



Quality control- the hows and whys

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Standardisation of disk diffusion

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

Routine internal quality control (QC)

- Control of materials and equipment
 - Medium
 - Antimicrobial disks
 - Densitometers
 - Incubators
- Control of the procedures
 - Inoculum preparation and inoculation
 - Incubation
 - Reading of results

Why is routine QC important?

- QC is performed to monitor the accuracy and reproducibility of susceptibility testing.
- QC mirrors testing of clinical isolates. QC is performed to avoid:
 - Reporting clinical isolates as false susceptible or false resistant.
 - Treatment failure and poor outcome for patients due to inaccurate AST results.

QC responsibility

- Manufacturers:
 - To ensure that products have been appropriately manufactured and validated against reference methodology.
- Laboratories/users:
 - To ensure that products are maintained properly (storage and handling).
 - To ensure that testing is performed according to methodology.

EUCAST QC recommendations

- Perform routine quality control daily, or at least four times a week.
- Use an overnight culture of the QC strain and follow the same testing procedure as for clinical isolates.
- Include antimicrobial agents which are part of routine panels.
- Read and evaluate QC results before reporting AST results for clinical isolates.
- EUCAST Disk Diffusion Manual, section 9
www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/
- EUCAST QC Tables
www.eucast.org/ast_of_bacteria/qc_tables/

Recommended control strains: Routine quality control

Organism	Number	Characteristics
<i>Escherichia coli</i>	ATCC 25922	Susceptible, wild type
<i>Pseudomonas aeruginosa</i>	ATCC 27853	Susceptible, wild type
<i>Staphylococcus aureus</i>	ATCC 29213	Weak β -lactamase producer
<i>Enterococcus faecalis</i>	ATCC 29212	Susceptible, wild type
<i>Streptococcus pneumoniae</i>	ATCC 49619	Reduced susceptibility to benzylpenicillin
<i>Haemophilus influenzae</i>	ATCC 49766	Susceptible, wild type
<i>Campylobacter jejuni</i>	ATCC 33560	Susceptible, wild type
<i>Bacteroides fragilis</i>	ATCC 25285	β -lactamase producer
<i>Clostridium perfringens</i>	ATCC 13124	Susceptible, wild type

Recommended control strains: Routine quality control

Control of the inhibitor component in β -lactam- β -lactamase inhibitor disks

Organism	Number	Characteristics
<i>Escherichia coli</i>	ATCC 35218	TEM-1 β -lactamase
<i>Klebsiella pneumoniae</i>	ATCC 700603	SHV-18 ESBL
<i>Klebsiella pneumoniae</i>	ATCC BAA-2814	KPC-3, SHV-11 and TEM-1
<i>Escherichia coli</i>	NCTC 13353	CTX-M-15 and OXA-1
<i>Staphylococcus aureus</i>	ATCC 29213	β -lactamase
<i>Bacteroides fragilis</i>	ATCC 25285	β -lactamase

Evaluation of QC results

- Check that results are within the published QC ranges.
- Mean values of repeat testing should optimally be close to the target value.
- Troubleshoot before reporting results for clinical isolates if:
 - Two consecutive results are out of range
 - Multiple disks are out of range on the same day

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

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Range

Used to allow for random variation

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Range

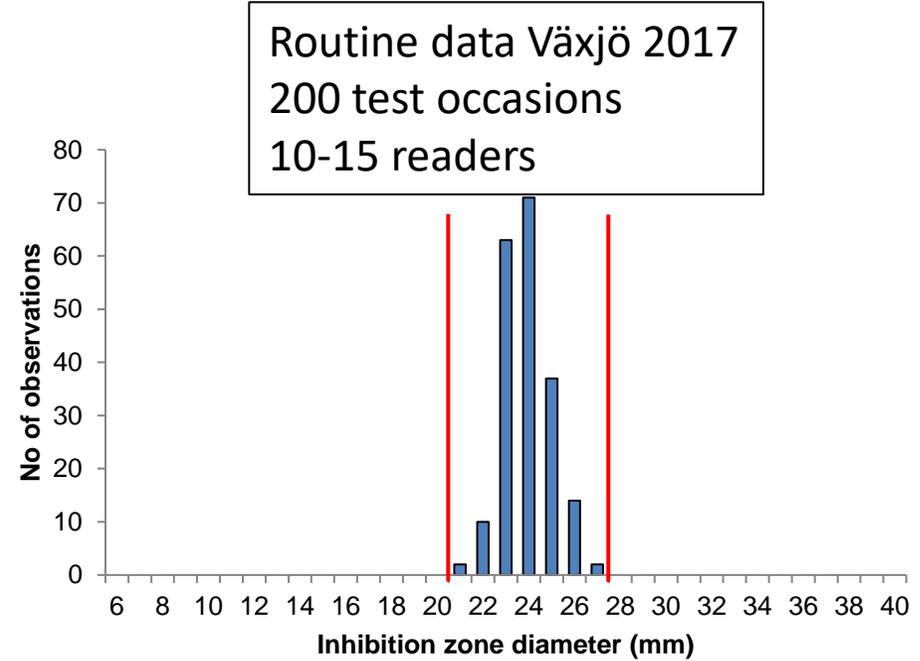
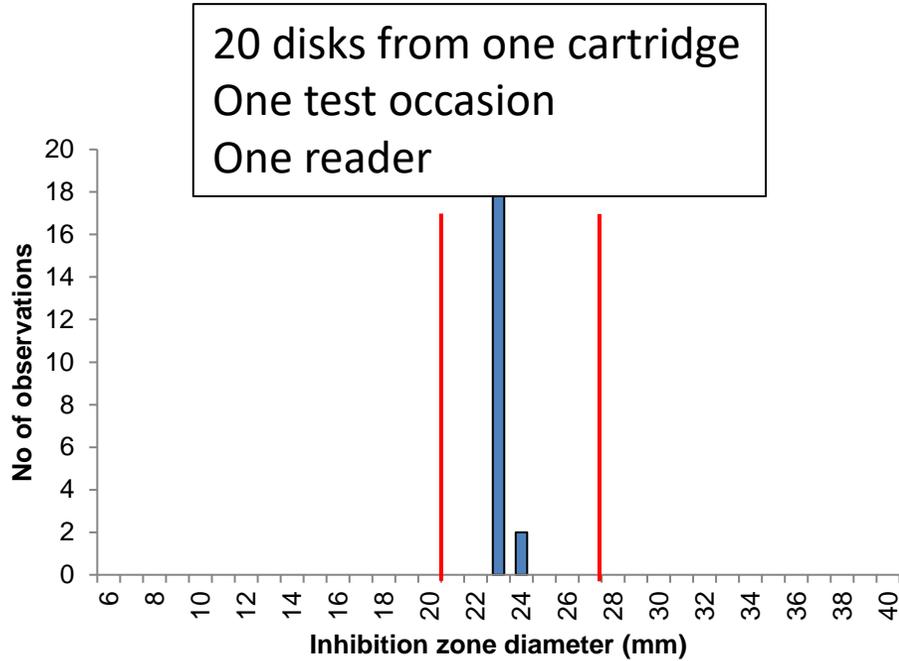
Used to allow for random variation

Target

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

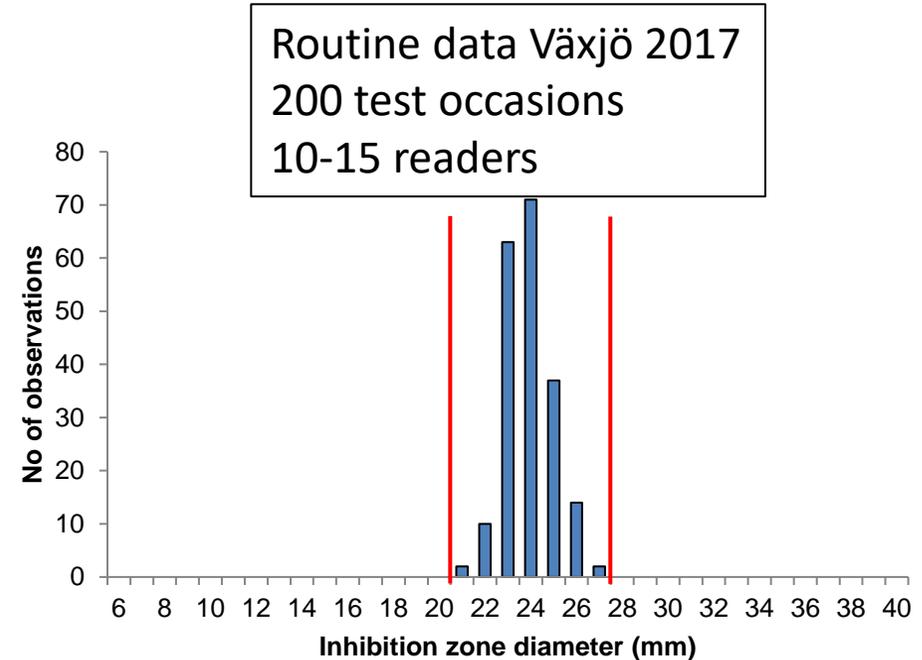
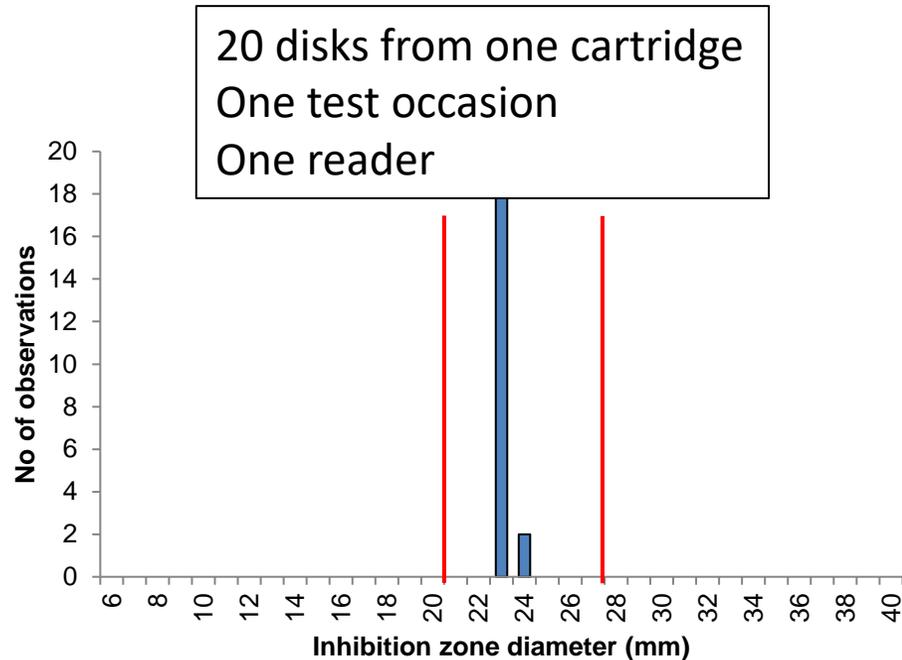
Product accuracy vs. day-to-day variation

