Lessons learned from internal quality control

Erika Matuschek, EUCAST Development Laboratory, Växjö, Sweden
Dennis Back Holmgaard, Dept. Clinical Microbiology, Region Zealand, Denmark
Anna Hill, Dept. Clinical Microbiology, Västerås Region County Hospital, Sweden

NordicAST workshop 12-13 May 2026

Internal quality control (QC)

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

NordicAST

Why is internal QC important?

- 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 antimicrobial susceptibility test results

NordicAST

EUCAST 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***

NordicAST

EUCAST 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
Used to allow for random variation

NordicAST

EUCAST 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***

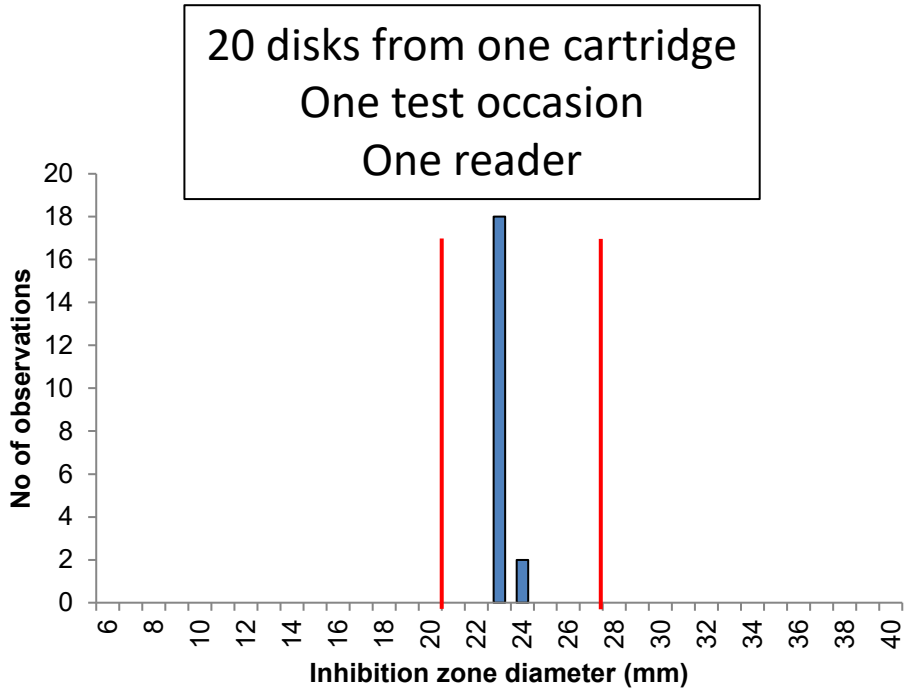
Range
Used to allow for random variation

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

NordicAST

QC ranges and targets

Example *E. coli* ATCC 25922 and piperacillin-tazobactam

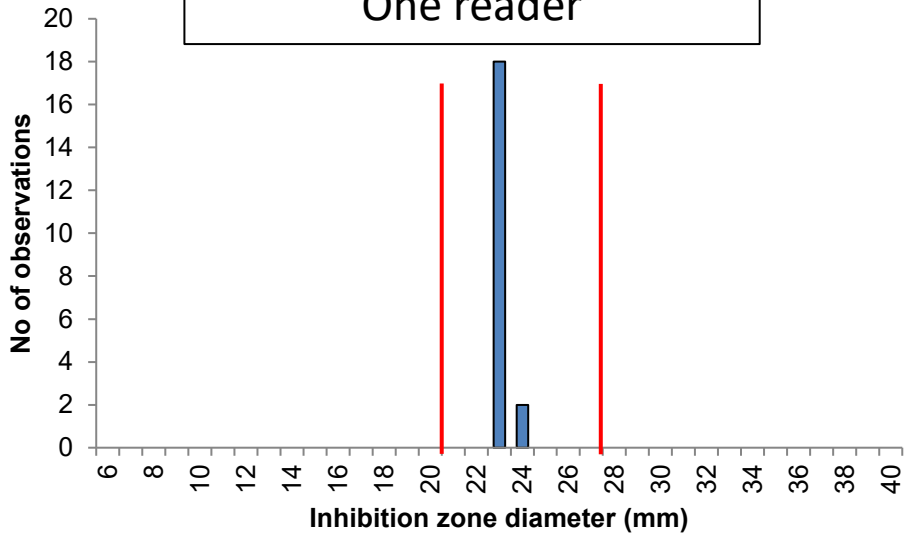


NordicAST

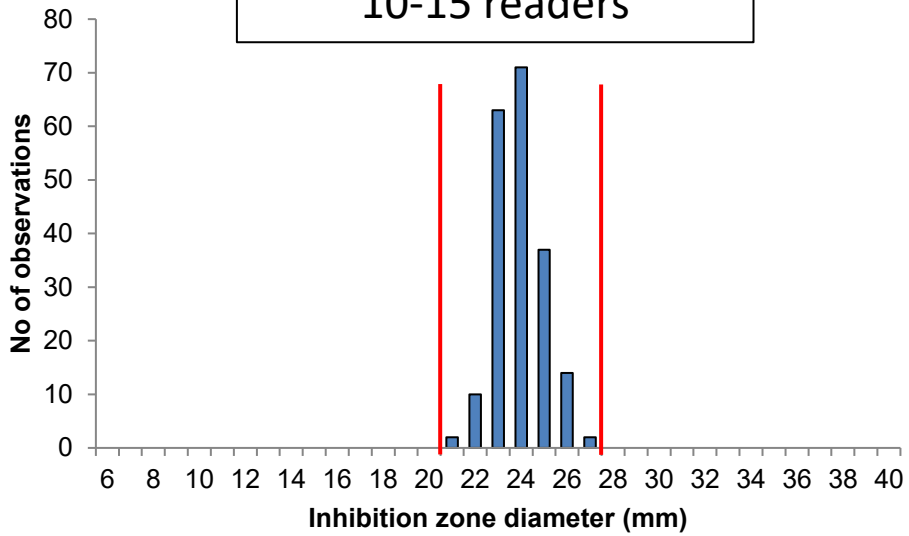
QC ranges and targets

Example *E. coli* ATCC 25922 and piperacillin-tazobactam

20 disks from one cartridge
One test occasion
One reader



Routine data Växjö 2022
200 test occasions
10-15 readers

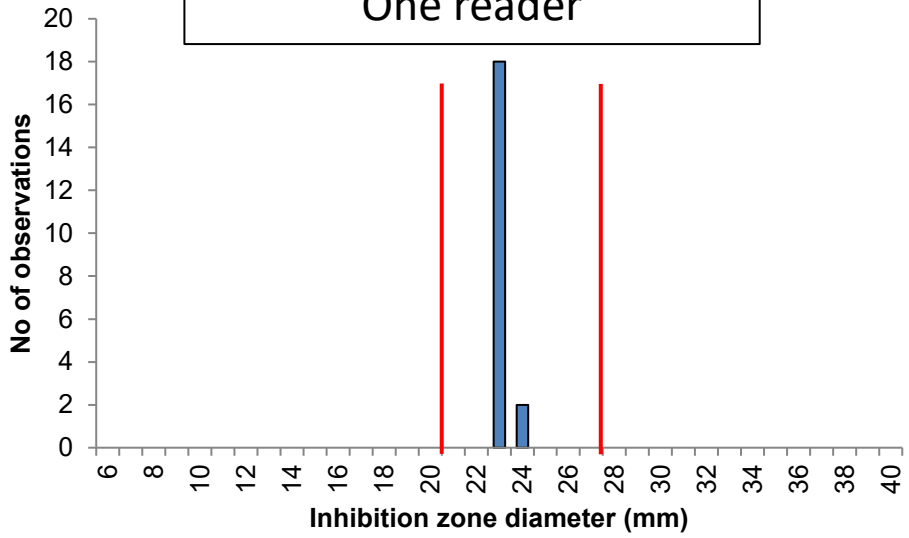


NordicAST

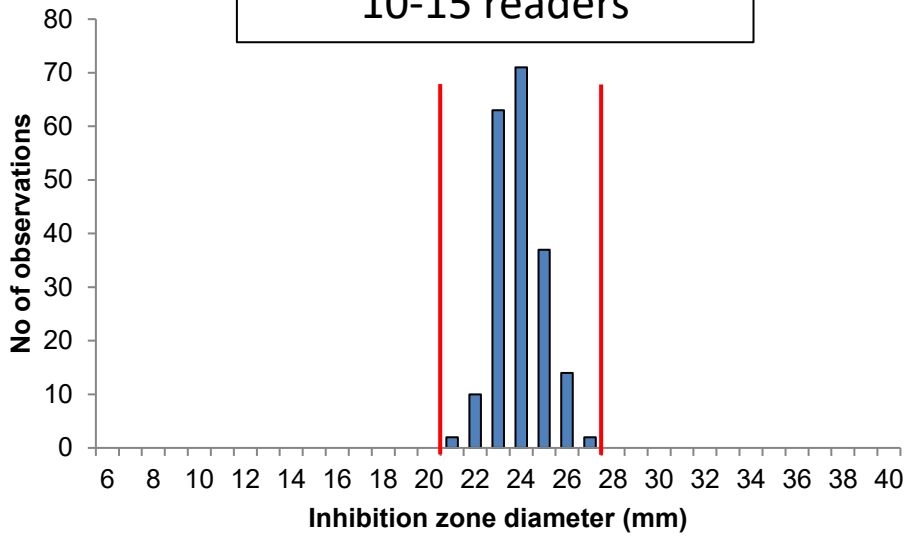
QC ranges and targets

Example *E. coli* ATCC 25922 and piperacillin-tazobactam

20 disks from one cartridge
One test occasion
One reader



Routine data Växjö 2022
200 test occasions
10-15 readers



Day-to-day variation due to small differences in:

- Inoculum preparation and plate inoculation
- Incubation time and temperature
- Reading of results

NordicAST

Frequency of internal QC

For disk diffusion, perform routine quality control daily, or at least four times a week

NordicAST

Analysis of disk diffusion QC results

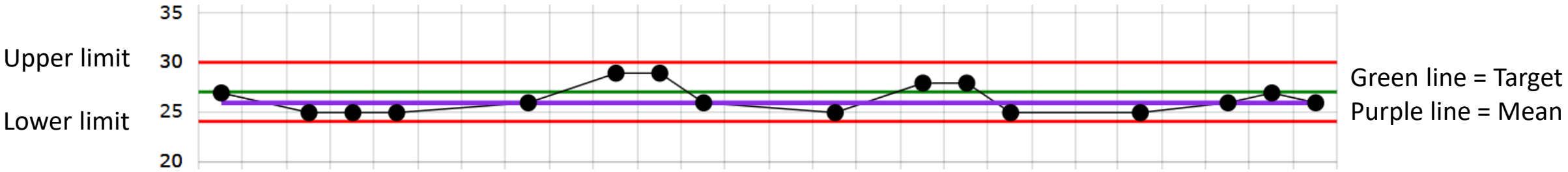
- Individual zone diameters out of range
- Evaluation of QC data over time
  - Comparison of mean values (≥10 tests) with EUCAST target values
  - Trends over time

NordicAST

Analysis of disk diffusion QC results

- Individual zone diameters out of range
- Evaluation of QC data over time
  - Comparison of mean values (≥10 tests) with EUCAST target values
  - Trends over time

