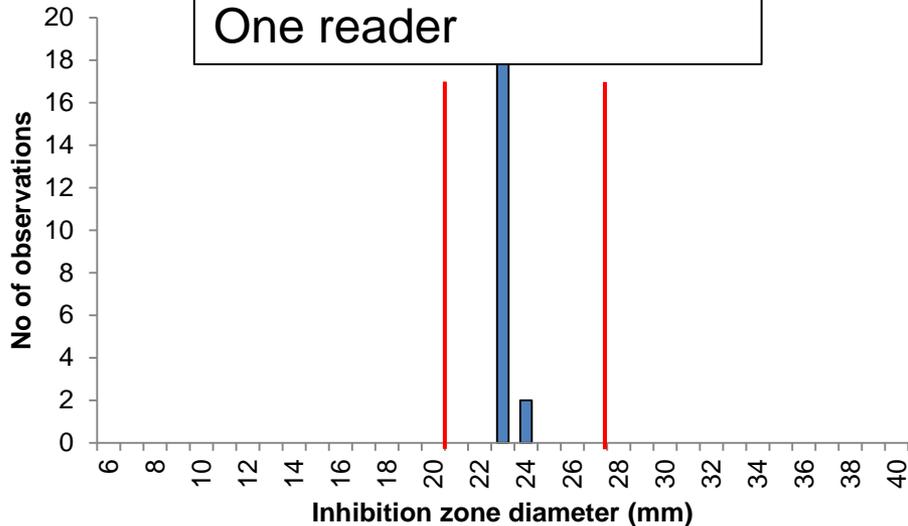
Target

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

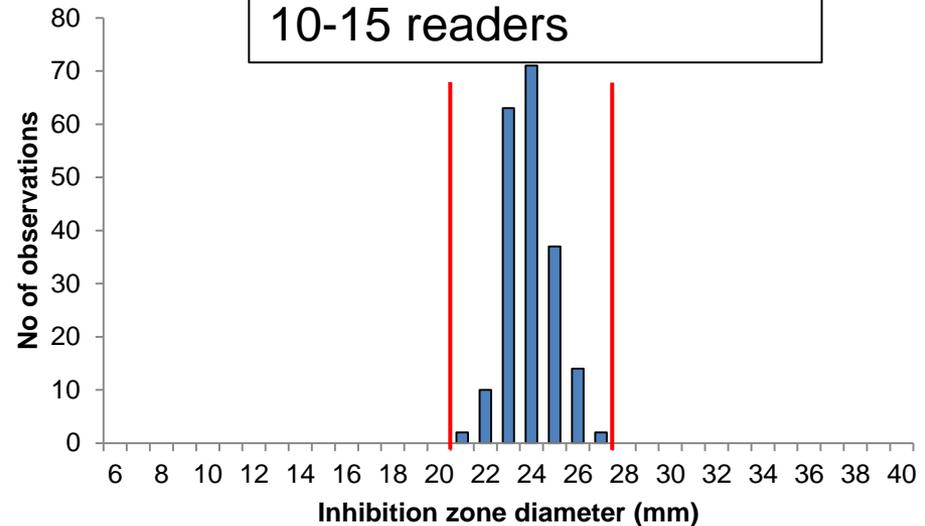
Product accuracy vs. day-to-day variation