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



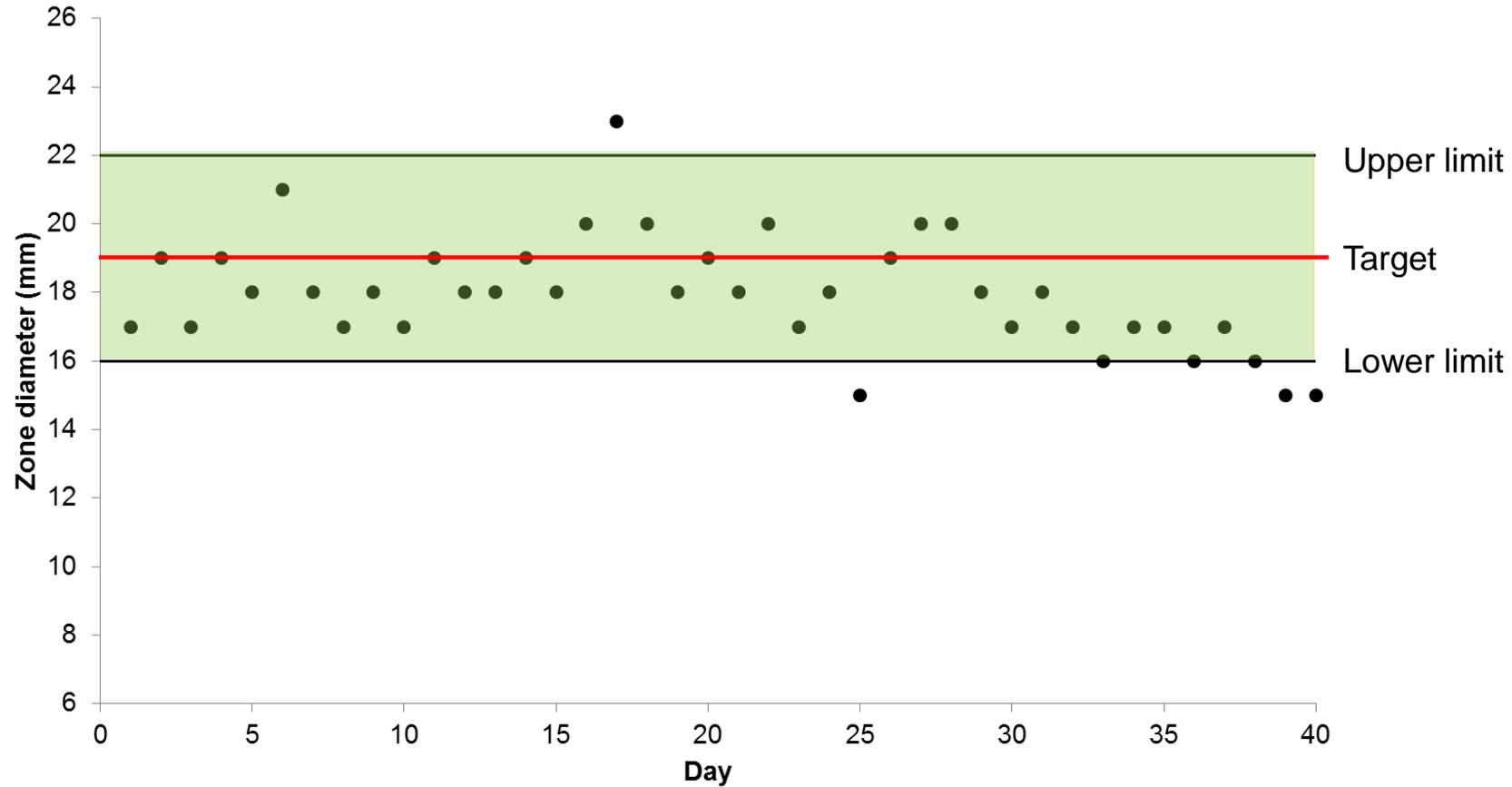
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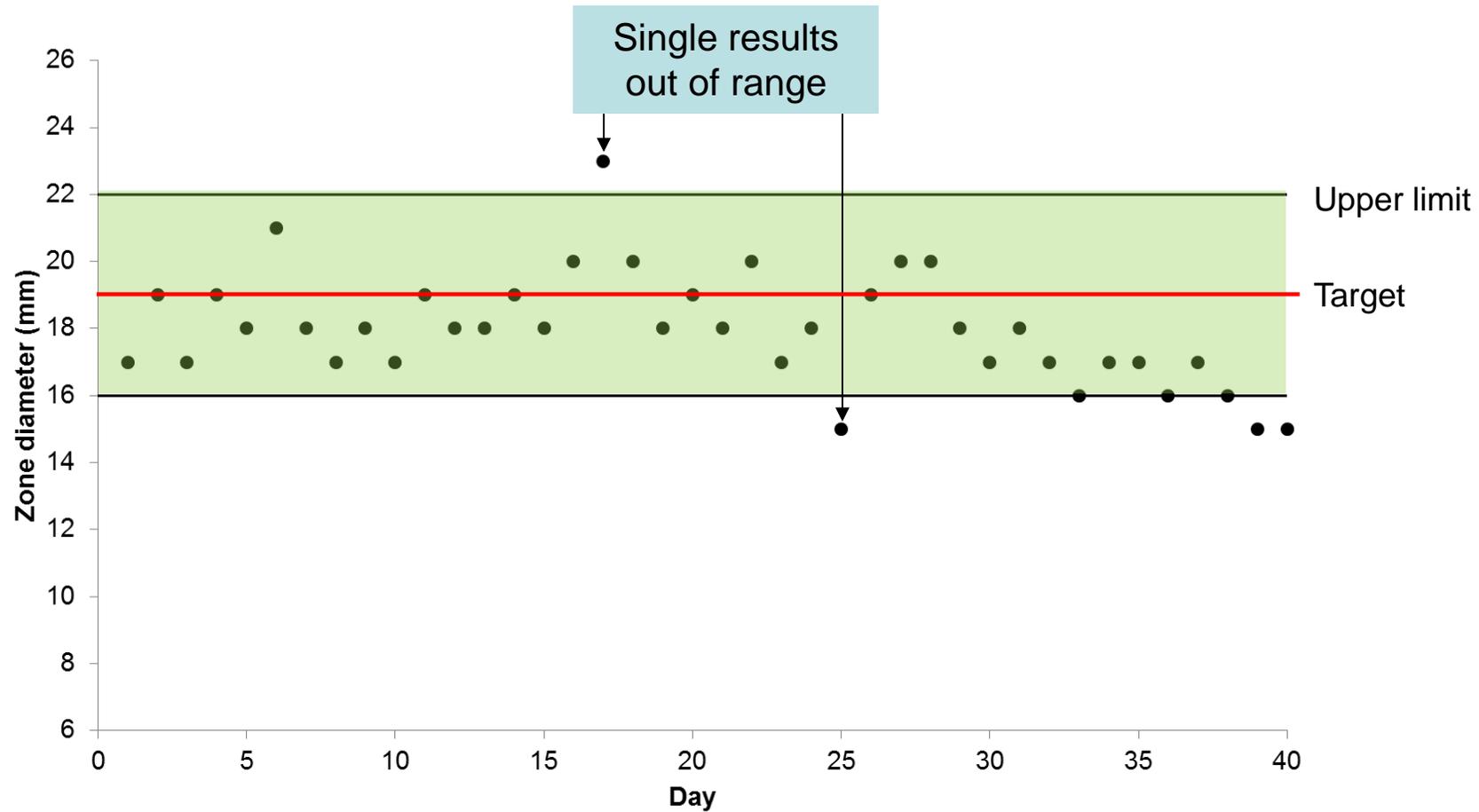