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



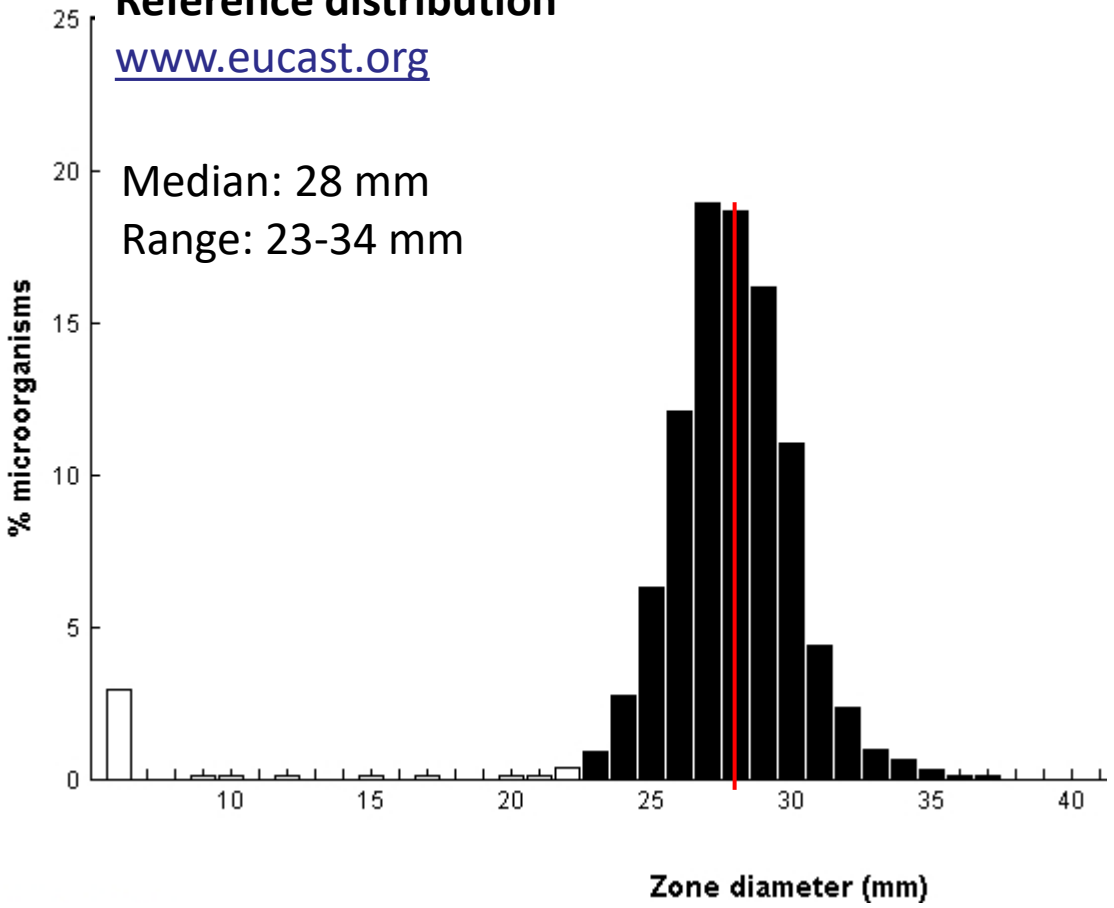
NordicAST

Comparison with reference distributions
Example *E. coli* and cefotaxime 5 µg

Reference distribution

www.eucast.org

Median: 28 mm
Range: 23-34 mm



Median of wild-type distribution should be at median for reference distribution ± 1 mm.

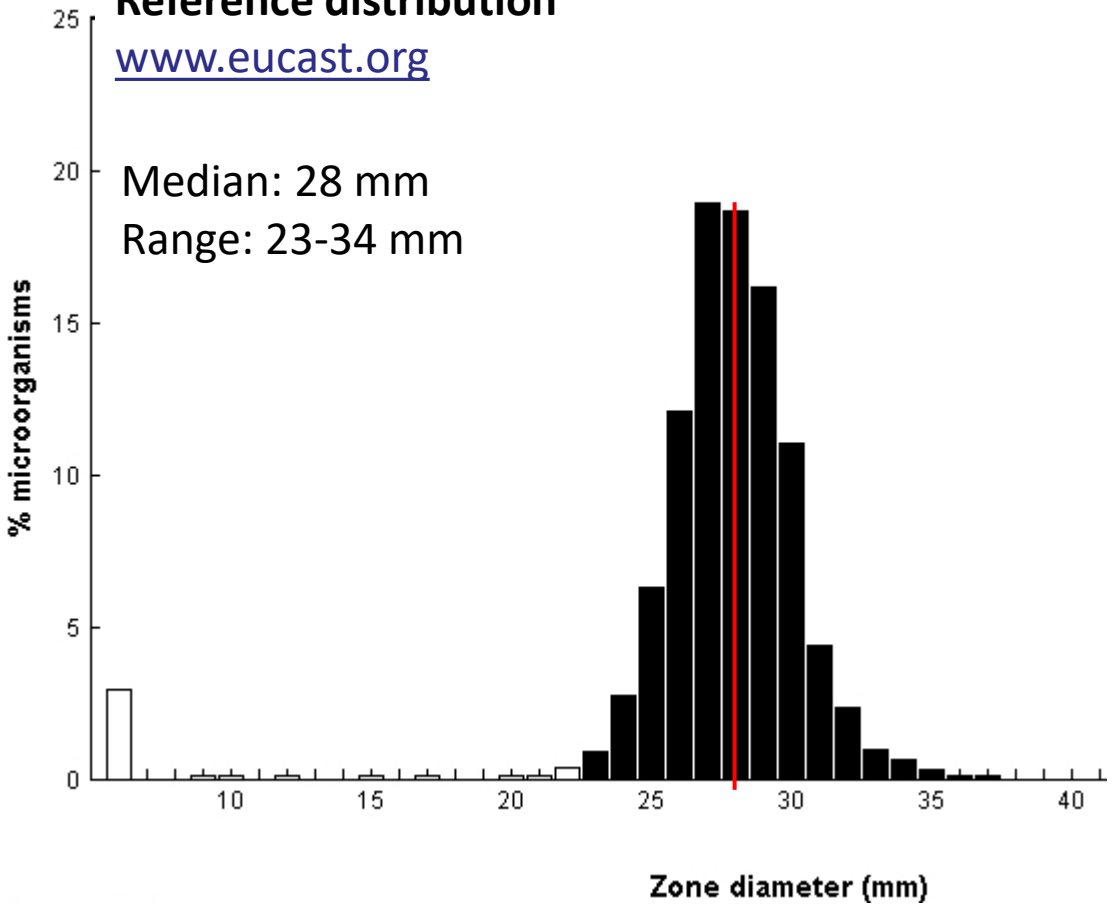
Comparison with reference distributions
Example *E. coli* and cefotaxime 5 µg

Reference distribution

www.eucast.org

Median: 28 mm

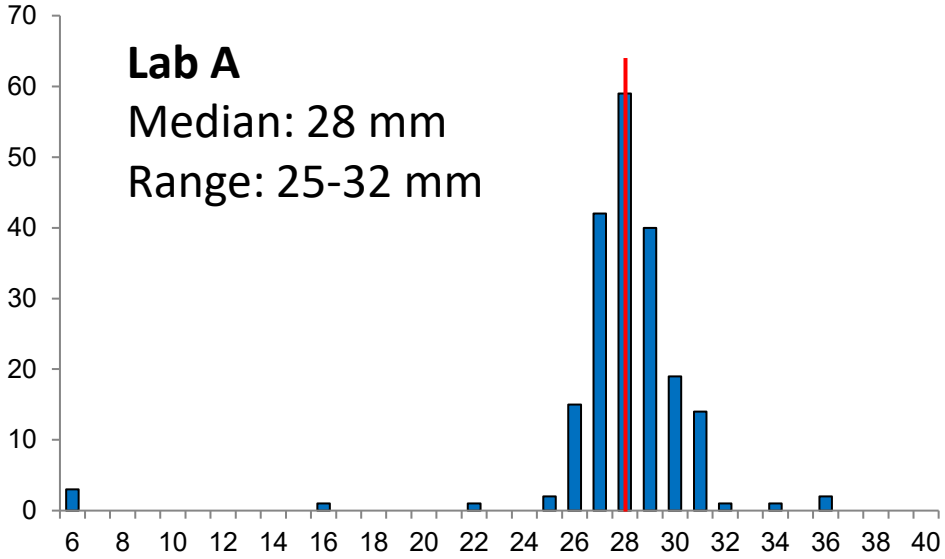
Range: 23-34 mm



Lab A

Median: 28 mm

Range: 25-32 mm



Median of wild-type distribution should be at median for reference distribution ± 1 mm.

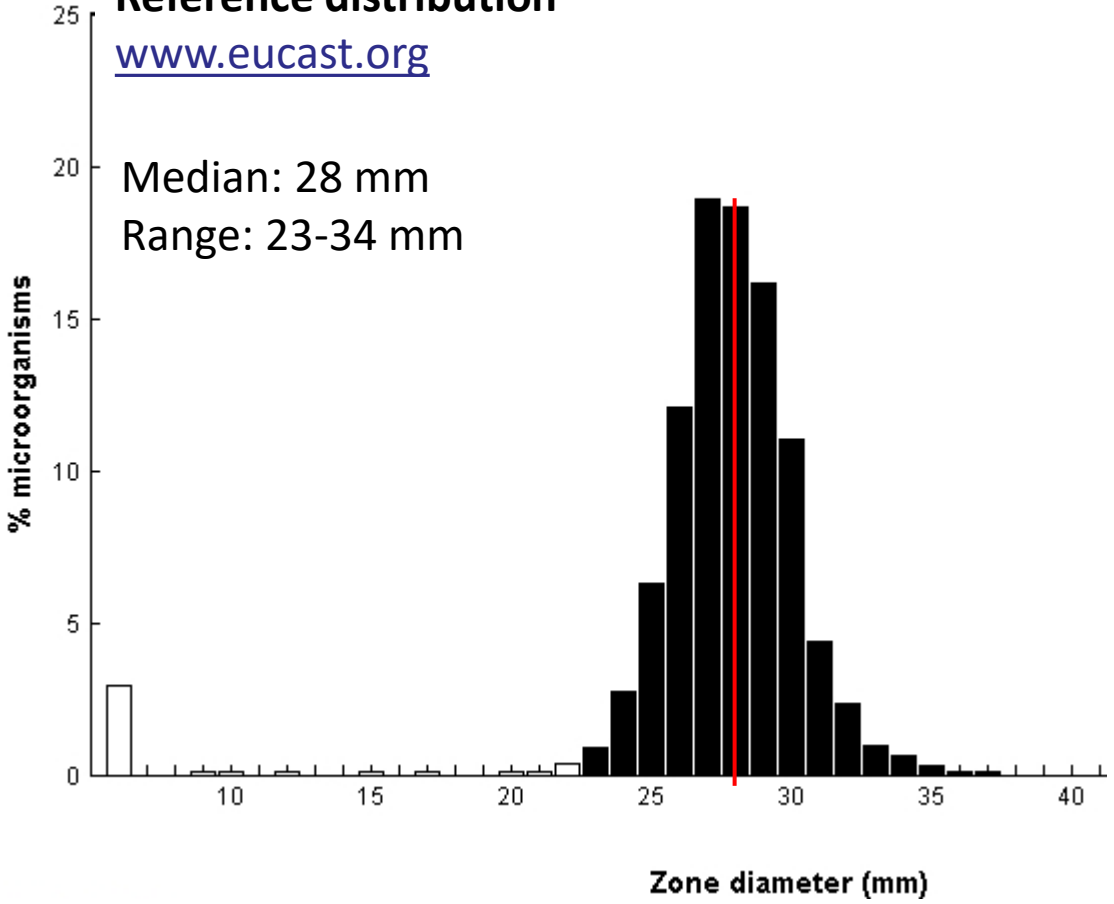
NordicAST

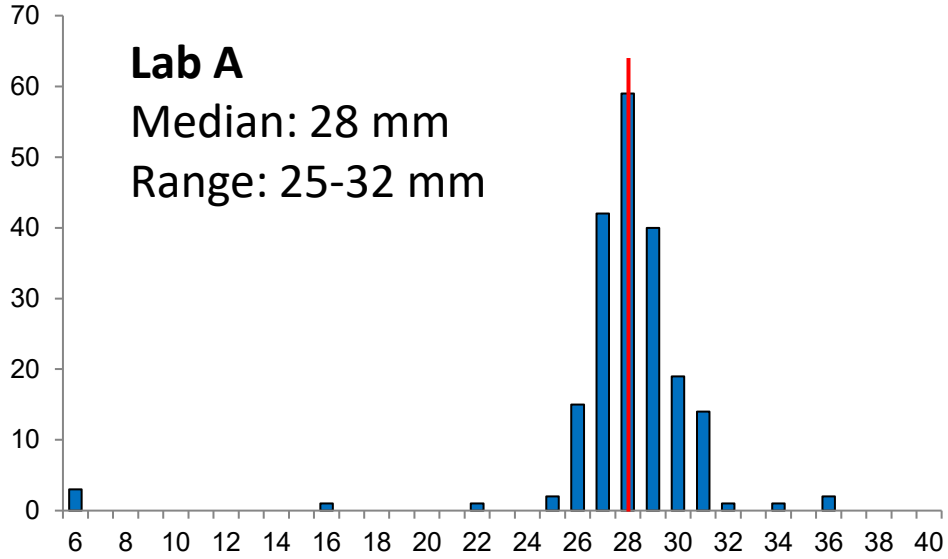
Comparison with reference distributions
Example *E. coli* and cefotaxime 5 µg

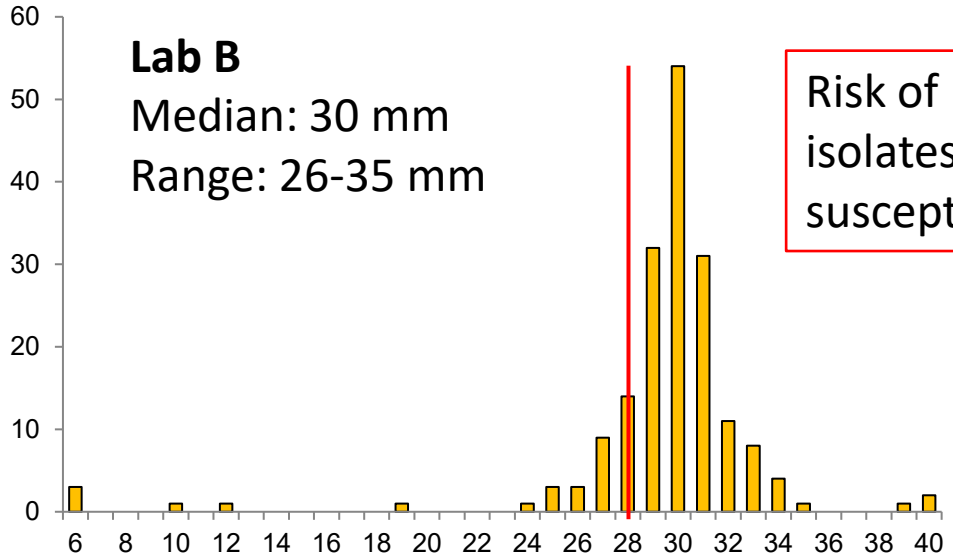
Reference distribution

www.eucast.org

Median: 28 mm
Range: 23-34 mm







Risk of reporting isolates as false susceptible!

Median of wild-type distribution should be at median for reference distribution ± 1 mm.

Potential sources of errors

- Disks
  - Poor quality, disks lost potency during storage and handling
- Media
  - Poor quality, medium components (cations, thymidine, pH), too humid agar plates, wrong agar depth
- Not adhering to methodology
  - 15-15-15 minute rule, inoculum, inoculation, incubation, reading of zone diameters
- Equipment
  - Incubators, densitometers
- QC strain
  - Old culture, contamination, mutation

NordicAST

Case 1

You have noted an increase in the number of mecillinam resistant isolates of *Enterobacterales* in a satellite lab

The lab is primarily manned by junior lab technicians and its primary function is to alleviate pressure on the main lab by performing AST on urine samples

Image generated using chatgpt

NordicAST

Case 1

You do a thorough inspection of the satellite lab

There have been no changes to plates or disks used - and the results have been consistent through several lots

There have been no changes to the used equipment

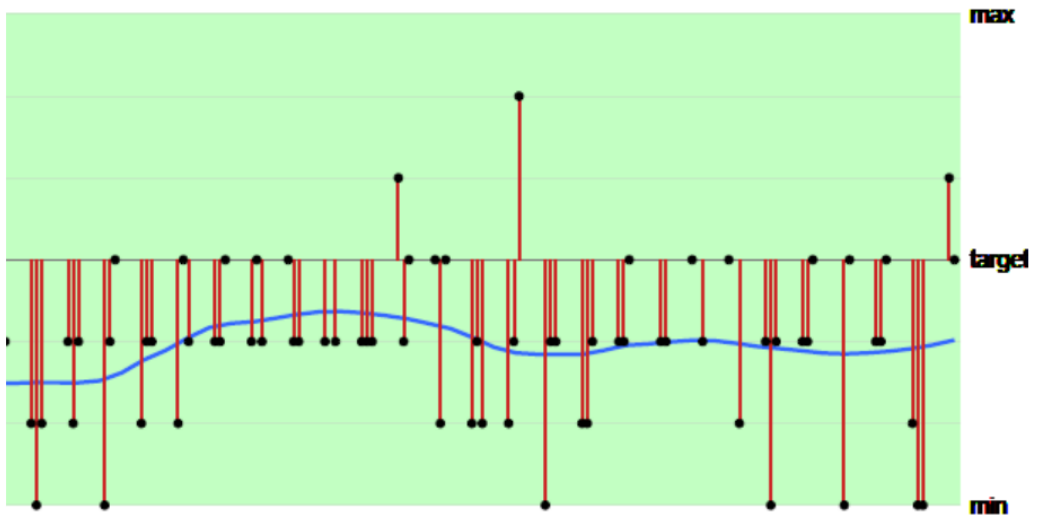
There have been no changes in methods

Results for *E. coli* ATCC 25922 are reportedly all within range…

Case 1

How do you interpret the results?

- Values are within range
- Values are mostly below target
- The underestimation is sustained



Run chart of mecillinam susceptibility on ATCC 25922 *E. coli*

The same pattern is not seen for other drugs examined

NordicAST

Case 1

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 1

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

Enterobacterales and mecillinam

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

NordicAST

Case 2

You have noticed an increase in metronidazole resistant/borderline resistant anaerobe isolates in your monthly AST report.

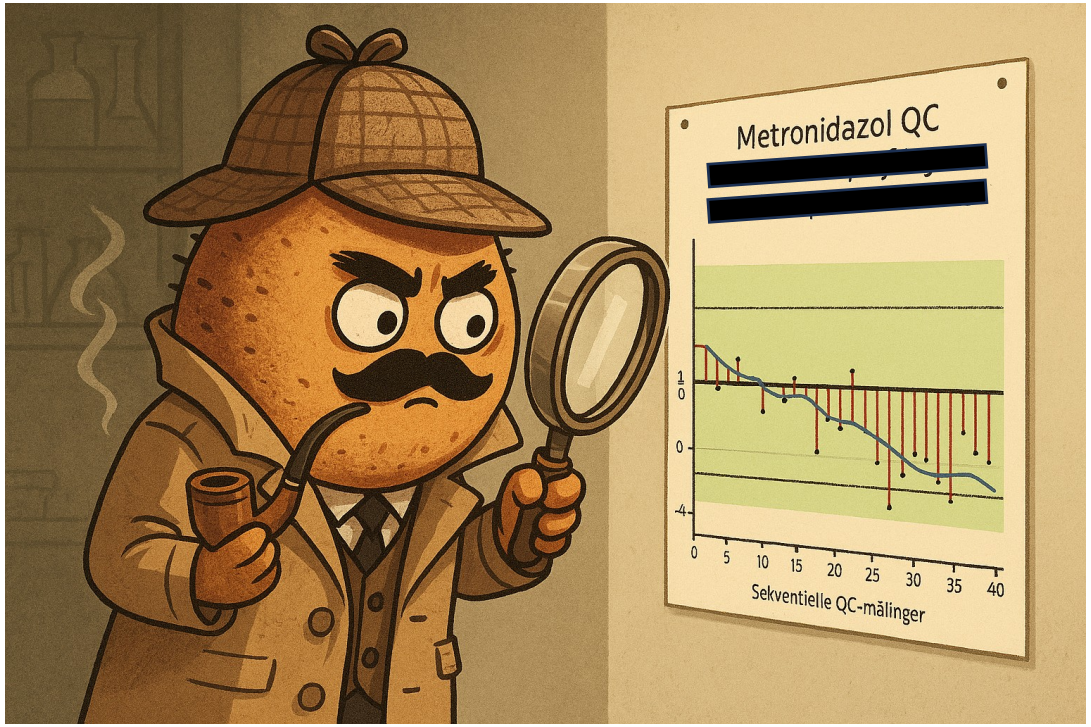


Illustration generated by Microsoft Copilot (AI)

NordicAST

Case 2

Anaerobic strains have in several cases shown poor, patchy growth with small colonies.

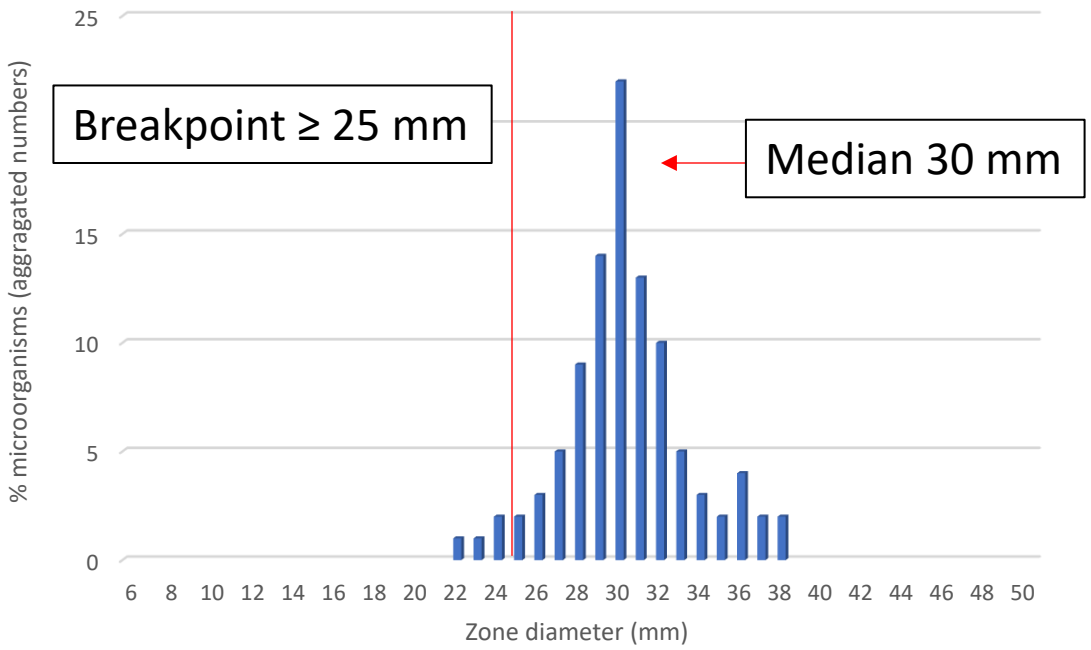
You have found 4 isolates of *B. fragilis t*hat has been reported resistant

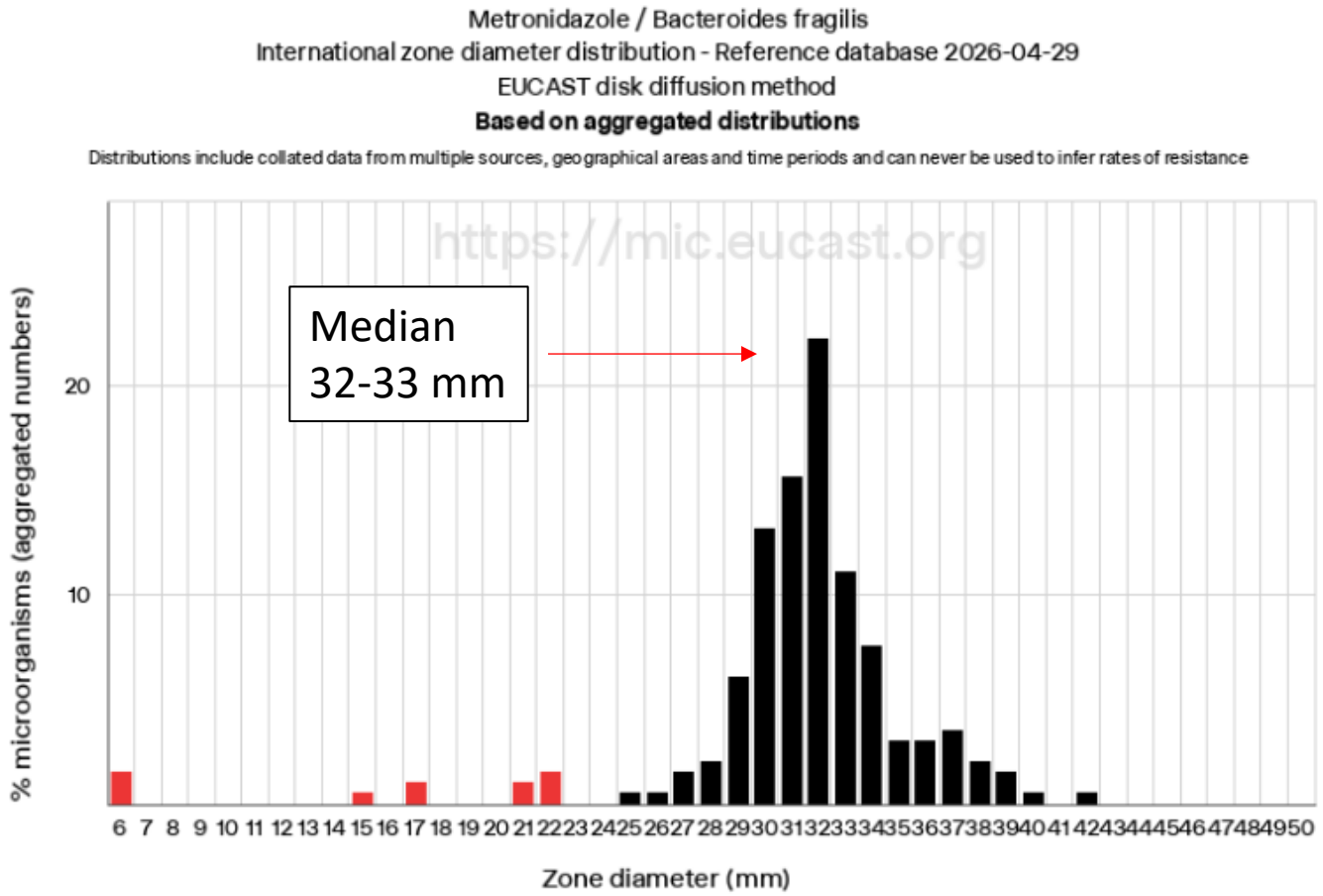
Subsequent analysis on reference labs have failed to show phenotypical resistance and WGS have shown no resistance genes

Illustration generated by Microsoft Copilot (AI)

Case 2

You decide to compare distributions:





Case 2

You suspect an old anaerobic incubator to be the cause. How do you determine if anaerobic growth conditions are met?

- Inspect the *Bacteroides fragilis* ATCC 25285 run chart
- Inspect the *Bacteroides thetaiotaomicron* ATCC 29741 run chart
- Inspect the zone diameter of *Clostridium perfringens* DSM 25589
- All of the above

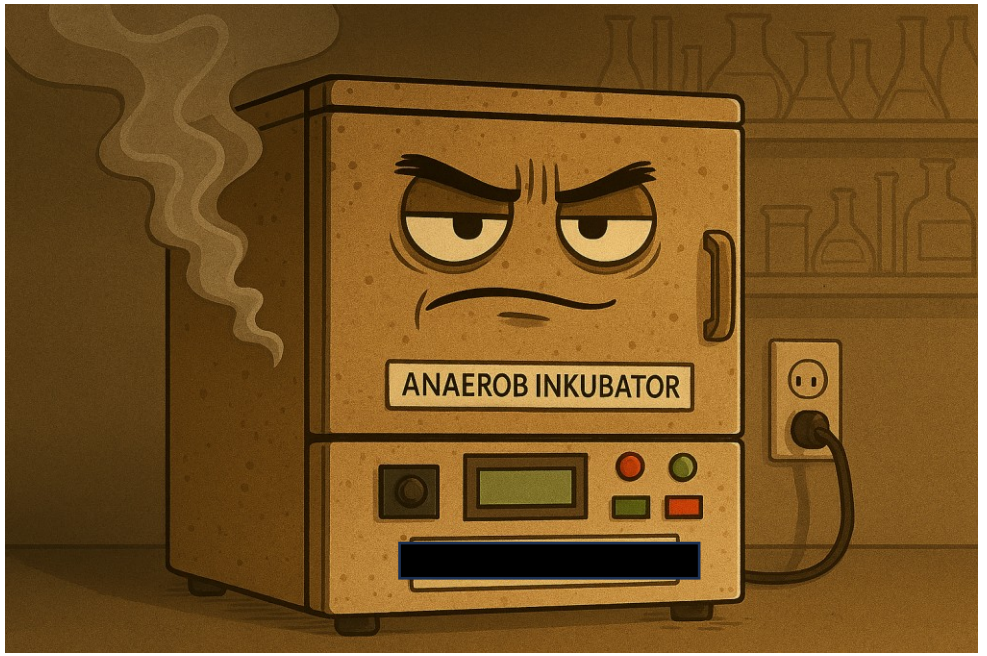


Illustration generated by Microsoft Copilot (AI)

Case 2

You suspect an old anaerobic incubator to be the cause. How do you determine if anaerobic growth conditions are met?

- Inspect the *Bacteroides fragilis* ATCC 25285 run chart
- Inspect the *Bacteroides thetaiotaomicron* ATCC 29741 run chart
- Inspect the zone diameter of *Clostridium perfringens* DSM 25589
- All of the above

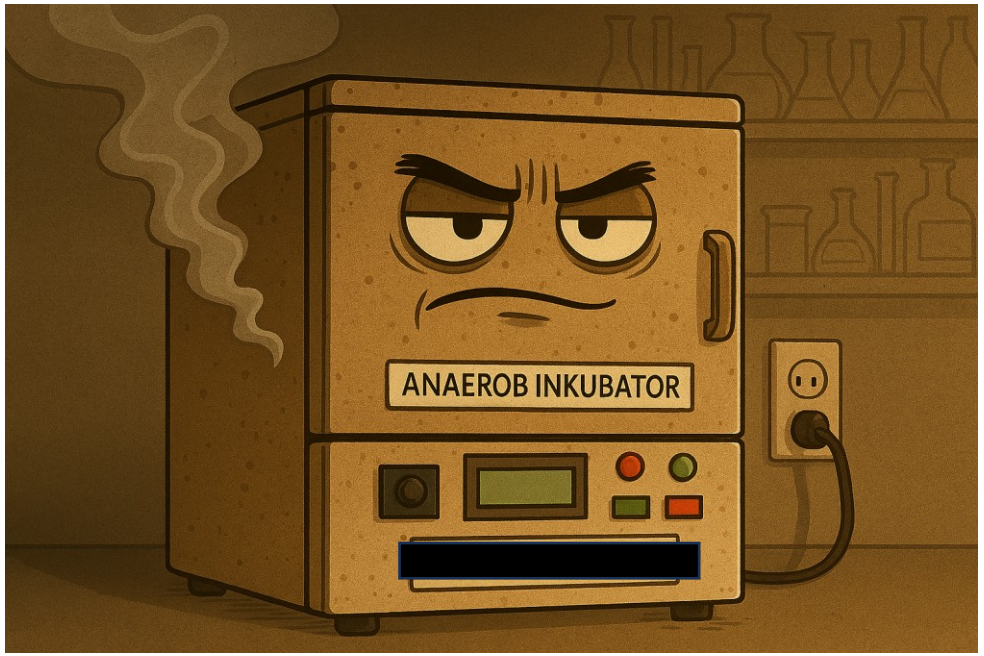
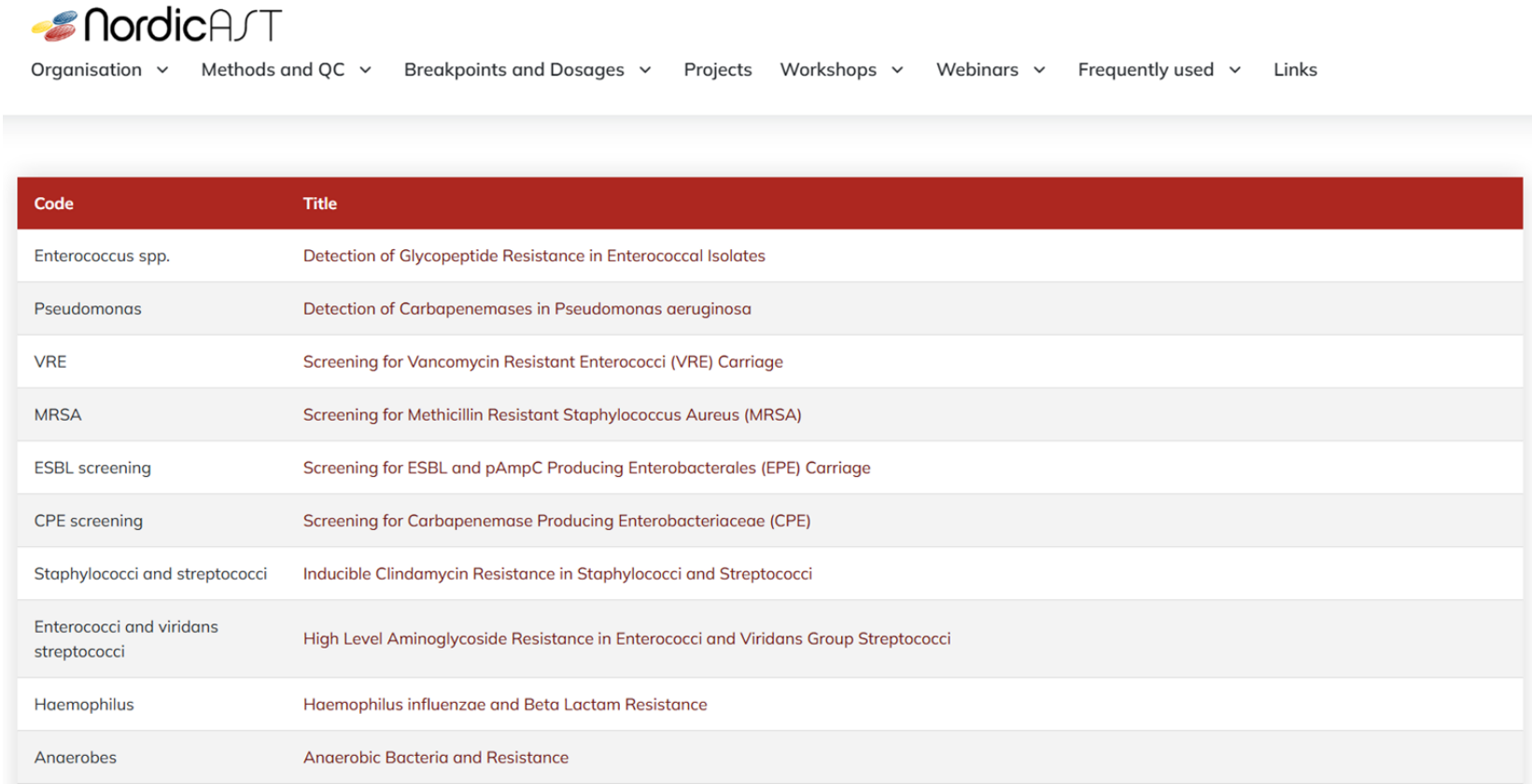


Illustration generated by Microsoft Copilot (AI)

Case 2



Code	Title
Enterococcus spp.	Detection of Glycopeptide Resistance in Enterococcal Isolates
Pseudomonas	Detection of Carbapenemases in Pseudomonas aeruginosa
VRE	Screening for Vancomycin Resistant Enterococci (VRE) Carriage
MRSA	Screening for Methicillin Resistant Staphylococcus Aureus (MRSA)
ESBL screening	Screening for ESBL and pAmpC Producing Enterobacterales (EPE) Carriage
CPE screening	Screening for Carbapenemase Producing Enterobacteriaceae (CPE)
Staphylococci and streptococci	Inducible Clindamycin Resistance in Staphylococci and Streptococci
Enterococci and viridans streptococci	High Level Aminoglycoside Resistance in Enterococci and Viridans Group Streptococci
Haemophilus	Haemophilus influenzae and Beta Lactam Resistance
Anaerobes	Anaerobic Bacteria and Resistance

NordicAST anaerobe method document:
When metronidazole resistance is observed in the laboratory, it can be due to pseudo-resistance caused by insufficient anaerobic conditions (small amounts of oxygen in the atmosphere). EUCAST recommends
that the anaerobic environment is tested with the aerotolerant Clostridium perfringens DSM 25589 strain and a metronidazole 5 µg disk.

Case 2

EUCAST European Committee on Antimicrobial Susceptibility Testing

European Committee on Antimicrobial Susceptibility Testing

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

Version 16.0, valid from 2026-01-01

Control of the anaerobic environment when performing antimicrobial susceptibility testing for anaerobic bacteria using EUCAST methods

Test according to EUCAST disk diffusion methodology for anaerobic bacteria (FAA-HB medium). See EUCAST Breakpoint Tables for short description of disk diffusion methodology.

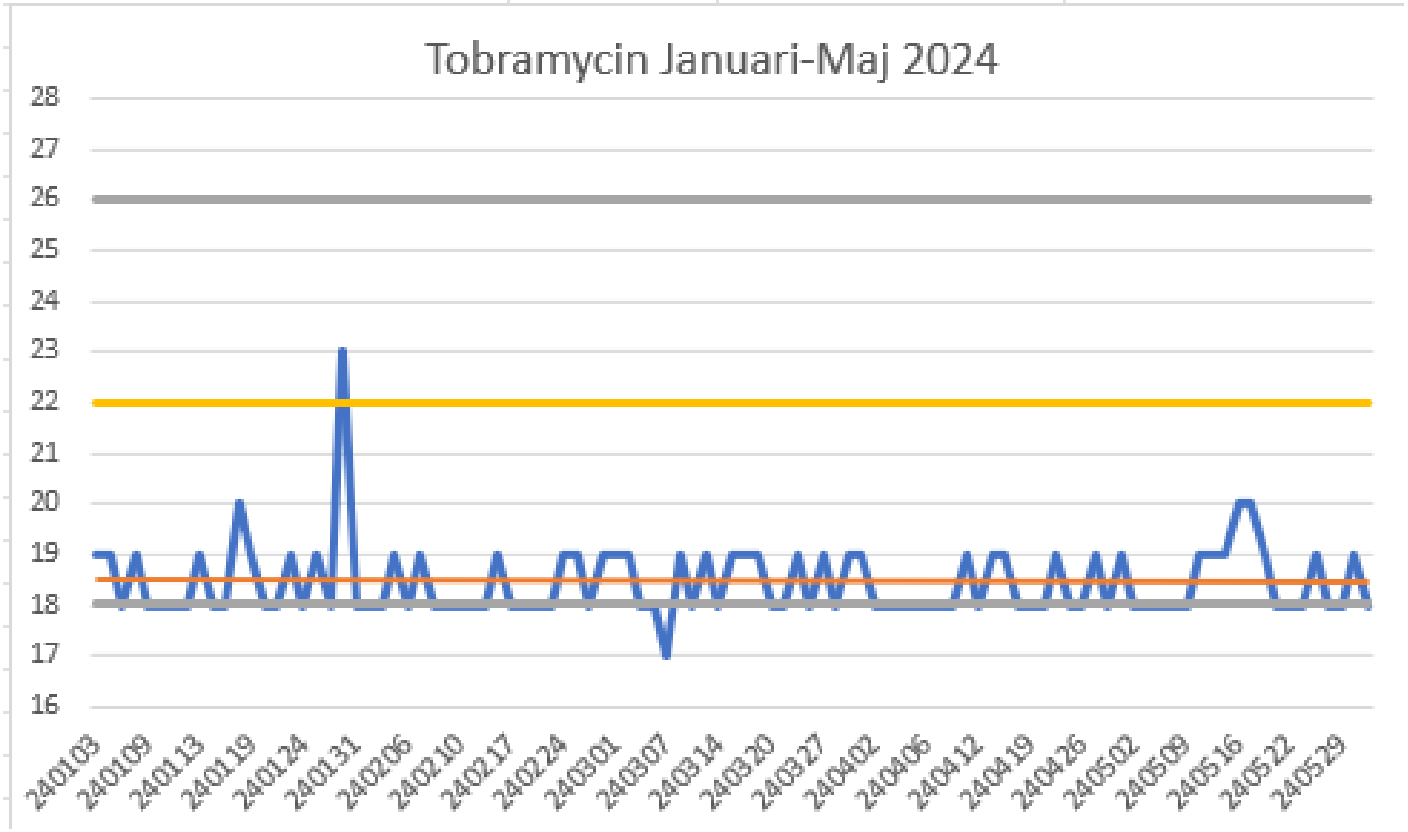
Clostridium perfringens **DSM 25589**
(NCTC 14679, CCUG 75076)

Antimicrobial agent	Disk content (µg)	Cut-off value[1] (mm)
Metronidazole	5	*<25*

1. A zone diameter of <25 mm indicates an insufficient anaerobicity. This may affect growth and susceptibility test results of anaerobic bacteria.