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

Disks from one cartridge
One test occasion
One reader



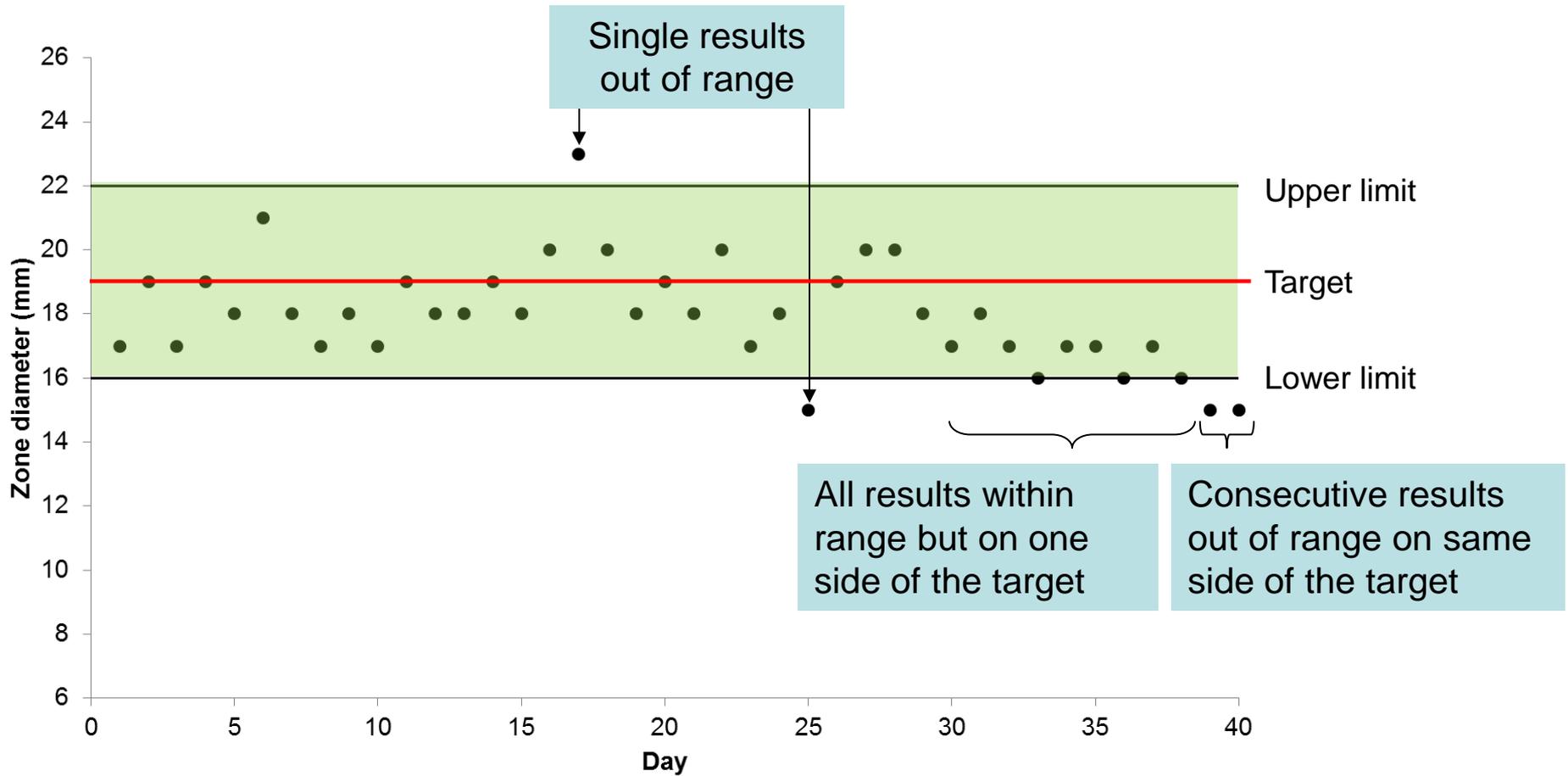
Routine data Växjö 2017
200 test occasions
10-15 readers



