EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Quality control of susceptibility testing

Erika Matuschek, Ph D

EUCAST Development Laboratory (EDL)

Växjö, Sweden

ICARS - ILRI webinar series

6 April 2022

Routine quality control (QC)

- Monitors day-to-day reliability and reproducibility
- Control of materials and equipment
  - Medium
  - Antibiotic disks/concentrations in panels
  - Incubators
  - Etc.
- Control of the procedures
  - Inoculum and inoculation
  - Incubation time
  - 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
Used to allow for random variation

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

Using a complete system

- Disk diffusion methodology
  - Media, inoculum, incubation time, disk potencies, reading instructions
- QC criteria
- Breakpoints
  - Zone diameter breakpoints are calibrated to clinical MIC breakpoints using the recommended methodology

- Never mix systems/guidelines!
  - EUCAST breakpoints are only valid if EUCAST methodology is used!

Maintainance of QC strains

Routine quality control

Recommended control strains

Organism	Number	Characteristics
E. coli	ATCC 25922	Susceptible, wild type
P. aeruginosa	ATCC 27853	Susceptible, wild type
S. aureus	ATCC 29213	Weak β-lactamase producer
E. faecalis	ATCC 29212	Susceptible, wild type
S. pneumoniae	ATCC 49619	Reduced susceptibility to benzylpenicillin
H. influenzae	ATCC 49766	Susceptible, wild type
Campylobacter jejuni	ATCC 33560	Susceptible, wild type

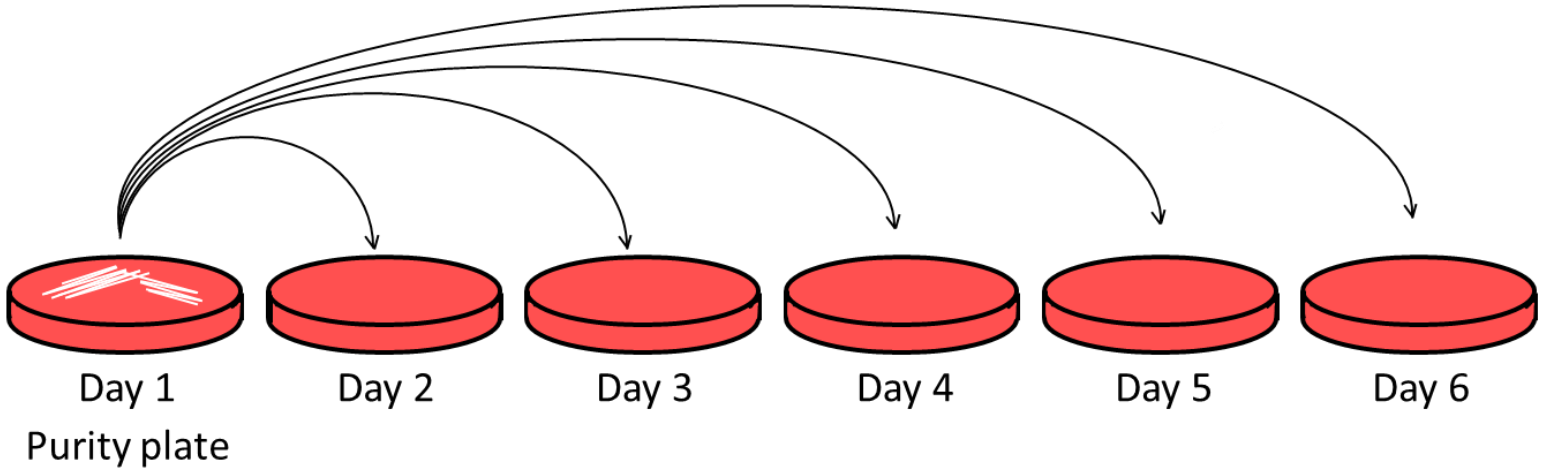
Organism	Number	Characteristics
E. coli	ATCC 35218	TEM-1 β-lactamase
K. pneumoniae	ATCC 700603	SHV-18 ESBL
K. pneumoniae	ATCC BAA-2814	KPC-3 carbapenemase
S. aureus	ATCC 29213	β-lactamase