- Routine QC data
 - Results randomly distributed within the QC range
 - Mean value close to the target value
 - Day-to-day variation due to small differences in
 - Inoculum preparation and plate inoculation
 - Incubation time and temperature
 - Reading of results

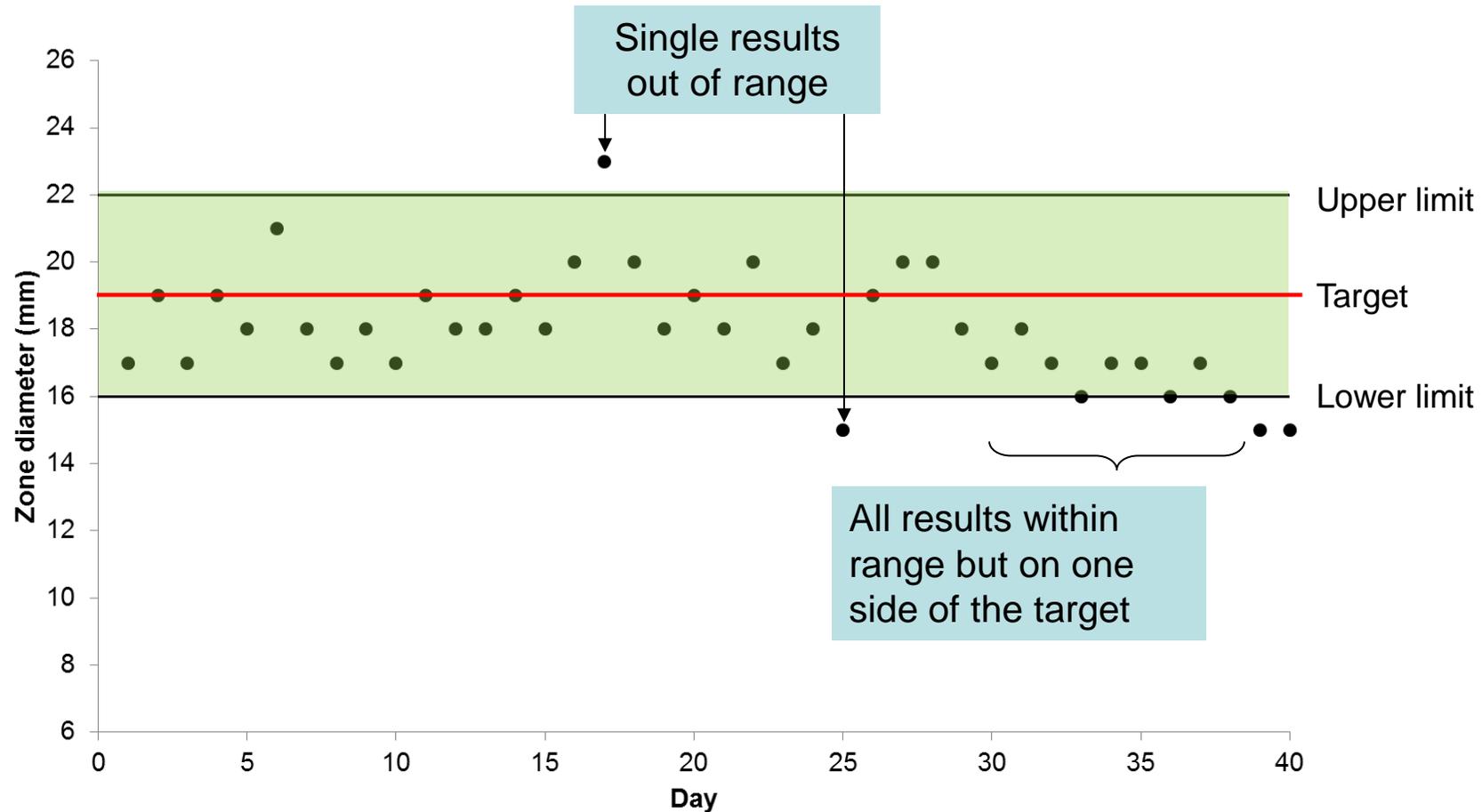
Monitoring Laboratory QC results



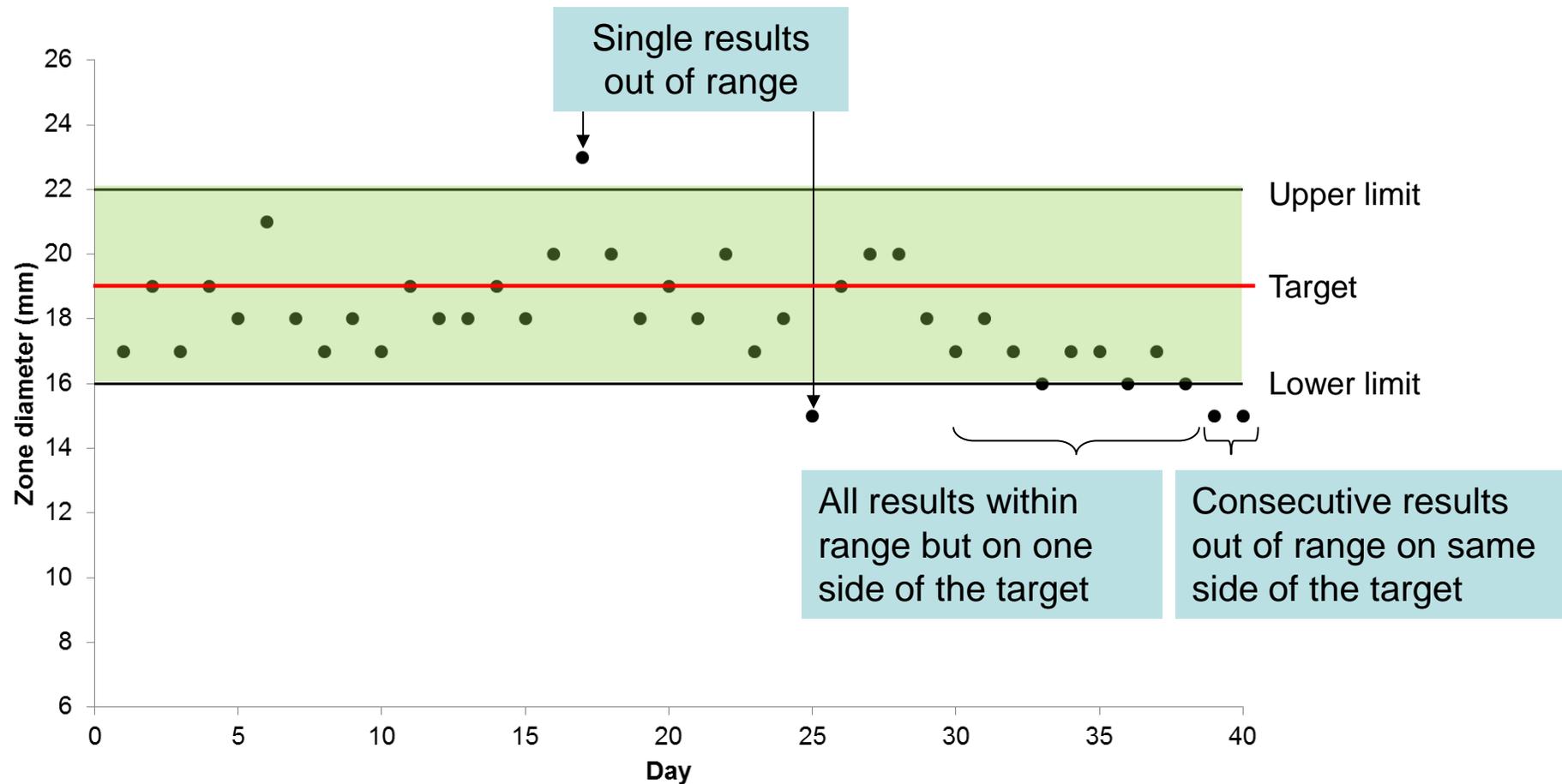
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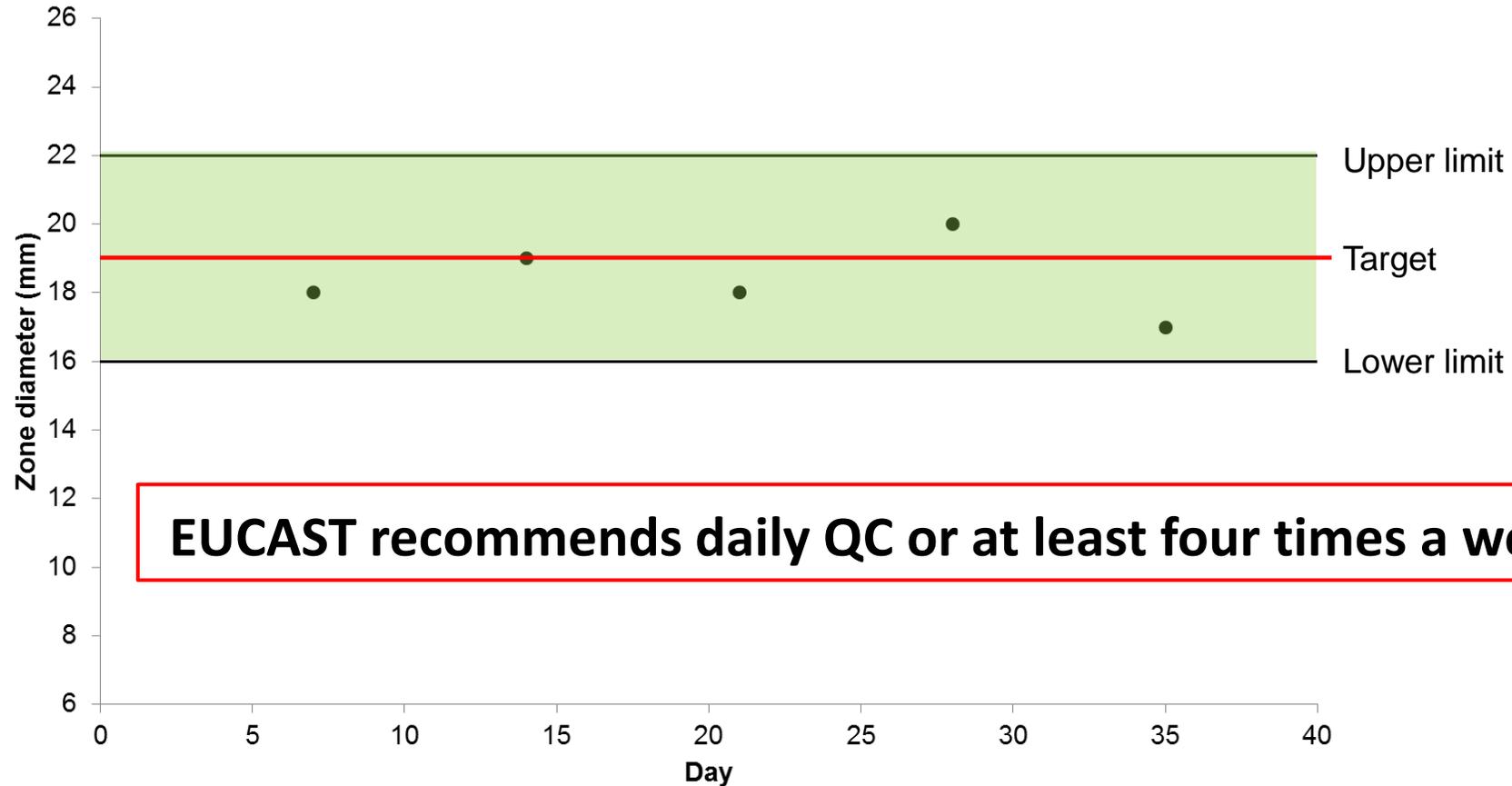
Monitoring Laboratory QC results