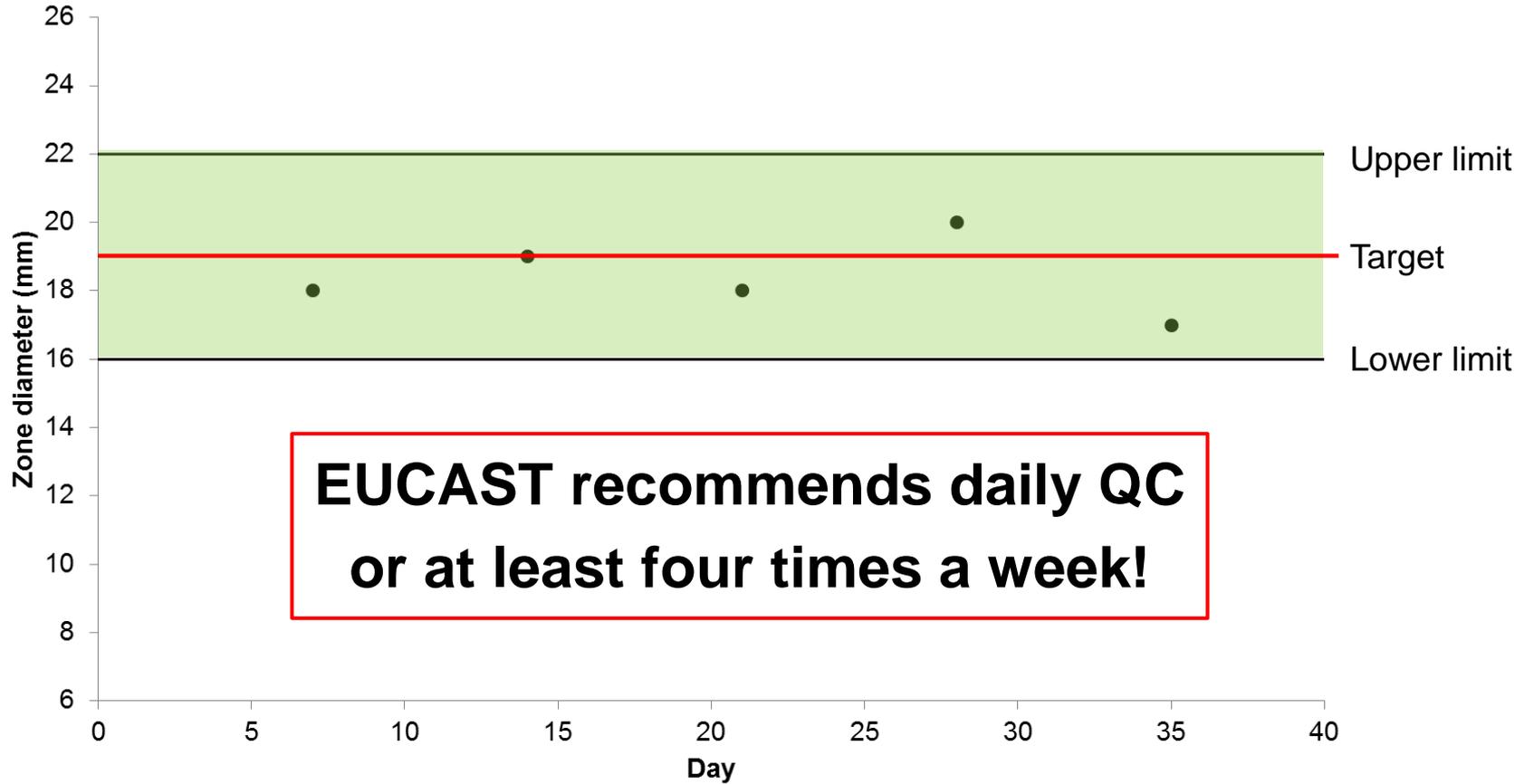
- Routine QC data

- Results randomly distributed within the QC range
- Mean value close to the target value
- Day-to-day variation due to small differences in
 - Inoculum preparation and plate inoculation
 - Incubation time and temperature
 - Reading of results