Quality control strains

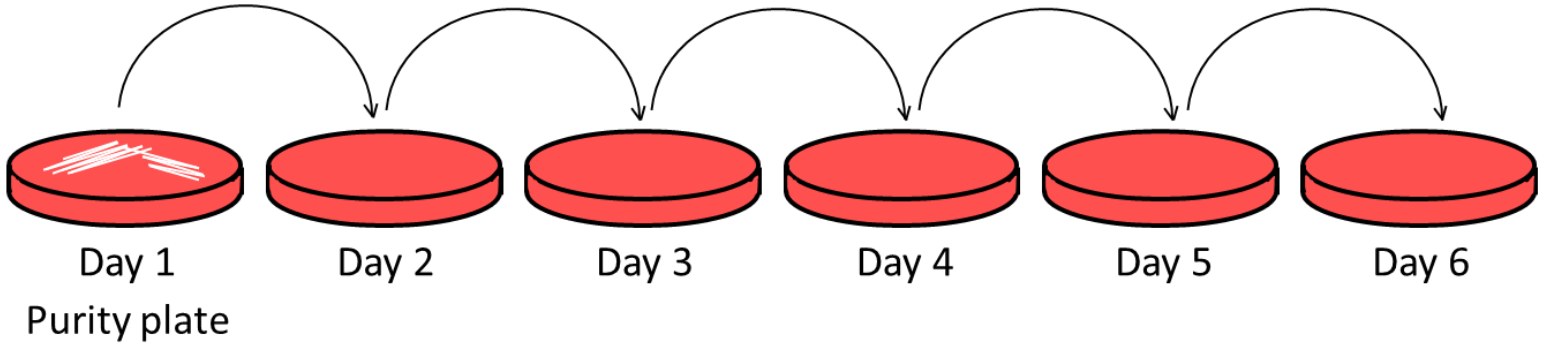
- Available from culture collections or commercial sources.
- Store by a method that minimises the risk of mutations, for example at -70°C on beads in glycerol broth.
- Store two vials of each control strain:
  1. In-use supply
  2. Archive
- Always pick several colonies when subculturing a QC strain, 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



Evaluation and presentation of QC results

EUCAST QC recommendations

- Control tests should be set up and checked daily, or at least four times per week, for antibiotics which are part of routine panels.
- Control tests should always be read and evaluated before reporting results for clinical isolates.
- Each day that tests are set up, examine the results of the last 20 consecutive tests.
- Examine results for trends and for zones falling consistently above or below the target.
- If two or more of 20 tests are out of range investigation is required.