Monitoring Laboratory QC results



Daily vs. weekly QC

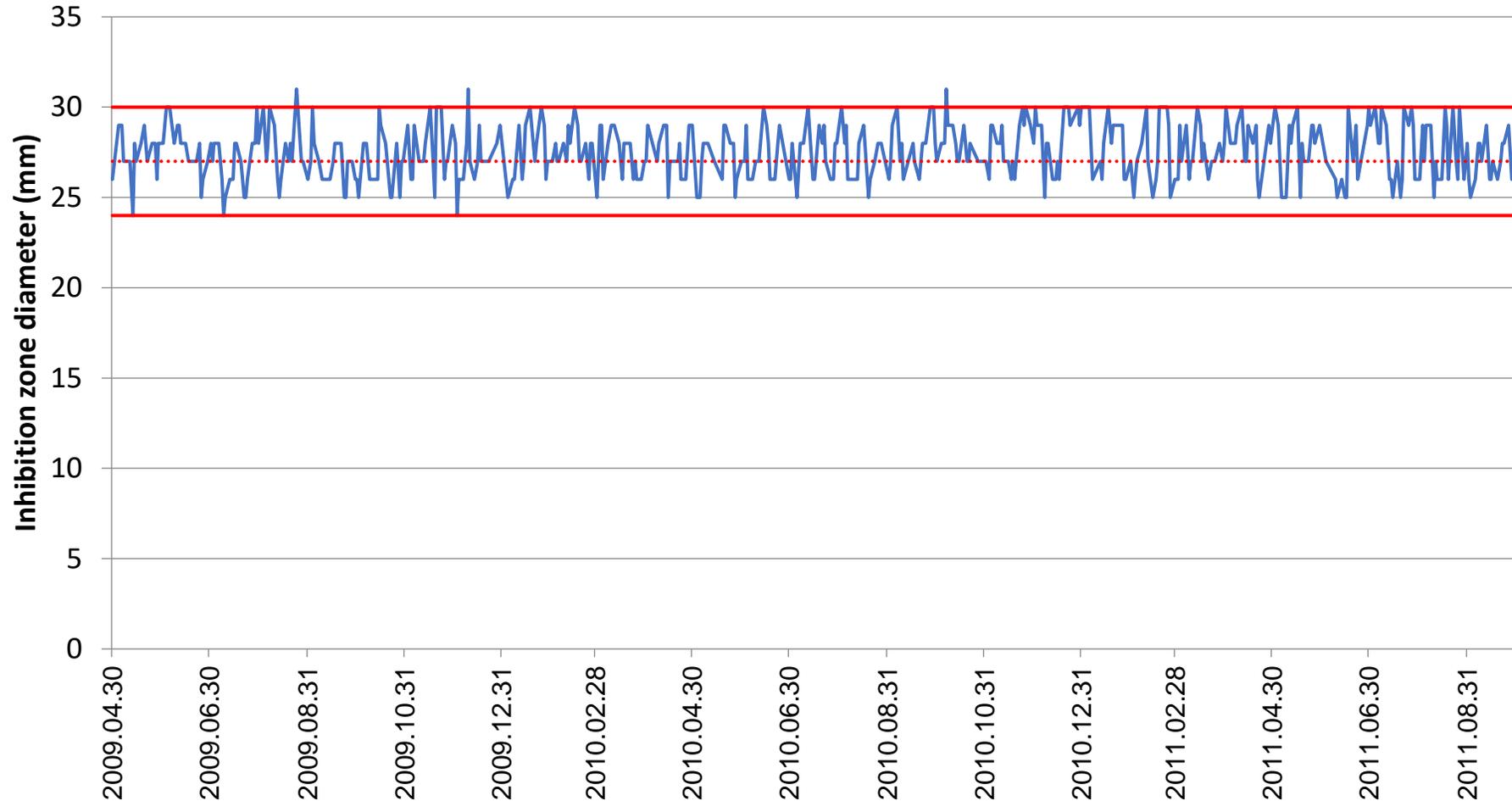


EUCAST recommends daily QC or at least four times a week!

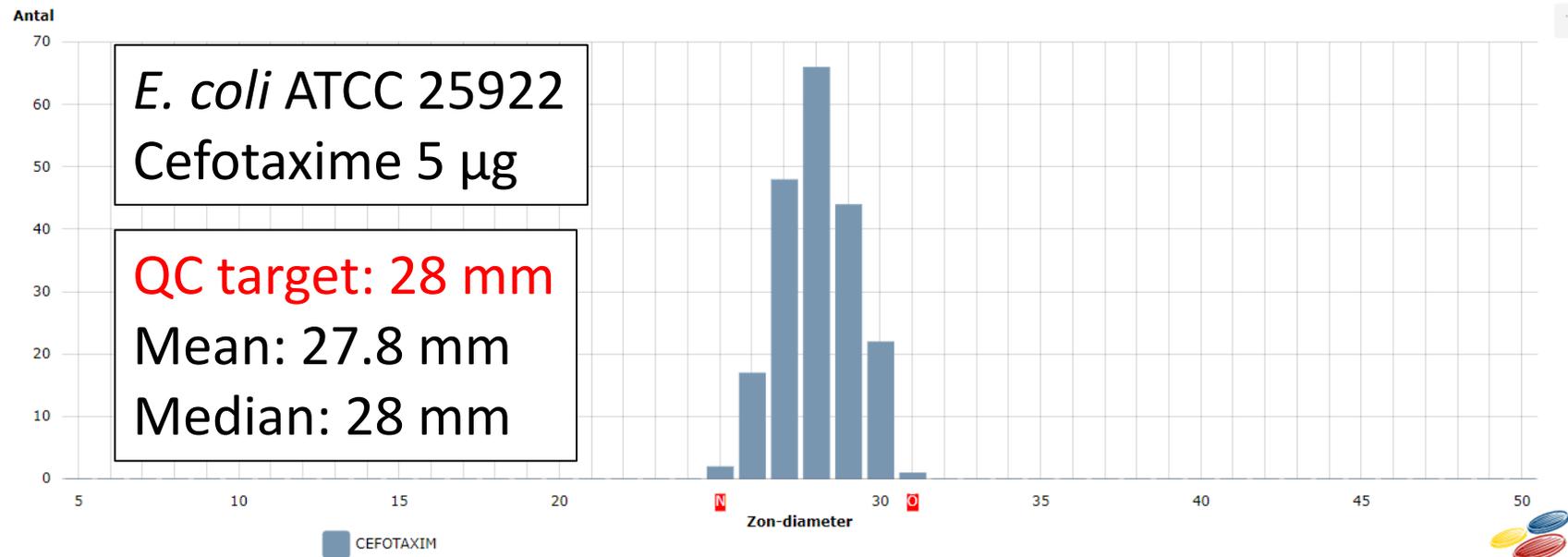
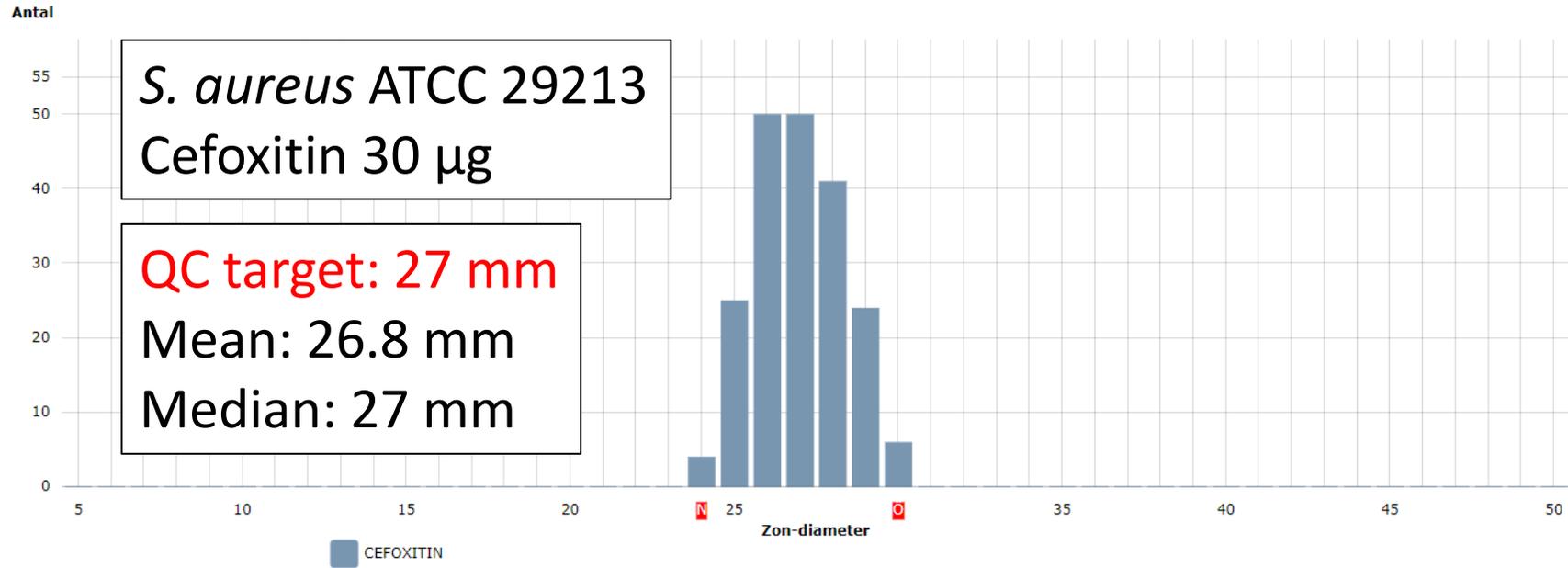
Example routine data Växjö