Monitoring Laboratory QC results



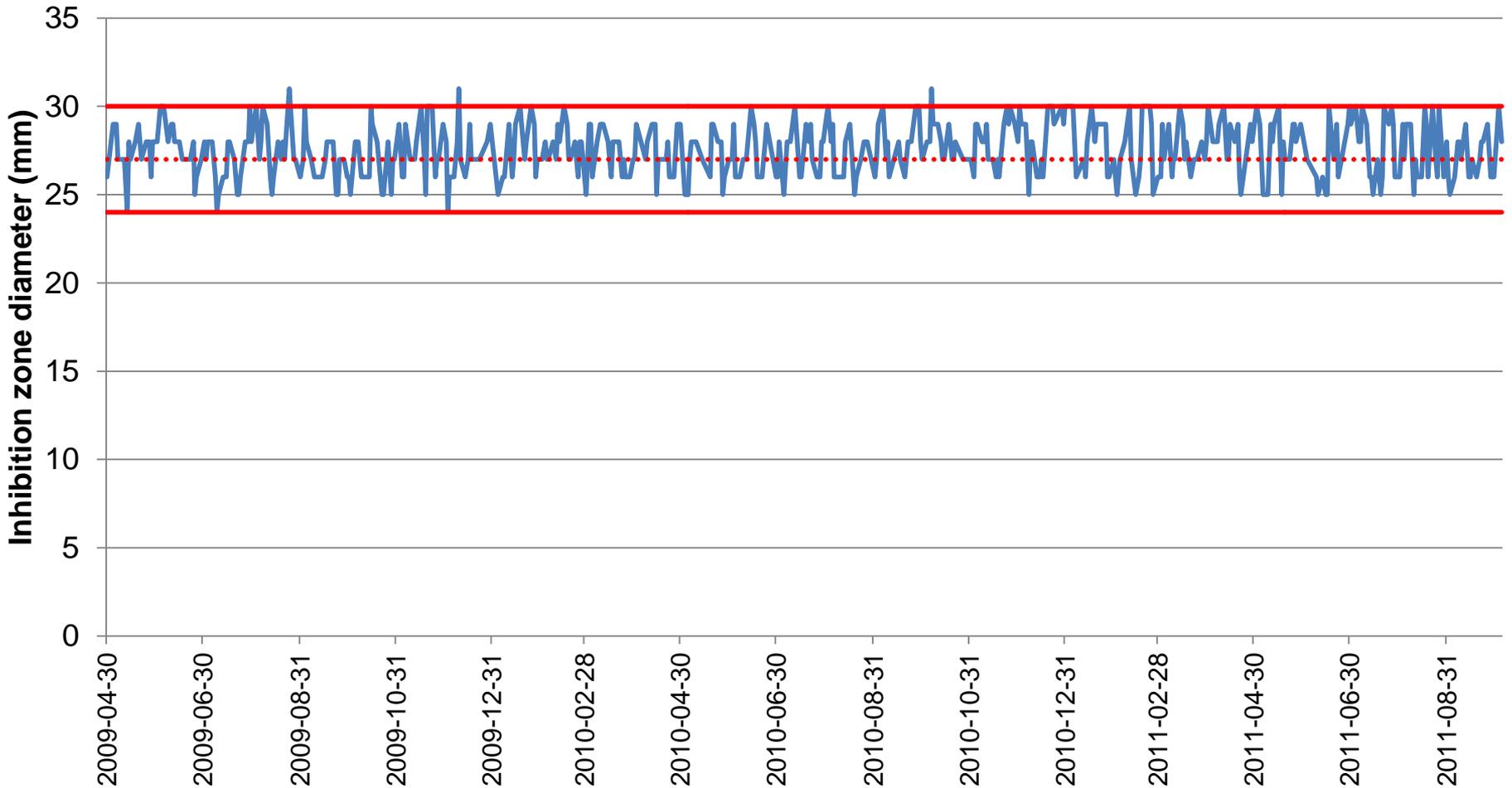
Daily vs. weekly QC



Example routine data Växjö

MH from 3 manufacturers, 10-15 technicians

***S. aureus* ATCC 29213 with cefoxitin 30 µg**



Potential sources of errors in disk diffusion

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



QC of β -lactam- β -lactamase inhibitor combinations

- The active component is checked with a susceptible QC strain.
 - Specific β -lactamase-producing strains are used to check the inhibitor:
 - Clavulanic acid: *E. coli* ATCC 35218
 - Avibactam: *K. pneumoniae* ATCC 700603
 - Tazobactam: *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603
- Both tests should be part of the routine QC.

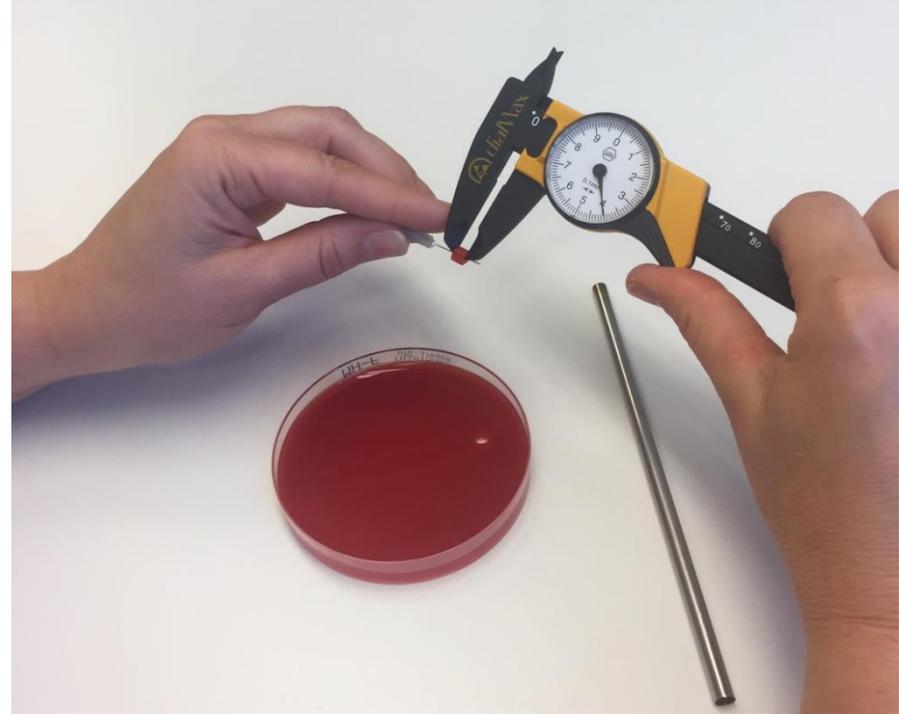
Potential sources of error (II)