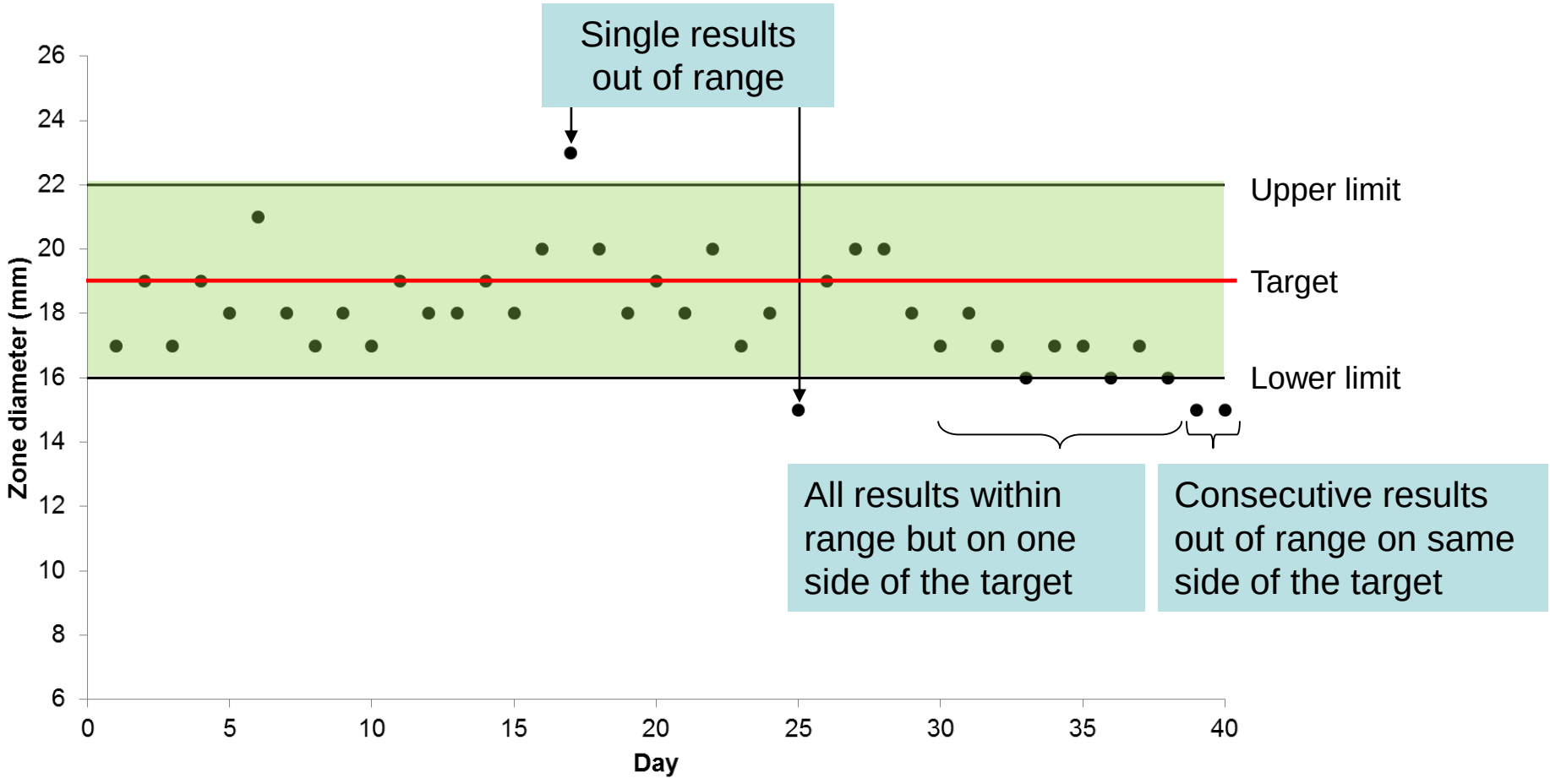
EUCAST Disk Diffusion Manual, Section 9

Response to QC results out of range

- If two non-consecutive control zone diameters of 20 tests are outside the acceptable range – then report susceptibility test results and investigate.
- If two consecutive control zone diameters of 20 tests are outside the acceptable range – then investigate before reporting susceptibility test results. The tests may have to be repeated.
- If multiple disks (>2) are out of range on one day – then investigate before reporting susceptibility test results. The tests may have to be repeated.
- If resistance in a resistant control strain is not recognised – then suppress susceptibility test results, investigate and retest.