MH from 3 manufacturers, 10-15 technicians

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



Routine QC data, Clinical microbiology Växjö, 2023



Case 1

- You receive in your lab a new batch of MH plates. Do you control this new batch?
 - No, we already used and controlled plates from this manufacturer
 - No, the plates are CE/IVD labelled
 - Yes, every batch should be controlled

Case 1

- How should you control this new batch (choose correct answers) ?
 - By using EUCAST QC strains
 - By using clinical isolates
 - By testing new lots of disks
 - By testing already controlled disks

Case 1

- During the testing you noticed that all inhibition zone diameters are bigger than expected.
- What could be the reason(s)?
 - Wrong storage of disks
 - Wrong disks potencies
 - Too thick agar
 - Too thin agar
 - Too old plates

Case 1

- You rejected the previous plates because the agar was too thin and ordered a new batch. The results for tobramycin and *P. aeruginosa* are out of range.
- What would be the reason(s)?
 - Excess moisture in the plate
 - Too long incubation time
 - Too heavy inoculum
 - Too much cations in the agar
 - Too little cations in the agar

Case 1

- High level of thymidine in MH media affects the results of trimethoprim-sulfamethoxazole (SXT).
- How do you control the thymidine level in MH plates
 - Ask the manufacturer to provide info on thymidine concentration in their plate
 - Test SXT with *S. aureus* QC strain
 - Test SXT with *E. coli* QC strain
 - Test SXT with both *S. aureus* and *E. coli* QC strains
 - Test SXT with *E. faecalis* QC strain

Case 2

- You have implemented in your laboratory DD for cefiderocol. The manufacturer informs you that the next delivery will be delayed at least for 6 months
- You order cefiderocol disks from 2 other manufacturers to make sure that the cefiderocol AST will be possible
- Do you need to control the new disks?
 - Yes
 - No

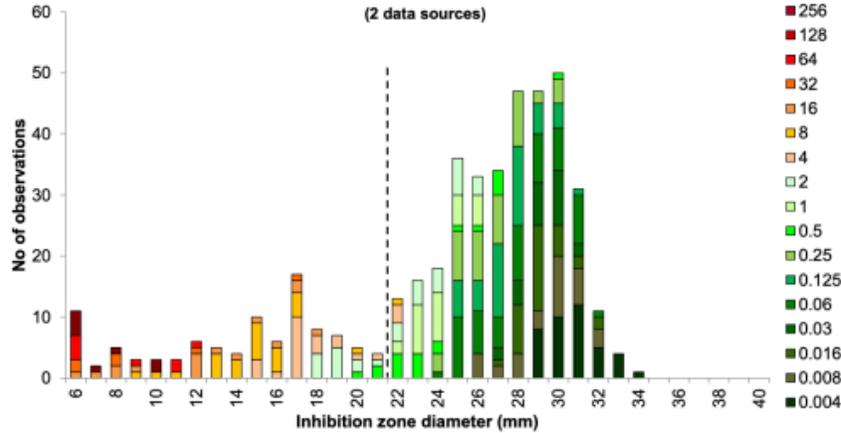
Case 2

- EUCAST has established cefiderocol targets and ranges for 2 QC strains:
E. coli and *P. aeruginosa*
- What would you test?
 - Only *E. coli*
 - Only *P. aeruginosa*
 - Both QC strains

Case 2

- The QC results show a clear difference between the 3 manufacturers: though the results are into the range, one disk gives bigger and the other smaller inhibition zone than the old disk
- What could be the reason(s)?
 - Disk batch variation
 - Too short disk shelf life
 - MH batch variation
 - MH-disk combination
 - I would repeat the testing

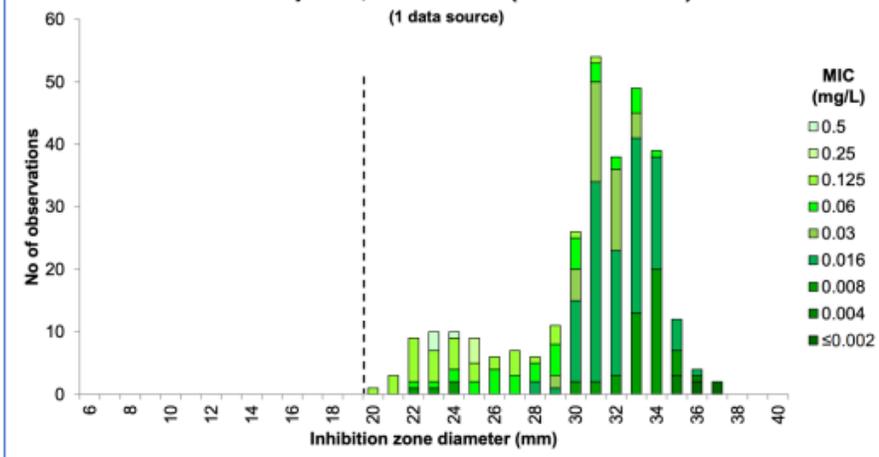
Cefiderocol 30 µg vs. MIC
E. coli, 142 isolates (440 correlates)



Breakpoints
MIC S≤2, R>2 mg/L
Zone diameter S≥22, R<22 mm

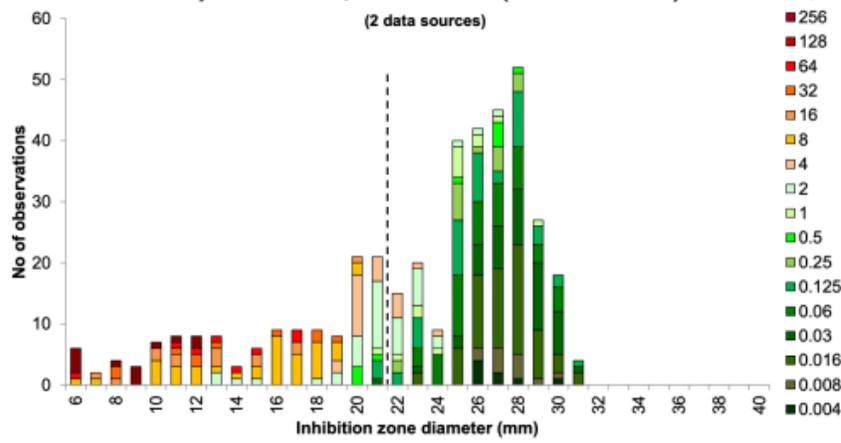
These zone diameter histograms can be used for quality assessment of individual laboratory disk diffusion results. When testing 25-50 isolates of respective species the "mid-point" of the most sensitive part of respective distribution should be on or close to 29 mm for *E. coli*, 27-28 mm for *K.pneumoniae* and *Ps. aeruginosa*, 32 mm for *S. maltophilia* and 24 mm for *Acintebacter*. Training an assessment is best performed with sensitive isolates.

Cefiderocol 30 µg vs. MIC
S. maltophilia, 74 isolates (296 correlates)



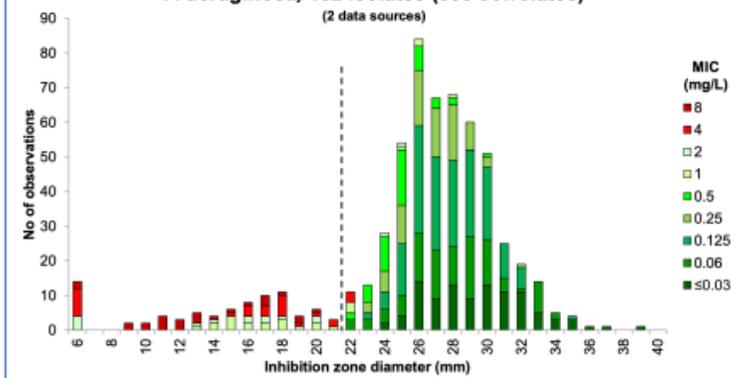
Zone diameters of ≥20 mm correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L.

Cefiderocol 30 µg vs. MIC
K. pneumoniae, 114 isolates (404 correlates)



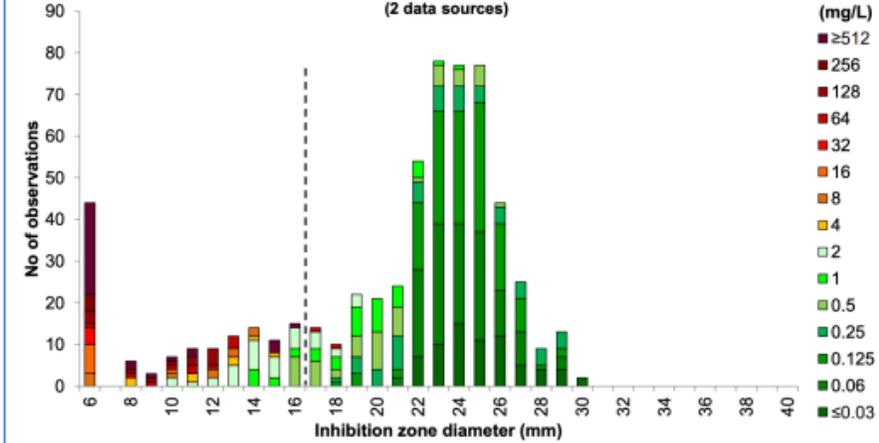
Breakpoints
MIC S≤2, R>2 mg/L
Zone diameter S≥22, R<22 mm

Cefiderocol 30 µg vs. MIC
P. aeruginosa, 152 isolates (588 correlates)



Breakpoints
MIC S≤2, R>2 mg/L
Zone diameter S≥22, R<22 mm

Cefiderocol 30 µg vs. MIC
Acinetobacter spp., 210 isolates (600 correlates)



Zone diameters of ≥17 mm correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L.

Case 2

- The same manufacturer informs you that there will be also problems for the delivery of ceftazidime-avibactam and ceftolozane-tazobactam. You order disks from another manufacturer and test them.
- Which QC strains would you use?
 - QC *E. coli* (susceptible strain)
 - QC *K. pneumoniae* (ESBL strain)
 - QC *K. pneumoniae* (KPC strain)
 - QC *E.coli* and *K. pneumoniae* (ESBL strain)

Case 2

- The QC results are acceptable for ceftolozane-tazobactam but repeatedly out of range with too large inhibition zone for ceftazidime-avibactam.
- What could be the reason(s)?
 - Too old disks
 - Too old MH plates
 - Wrong disc potency
 - MH plate-disc combination

Case 3

- You have noted an increase in the number of mecillinam resistant isolates in *Enterobacterales* in your lab
- How do you respond?
 - Accept that some variation is to be expected
 - Accept that increasing resistance is to be expected
 - Go on vacation and let someone else deal with it
 - Examine the numbers

Case 3

- Numbers reveal a significant increase in reported mecillinam resistance in *Enterobacterales*
- What do you do next?
 - Control of materials and equipment
 - Control of internal procedures
 - Control ATCC run charts
 - All of the above

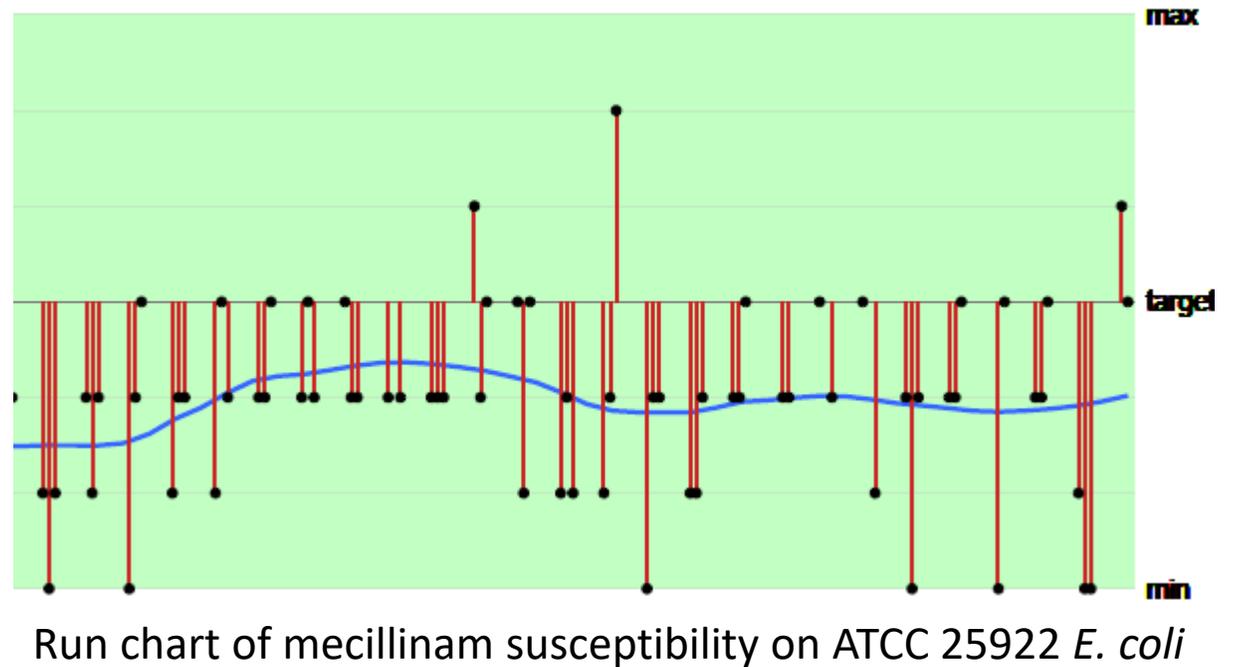
Case 3

You do a thorough inspection of the lab

- There have been no changes to plates or disks used
- There have been no changes to the used equipment
- There have been no changes in methods
- Results for E. coli ATCC 25922 are all within range

Case 3

- How do you interpret results?
 - Values are within range
 - Values are consistently below target
 - The underestimation is sustained



Case 3

- The reading of mecillinam in ATCC 25922 *E. coli* is consistently below target, but within range
- What is the most likely cause?
 - Expired disks
 - Expired MH plates
 - Wrong disc potency
 - Training of staff

Case 3

Enterobacteriales and mecillinam

Ignore isolated colonies within the inhibition zone and read the outer zone edge.



Source: Disk diffusion – Reading guide v 11.0 (January 1st, 2025)

Case 4

You have noticed an increased number of meropenem screening positive isolates in *Enterobacterales* for CPE

Subsequent analysis reveals no underlying resistance mechanism

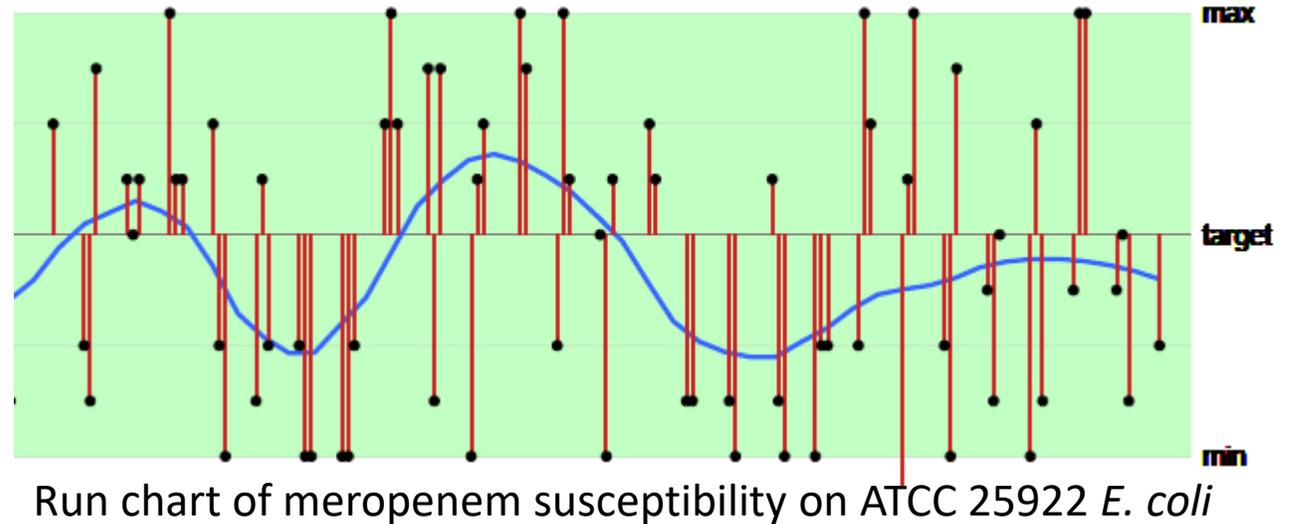
- There has been no changes to plates or disks used
- There has been no changes to the used equipment
- There has been no changes in methods
- ATCC strains are reportedly all within range

Case 4

The same pattern is apparent every week for the last month

What is the most likely cause ?

- Disks lost potency
- Too thin agar
- Wrong disc potency
- Training



Case 4

Store and use disks in accordance with manufacturers instructions



condensation. Once opened from their desiccant-containing packaging, discs should be used within 3 days and only if stored as described in this IFU.

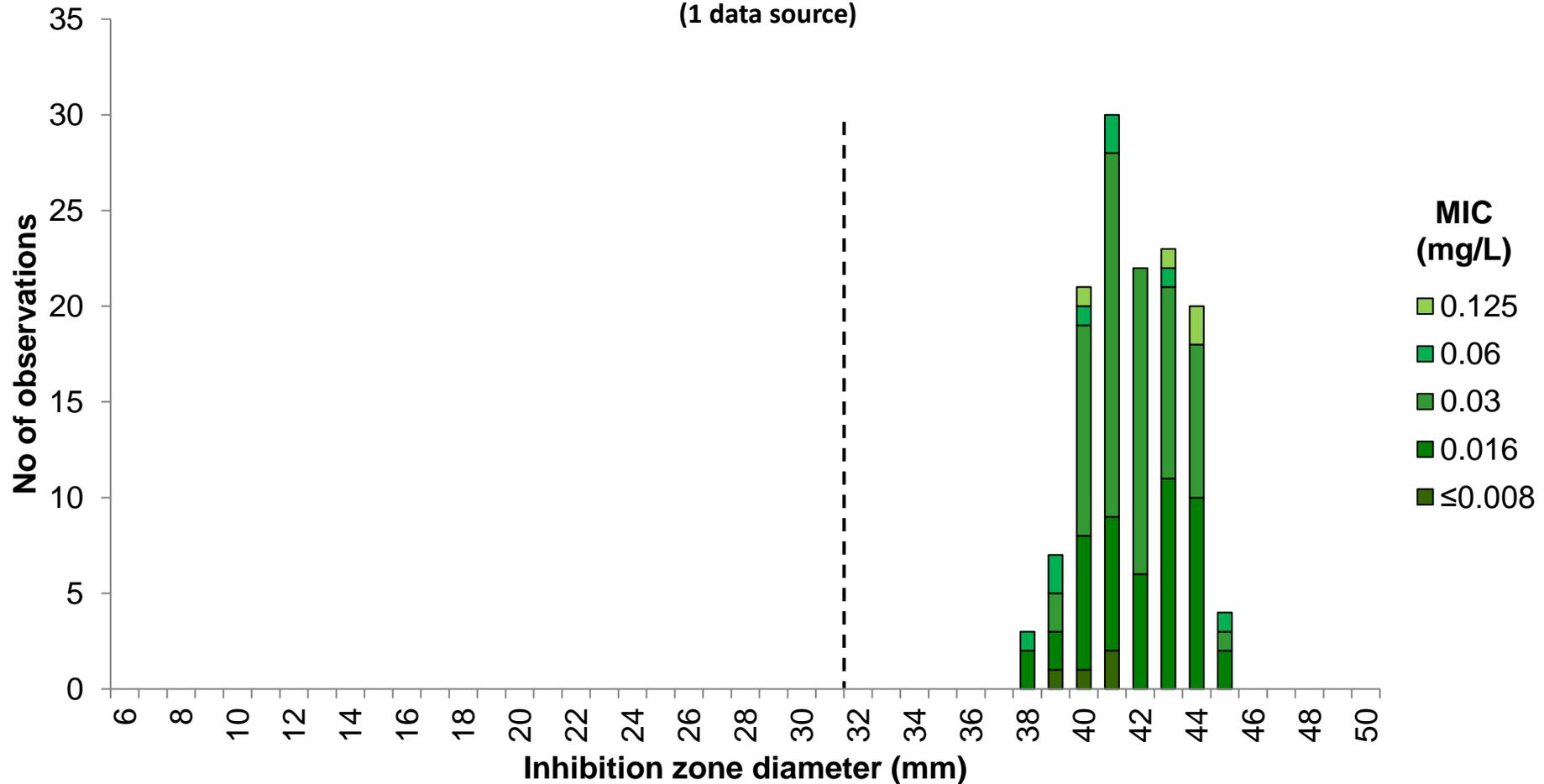
Source: Oxoid Instructions for meropenem AST discs

Case 5

- *Fusobacterium necrophorum* in a positive blood culture which is resistant to piperacillin-tazobactam with disk diffusion. Explanation?
 - Piperacillin component degraded
 - Tazobactam component degraded
 - Over-inoculation of plates
 - Incubation time exceeded
 - FAA-HB plates too humid
 - Zone diameter difficult to read

Piperacillin-tazobactam 30-6 µg vs. MIC *F. necrophorum*, 51 isolates (130 correlates)

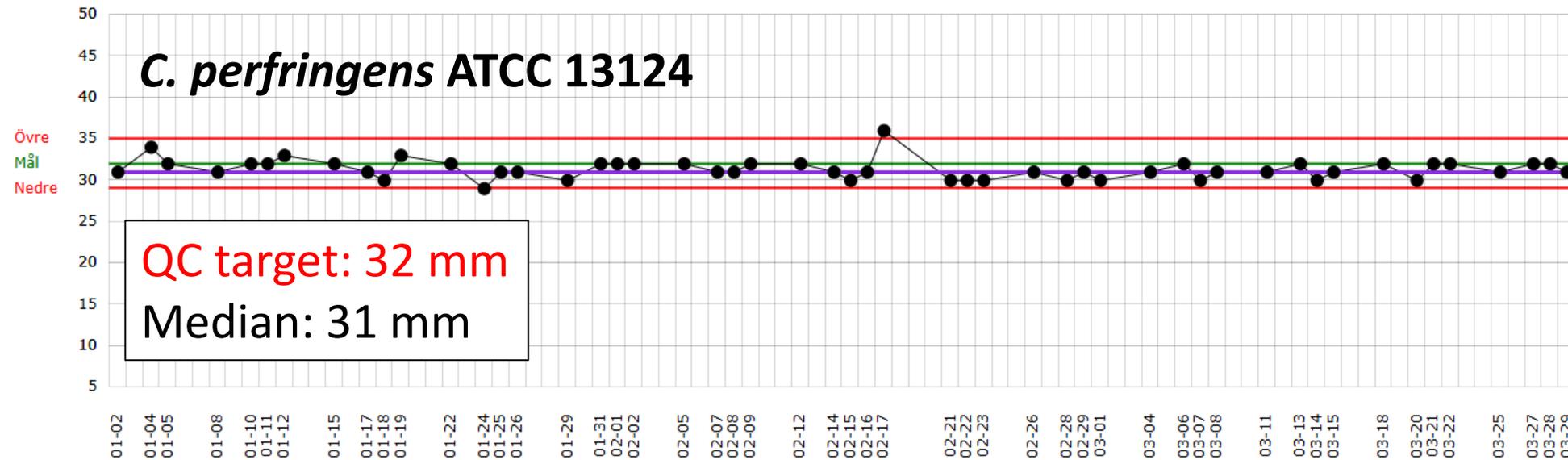
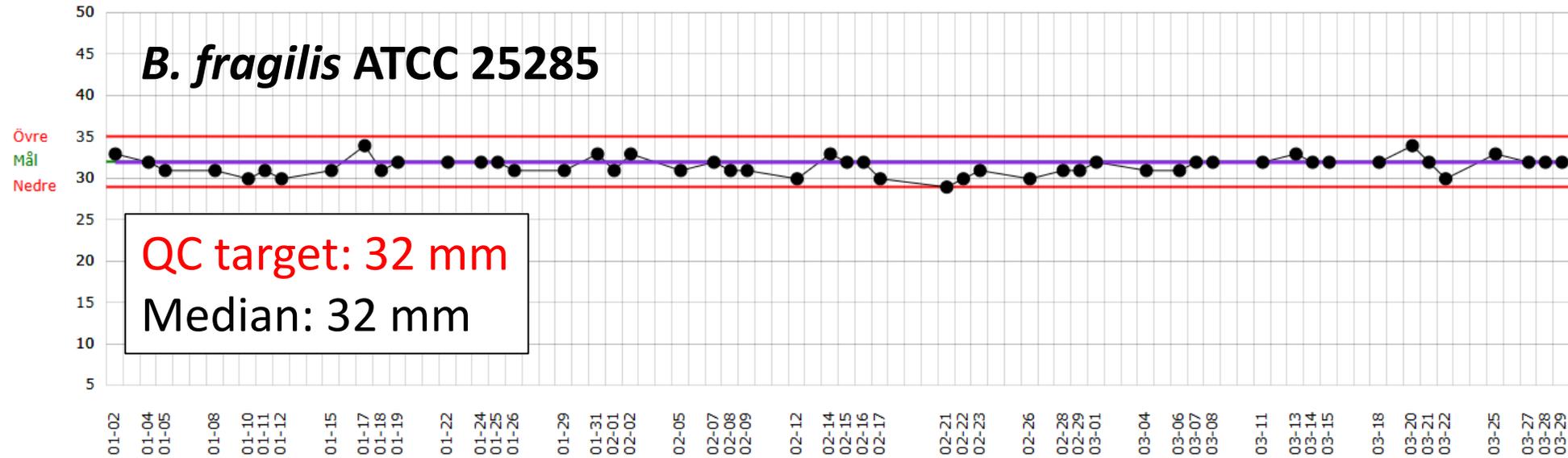
(1 data source)



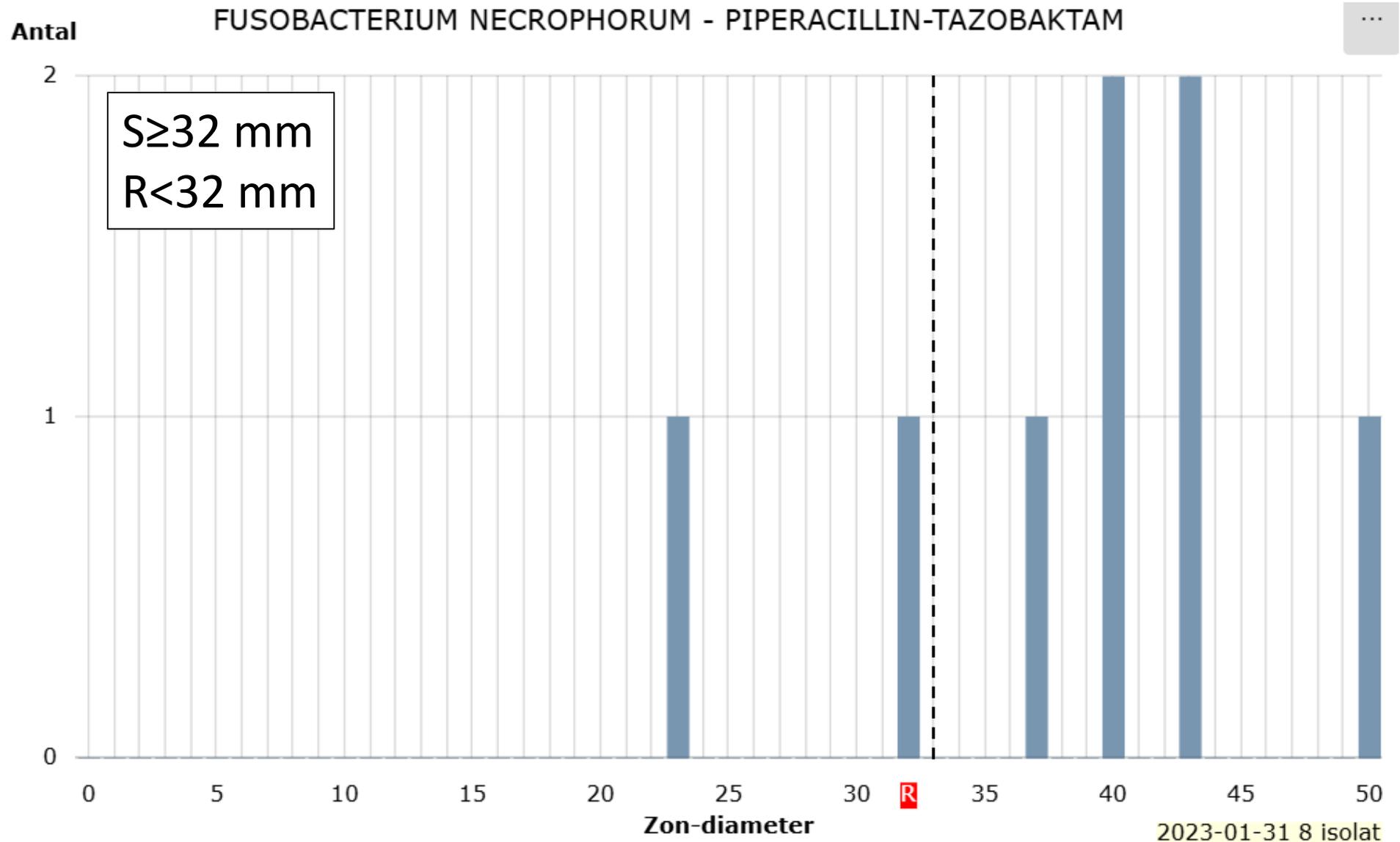
Breakpoints

MIC	S ≤ 0.5, R > 0.5 mg/L
Zone diameter	S ≥ 32, R < 32 mm

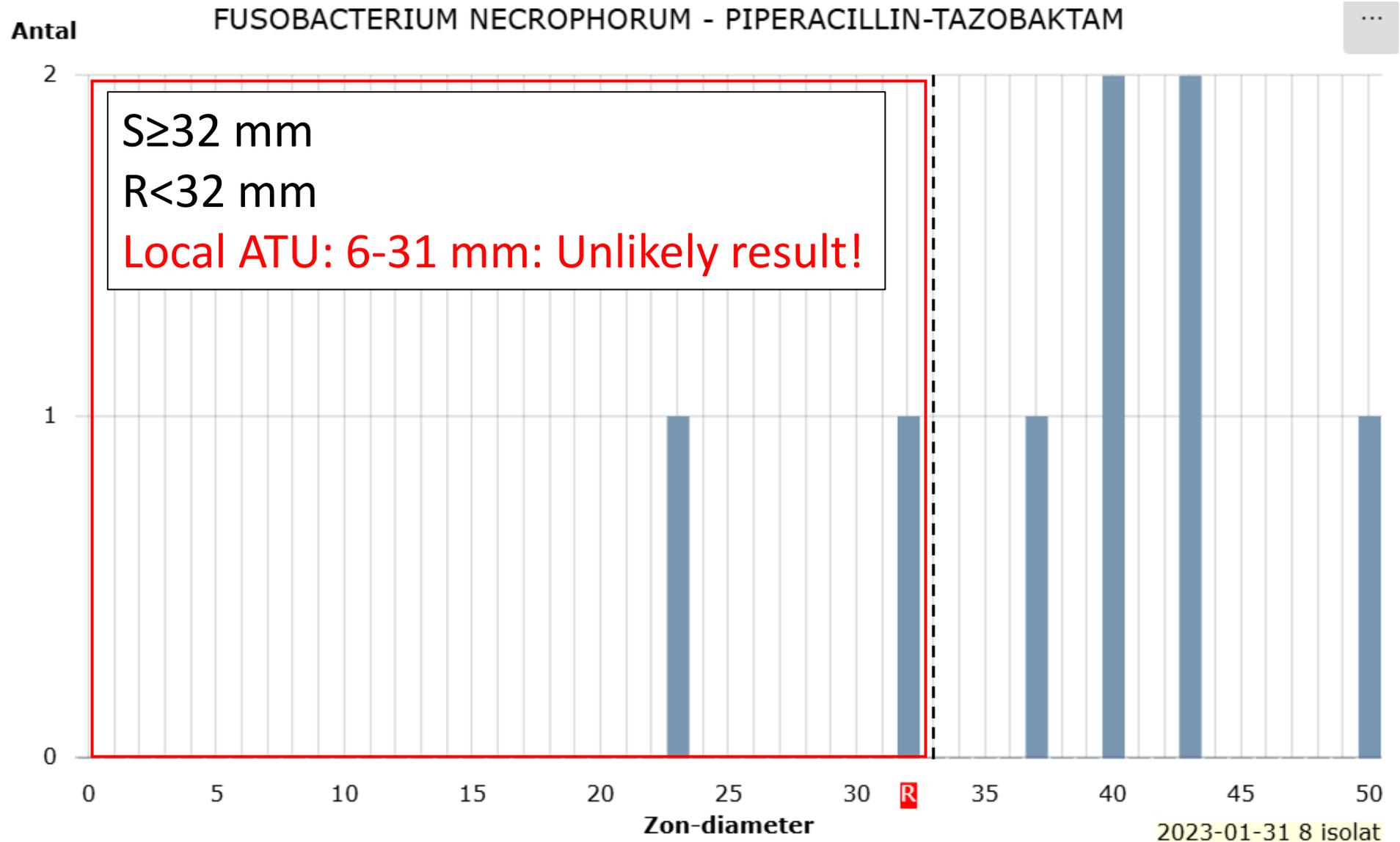
QC results



Clinical isolates



Clinical isolates



Important aspects of methodology

- Dry the FAA-HB plates before inoculation
- Streak plates carefully to ensure that there are no gaps between streaks.
- Limit the number of disks on each plate to allow good growth and to avoid overlapping of zones.
- Control the anaerobic environment and do not prolong the incubation time.
- Follow the reading instructions.

Reading Guide for anaerobic bacteria



Reading guide

EUCAST disk diffusion for selected rapidly growing anaerobic bacteria on Fastidious Anaerobe Agar (FAA)

Bacteroides spp.



Clostridium perfringens