- Media
 - Divalent cations, thymidine, pH
 - Supplements: Horse blood and β -NAD
 - Agar depth ($4 \text{ mm} \pm 0.5 \text{ mm}$)
 - Preparation and storage
 - Shelf life?
 - Humid agar plates?
 - Media of poor quality



- Batch control for each new (powder) batch
- Check agar depth for each batch
- Check routines for preparation and storage

Measuring agar depth



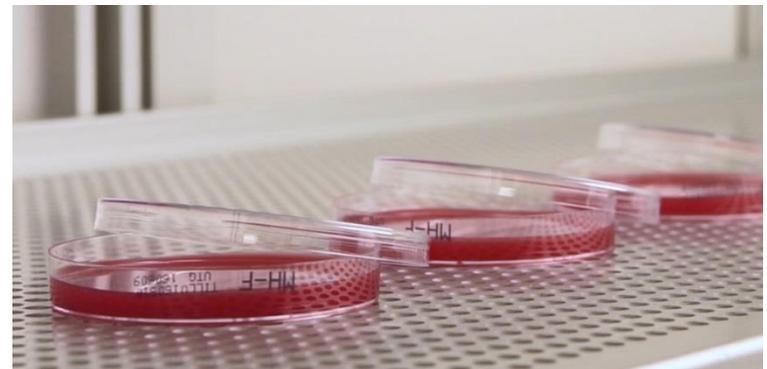
Adjust the volume if the agar depth is repeatedly above or below 4.0 mm!

Drying and storage of agar plates

- Store plates at 4-8°C
- Make sure agar plates are at room temperature prior to inoculation.
- Excess moisture may cause fuzzy zone edges and/or haze within zones.

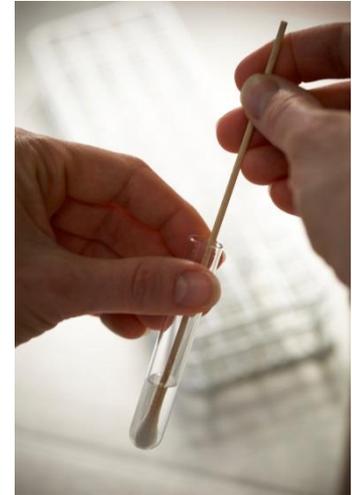


- If necessary, dry plates
 - 20-25°C overnight or
 - 35°C for 15 min
with the lid removed



Potential sources of error (III)

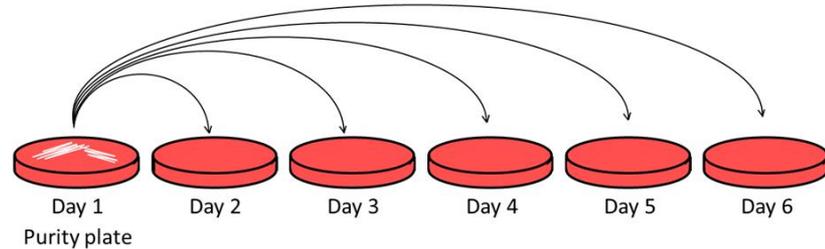
- Not adhering to methodology
 - 15-15-15 minutes rule
 - Wrong inoculum
 - Incubation temperature
 - Incubation atmosphere
 - Incubation time (16-20 h)



- Review AST routines
- Educate staff

Potential sources of error (IV)

- QC strains
 - Wrong strain
 - Old culture
 - Contamination
 - Mutation



- Review routines for QC strains
- If you suspect a problem, prepare a new subculture from freezer

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

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MIC and zone diameter distributions and ECOFFs

 [Link to MIC and zone diameter distributions and ECOFFs](#)

The website gives MIC distributions (and since 2010 inhibition zone diameter distributions generated with the EUCAST disk diffusion method) for a wide range of organisms and antimicrobial agents, including antifungals.

The distributions are based on collated data from a total of more than 30 000 MIC distributions containing more than several million MICs from worldwide sources. The distributions include MICs from national and international studies such as resistance surveillance programs (Alexander, BSAC, ECO-SENS, MYSTIC, NORM and SENTRY), as well as MIC distributions from published articles, the pharmaceutical industry drug development programmes, veterinary programmes and from individual laboratories. Histograms display wild type organisms, together with EUCAST epidemiological cut-off values (ECOFFs) and tentative ECOFFs (TECOFF).

The distributions should **never be referred to in any epidemiological context** since data from many time periods and many countries have been aggregated.