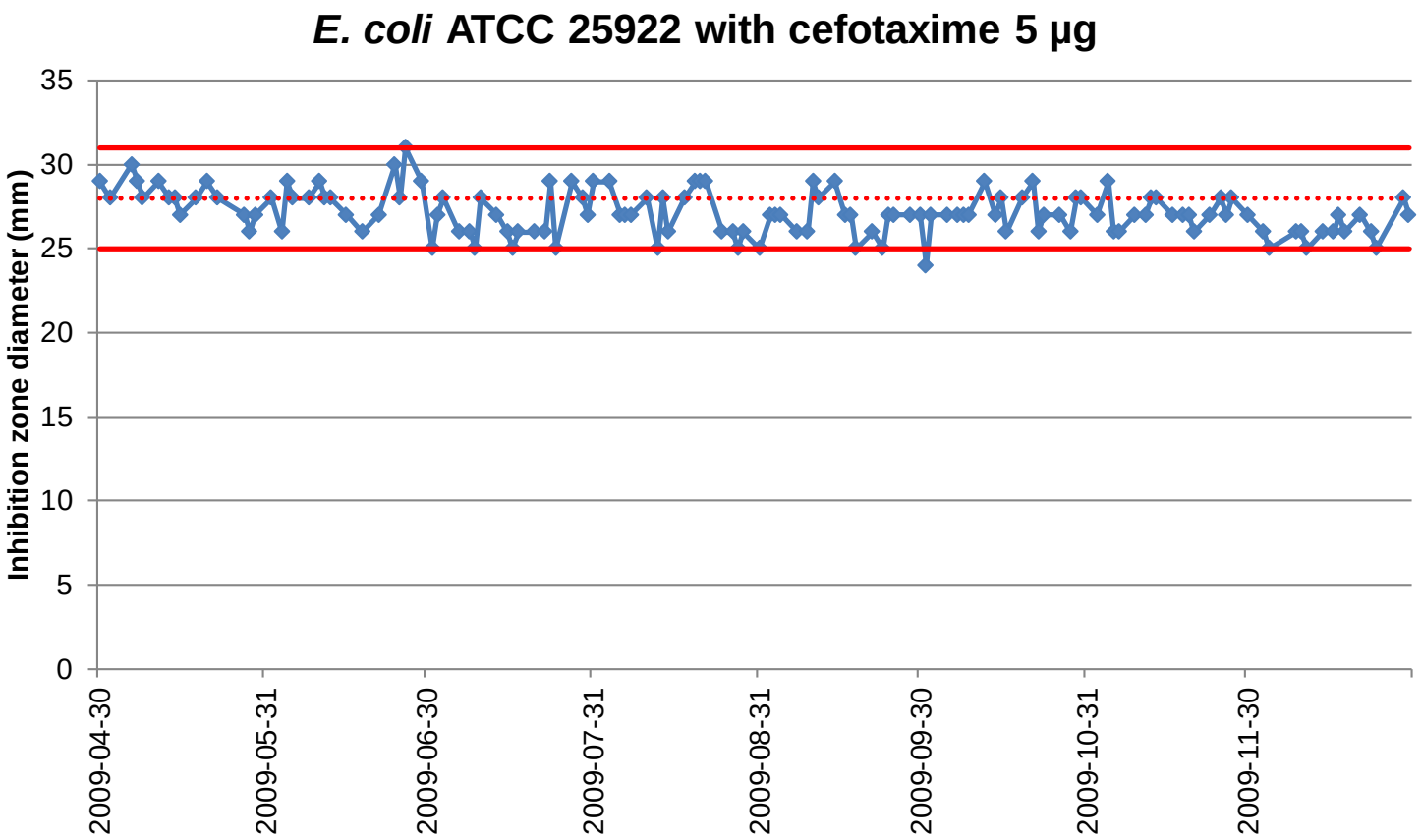
EUCAST Disk Diffusion Manual, Section 9

Monitoring Laboratory QC results



Example of registration of disk diffusion QC data Excel

Date	Zone	Lower	Target	Upper
2009-04-30	29	25	28	31
2009-05-02	28	25	28	31
2009-05-06	30	25	28	31
2009-05-07	29	25	28	31
2009-05-08	28	25	28	31
2009-05-11	29	25	28	31
2009-05-13	28	25	28	31
2009-05-14	28	25	28	31
2009-05-15	27	25	28	31
2009-05-18	28	25	28	31
2009-05-20	29	25	28	31
2009-05-22	28	25	28	31
2009-05-27	27	25	28	31
2009-05-28	26	25	28	31
2009-05-29	27	25	28	31
2009-06-01	28	25	28	31
2009-06-03	26	25	28	31
2009-06-04	29	25	28	31
2009-06-05	28	25	28	31
2009-06-08	28	25	28	31
2009-06-10	29	25	28	31
2009-06-11	28	25	28	31
2009-06-12	28	25	28	31
2009-06-15	27	25	28	31
2009-06-18	26	25	28	31
2009-06-21	27	25	28	31
2009-06-24	30	25	28	31
2009-06-25	28	25	28	31
2009-06-26	31	25	28	31
2009-06-29	29	25	28	31
2009-07-01	25	25	28	31
2009-07-02	27	25	28	31
2009-07-03	28	25	28	31
2009-07-06	26	25	28	31
2009-07-08	26	25	28	31



Example of registration of disk diffusion QC data LIMS

ESCHERICHIA COLI ATCC 25922 MECILLINAM 27 (24 - 30 enh)

220323	29
220321	26
220318	28
220317	29
220316	28
220314	29
220311	26
220310	27
220309	27
220307	29
Medelvärde	27.8 CoV: 4.4 %

ESCHERICHIA COLI ATCC 25922 AMPICILLIN (iv adm) 18.5 (15 - 22 enh)

220323	16
220321	18
220318	20
220317	20
220316	18
220314	20
220311	19
220310	18
220309	16
220307	18
Medelvärde	18.3 CoV: 8.2 %

Example of registration of disk diffusion QC data Project

Strain	CTX5	CAZ10	MER10	TZP36	SXT25	CIP5	LEV5	PEF5
E. coli ATCC 25922	28	27	32	25	27	35	34	30
E. coli ATCC 25922	27	26	31	23	26	33	31	30
E. coli ATCC 25922	27	27	32	25	27	33	31	29
E. coli ATCC 25922	27	26	33	24	28	31	29	28
E. coli ATCC 25922	27	26	33	24	26	33	31	29
E. coli ATCC 25922	26	25	31	22	27	32	30	29
E. coli ATCC 25922	27	27	32	25	27	34	33	31
E. coli ATCC 25922	28	26	33	23	27	34	32	29
E. coli ATCC 25922	29	28	33	25	28	35	34	30
E. coli ATCC 25922	27	27	33	24	29	33	33	30
Mean	**27**	**27**	**32**	**24**	**27**	**33**	**32**	**30**
Target	**28**	**26**	**31-32**	**24**	**26**	**33**	**33**	**29**
Range	**25-31**	**23-29**	**28-35**	**21-27**	**23-29**	**29-37**	**29-37**	**26-32**

Strain	CTX5	CAZ10	MER10	TZP36	SXT25	CIP5	LEV5	PEF5
E. coli ATCC 35218	31	29	35	27	25	34	32	31
E. coli ATCC 35218	29	28	35	23	25	31	29	28
E. coli ATCC 35218	31	30	35	27	22	33	31	30
E. coli ATCC 35218	28	28	35	25	25	32	30	28
E. coli ATCC 35218	30	29	34	26	21	31	29	28
E. coli ATCC 35218	29	28	34	23	24	31	30	28
E. coli ATCC 35218	31	30	35	27	21	32	31	30
E. coli ATCC 35218	29	29	34	23	26	32	31	27
E. coli ATCC 35218	29	29	34	25	21	32	31	27
E. coli ATCC 35218	29	28	33	23	24	31	30	27
Mean	**30**	**29**	**34**	**25**	**23**	**32**	**30**	**28**
Target	**-**	**-**	**-**	**24**	**-**	**-**	**-**	**-**
Range	**-**	**-**	**-**	**21-27**	**-**	**-**	**-**	**-**

On target ± 1 mm
Upper limit
Lower limit
Out of range

QC testing scheme

- