



Troubleshooting in disk diffusion testing

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QC responsibility

- Manufacturers of AST materials:
 - To ensure that products have been appropriately manufactured and comply with published QC criteria.
- Laboratories/users:
 - To ensure that products are maintained properly (storage and handling).
 - To ensure that testing is performed according to the standardised methodology.

The final responsibility for AST results
lies with the laboratory!

Troubleshooting in disk diffusion

- Quality control (QC)
 - QC results out of range
 - Investigate if two consecutive tests are out of range or multiple disks out of range on one day.
 - Evaluation of QC data over time
 - Look for trends and zones falling consistently above or below the target.
- Zone distributions for clinical isolates not in agreement with reference distributions.

Routine quality control

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

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

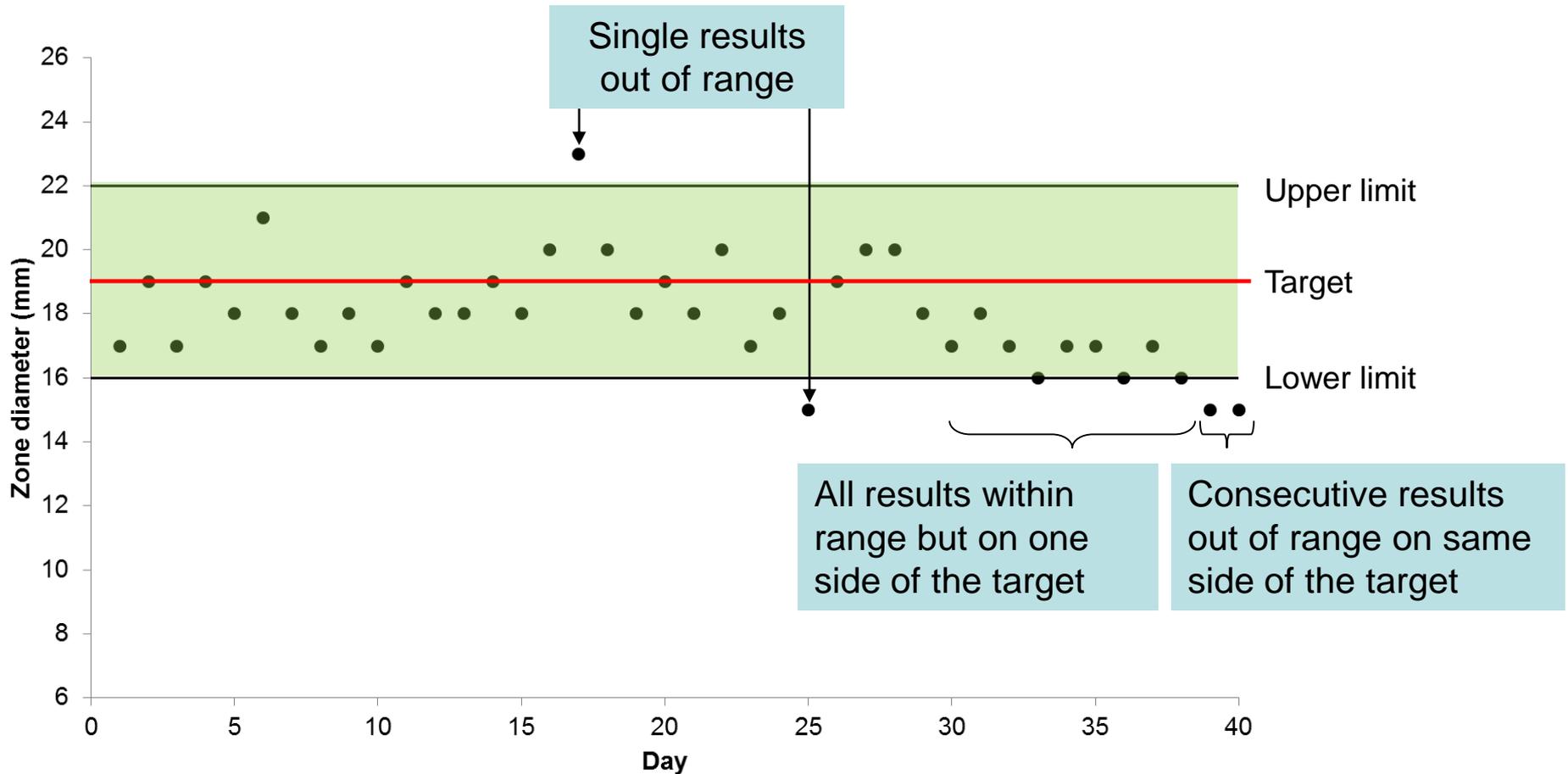
Range

Set to allow for random variation

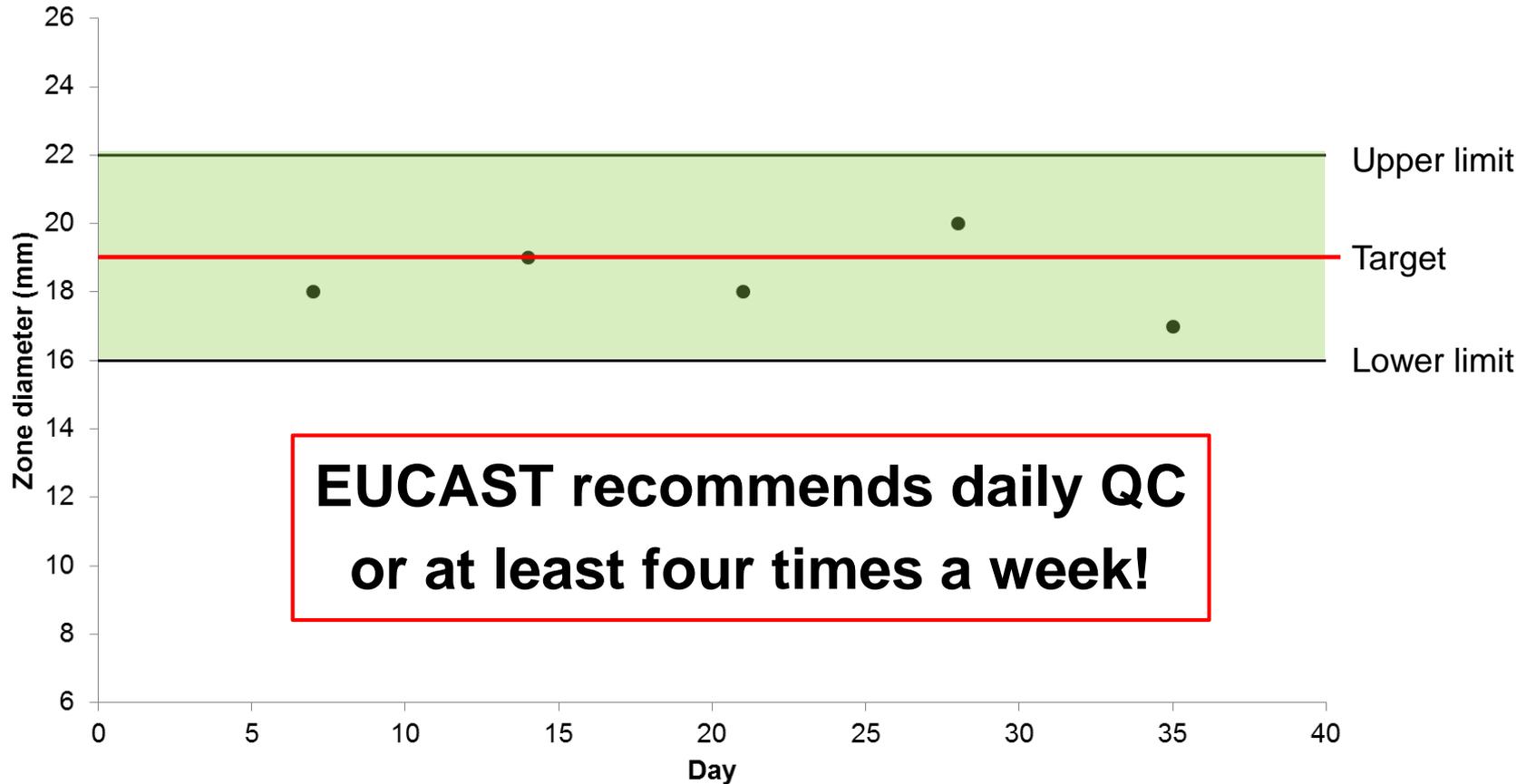
Target

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

Monitoring Laboratory QC results



Daily vs. weekly QC



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

[Organization](#)