Using agreed criteria for the acceptance of MIC distributions, the database and ECOFFs are being critically evaluated during 2018 and 2019. Official ECOFFs are determined by EUCAST, adhering to protocols generated by the "EUCAST Subcommittee on MIC distributions and the determination of ECOFFs" (SOP10 and the document "[Considerations in the numerical estimation of ECOFF values](#)" - which will be integrated in SOP10).

The [ECOFFfinder program](#) is now available from the EUCAST website. This is a tool made available by EUCAST Scientific Secretary John Turnidge for people wanting to assess MIC distribution data.

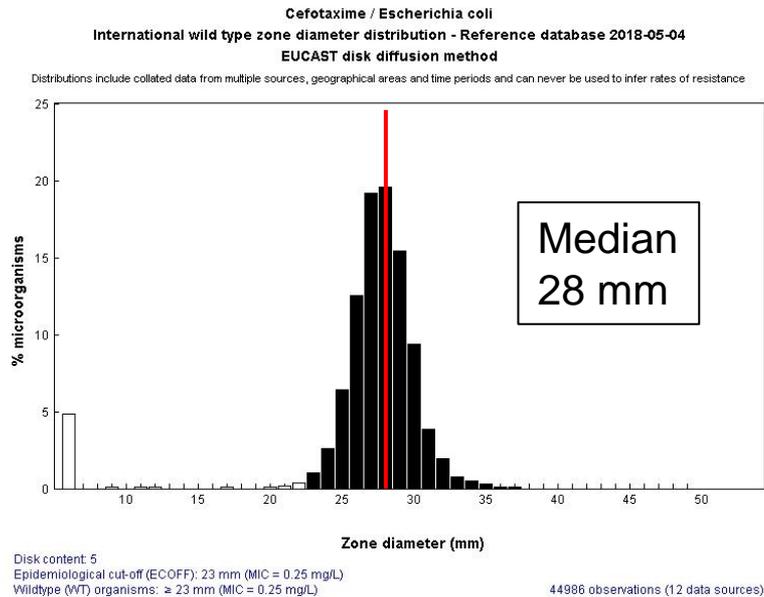
Contributions of **MIC and Zone diameter distributions** can be made using the following Excel file templates:

- **MIC distributions** organised by
 - **agent**
 - **organism**
- **Zone diameter** distributions organised by