NordicAST

Case 3: Zones below range for tobramycin 10 µg

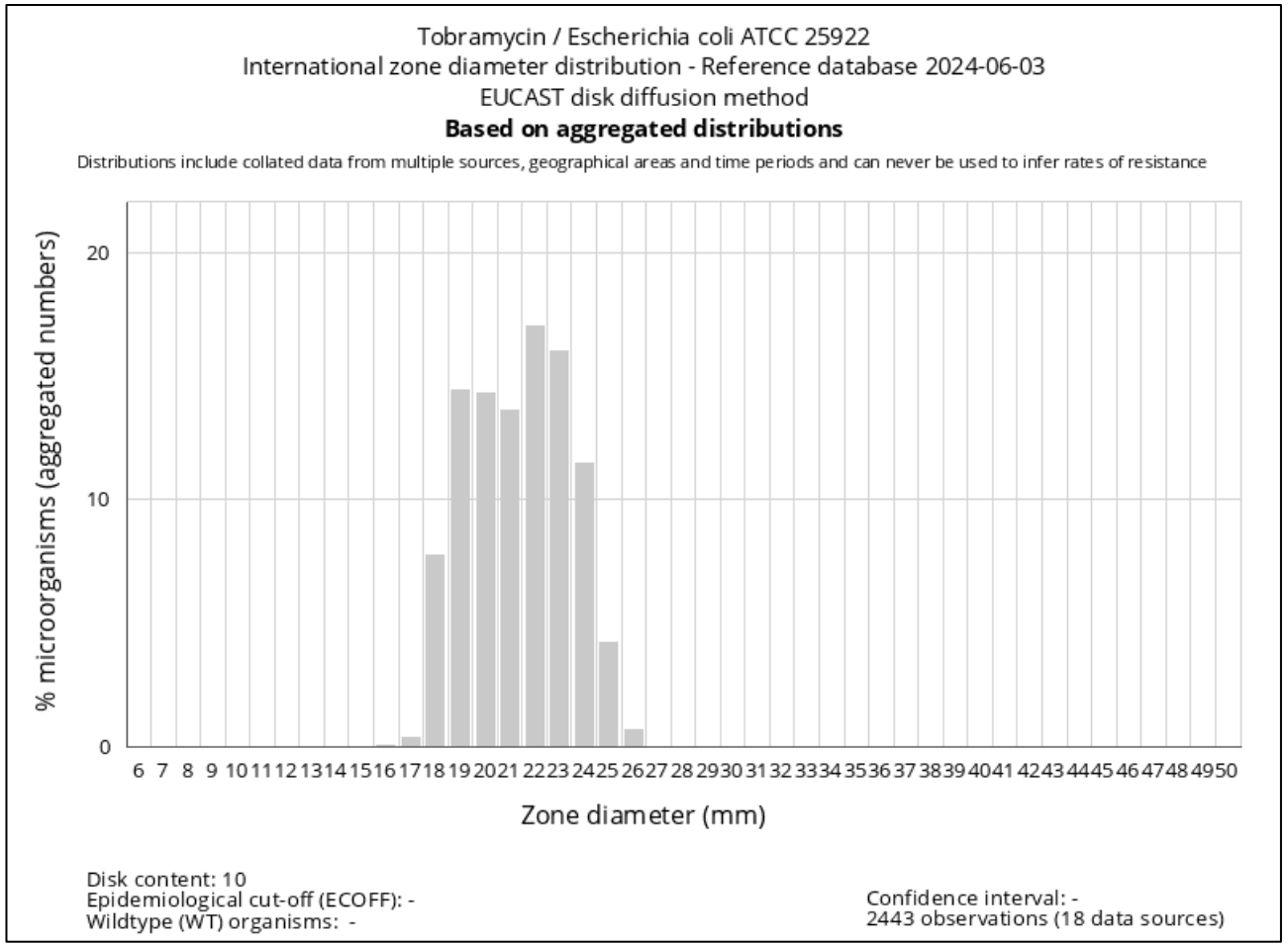


- *E. coli* CCUG17620/ATCC25922
- Local QC data close to the lower limit

Case 3: Zones below range for tobramycin 10 µg

Target: 22 mm
Local median: 18 mm
Discrepancy -4 mm

What could be possible reasons?



Case 3: Zones below range for tobramycin 10 µg

Most likely reasons (several options possible)?

1. Too low agar depth
2. Too high agar depth
3. Poor disk quality
4. Disks lost potency over time
5. MH agar

Case 3: Zones below range for tobramycin 10 µg

Most likely reasons (several options possible)?

1. Too low agar depth
2. Too high agar depth
3. Poor disk quality
4. Disks lost potency over time
5. MH agar

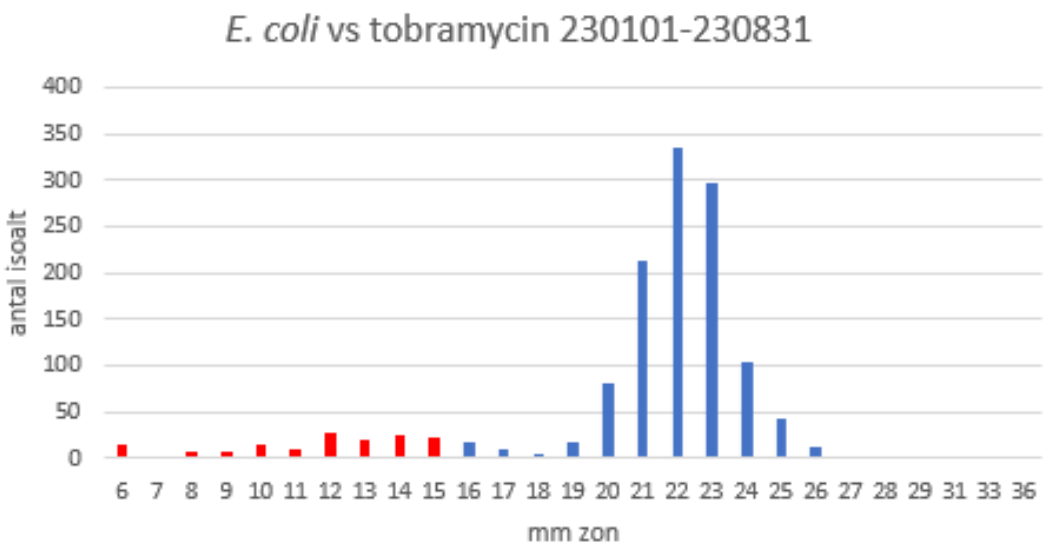
Case 3: Investigation

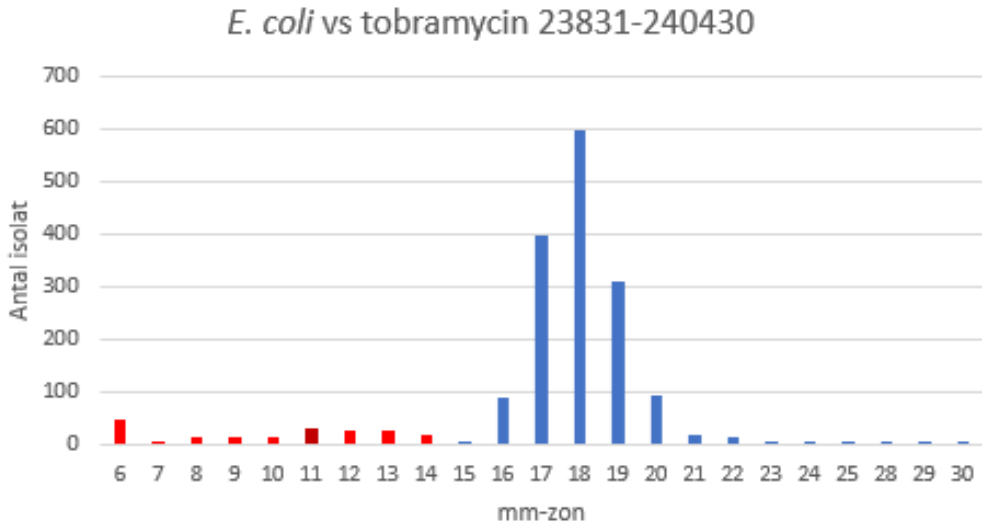
- Disks: storage, handling, antibiotic content – no problem
- Media: *change of agar distributor in fall 2023 to Oxoid*
- No problems adhering to methodology

  Zones with clear edges

- Equipment -
- QC strain -

Case 3: Clinical consequence?





Resistance rate before change of agar: 6,3 %
Resistance rate after change of agar: 8,45 % +2%

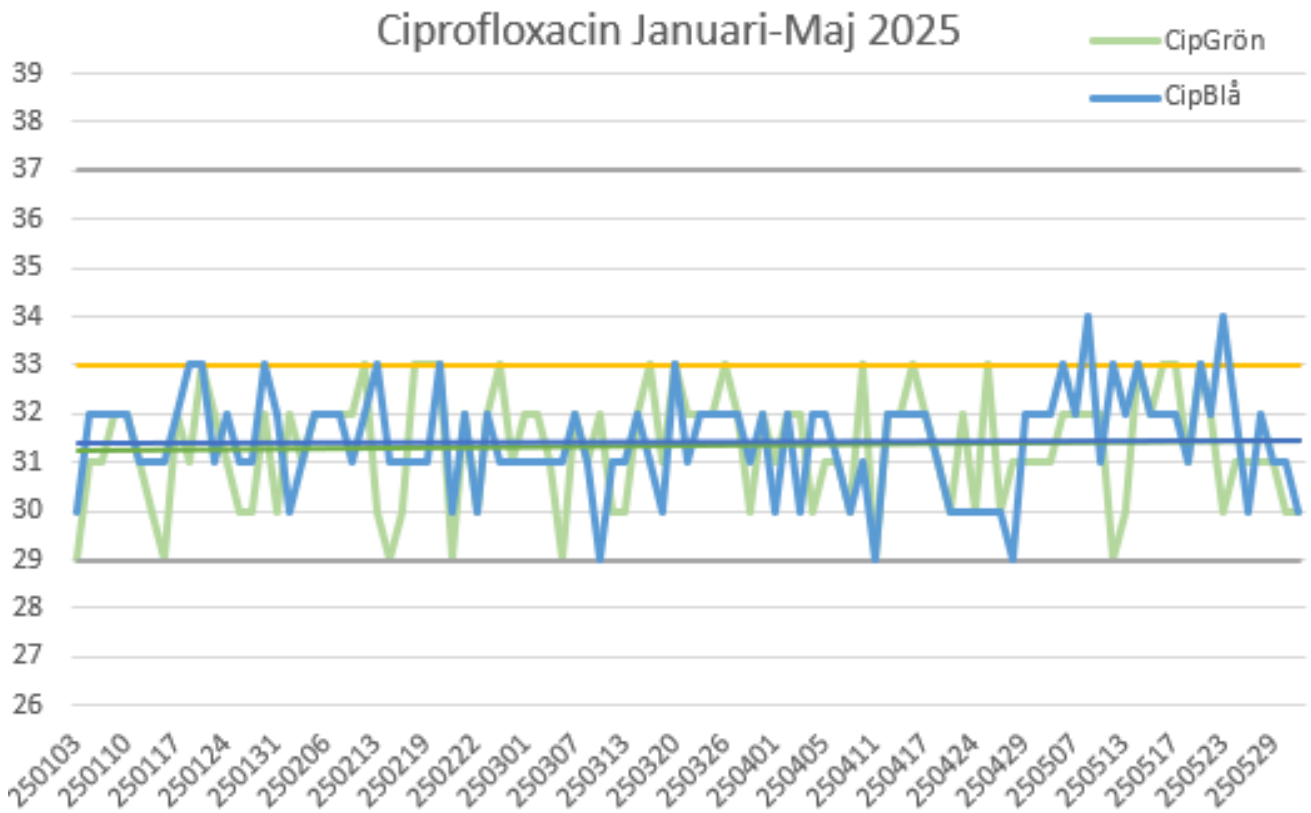
Risk assessment Agent not commonly used in Sweden, if so, mostly as single dose empirically and in combination with a betalactam antibiotic. ATU confusing for clinicians. Resistance rate not that much increased. Report as tested.

Case 4: *E. coli* and ciprofloxacin 5 µg

Local QC data for ciprofloxacin and *E. coli* CCUG17620/ATCC25922

What do you think about it?

- All results are within range, it's ok
- Results within range but consistently low, further investigation needed

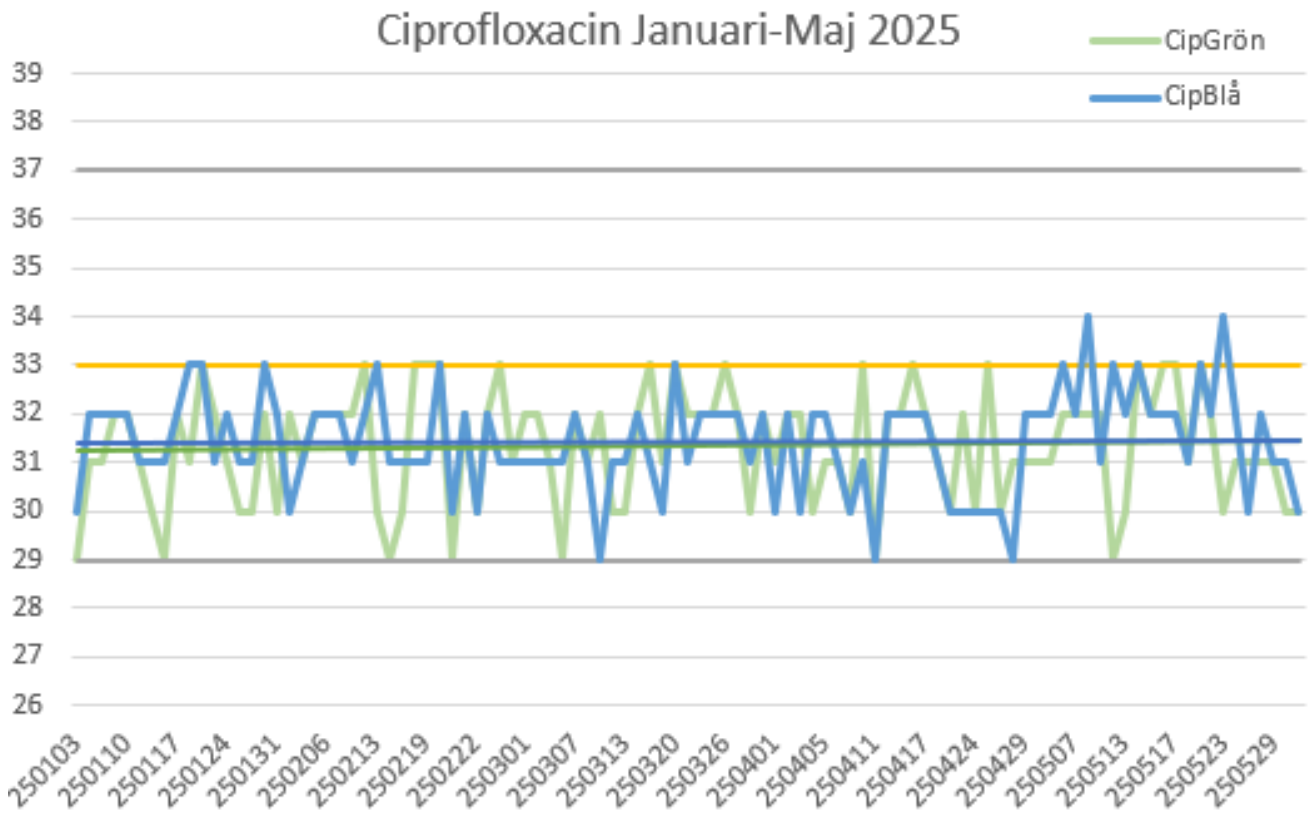


Case 4: *E. coli* and ciprofloxacin 5 µg

Local QC data for ciprofloxacin and *E. coli* CCUG17620/ATCC25922

What do you think about it?

- All results are within range, it's ok
- Results within range but consistently low, further investigation needed

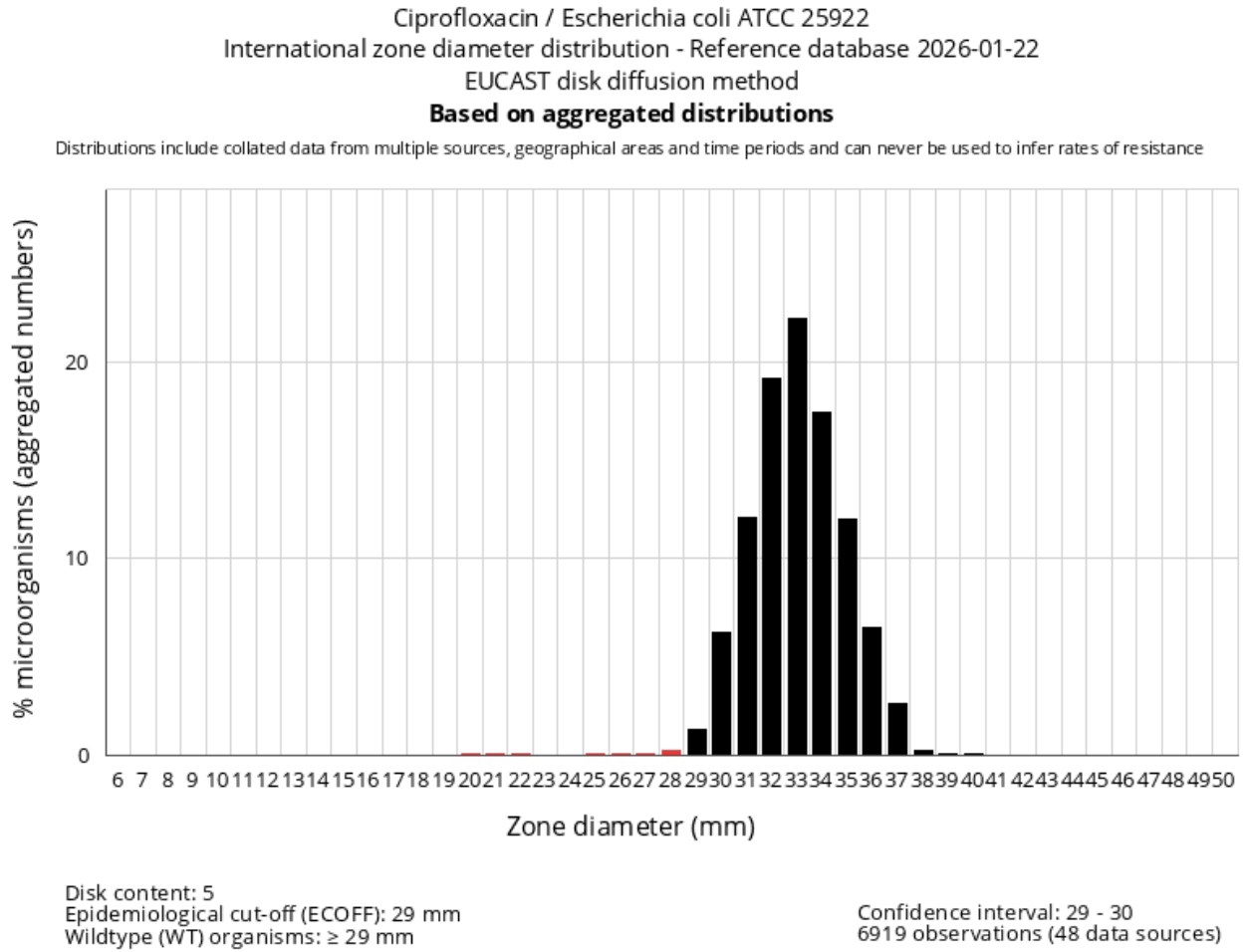


Case 4

You start with checking local wildtype distribution compared to EUCAST reference

Median target EUCAST: 33 mm
Local median: 31 mm
Discrepancy -2 mm

Median of wild-type distribution should be at median for reference distribution ± 1 mm.



What could be possible reasons?

***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]
Ciprofloxacin	0.008	0.004-0.016	5	***33***	***29-37***

Case 4 Zones below range for ciprofloxacin 5 µg

Most likely reasons (several options possible)?

1. Too humid agar plates
2. Too low agar depth
3. Reading difficulties
4. Too high agar depth
5. Poor disk quality
6. Disks lost potency over time

NordicAST

Case 4 Zones below range for ciprofloxacin 5 µg

Most likely reasons (several options possible)?

1. Too humid agar plates
2. Too low agar depth
3. Reading difficulties
4. Too high agar depth
5. Poor disk quality
6. Disks lost potency over time

NordicAST

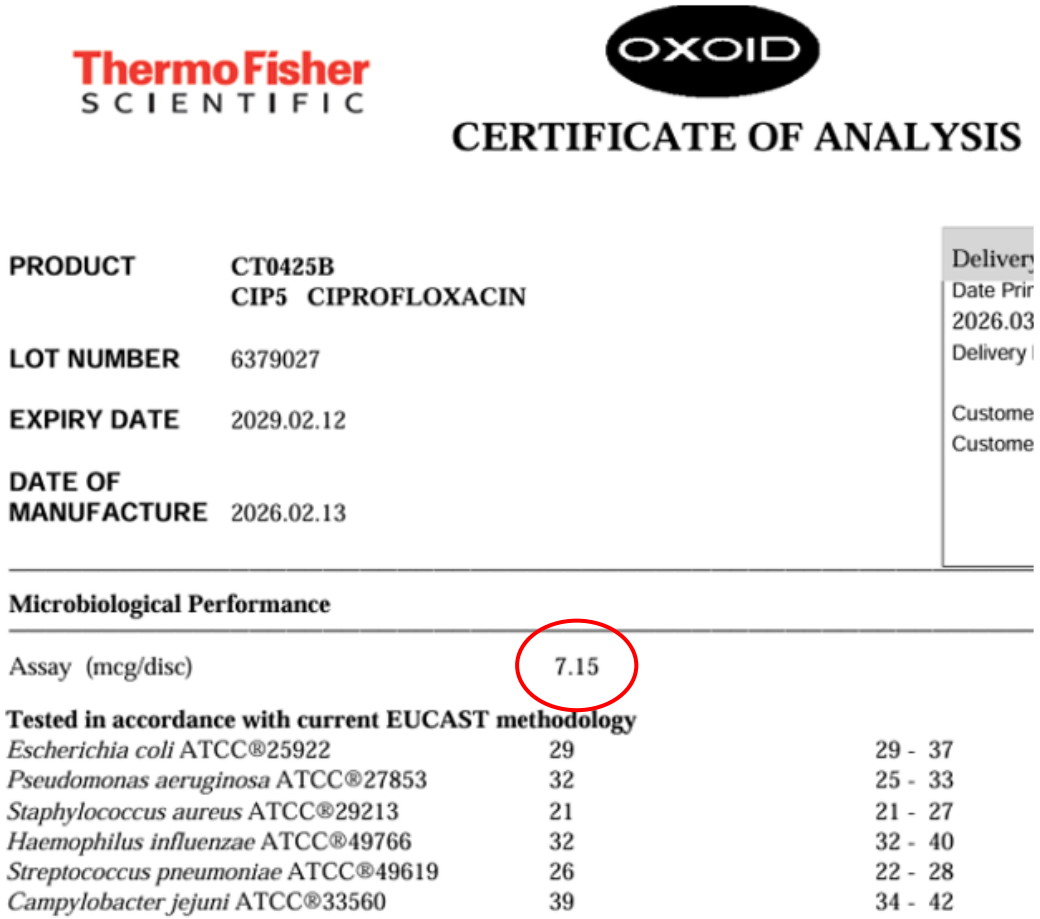
Case 4 Zones below range for ciprofloxacin 5 µg

Disks

- Storage: according to manufacturer
- Expiry date: not exceeded
- Tubes had not been opened for to long
- Antibiotic content in the discs of actual badge was a bit low

Media

- MH-agar from Oxoid produced locally
- Room temperature prior to adding antibiotic disks
- Agar depth measured with each new batch



ThermoFisher SCIENTIFIC

OXOID

CERTIFICATE OF ANALYSIS

PRODUCT	CT0425B CIP5 CIPROFLOXACIN
LOT NUMBER	6379027
EXPIRY DATE	2029.02.12
DATE OF MANUFACTURE	2026.02.13

Delivery
Date Prir
2026.03
Delivery
Custome
Custome

Microbiological Performance

Assay (mcg/disc)	7.15	
Tested in accordance with current EUCAST methodology		
Escherichia coli ATCC®25922	29	29 - 37
Pseudomonas aeruginosa ATCC®27853	32	25 - 33
Staphylococcus aureus ATCC®29213	21	21 - 27
Haemophilus influenzae ATCC®49766	32	32 - 40
Streptococcus pneumoniae ATCC®49619	26	22 - 28
Campylobacter jejuni ATCC®33560	39	34 - 42

Case 4 Zones below range for ciprofloxacin 5 µg

Testing procedure

Plates stored in roomtemperature prior to AST

Plate rotator used to evenly streak the plates

15-15-15 minute rule applied

How about the reading of zones?

Fuzzy zone edges – harmonization exercise, results good

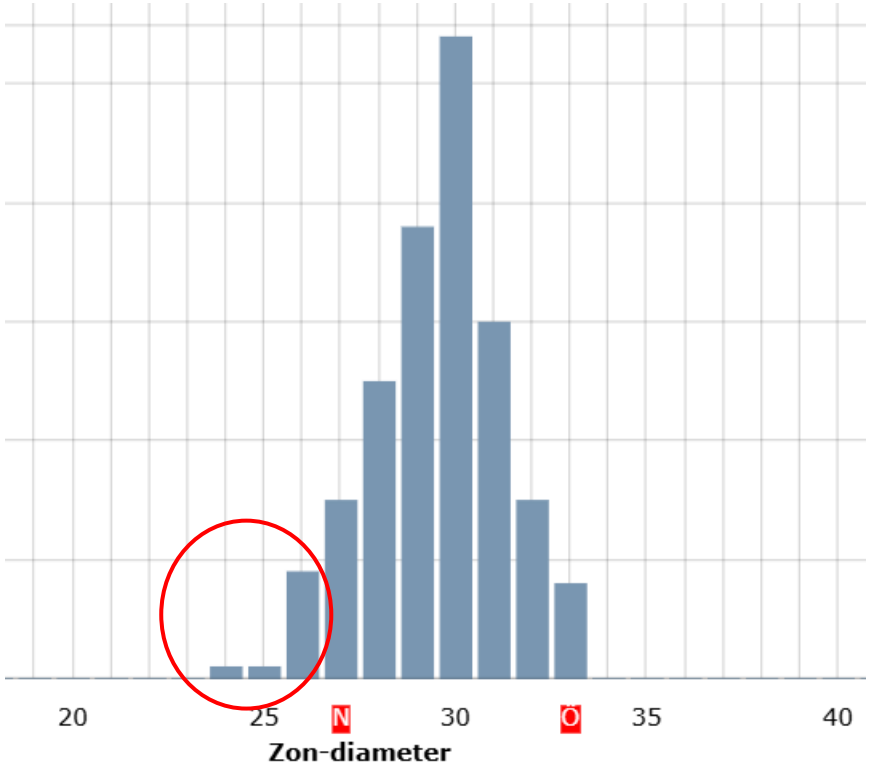
Case 4 Risk assessment

- Antibiotic concentration in discs slightly lower than target, reproducibility of manufacturers results
- Investigation leads to no other explanation
- Clinical consequence?
  - CIP is an important agent on UTI and other indications
  - Borderline isolates ends up in ATU. In our lab answered with a comment (SIR-result cannot be determined, if treatment failure, consider changing therapy)
- Change of MH-agar planned during 2026
- No intervention, active surveillance

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Ciprofloxacin, *Salmonella* spp.	0.06	0.06			Note	Note	
Ciprofloxacin (indications other than meningitis)	0.25	0.5	0.5	5	25	22	22-24
Ciprofloxacin (meningitis)	0.125	0.125			Note	Note	

Case 5: Zones below range for meropenem 10 µg

***P. aeruginosa* ATCC 27853 and meropenem 10 µg (2017)**



Internal QC 2017

- *P. aeruginosa* ATCC 27853
  - 11/196 (5.6%) zones below range
- *H. influenzae* ATCC 49766
  - 10/201 (5.0%) results below range
- *E. coli* ATCC 25922
  - No zones below range

NordicAST

Case 5: Zones below range for meropenem 10 µg

Most likely reasons (several options possible)?

1. Too humid agar plates
2. Too low agar depth
3. Too high agar depth
4. Poor disk quality
5. Disks lost potency

Case 5: Zones below range for meropenem 10 µg

Most likely reasons (several options possible)?

1. Too humid agar plates
2. Too low agar depth
3. Too high agar depth
4. Poor disk quality
5. Disks lost potency

NordicAST

Case 5: Zones below range for meropenem 10 µg

EUCAST disk diffusion manual section 5 (Antimicrobial disks)

- Allow disks to reach room temperature before opening cartridges or containers used for disk storage. This is to prevent condensation, leading to rapid deterioration of some agents.
- Loss of potency of antimicrobial agents in disks results in reduced inhibition zone diameters and is a common source of error. Store disks in use in sealed containers with a moisture-indicating desiccant and protected from light.
- Store disk stocks according the manufacturers' instructions. Some agents are more labile than others (e.g. amoxicillin-clavulanic acid, cefaclor and carbapenems) and specific recommendations may be available from the manufacturers.
- **Perform frequent quality control of working supplies to control that the antimicrobial disks have not lost potency during storage.**

NordicAST

Case 5: Zones below range for meropenem 10 µg

Customer Notification

We have made a change in the storage period for Oxoid Antimicrobial Susceptibility Testing Discs **Meropenem MEM 10 CT0774B, CT1548S, CT1885S, CT1914S, CT1918S and CT1945S**.

The stipulated usage, one opened, has changed from **7 days to 3 days**. All new batches will be labeled accordingly.

Case 5: Zones below range for meropenem 10 µg

Customer Notification

We have made a change in the storage period for Oxoid Antimicrobial Susceptibility Testing Discs **Meropenem MEM 10 CT0774B, CT1548S, CT1885S, CT1914S, CT1918S and CT1945S**.

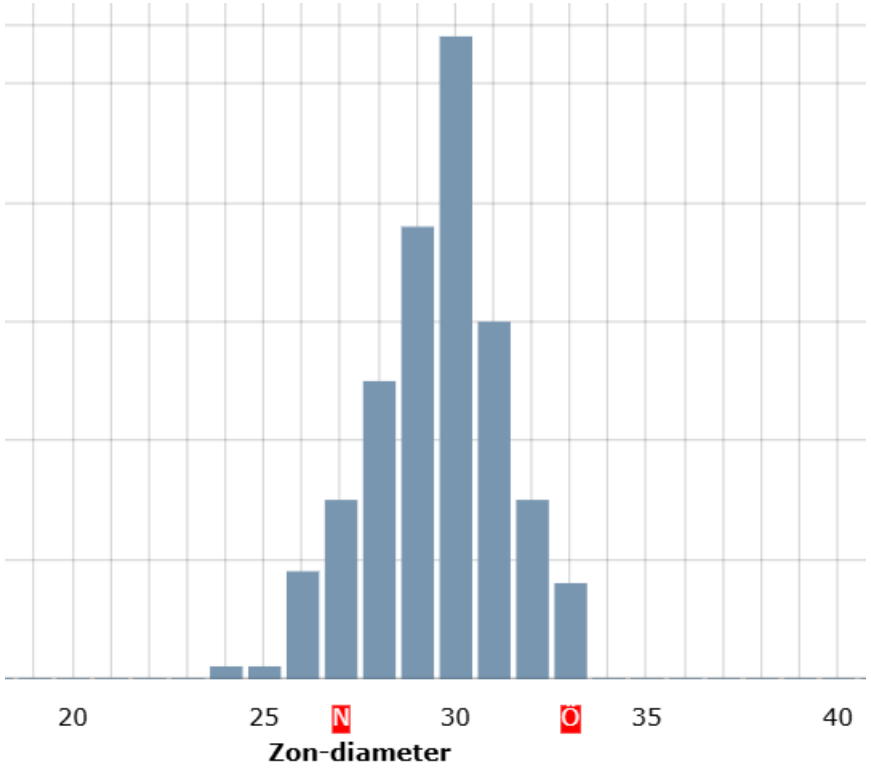
The stipulated usage, one opened, has changed from **7 days to 3 days**. All new batches will be labeled accordingly.

- Updated local routines for amoxicillin-clavulanic acid and carbapenem disks (ertapenem, imipenem and meropenem)
  - Change to new cartridge each Monday
  - Local warnings for meropenem R
    - *H. influenzae*, Enterobacterales, anaerobic bacteria

NordicAST

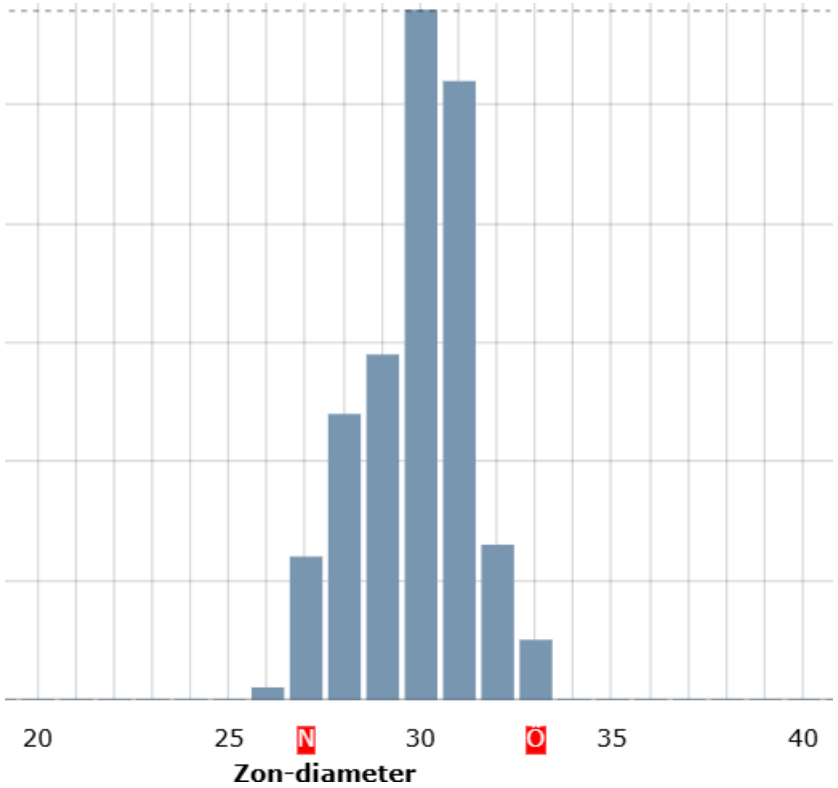
Case 5: Zones below range for meropenem 10 µg

P. aeruginosa ATCC 27853 and meropenem 10 µg (2017)



11/196 values out of range (low)
Median 30 mm (target 30 mm)

P. aeruginosa ATCC 27853 and meropenem 10 µg (2018)



1/194 values out of range (low)
Median 30 mm (target 30 mm)

NordicAST

Summary of internal quality control

- Perform frequent QC to detect problems with the materials and procedures used
  - Disk diffusion: daily or at least four times a week
- Analyse QC data against EUCAST published ranges and target values
  - Aim for the target!
- Compare local data with EUCAST reference distributions to investigate if there are systematic deviations
- When materials don’t perform according to the standards:
  - The EDL can help with troubleshooting
  - Contact the manufacturers and complain!

NordicAST


NordicAST