[Public consultations](#)

[EUCAST News](#)

[New definitions of S, I and R](#)

[Clinical breakpoints and dosing](#)

[Rapid AST in blood cultures](#)

[Expert rules and expected phenotypes](#)

[Resistance mechanisms](#)

[Guidance documents](#)

[SOP](#)

[MIC and zone distributions and ECOFFs](#)

[New and revised ECOFFs](#)

[AST of bacteria](#)



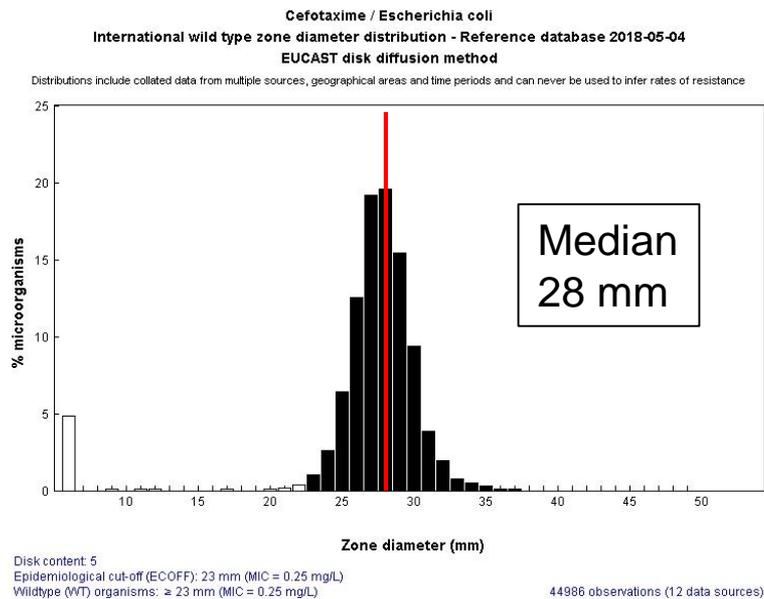
MIC and zone diameter distributions and ECOFFs

1. [EUCAST MIC and zone diameter distributions and ECOFFs](#)
2. [Correlation between MIC values and inhibition zone diameters](#)

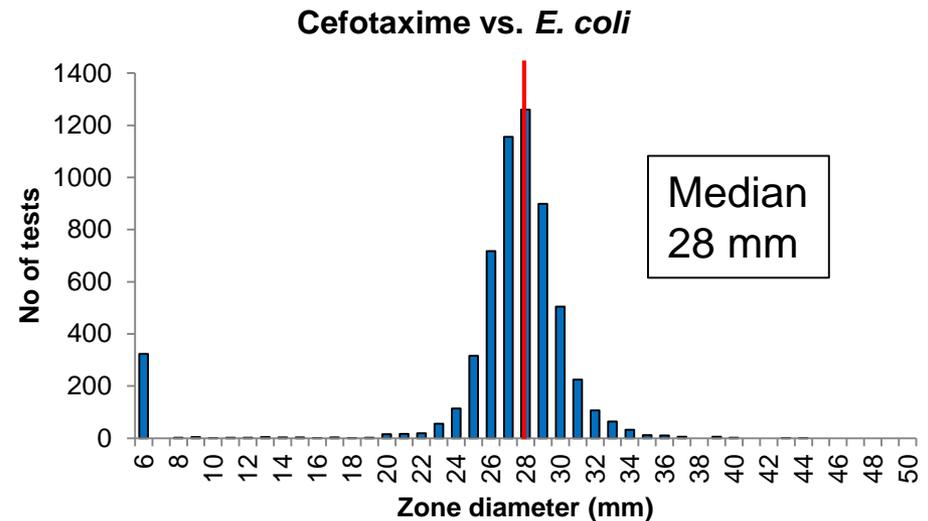
The EUCAST software, originally created in 2003, for displaying distributions of MIC-values (generated with methods calibrated to reference broth microdilution or for some agents and species, agar dilution) and inhibition zone diameters (generated with EUCAST standardised disk diffusion methodology) was re-programmed during 2020 and re-launched on 24 November, 2020. Each graph is shown in two versions where one is constructed by just adding up all approved distributions, and the other by giving each distribution equal weight before constructing the aggregated distribution. The later is generated through converting numbers to per cent before aggregation. This prevents large distributions from dominating or even "obliterating" smaller distributions and allows all distributions equal weight. During

Comparison with reference distributions for clinical isolates

Reference distribution



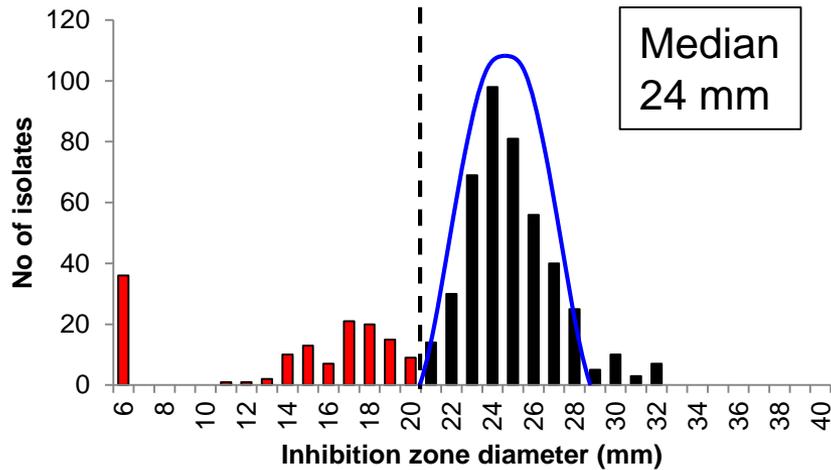
Routine distribution



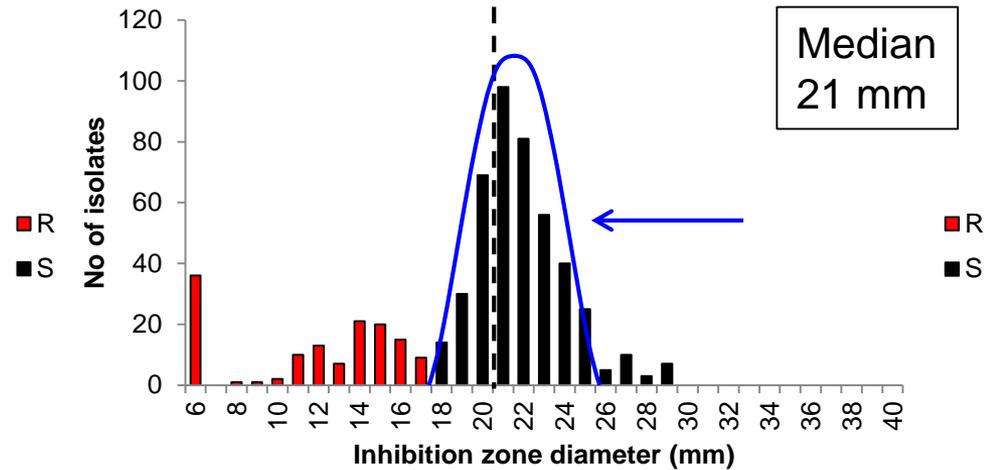
Compare the median values for wild-type isolates!

Example – too small zones

Reference distribution



Routine distribution



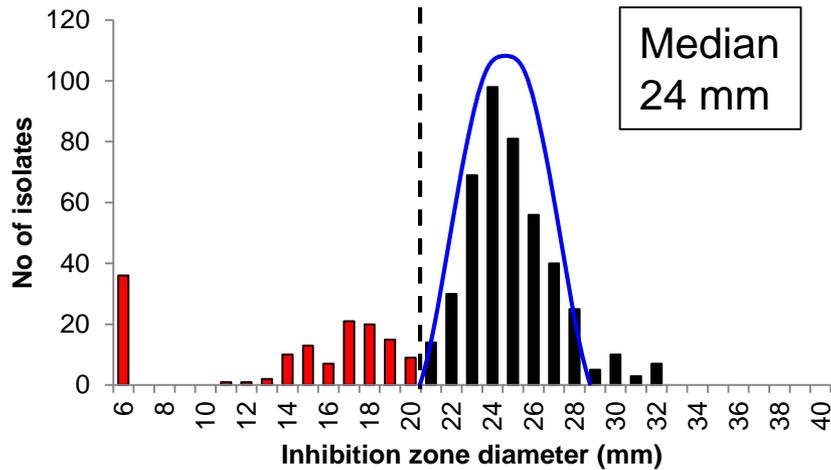
**Risk of reporting isolates
as false resistant!**

Potential errors:

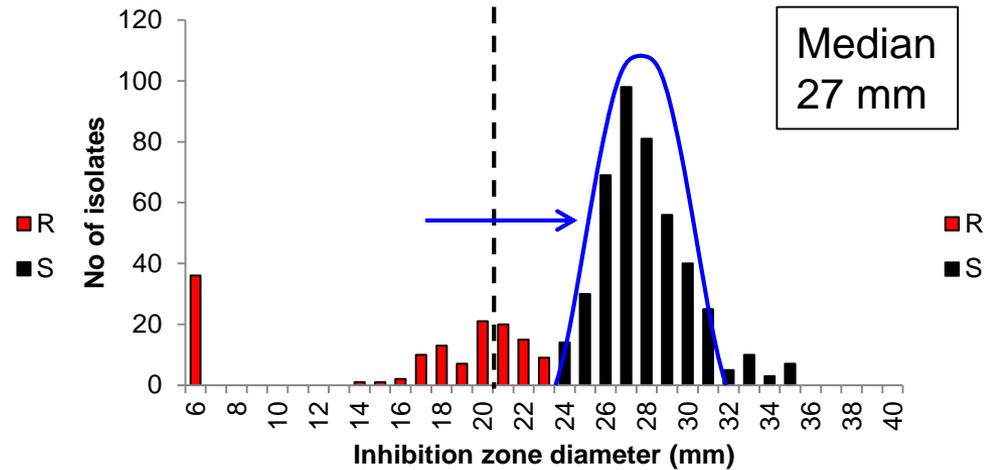
- Disk lost potency
- Over-incubation
- Over-inoculation
- Humid agar plates

Example – too large zones

Reference distribution



Routine distribution



Risk of reporting isolates as false susceptible!

Potential errors:

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

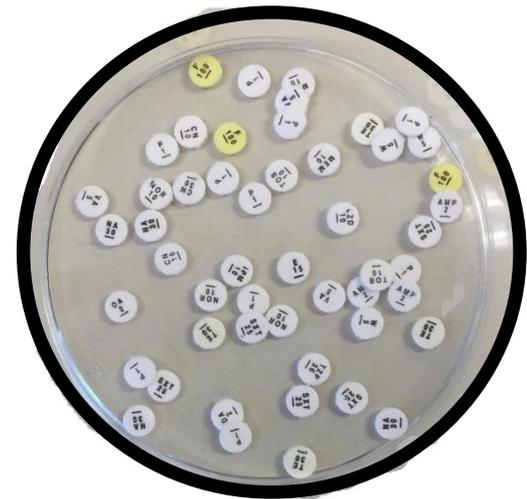
Potential sources of errors

1. Disks
2. Media
3. Not adhering to methodology
4. Equipment
5. QC strain



Example 1

- **Problem:** Ampicillin zone diameters above QC range for *H. influenzae* ATCC 49766.
 - Correct disk potency?
 - No, ampicillin 10 μg -disk used!
- **Solution:** Change to correct disk potency: ampicillin 2 μg



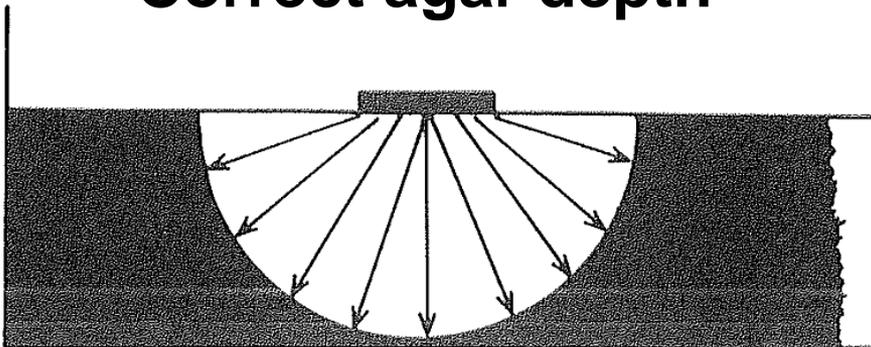
Example 2

- **Problem:** Ampicillin zone diameters above QC range for *H. influenzae* ATCC 49766.
 - Correct disk potency?
 - Yes, ampicillin 2 µg-disk used.
 - Other agents within range?
 - Yes, but some are in the upper part of the QC ranges.
 - Ampicillin zones for *S. pneumoniae* ATCC 49619 within range?
 - Yes, but in the upper part of the QC range.

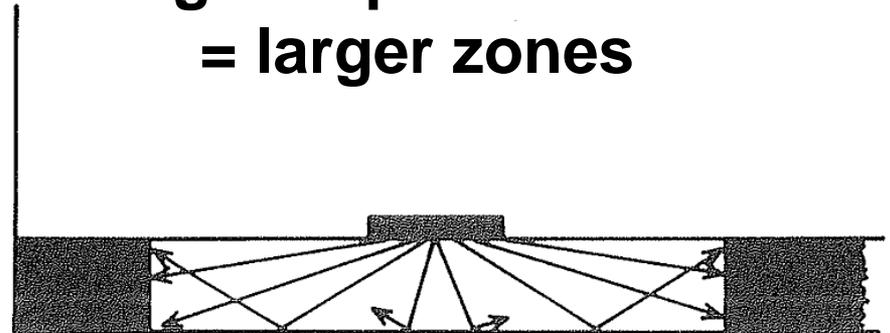
Example 2

- **Problem:** Ampicillin zone diameters above QC range for *H. influenzae* ATCC 49766.
 - Correct agar depth?
 - Agar depth 3.0 mm
- **Solution:** Adjust volume of agar to achieve agar depth of 4.0 mm.

Correct agar depth

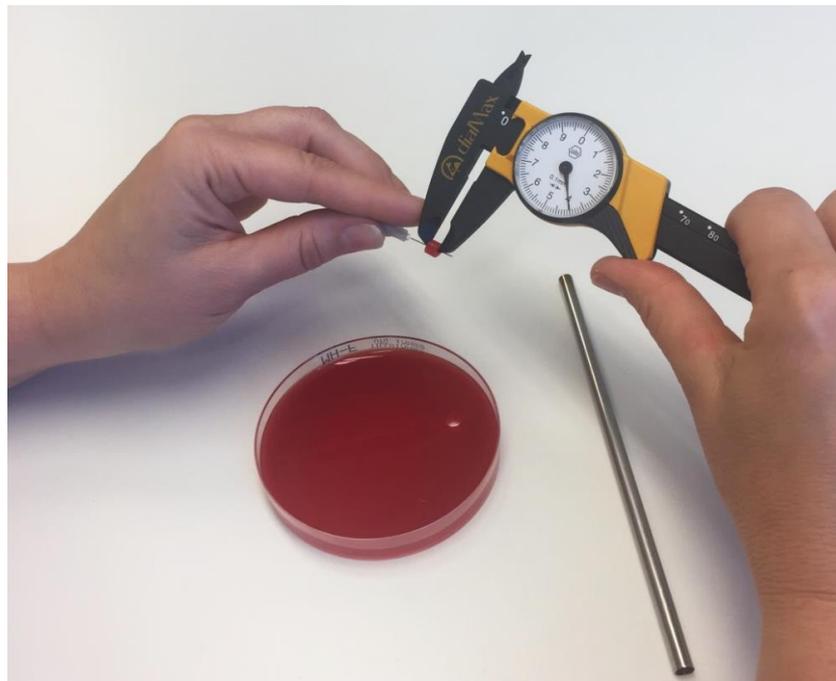


Agar depth too low
= larger zones



Measuring agar depth

- Check the agar depth for all new lots of plates, both commercial and in-house produced.



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

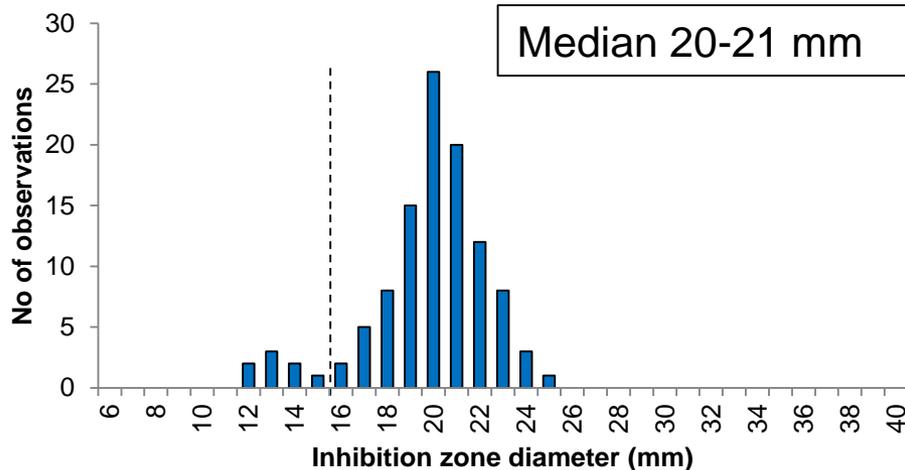
Example 3

- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
 - Disks ok?
 - Yes, within expiry date and correctly stored.
 - Media ok?
 - Yes, within expiry date and agar depth correct.
 - Tobramycin zones within range for other QC strains?
 - Yes, within range for *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213.
 - Are results related to the MH media used?
 - Yes, zones are larger when tested on agar from another manufacturer.

Example 3

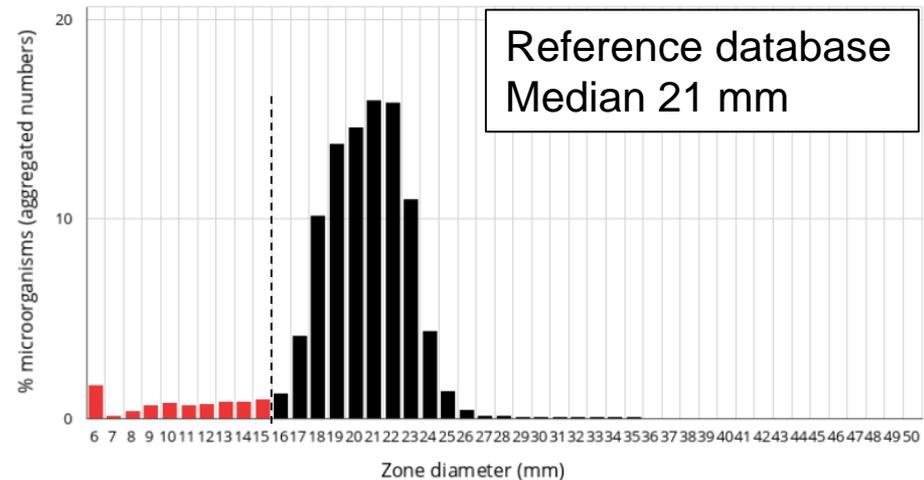
- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
 - Median for clinical isolates within ± 1 mm of the median of the reference distribution?
 - **Yes!**
 - **Solution:** Monitor data over time

E. coli with tobramycin 10 μ g



Tobramycin / Escherichia coli
International zone diameter distribution - Reference database 2022-03-23
EUCAST disk diffusion method
Based on aggregated distributions

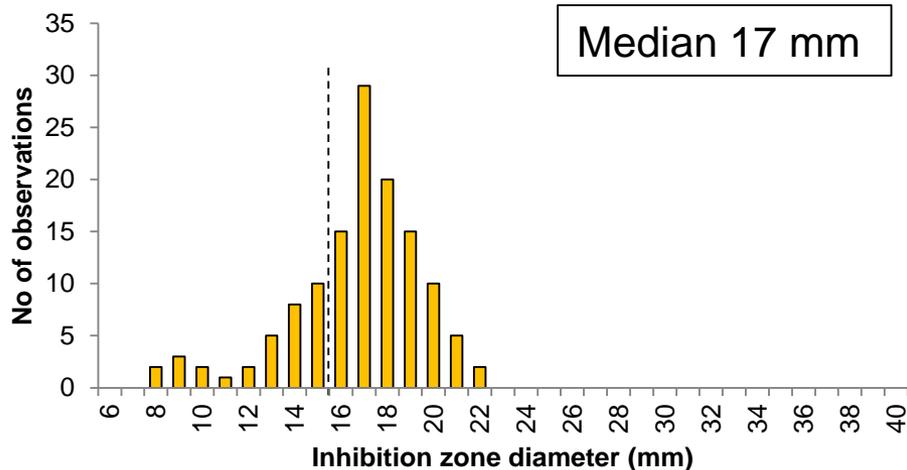
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



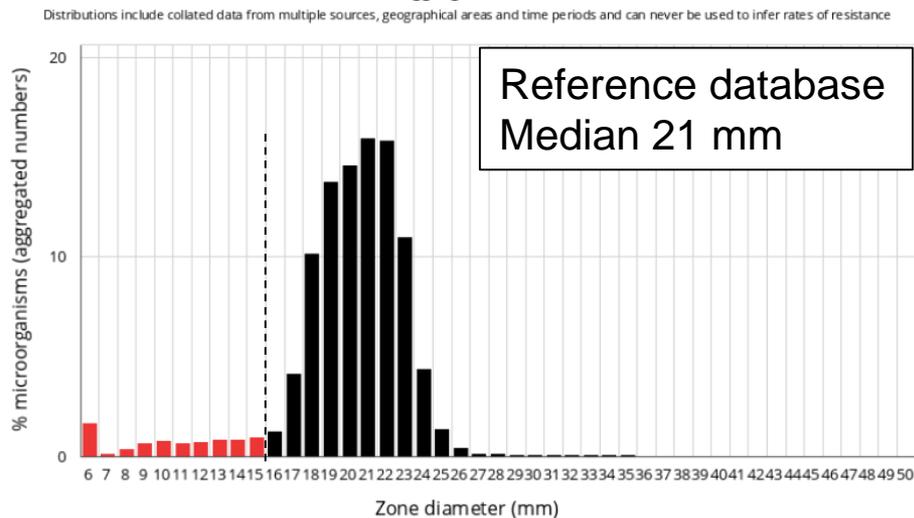
Example 3

- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
 - Median for clinical isolates within ± 1 mm of the median of the reference distribution?
 - **No!**
 - **Solution:** Introduce a local warning and contact the manufacturer.

E. coli with tobramycin 10 μ g



Tobramycin / Escherichia coli
International zone diameter distribution - Reference database 2022-03-23
EUCAST disk diffusion method
Based on aggregated distributions



21 brands of dehydrated Mueller-Hinton media

Total rating	Manufacturer	Product name	Percent zones on QC target ± 1 mm	Percent zones outside QC range	Agents outside range, high	Agents outside range, low
-4	Bio-Rad	MH Agar	86	0		
-10	Biolife	MH Agar II	81	1.1	TS	
-10	Oxoid	MH Agar	78	1.1	TS	
-11	Sigma	MH Agar 2	81	0		
-12	Becton Dickinson	BBL MH II Agar	73	0		
-12	Hardy Diagnostics	CRITERION MH Agar	71	0		
-13	Becton Dickinson	Difco MH Agar	70	3.3	AM	
-14	Alpha Biosciences	MH Agar	71	3.3	FQ	
-17	E&O Laboratories	MH Agar	82	8.9	CA, FQ, AM	TS
-18	Sigma	MH Agar	57	3.3	CS	
-20	HiMedia	MH Agar	56	0		
-21	bioMérieux	MHE Agar	64	3.3	TS	
-22	Acumedia	MH Agar	63	3.3	AM	
-24	Remel	MH Agar	64	6.7	AM	TS
-25	Lab M	MH Agar	69	6.7	AM	TS
-25	Merck	MH Agar CLSI	66	6.7	AM, TS	
-27	Mast Group	MH Agar	59	8.9	CA, FQ	TS
-31	Sifin	MH Agar	60	6.7	AM, TS	
-32	HiMedia	MH Agar No. 2	50	6.7	CA, AM	
-40	Biolab	MH II Agar	52	10	PC, MA, TE	TS
-55	Merck	MH Agar	44	23	CS, CA, FQ, AM	TE

Abbreviations

PC - Penicillins; CS - Cephalosporins; CA - Carbapenems; FQ - Flouroquinolones; AM - Aminoglycosides; MA - Macrolides; TE - Tetracyclines; TS - Trimethoprim-sulfamethoxazole

Example 4

- **Problem:** Meropenem zone diameters below QC range for *H. influenzae* ATCC 49766.
 - Disks lost potency?
 - Disks within the expiry date?
 - Yes!
 - Active desiccant?
 - Yes!
 - Correct storage?
 - Yes!
 - Meropenem zones for *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. pneumoniae* ATCC 49619 within range?
 - Yes, but in the lower part of the QC range for *S. pneumoniae*.

Example 4

- **Problem:** Meropenem zone diameters below QC range for *H. influenzae* ATCC 49766.
 - MH-F plates within the expiry date?
 - Yes!
 - MH-F plates sufficiently dry?
 - No, condensation inside the lid!



- **Solution:** Check storage conditions and/or dry plates prior to inoculation.

Drying and storage of MH-F



In-house produced
plate stored in
ventilated rack
- Clear zone edge

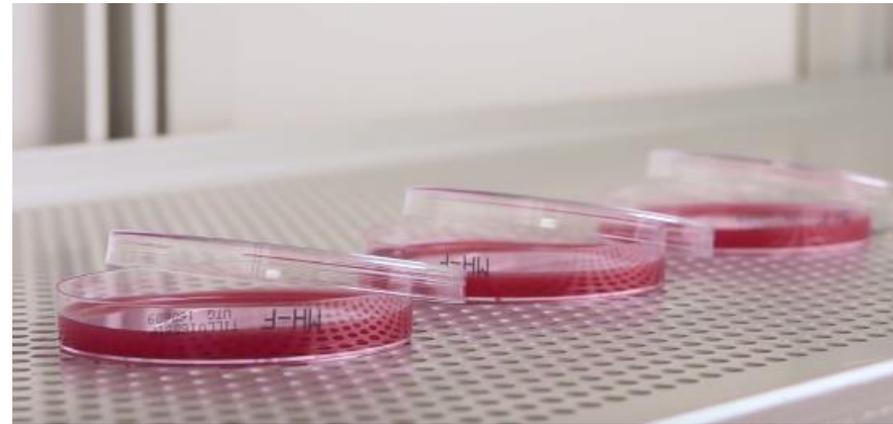
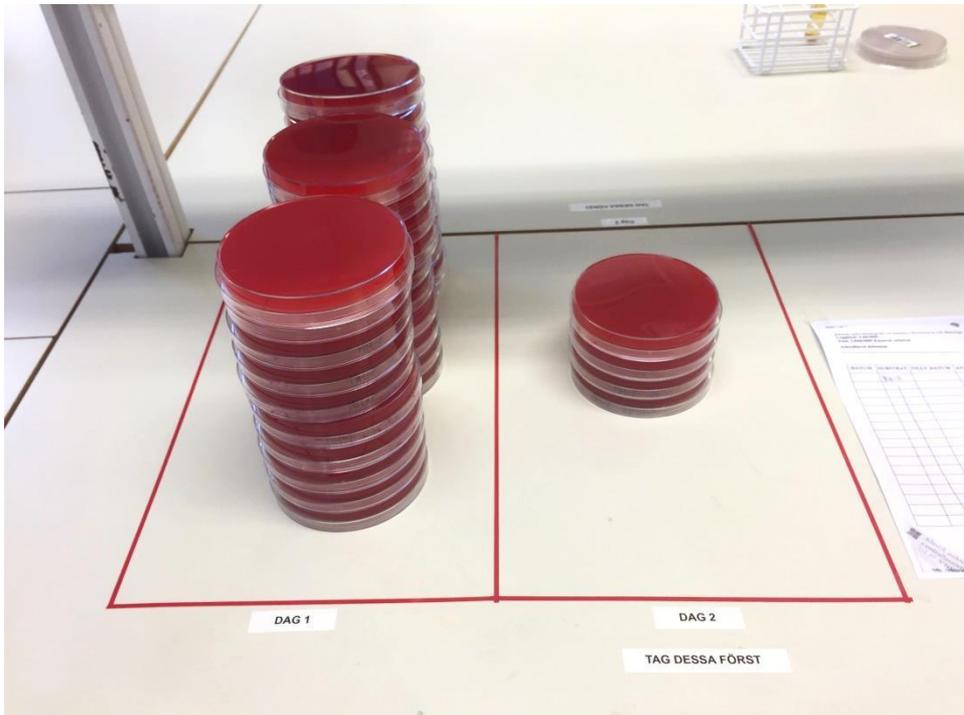
Commercial plate
stored in plastic bag
- Fuzzy zone edge
- Regrowth close to
disk

No drops of water should be visible on the surface of the agar or inside the lid.

- Store plates unpacked in the fridge
- Dry plates before inoculation
 - 20-25°C overnight, or at 35°C, with the lid removed, for 15 min.

Drying and storage of MH-F

For agar plates stored in plastic bags or sealed containers, it may be necessary to dry the plates prior to use. This is to avoid excess moisture, which may result in problems with fuzzy zone edges and/or haze within zones.

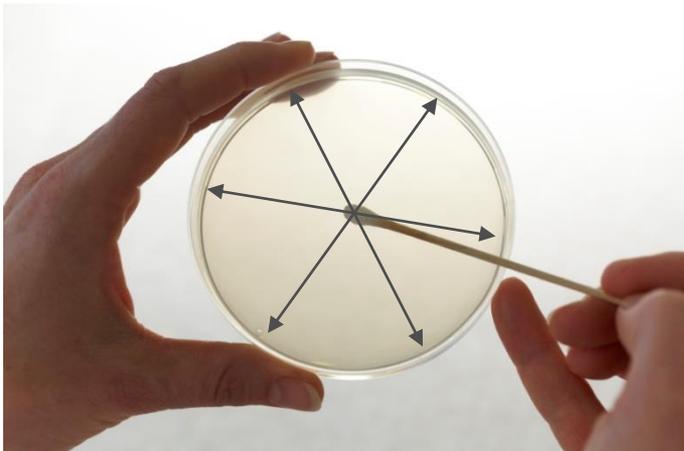


Example 5

- **Problem:** Zone diameters for multiple agents below QC ranges for *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922.
 - Correct agar depth?
 - Yes!
 - Agar plates correctly inoculated?
 - No, growth too heavy due to over-inoculation!
- **Solution:** Make sure that plates are correctly inoculated.

Inoculation of agar plates

- Spread the inoculum evenly over the entire agar surface.
- **Gram-negative bacteria:** remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation.
- **Gram-positive bacteria:** do not press or turn the swab against the inside of the tube. Take particular care to ensure that there are no gaps between streaks.



Example 6

- **Problem:** Colonies within zones for *H. influenzae* ATCC 49766 and several agents.
 - Pure culture?
 - Yes!
 - Agar plates correctly inoculated?
 - Yes!
 - MH-F plates dry?
 - Yes!
 - 15-15-15 minute rule adhered to?
 - Yes!
 - Correct incubation time, atmosphere and temperature?
 - 35°C and 5% CO₂, but 20-22 hours incubation!
- **Solution:** Follow recommendations and incubate for 16-20 hours.

Example 7

- **Problem:** Ciprofloxacin zone diameters below QC range for *E. coli* ATCC 25922.
 - Disks lost potency?
 - Disks within the expiry date?
 - Yes!
 - Correct storage of disks?
 - Yes!
 - Media within the expiry date?
 - Yes!
 - Ciprofloxacin zones within ranges for other QC strains?
 - Yes!

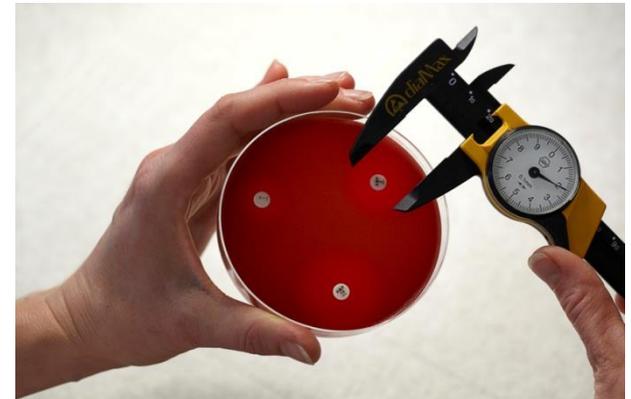
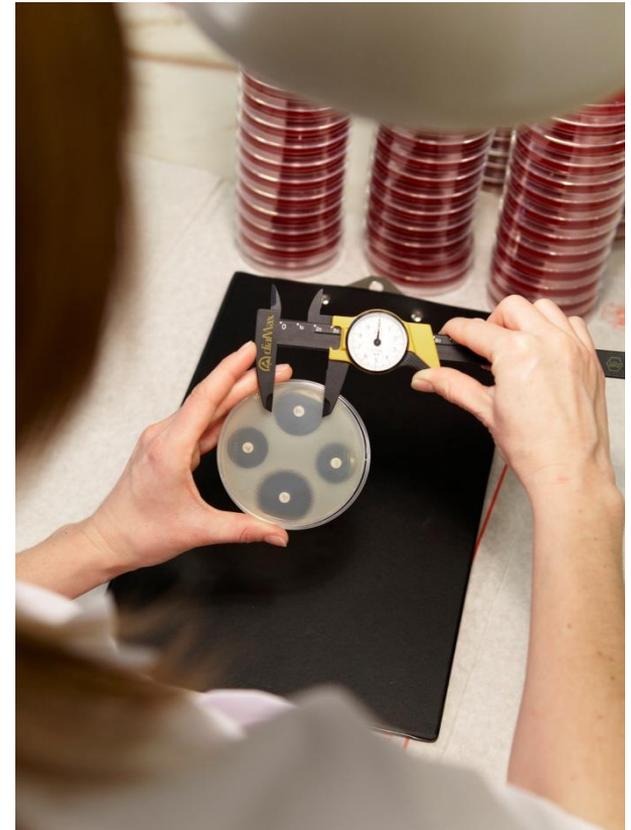
Example 7

- **Problem:** Ciprofloxacin zone diameters below QC range for *E. coli* ATCC 25922.
 - Agar plates correctly inoculated?
 - Yes!
 - Ciprofloxacin zones edges?
 - Fuzzy zone edges!
- **Solution:** Follow reading instructions, perform reading exercises.



Reading zones

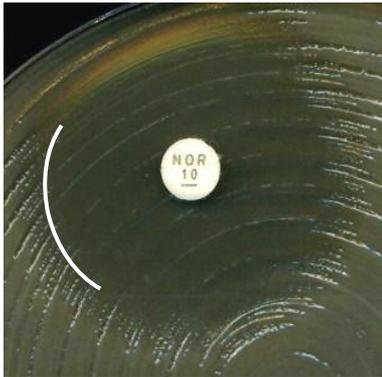
- Read **MH** plates from the back against a dark background illuminated with reflected light.
- Read **MH-F** and **FAA-HB** plates from the front with the lid removed illuminated with reflected light.



Fuzzy zone edges

Enterobacterales

- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is. Do not hold the plate up to light (transmitted light) or use a magnifying glass.



Reading of zones with fuzzy zone edges for *Enterobacterales*

Reading exercise, Växjö laboratory

S. aureus

First occasion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacilin	Rifampicin
26	27	26	29	20	30
26	28	28	29	22	31
26	29	28	29	25	31
29	28	28	30	23	31
26	27	27	28	19	30
26	28	29	30	23	32
26	29	29	30	21	31
26	27	27	29	23	29
25	27	26	29	20	32
25	25	26	28	25	32
28	27	26	27	22	30

Mean
SD

26	27	27	29	22	31
1.2	1.1	1.2	0.9	2.0	1.0

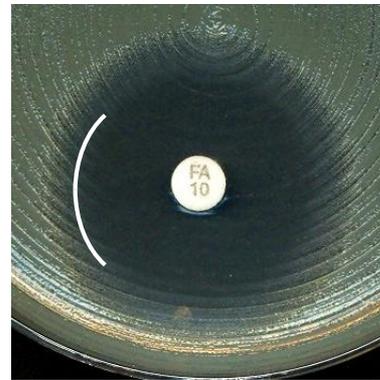
> 1 mm above mean

> 1 mm below mean

Discussion

S. aureus and fuzzy zone edges

- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is. Do not hold the plate up to light (transmitted light) or use a magnifying glass.



Reading exercise, Växjö laboratory

S. aureus

First occasion

	Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacine	Rifampicin
	26	27	26	29	20	30
	26	28	28	29	22	31
	26	29	28	29	25	31
	29	28	28	30	23	31
	26	27	27	28	19	30
	26	28	29	30	23	32
	26	29	29	30	21	31
	26	27	27	29	23	29
	25	27	26	29	20	32
	25	25	26	28	25	32
	28	27	26	27	22	30
Mean	26	27	27	29	22	31
SD	1.2	1.1	1.2	0.9	2.0	1.0

> 1 mm above mean

> 1 mm below mean

Discussion

S. aureus and norfloxacin

- If cultures are pure, read the inner zone.
- There is almost always a double zone for *S. aureus* and norfloxacin.



Reading exercise, Växjö laboratory

S. aureus

Following analysis and discussion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
27	24	25	26	18	28
27	25	26	28	18	29
27	25	26	27	17	27
27	25	26	27	16	27
27	25	26	27	19	28
28	24	24	26	17	25
27	26	27	29	19	29
27	24	26	28	18	28
27	25	23	26	18	27
28	25	26	27	18	30
27	24	24	25	17	27

Mean

27

25

25

27

18

28

SD

0.4

0.6

1.2

1.1

0.9

1.3

> 1 mm above mean

> 1 mm below mean

Example 8

- **Problem:** Meropenem zone diameters below QC range for *B. fragilis* ATCC 25285.
 - Other agents out of range for this strain?
 - No, but some are in the lower part of the QC ranges.
 - Meropenem within range for aerobic QC strains? (e.g. *E. coli* ATCC 25922)?
 - Yes!
 - Are FAA-HB plates humid?
 - Are zone edges fuzzy or difficult to read?

Specific instructions for anaerobes

- *Bacteroides* spp. and *C. perfringens*
 - Remove excess fluid from the cotton swab before inoculation to avoid over-inoculation
- FAA-HB plates must always be dried prior to inoculation
 - 20-25°C overnight or
 - 35°C for 15 min with the lid removed.
- Do not prolong incubation beyond 20 h!
 - Breakpoints and QC criteria are only valid for 16-20 h incubation.
- Follow specific reading instructions for anaerobes

Reading instructions for anaerobes

- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- Ignore any faint haze within the inhibition zone and read the most obvious zone. Tilt the plate towards you to better define the obvious zone edge.
- Ignore haemolysis and swarming and read inhibition of growth.

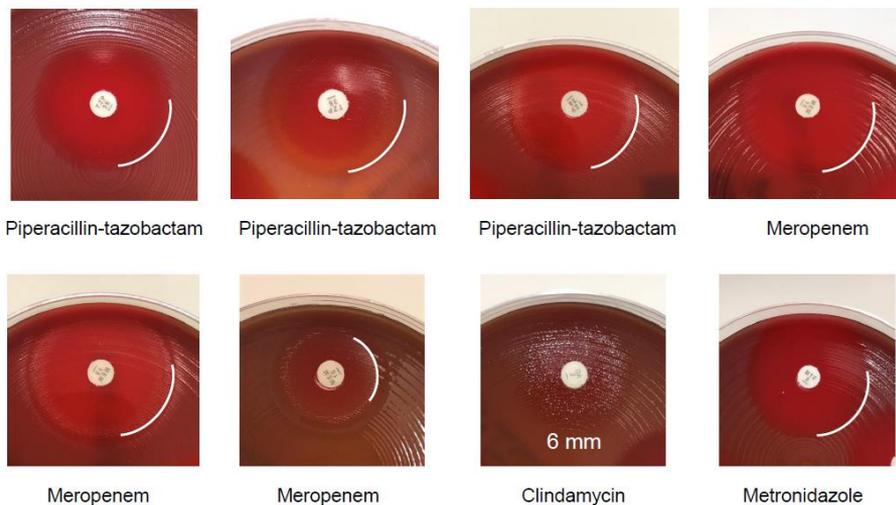


Reading guide

EUCAST disk diffusion for selected rapidly growing anaerobic bacteria on Fastidious Anaerobe Agar with 5% horse blood (FAA-HB)

Version 2.0
January 2023

Bacteroides spp.



Fusobacterium necrophorum



Clostridium perfringens



Control the anaerobic atmosphere

- ***C. perfringens* DSM 25589** is an aerotolerant strain.
- In combination with metronidazole 5 µg disk it is a sensitive indicator of the anaerobic atmosphere.
- Metronidazole zone < 25 mm indicates insufficient anaerobicity. This may affect growth and susceptibility test results.

Metronidazole zone diameters at different oxygen levels

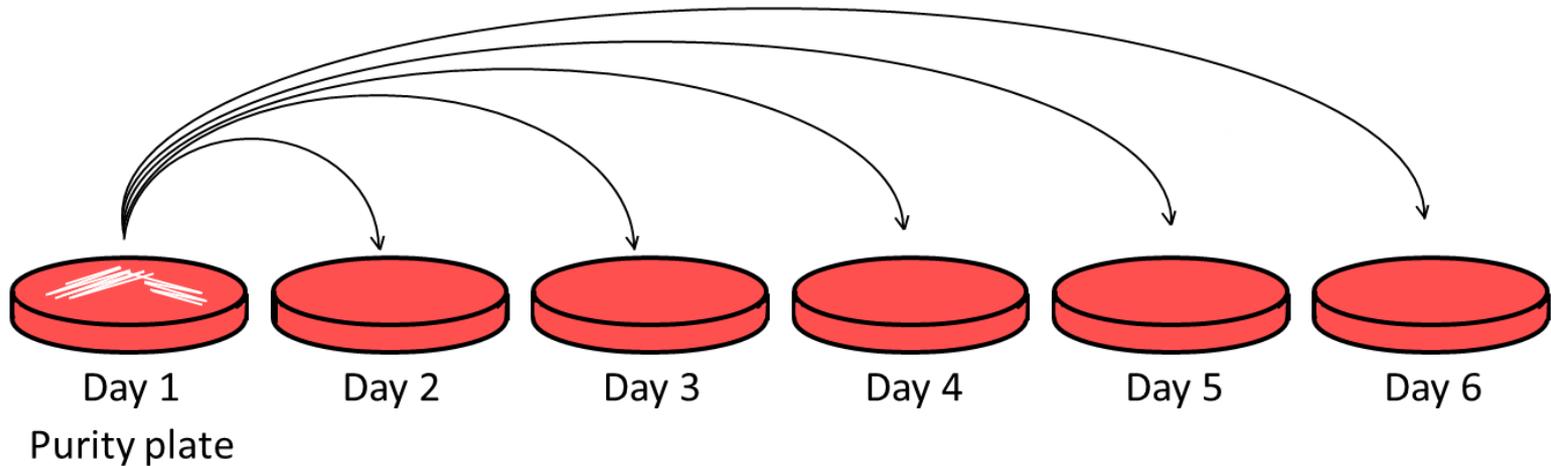
Oxygen	0%	0.16%	1%	2%	4%
	30	23	16	16	15
	31	22	17	15	15
	29	18	16	16	15
	30	21	18	16	14
Mean	30	21	17	16	15

Example 9

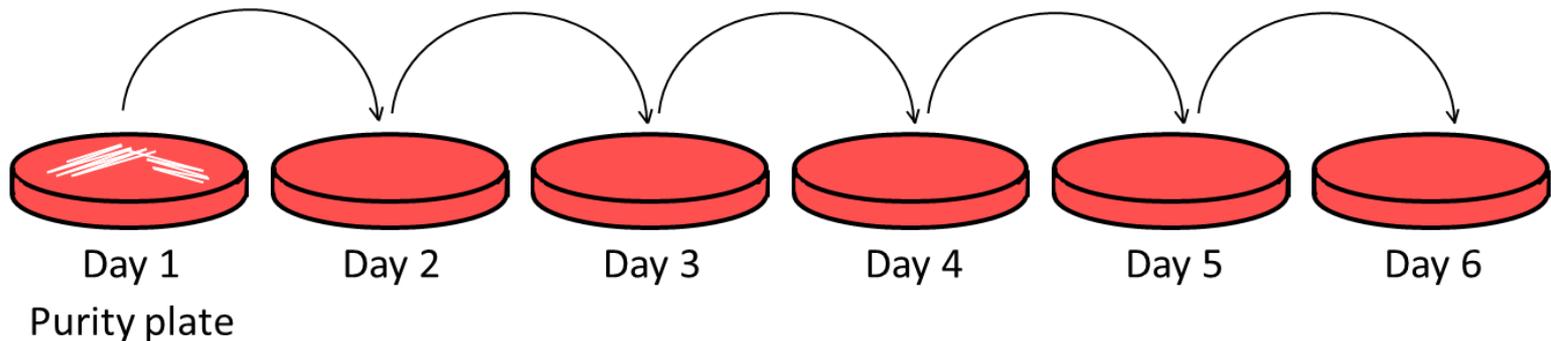
- **Problem:** Piperacillin-tazobactam zone diameters above QC range for *K. pneumoniae* ATCC 700603.
 - Pip-tazo zones within range for *E. coli* ATCC 25922?
 - Yes, piperacillin content ok!
 - Pip-tazo zones within range for *E. coli* ATCC 35218?
 - Yes, tazobactam content ok!
 - QC strain lost resistance?
- **Solution:** Prepare a new subculture of the QC strain from frozen stock.

Handling of QC strains

Non-fastidious QC strains



Fastidious QC strains



QC of β -lactam- β -lactamase inhibitor combinations

- Control the active component with a susceptible QC strain.
- Control the inhibitor with specific β -lactamase-producing QC strains.
 - *E. coli* ATCC 35218: clavulanic acid, tazobactam
 - *K. pneumoniae* ATCC 700603: avibactam, tazobactam
 - *K. pneumoniae* ATCC BAA-2814: relebactam, vaborbactam
- Both tests should be part of the routine QC.

Potential sources of error (1)

Medium	Storage of plates
	Not prepared according to instructions
	Batch to batch variation or change of supplier of agar
	Supplements (batch to batch variations, incorrect amount or expired)
	pH
	Agar depth/Agar volume
	Expiry date
Test conditions	“15-15-15 minute”-rule not adhered to (suspension used within 15 min, disks applied within 15 min, incubation within 15 min)
	Incubation (temperature, atmosphere and time)
	Incorrect inoculation (too light, too heavy or uneven)
	Reading conditions (background, light)
	Reading zone edges

EUCAST Disk Diffusion – Slide show

https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology

Potential sources of error (2)

Disks	Incorrect disk (wrong agent or wrong disk strength)
	Disk potency (incorrect storage, labile agent, expiry date)
	Disks not at room temperature when containers opened
	Too many disks on plate (interference between agents)
Control organisms	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

EUCAST Disk Diffusion – Slide show

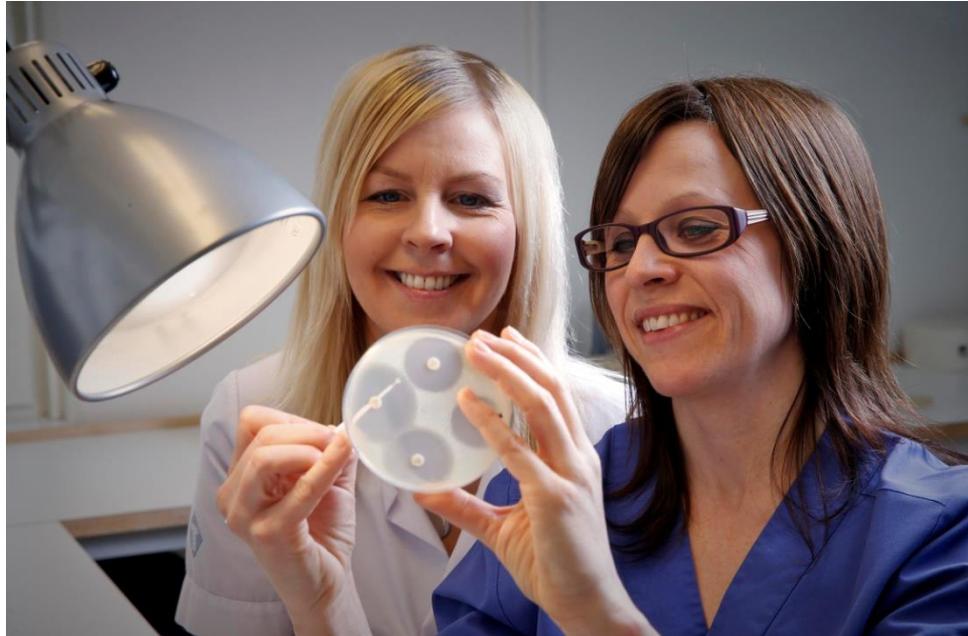
https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology

Questions and/or problem?

Contact us!

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Frequently Asked Questions (FAQ)

The EUCAST secretariat receives many questions on subjects ranging from how we determine breakpoints, how to use the MIC-distribution website, how ECOFFs are determined, disk diffusion methodolog etc. 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](#) - updated 2023-06-14

[Frequently asked questions related to EUCAST RAST](#) (Rapid AST directly from blood culture bottles).

Previous versions of FAQ:

- [Frequently Asked Questions](#) - valid from 2021-06-04 - 2023-06-14
- [Frequently Asked Questions](#) - valid from 2019-08-12 - 2021-04-03
- [Frequently Asked Questions](#) (and an **update** on question 8) - valid from 2018-02-18 - 2019-08-12
- [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

Sub-headings in the FAQ document

- EUCAST Disk Diffusion Test
 - Medium
 - Disks
 - Inoculum preparation
 - Reading zones of inhibition
 - General methodology
- Breakpoints –general
- Breakpoints –zone diameter
- Quality Control
- Other questions



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?](#)
9. [We have problems with fuzzy zone edges and haze within zones when testing anaerobic bacteria on FAA-HB media\). How can we improve this?](#)

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.

A more extensive evaluation of MH agar from several manufacturers (21 brands from 17 manufacturers) performed by the EUCAST Development Laboratory is available in Clin Microbiol Infect. 2020 Oct;26(10):1412.e1-1412.e5. (also available at https://www.eucast.org/publications_and_documents/publications_in_journals/).

2. What is the difference between Mueller-Hinton agar and Mueller-Hinton II agar?

The original specification of Mueller-Hinton agar did not define cation content, which is known to affect the activity of several agents, particularly aminoglycosides. Furthermore, the content of thymidine, which affects trimethoprim and trimethoprim-sulfamethoxazole activity, was undefined. Mueller-Hinton II agar is manufactured to contain a low concentration of thymidine and controlled concentrations of calcium and magnesium ions. Today, all Mueller-Hinton agars for susceptibility testing should be produced to meet the ISO technical specification 16782. Therefore, all Mueller-Hinton agars that yield inhibition zones within the acceptable ranges for EUCAST internal quality control strains can be used and EUCAST does not distinguish between MH and MH II.

An evaluation of MH agar from several manufacturers (21 brands from 17 manufacturers) performed by the EUCAST Development Laboratory is available in Clin Microbiol Infect. 2020 Oct;26(10):1412.e1-1412.e5. (also available at https://www.eucast.org/publications_and_documents/publications_in_journals/).

Warnings on www.eucast.org

- When AST products (disks, media, gradient tests etc.) do not perform to the expected standard
 - EDL informs the manufacturer and publish a warning on www.eucast.org
- EDL does not systematically test all products
 - The lack of a warning does not imply that there is no problem with the product in question
- Contact the EDL for advice if you suspect that problems with AST is related to a particular product!

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AST of bacteria

IVDR

Media preparation

MIC determination

Disk diffusion methodology

Disk diffusion implementation

Breakpoint tables

Quality Control

Strains with defined susceptibility

Calibration and validation

Warnings!

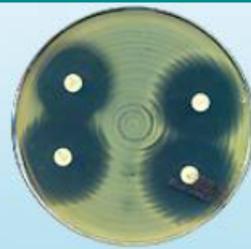
MIC testing services from EUCAST

Previous versions of documents

AST of mycobacteria

AST of fungi

AST of veterinary pathogens



Warnings! ▾

www.eucast.org

EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST Development Laboratories, from time to time discover products (disks, media batches, gradient tests, devices and/or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page. We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question. Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice. Use the [EUCAST subject related contact form](#).

1. Warnings against piperacillin-tazobactam gradient tests from two manufacturers removed.
2. Wide variation in disk quality in 16 disks from nine manufacturers
3. Problems with colistin susceptibility testing and several commercially available products
4. Vancomycin susceptibility testing in *Enterococcus faecalis* and *E. faecium* using MIC gradient tests
5. A problem with meropenem in a Thermofisher Sensititre panel (DKMGN) discovered.
6. Variable performance of the itraconazole agar in the azole resistance screening plate from VipCheck™
7. Warning against the use of gradient tests for determining benzylpenicillin MICs in *S. pneumoniae*
8. Mueller-Hinton media - EUCAST has evaluated 21 brands of commercially available MH powders
9. Mueller-Hinton pre-poured media - EUCAST has now evaluated commercially available pre-poured MH plates.
10. Cefiderocol MIC by broth microdilution on freeze dried panels from Thermofisher
11. Incomplete species validation and concentration range validation of commercially available testing material and devices.
12. Cefiderocol - advice pertaining to AST and the use of commercial panels
13. VIPcheck - variable performance with several batches.
14. Disk diffusion of anaerobe bacteria and the FAA-HB medium.
15. Thermo Fisher Scientific Sensititre broth external contamination.
16. Antifungal susceptibility testing of amphotericin B – problems detected with several commercially available products.

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Videos and Online seminars from EUCAST

Online seminars

- future seminars and recordings of previous seminars

[Online seminars and presentations.](#)

Instruction videos

In collaboration with the World Health Organisation (WHO), EUCAST publishes instruction videos on how to perform antimicrobial susceptibility testing (AST) using EUCAST recommended methods and interpretation.

The videos are published on Youtube™ and have an English speaker voice and English subtitles. Since not all countries may access Youtube™ videos in some languages are made available directly on the EUCAST web page.

Information added 2014-01-01: In some of the informational videos colleagues are advised to categorise the susceptibility of bacteria without breakpoints using the PK/PD breakpoints (previously available from the EUCAST breakpoint tables). This is no longer acceptable since the current opinion is that the PK/PD cut-off values may vary between species and EUCAST has therefore removed the PK/PD tab from the breakpoint table 14.0 (1 January, 2024). Instead we refer the EUCAST user to the guidance document "When there are no breakpoints" among the [guidance documents](#).

The videos cover the following topics:

1. Preparation of inoculum (English).
2. Inoculation of agar plates for disk diffusion (English).
3. Application of antibiotic disks and incubation of plates (English).
4. Reading of inhibition zone diameters (English).
5. Guidance on the use of the breakpoint table (English). See comment above added 2024.
6. Storage and handling of media and disks (English) - subtitles in other languages pending.
7. Quality control of AST in clinical microbiology (English) - subtitles in other languages pending.

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