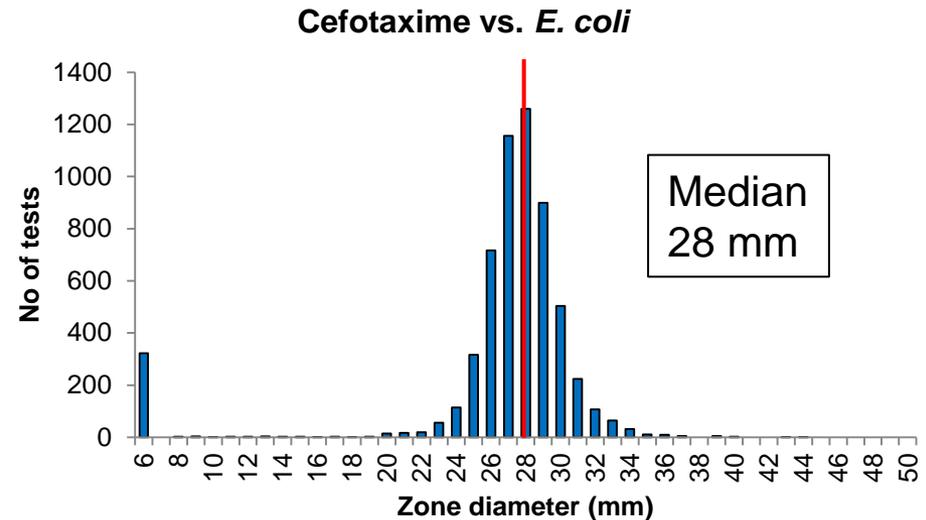
MIC and zone distributions and ECOFF ▾

Comparison with reference distributions for clinical isolates

Reference distribution



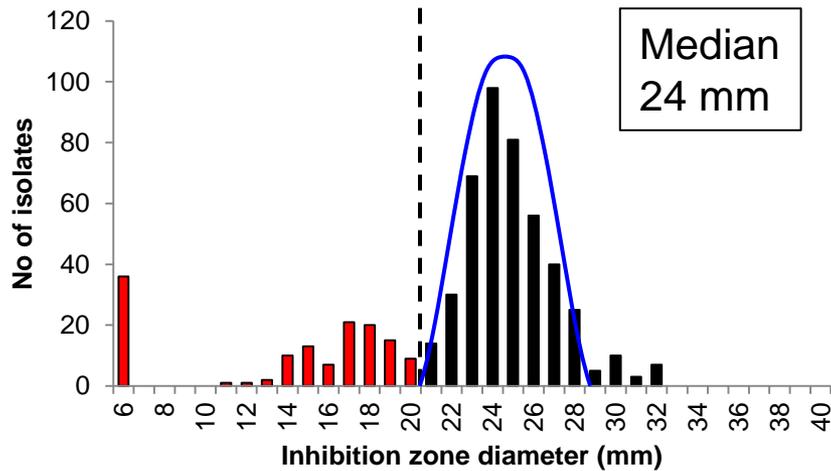
Routine distribution



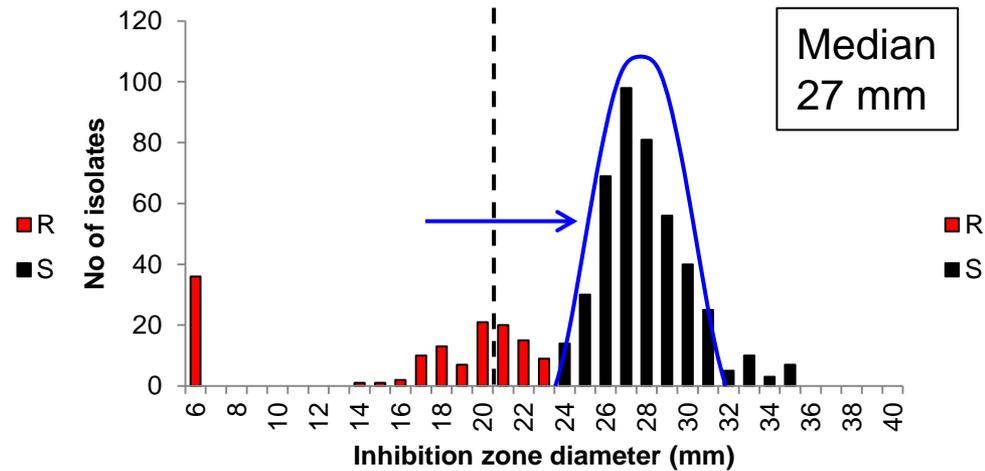
Compare the median values for wild-type isolates!

Example – too large zones

Reference distribution



Routine distribution



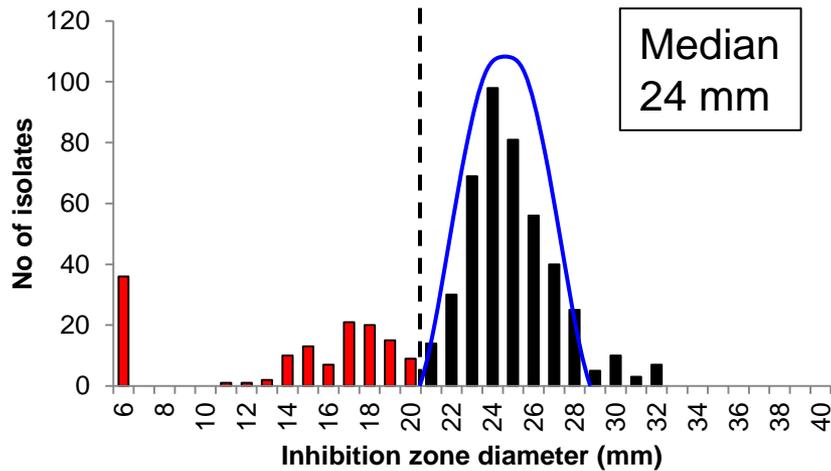
Potential errors:

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

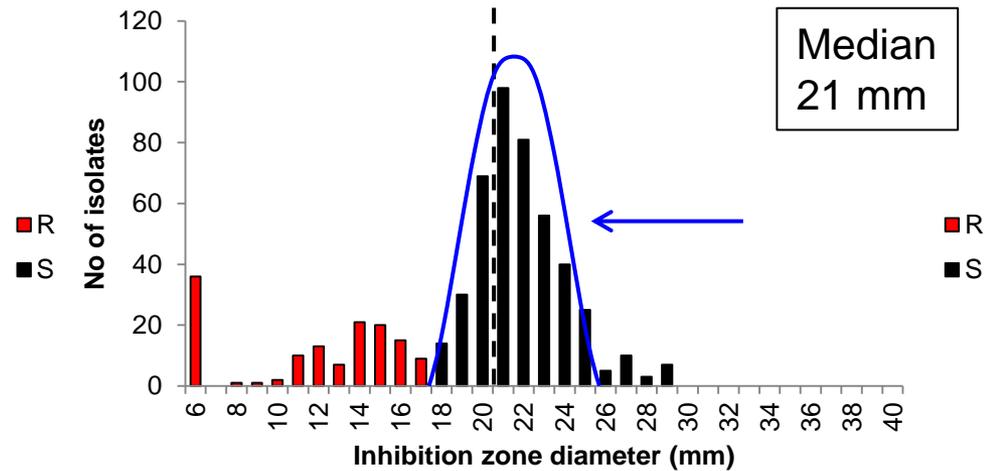
Risk of reporting isolates false susceptible!

Example – too small zones

Reference distribution



Routine distribution



Potential errors:

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

**Risk of reporting isolates
false resistant!**

EUCAST clinical breakpoints

Enterobacterales*

Expert Rules and Intrinsic Resistance Tables

EUCAST Clinical Breakpoint Tables v. 9.0, valid from 2019-01-01

MIC determination (broth microdilution according to ISO standard 20776-1 except for mecillinam and fosfomycin where agar dilution is used)
Medium: Mueller-Hinton broth
Inoculum: 5×10^5 CFU/mL
Incubation: Sealed panels, air, $35 \pm 1^\circ\text{C}$, 18±2h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.
Quality control: *Escherichia coli* ATCC 25922. For agents not covered by this strain and for control

McF 0.5

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, $35 \pm 1^\circ\text{C}$, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Escherichia coli* ATCC 25922. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor-combination disks, see EUCAST QC Tables.

35°C

* Recent taxonomic changes have a major redefining of the family Enterobacteriaceae. Some previous members of this family are now included in other families within the Order

Penicillins ¹	MIC breakpoint			Disk content	Zone diameter			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	
Benzylpenicillin	-	-	-	-	-	-	-	Numbered notes relate to general comments and/or MIC breakpoints.
Ampicillin	8 ¹	8	-	10	14 ^{A,B}	14 ^B	-	1/A. Wild type Enterobacterales are categorised as susceptible to aminopenicillins. Some countries prefer to categorise wild-type isolates of <i>E. coli</i> and <i>P. mirabilis</i> as "Susceptible, increased exposure". When this is the case, use the MIC breakpoint S ≤ 0.5 mg/L and the corresponding zone diameter breakpoint S ≥ 50 mm.
Ampicillin-sulbactam	8 ^{1,2}	8 ²	-	10-10	14 ^{A,B}	14 ^B	-	2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Amoxicillin	8 ¹	8	-	-	Note ^C	Note ^C	-	3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin-clavulanic acid	8 ^{1,3}	8 ³	-	20-10	19 ^{A,B}	19 ^B	19-20	4. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin-clavulanic acid (uncomplicated UTI only)	2 ¹	2 ³	-	20-10	16 ^A	16 ^B	-	5. Breakpoints still under consideration.
Piperacillin	8	16	-	30	20	17	-	6. Agar dilution is the reference method for mecillinam MIC determination.
Piperacillin-tazobactam	8 ⁴	16 ⁴	16	30-6	20	17	17-19	B. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
Ticarcillin	8	16	-	75	23	20	-	C. Susceptibility inferred from ampicillin.
Ticarcillin-clavulanic acid	8 ³	16 ³	-	75-10	23	20	-	D. Ignore isolated colonies within the inhibition zone for <i>E. coli</i> .
Temocillin	Note ⁵	Note ⁵	-	-	Note ⁵	Note ⁵	-	
Phenoxymethylpenicillin	-	-	-	-	-	-	-	
Oxacillin	-	-	-	-	-	-	-	
Cloxacillin	-	-	-	-	-	-	-	
Dicloxacillin	-	-	-	-	-	-	-	
Flucloxacillin	-	-	-	-	-	-	-	
Mecillinam (uncomplicated UTI only) <i>E. coli</i> , <i>Klebsiella</i> spp. (except <i>K. aerogenes</i>), <i>Raoultella</i> spp. and <i>P. mirabilis</i>	8	8	10	10	15	15	-	

Confluent growth

16-20 hours

High quality media

4 mm

High quality disks

Standardised reading

QC!

Summary of internal QC

- Perform routine quality control daily, or at least four times a week.
- Evaluate QC data against EUCAST published ranges and target values
- Follow EUCAST disk diffusion methodology
- Use materials of good quality
- Correct storage and handling of disks and media
- When materials don't perform according to the standards
 - Contact the manufacturer!

Questions or problems?

Contact us!

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