



Frequently Asked Questions and troubleshooting in AST

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EUCAST

EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Frequently Asked Questions (FAQ)

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The European Committee on Antimicrobial Susceptibility Testing – EUCAST

[Frequently Asked Questions \(FAQ\)](#)

Frequently Asked Questions (FAQ)

The EUCAST secretariat receives many questions on subjects ranging from how we determine breakpoints, the MIC-distribution website, and the new disk diffusion methodology. We try to answer each question individually but also publish frequently asked questions and answers in a classical FAQ document. The file is updated at regular intervals.

[Frequently Asked Questions](#) - valid from 2018-02-18 ([update](#) 11 April, 2018; see question 8 on temocillin)

Previous versions of FAQ:

[Frequently Asked Questions](#) - valid from 2016-03-29

[Frequently Asked Questions](#) - valid 2015-03-23 - 2016-03-29

[Frequently Asked Questions](#) - valid 2014-02-26 - 2015-03-23

[Frequently Asked Questions](#) - valid 2013-04-24 - 2014-02-26



Questions? Please contact
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EUCAST Frequently Asked Questions

Click on question
to read answer

1. EUCAST Disk Diffusion Test - Medium

- [1. Which manufacturer of Mueller-Hinton agar does EUCAST recommend?](#)
- [2. What is the difference between Mueller-Hinton agar and Mueller-Hinton II agar?](#)
- [3. Do we need to quality control each new batch of Mueller-Hinton agar?](#)
- [4. Can we use sheep blood instead of horse blood for the MH-F medium?](#)
- [5. Which \$\beta\$ -NAD should we use?](#)
- [6. Can MH-F be used as medium for gradient tests?](#)
- [7. It is stated in the EUCAST disk diffusion manual that the agar depth should be \$4.0 \pm 0.5\$ mm. Does this mean that it is acceptable to use plates with an agar depth of 3.5-3.7 mm?](#)
- [8. We have problems with haze within the inhibition zones and growth of colonies close to the zone edge, particularly on the MH-F media. Can we do something to improve this?](#)

1. EUCAST disk diffusion test - Medium

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1 Which manufacturer of Mueller-Hinton agar does EUCAST recommend?



EUCAST does not recommend a particular manufacturer of Mueller-Hinton agar. We have tested batches of Mueller-Hinton agar from four manufacturers (BBL, Oxoid, bioMérieux and Bio-Rad) repeatedly and have evaluated other media occasionally. We have also tested batches of pre-poured commercial MH-F (Mueller-Hinton Fastidious organisms; which is Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L β -NAD) from the manufacturers mentioned above. Irrespective of the manufacturer used, each user should ensure that batches of media meet the internal quality control ranges published by EUCAST. These ranges have been checked with media from several manufacturers. For users of pre-poured commercial plates, it should be noted that the plate manufacturer not necessarily is the same as the Mueller-Hinton powder manufacturer.

Question no 1

In the Breakpoint Tables v. 8.0 EUCAST recommends broth microdilution for MIC determination.

Does this mean that we can no longer use automated systems or gradient tests?

Reference methodology for MIC determination

ISO standard 20776-1, 2006

Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —

Part 1:

Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

BMD of fastidious organisms

- MH-F broth
 - Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L β -NAD

AST of bacteria

Organization

EUCAST News

Clinical breakpoints

Expert rules and intrinsic resistance

Resistance mechanisms

Guidance documents

Consultations

MIC and zone distributions and ECOFFs

AST of bacteria

Media preparation

MIC determination



... Media preparation

Preparation of MH plates and broth

EUCAST antimicrobial susceptibility testing is based on MH agar and MH broth, without supplements for non-fastidious organisms and with supplements for fastidious organisms (Streptococci, Haemophilus influenzae, Campylobacter and others). The plate and broth for fastidious organisms are named MH-F plates and MH-F broth.

Preparation of agar plates and broth for EUCAST AST v 5.0 (9 January, 2017)

EDL validation of MH-F broth

S. pneumoniae ATCC 49619: 99.4% within range compared with ISO (MH-LHB)

Antimicrobial agent	MIC panel	No of tests within concentration interval	No of tests with QC range	No of tests out of range	Comment
Amoxicillin	Streptococcus	68	68	0	
Ampicillin	Streptococcus	68	68	0	
Azithromycin	Streptococcus	63	61	2	Above range
Benzylpenicillin	Streptococcus	68	68	0	
Cefotaxime	Streptococcus	68	68	0	
Ceftriaxone	Streptococcus	68	68	0	
Ceftriaxone	Haemophilus	48	48	0	
Cefuroxime	Streptococcus	68	68	0	
Cefuroxime	Haemophilus	29	29	0	
Clarithromycin	Streptococcus	51	51	0	
Clindamycin	Streptococcus	54	54	0	
Doxycycline	Streptococcus	31	30	1	Above range
Imipenem	Streptococcus	68	63	5	Below range
Levofloxacin	Streptococcus	68	68	0	
Levofloxacin	Haemophilus	48	48	0	
Linezolid	Streptococcus	48	48	0	
Meropenem	Haemophilus	46	46	0	
Meropenem	Streptococcus	68	68	0	
Moxifloxacin	Streptococcus	17	17	0	
Moxifloxacin	Haemophilus	48	48	0	
Rifampicin	Streptococcus	65	65	0	
Tetracycline	Streptococcus	68	68	0	
Trimethoprim-sulfametoxazole	Haemophilus	48	48	0	
Trimethoprim-sulfametoxazole	Streptococcus	36	36	0	
	No of tests	1312	1304	8	
	%		99.4%	0.6%	

EDL validation of MH-F broth

H. influenzae ATCC 49766: 100% within range compared with CLSI (HTM broth)

Antimicrobial agent	MIC panel	No of tests within concentration	No of tests with QC range	No of tests out of range	Comment
Cefuroxime	Haemophilus	22	22	0	
Cefuroxime	Streptococcus	8	8	0	
Imipenem	Haemophilus	7	7	0	
Imipenem	Streptococcus	8	8	0	
Meropenem	Streptococcus	8	8	0	
No of tests		53	53	0	
%			100%	0.0%	

H. influenzae ATCC 49247: 100% within range compared with CLSI (HTM broth)

Antimicrobial agent	MIC panel	No of tests within concentration	No of tests with QC range	No of tests out of range	Comment
Ampicillin	Haemophilus	52	52	0	
Cefepime	Haemophilus	21	21	0	
Cefotaxime	Haemophilus	52	52	0	
Ceftriaxone	Haemophilus	52	52	0	
Tetracycline	Haemophilus	16	16	0	
No of tests		193	193	0	
%			100%	0.0%	

Exceptions to BMD as reference

- For some agents, for which BMD does not give reliable results, agar dilution is the reference MIC method (as recommended in ISO 20776-1):
 - Fosfomycin
 - Mecillinam
- BMD has not been validated for some slow-growing organisms such as anaerobes and *Neisseria gonorrhoeae*.
 - Agar dilution is most commonly used (as recommended by CLSI)

Other MIC methods

- All other AST methods (disk diffusion and gradient tests, and semi-automated systems) must be calibrated to reference methodology.
- It is the responsibility of the **manufacturer** to develop commercial systems calibrated to BMD.
 - Gradient tests: Etest, MTS, M.I.C.E and others
 - Semi-automated systems: MicroScan, Phoenix, Vitek etc.
- It is the responsibility of the **user** to follow manufacturers' guidelines and to quality control the system in everyday use.

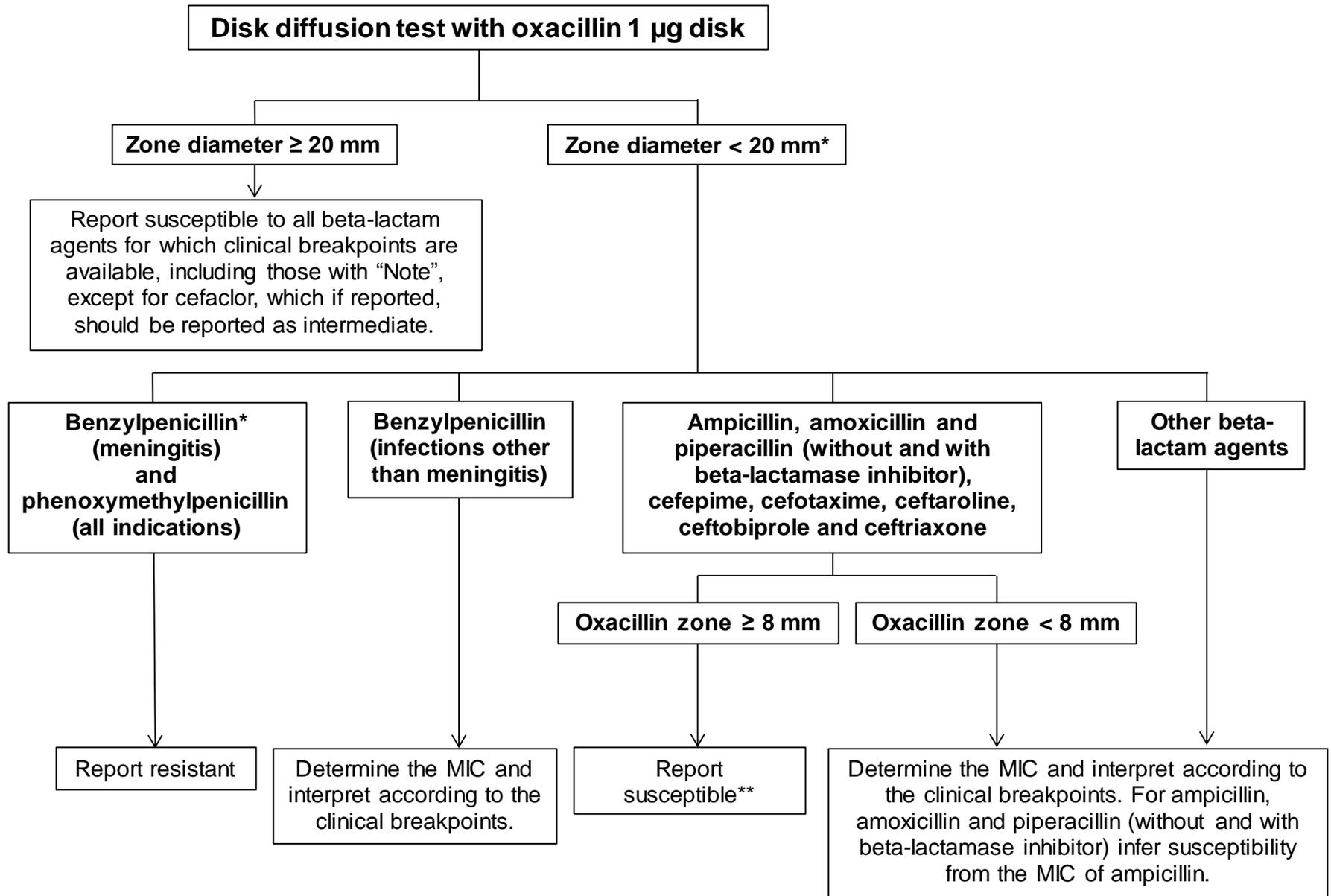
Quality control of AST methods

- Disk diffusion
 - Always on-scale results
- Gradient tests
 - Difficult to control the whole gradient (susceptible and resistant part)
- Semi-automated systems and commercial BMD products
 - Off-scale results for the standard QC strains due to short MIC ranges
 - Manufacturers must be tasked with providing QC recommendations (strains and expected results)

Question no 2

**We sometimes get benzylpenicillin
MIC susceptible results for oxacillin-
resistant *S. pneumoniae*.
Which result shall we trust?**

Screening for beta-lactam resistance in *S. pneumoniae*

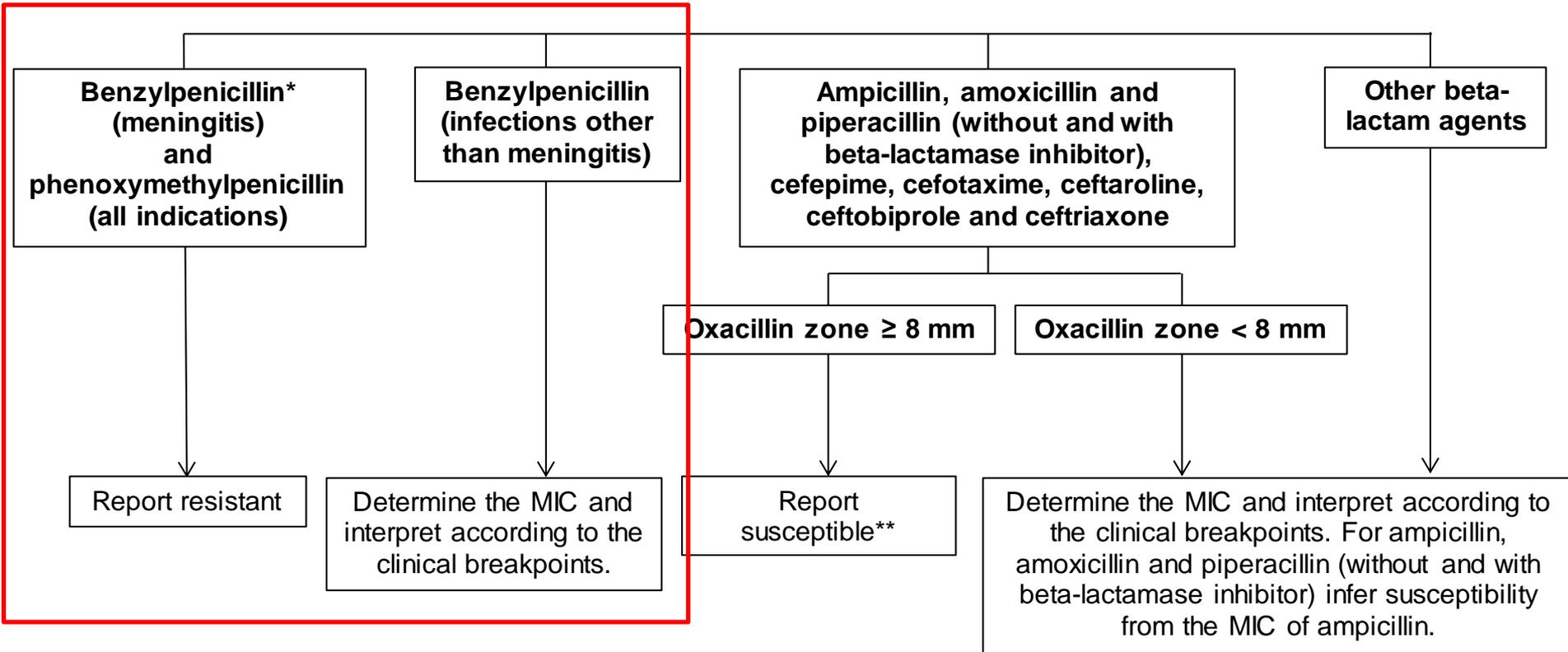


*Always determine the MIC of benzylpenicillin. Do not delay reporting resistance in meningitis.

** In meningitis confirm by determining the MIC for the agent considered for clinical use.

Screening for β -lactam resistance in *S. pneumoniae*

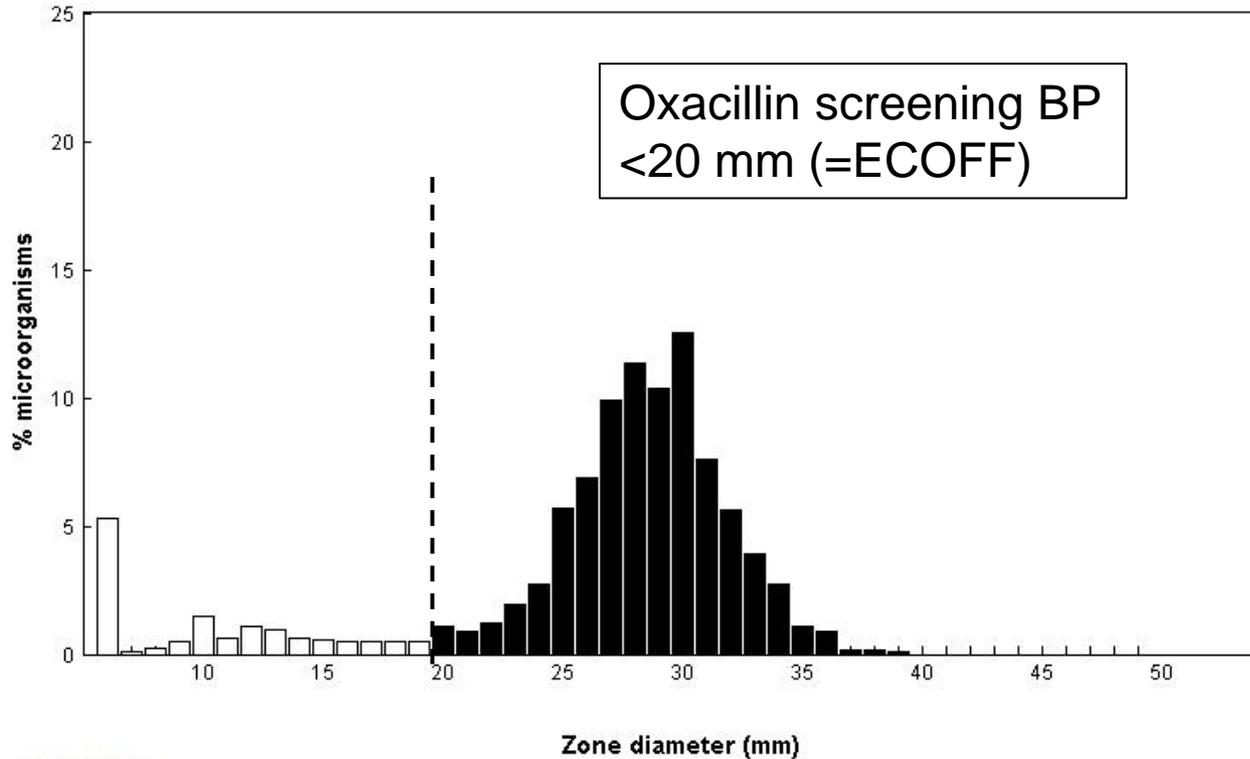
Oxacillin < 20 mm



*Always determine the MIC of benzylpenicillin. Do not delay reporting resistance in meningitis.

Oxacillin / *Streptococcus pneumoniae*
International wild type zone diameter distribution - Reference database 2018-03-27
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

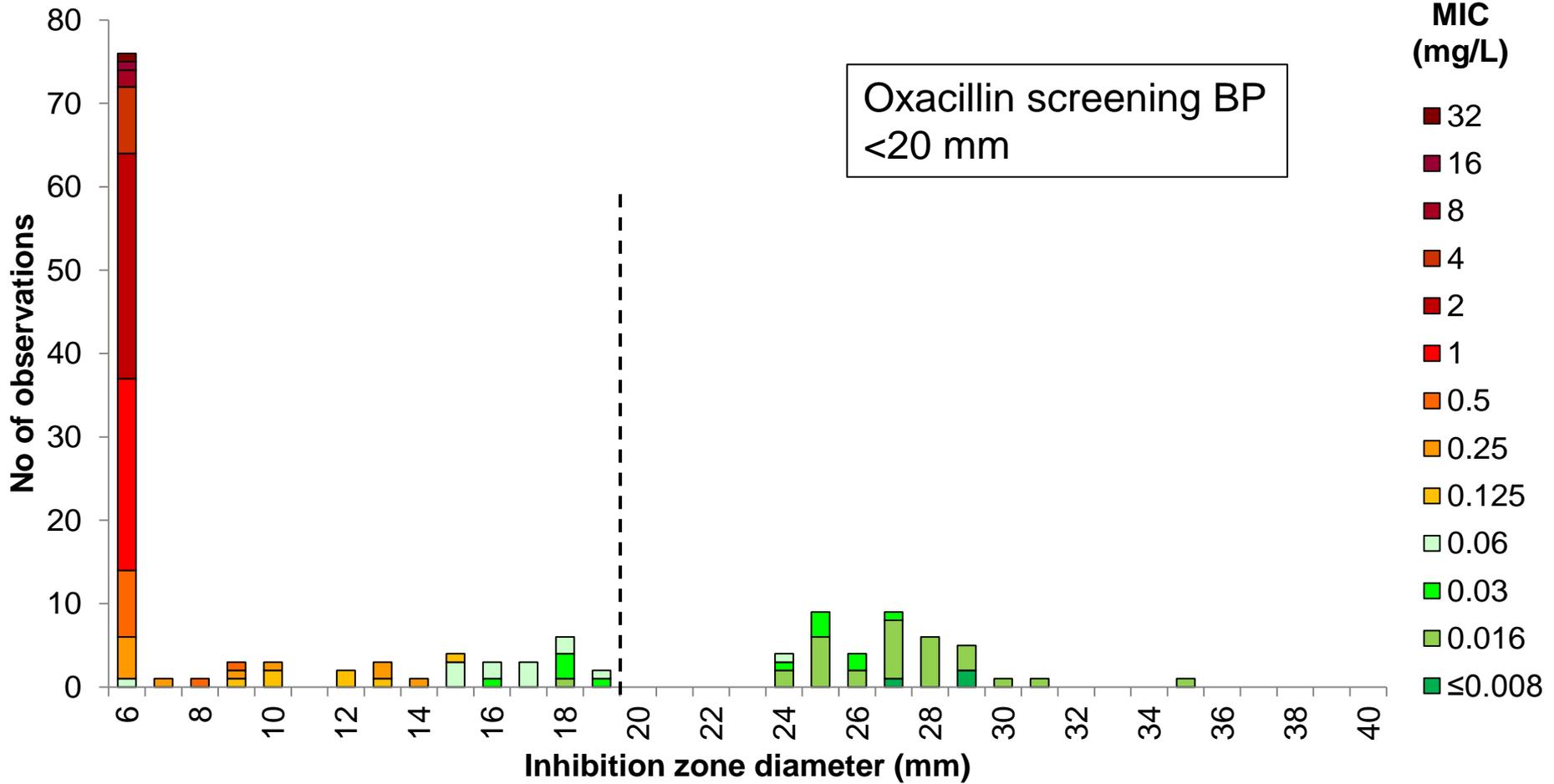


The oxacillin 1 μ g screening test is a sensitive method to detect β -lactam resistance mechanisms in *S. pneumoniae*.

Screen-positive isolates are resistant to one or several β -lactam agents, and always to phenoxymethylpenicillin.

Oxacillin 1 µg vs. Benzylpenicillin MIC

S. pneumoniae, 148 isolates



Evaluation of gradient tests for *S. pneumoniae* and β -lactam antibiotics

- Gradient tests from three manufacturers (Etest, M.I.C.E. and MTS) were evaluated vs. reference broth microdilution.
- Gradient tests generally underestimated MIC values for β -lactam antibiotics for *S. pneumoniae*, potentially giving false susceptible results.
 - Gradient test MICs were most commonly 1 doubling dilution lower.
 - Quality control with *S. pneumoniae* ATCC 49619 was not sufficient to disclose this.

Recent examples

S. pneumoniae sent for BMD testing at EDL during 2017

OXA 1 µg (mm)	PCG MIC (mg/L)	
	original data	BMD EDL
6	4	4
6	2	4
6	1	8
6	0.5	1
6	2	2
6	0.25	0.25
10	0.064	0.25

OXA 1 µg (mm)	PCG MIC (mg/L)	
	original data	BMD EDL
6	4	4
6	2	4
6	1	8
6	0.5	1
6	2	2
6	0.25	0.25
10	0.064	0.25

Same MIC
1 dilution lower
>1 dilution lower

S
S/I - dose dependent
R

4/7 tests are ≥ 1 dilution
lower than BMD

S. pneumoniae and benzylpenicillin AST

- Recommendations:
 - Perform frequent QC of oxacillin disks and benzylpenicillin MIC testing products.
 - If possible, use BMD for MIC testing.
 - Trust PCG-S results for OXA-R *S. pneumoniae* only if the PCG MIC has been verified with BMD.
 - In meningitis, report all OXA-R *S. pneumoniae* as resistant to PCG.
 - In other infections, isolates with OXA R and PCG S can be reported PCG S if verified with BMD, with a comment that β -lactam resistance mechanisms have been detected.

Question no 3

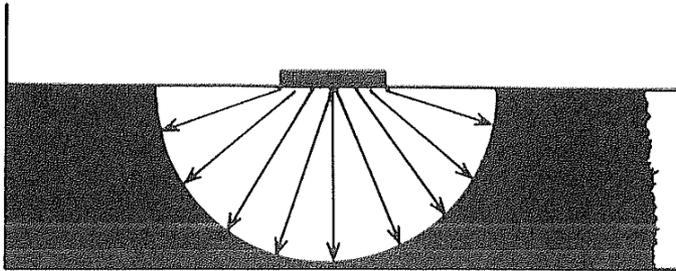
How can we measure agar depth and what are the acceptable limits?

Agar depth

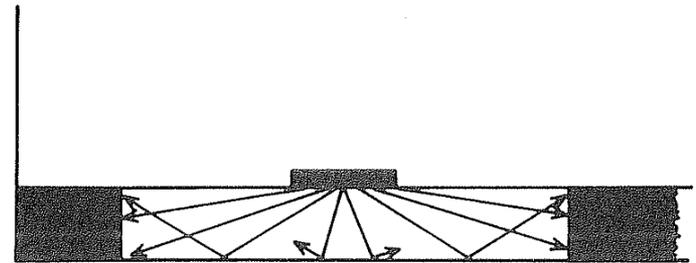
- Accepted limits: 4.0 ± 0.5 mm
- A level depth (not uneven)
- Measure for each new lot of plates, both for in-house prepared and commercial.
- For in-house production:
 - Note that plate dimensions may differ between manufacturers.
 - Calculate the correct volume based on the true dimensions of the Petri dishes
 - Pour plates on a level surface
 - Adjust the volume if the agar depth is within the acceptable range but repeatedly above or below 4 mm.

Why is the agar depth important?

- Antimicrobial agents diffuse into the agar
- Diffusion will be affected by the agar depth



Correct agar depth

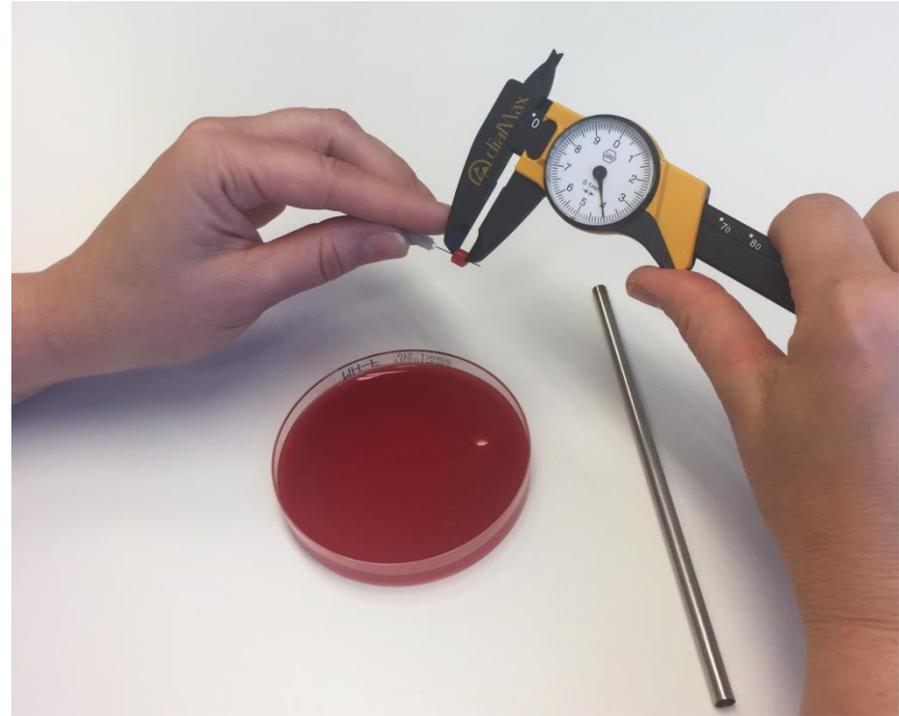


Agar too thin

- Agar depth < 4 mm: Larger zone diameters
- Agar depth > 4 mm : Smaller zone diameters

The method is calibrated to an agar depth of 4 mm!

Measuring agar depth



Correct agar depth: 4.0 mm, with occasional variation between 3.5 and 4.5 mm!

Question no 4

How can we minimise problems related to humid MH-F plates?

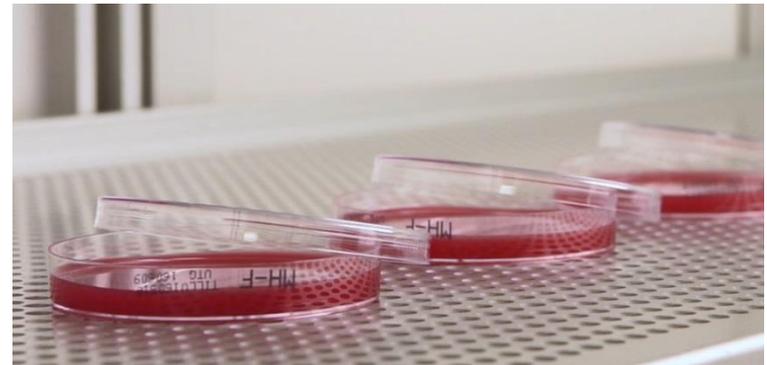
Drying and storage of agar plates

- Prior to use, make sure agar plates are at room temperature and have a dry surface.
- No drops of water should be visible on the surface of the agar or inside the lid.
- Excess moisture may cause fuzzy zone edges and/or haze within zones.



Drying and storage of agar plates

- **In-house production:**
 - Dry newly produced plates in room temperature over night to remove excess humidity, before storing them in the fridge.
- **Plates stored in plastic bags or sealed containers:**
 - Unpack plates to be used within a short time and store in ventilated racks in the fridge.
- If necessary, dry plates
 - 20-25°C overnight or
 - 35°C with the lid removed for 15 min.



Question no 5

Can we use antimicrobial disks from one manufacturer with Mueller-Hinton media from another manufacturer?

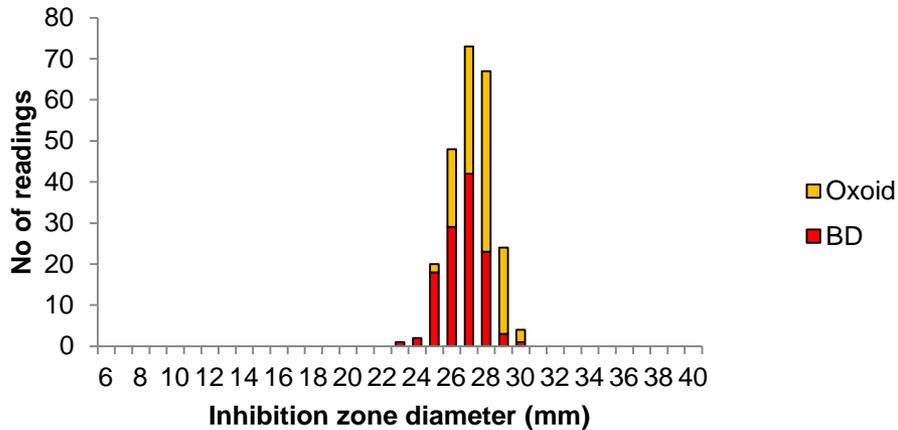
Disks and media

- Combining disk and media from different manufacturers should give accurate and reproducible results
 - Disks and media are not sold as a kit!
- As always, internal quality control should be performed to confirm accuracy of the test.
 - Results should be within EUCAST QC ranges and mean values from repeated tests should be close to the target values.
- EUCAST use data for disks and media from several manufacturers (this variation is built into the system) to produce :
 - QC criteria
 - Zone diameter breakpoints
 - Zone diameter reference distributions (EUCAST website)

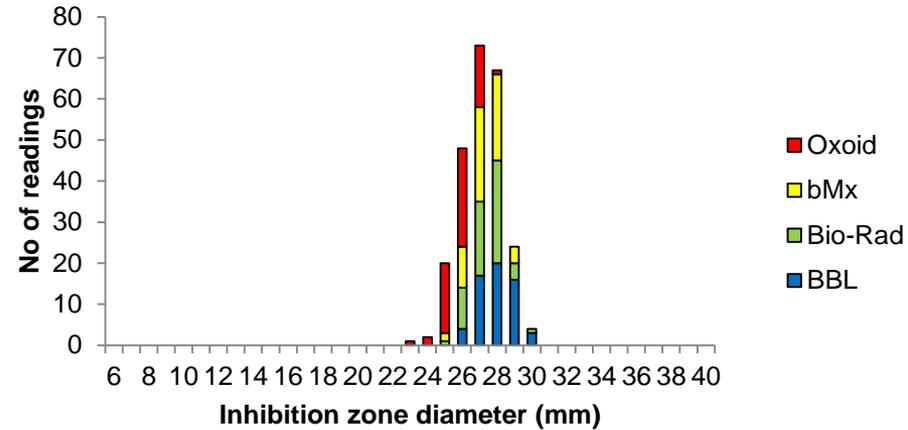
Establishment of QC criteria

Example *E. coli* ATCC 25922, Agent X

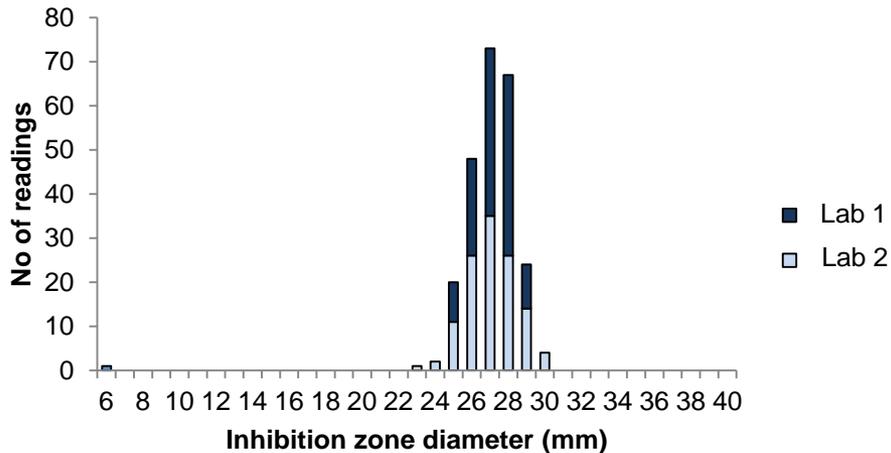
vs. disk manufacturer



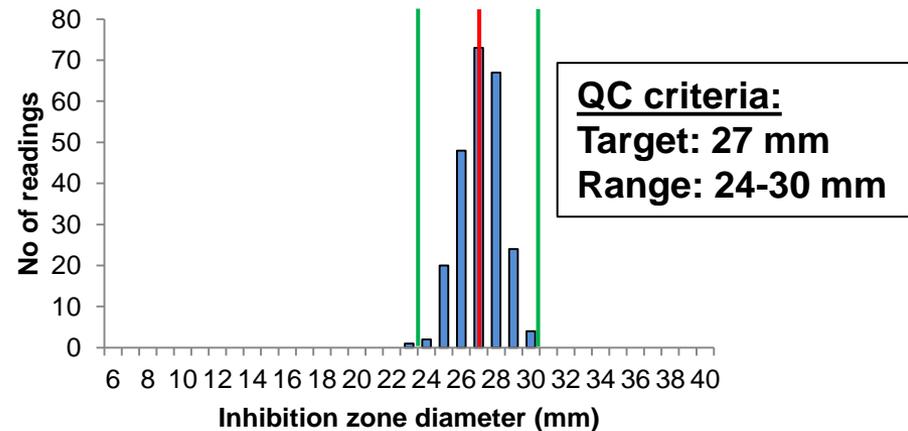
vs. MH manufacturer



vs. testing site



All data



Question no 6

How can we evaluate quality control results over time?

Quality control over time



Each day:

- Start with reading and evaluating QC results

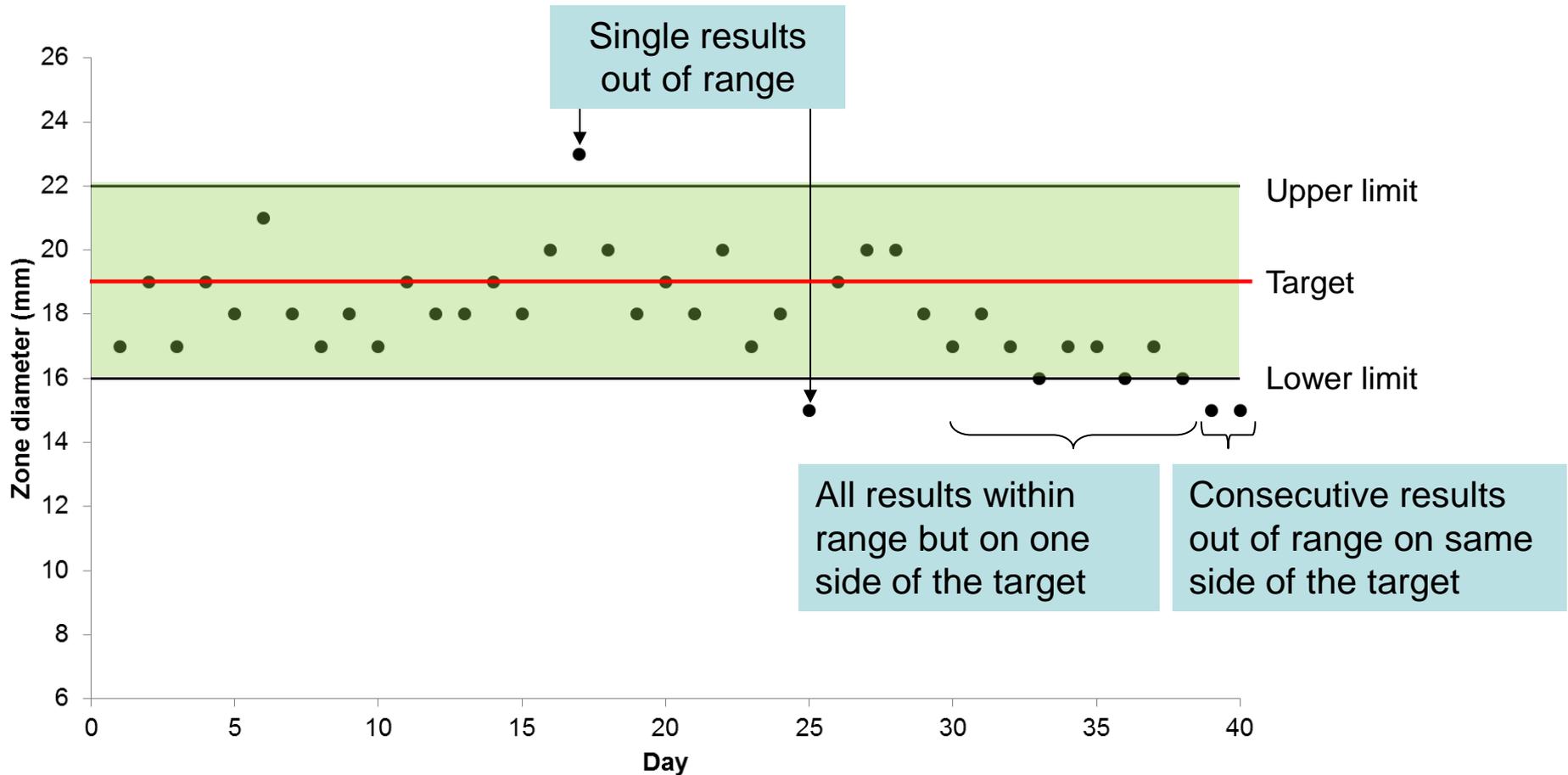
➔ “Green light” for your clinical isolates

or a



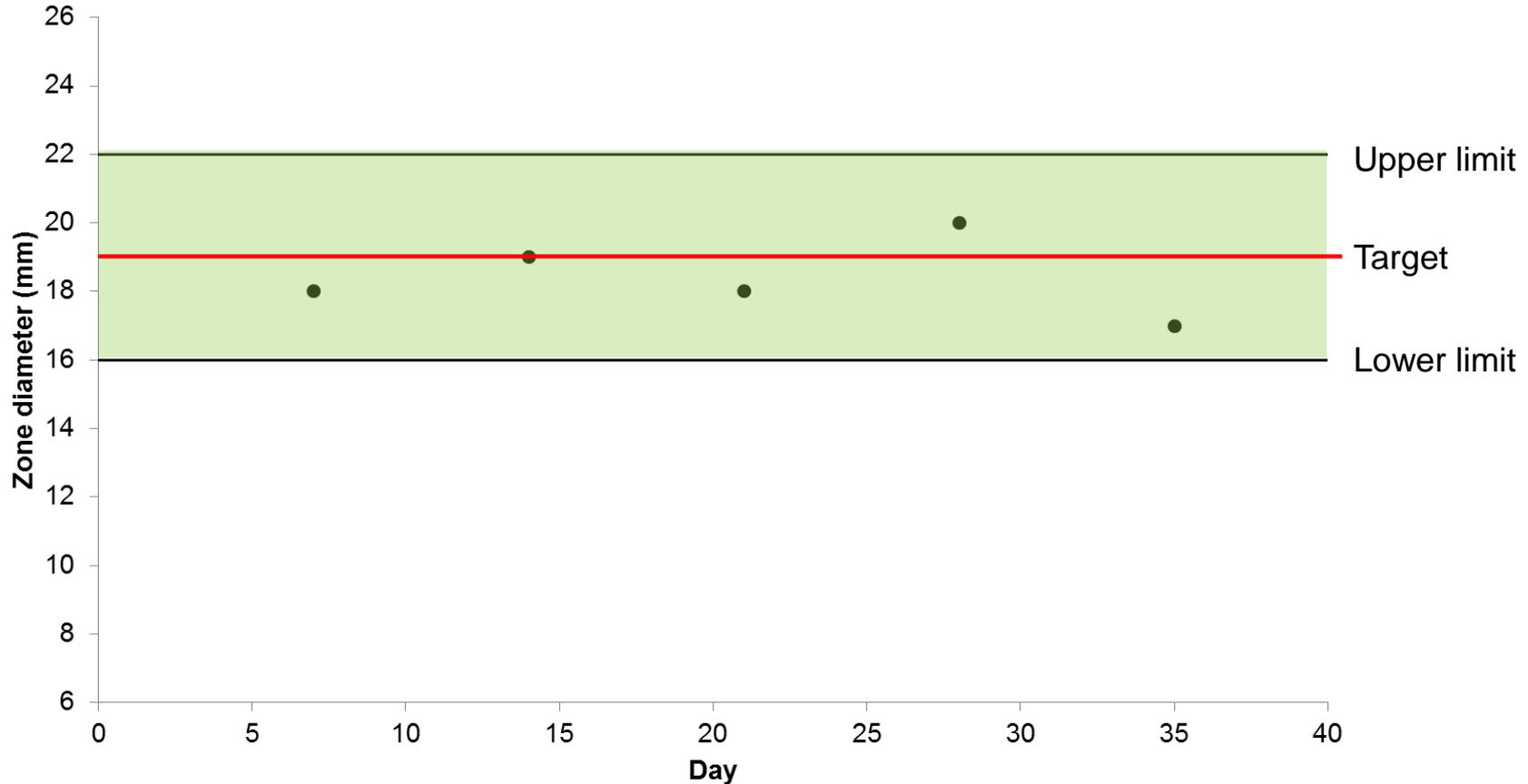
- Examine the results of the **last 20 consecutive tests**.
 - Look for trends and zones falling consistently above or below the target.
 - If two or more of 20 tests are out of range investigation is required.

Monitoring of QC results over time



This can reveal upcoming problems before they even appear!

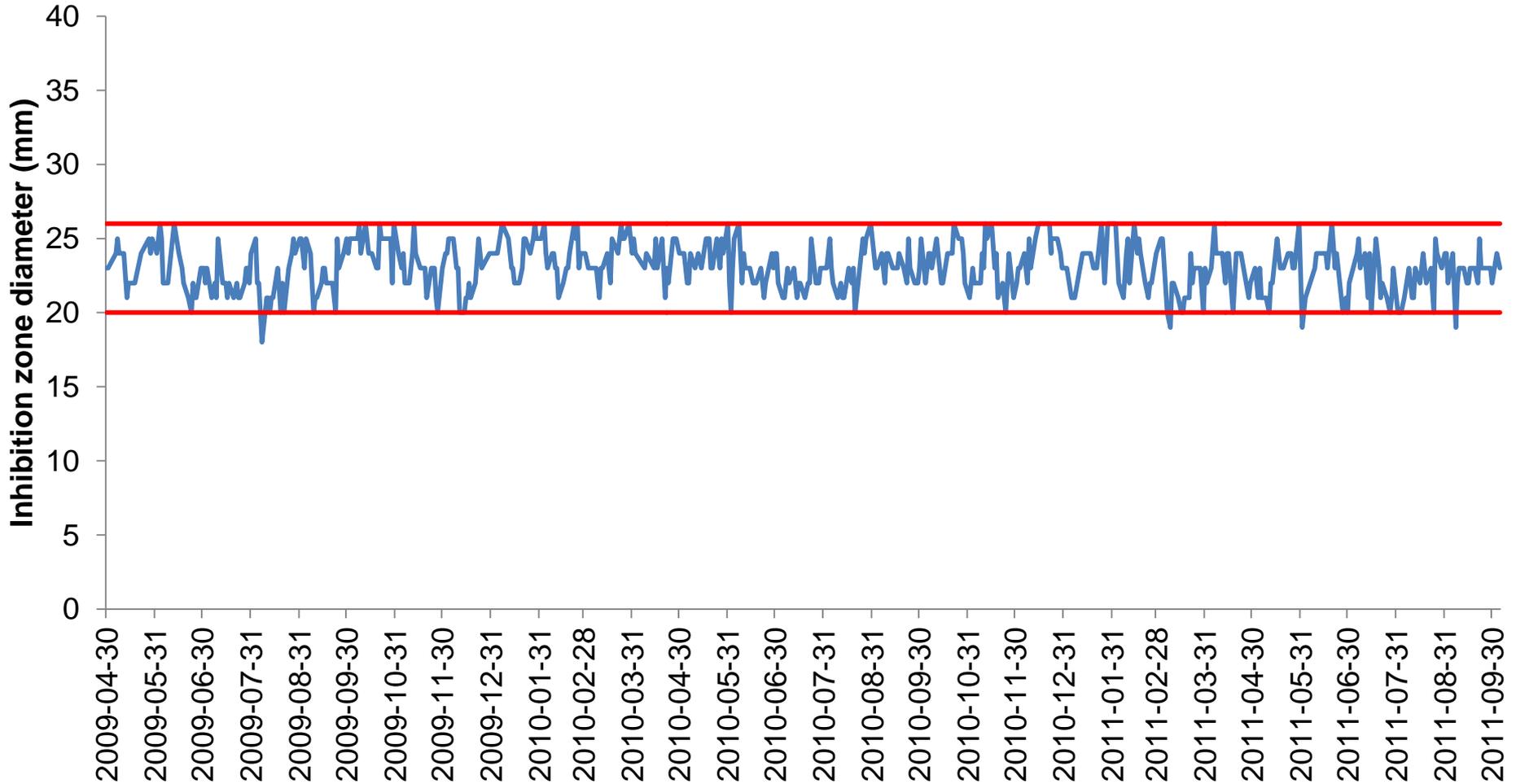
Monitoring of QC results over time



Daily QC versus weekly QC!

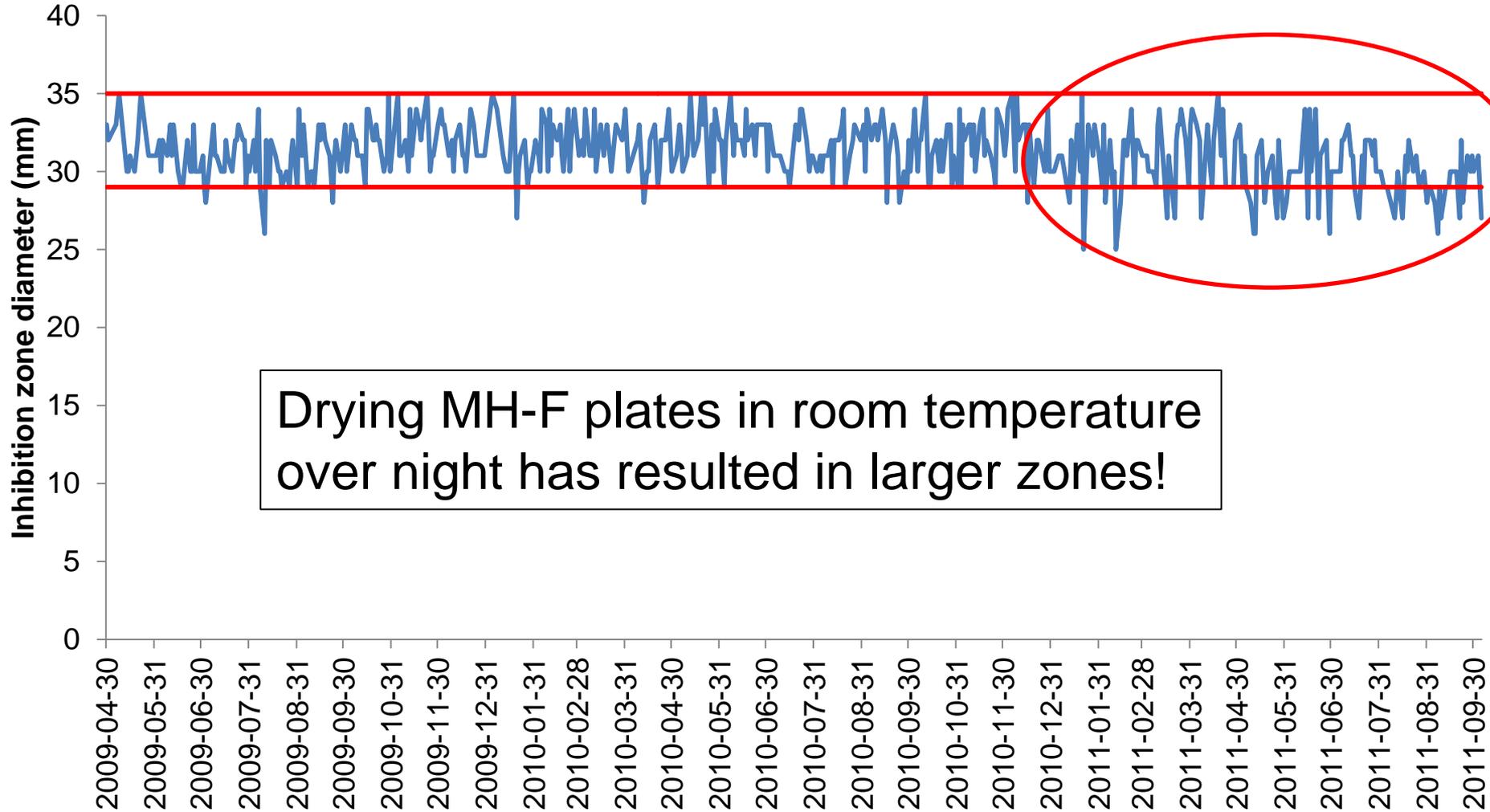
Routine QC: Agar from 3 manufacturers, 10-15 technicians

***S. pneumoniae* ATCC 49619
with trimethoprim-sulfamethoxazole 25 µg**



Routine QC: Agar from 3 manufacturers, 10-15 technicians

***H. influenzae* NCTC 8468 with cefotaxime 5 µg**



Question no 7

How can we compare our zone diameter distributions with reference distributions?

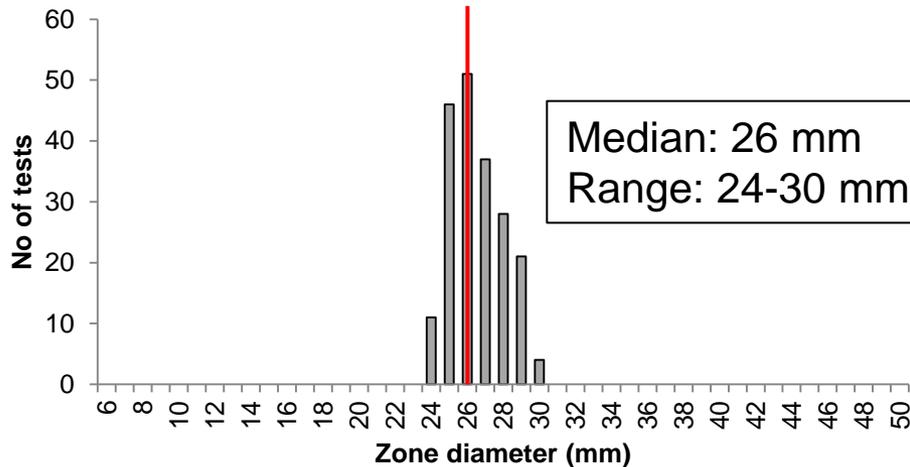
Comparison with reference distributions

- Used to detect
 - Systematic deviations
 - Problems with disks or media
 - Problems with specific drug-bug combinations
- Compare distributions for
 - QC strains
 - Clinical isolates

Comparison with reference distributions for QC strains

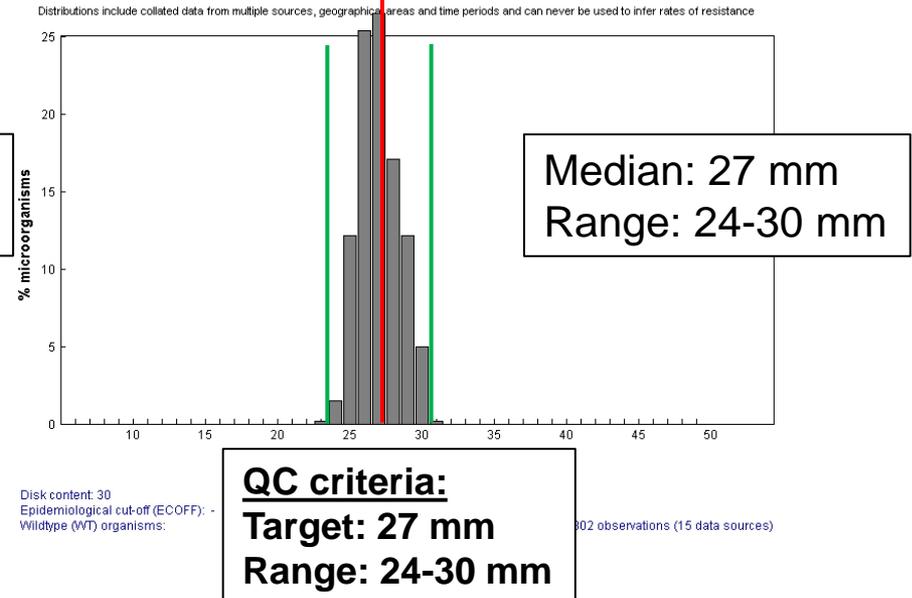
Routine distribution

Cefoxitin vs. *S. aureus* ATCC 29213



Reference distribution

Cefoxitin / *Staphylococcus aureus* ATCC 29213
International wild type zone diameter distribution - Reference database 2018-05-03
EUCAST disk diffusion method

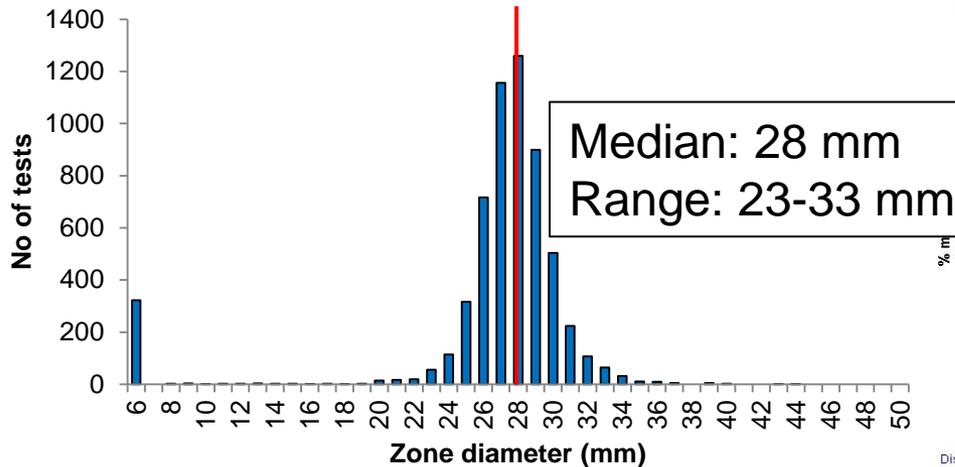


Compare the median values for the two distributions!

Comparison with reference distributions for clinical isolates

Routine distribution

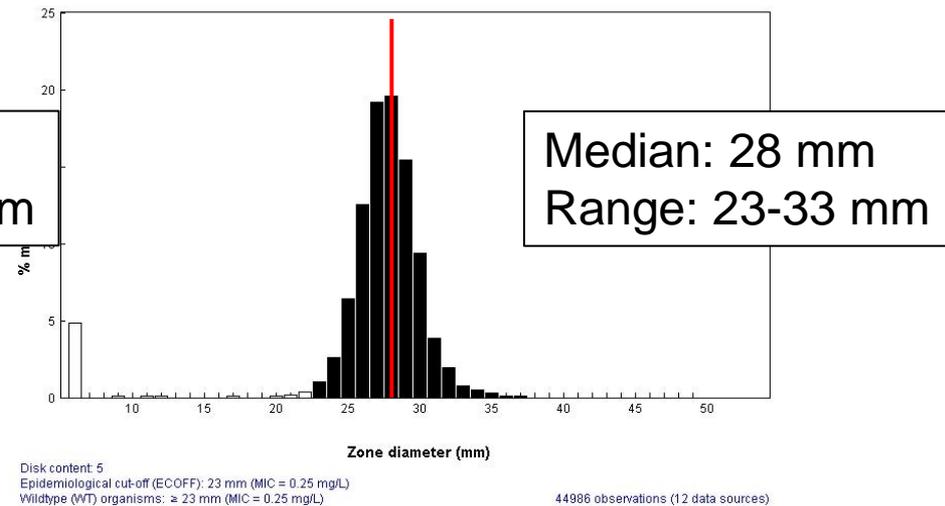
Cefotaxime vs. *E. coli*



Reference distribution

Cefotaxime / *Escherichia coli* International wild type zone diameter distribution - Reference database 2018-05-04 EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

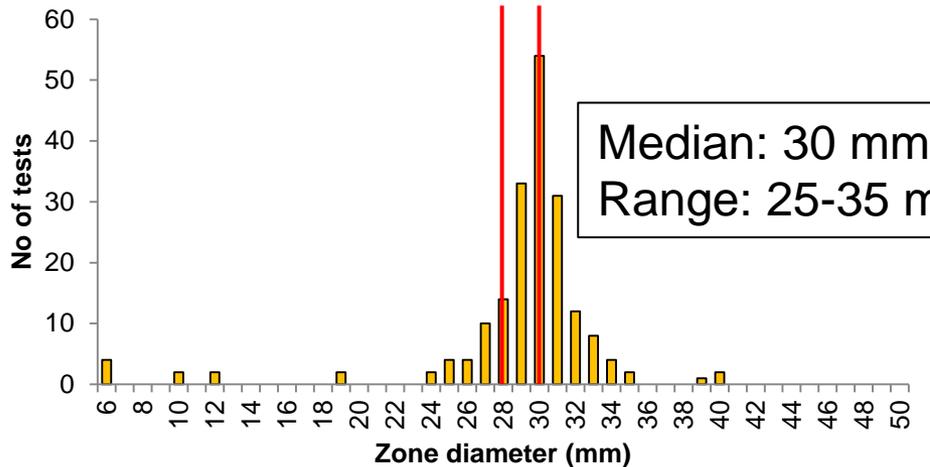


Compare the median values for wild-type isolates!

Comparison with reference distributions for clinical isolates

Routine distribution

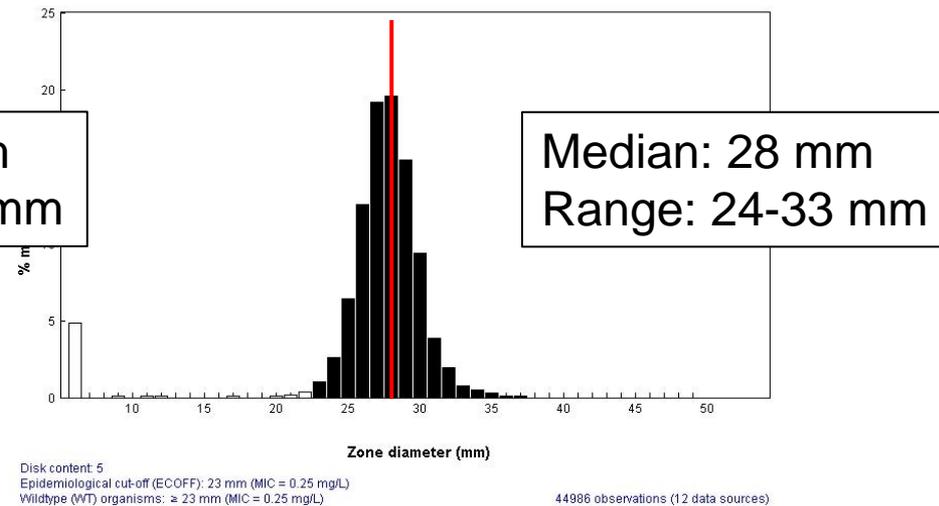
Cefotaxime vs. *E. coli*



Reference distribution

Cefotaxime / *Escherichia coli* International wild type zone diameter distribution - Reference database 2018-05-04 EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



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

Question no 8

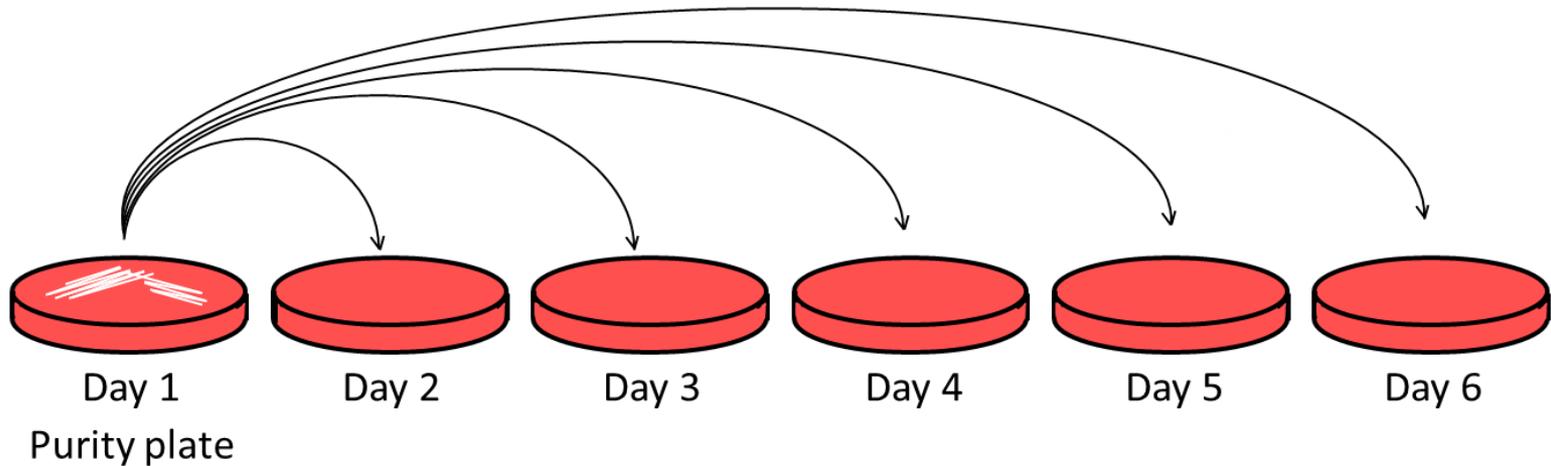
How should quality control strains be stored and subcultured?

Quality control strains

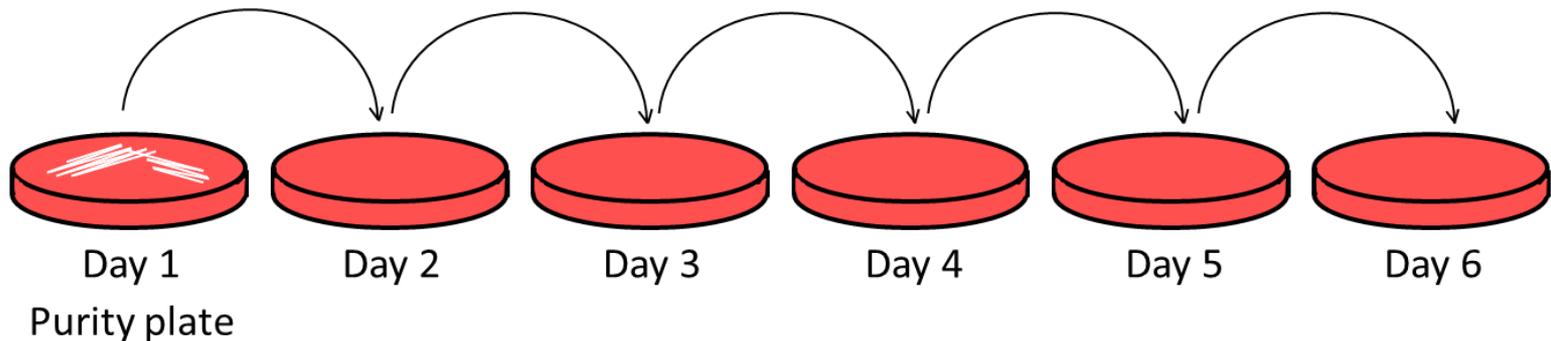
- Available from culture collections or commercial sources.
- Store by a method that minimises the risk of mutations, for example at -70°C on beads in glycerol broth.
- Store two vials of each control strain:
 1. In-use supply
 2. Archive
- Always pick several colonies when subculturing a QC strain, to avoid selecting a mutant.
- If mutation or contamination is suspected, subculture from the archive or buy a new QC strain.

Subculturing of QC strains

Non-fastidious QC strains



Fastidious QC strains



Questions and/or problem?

Contact us!

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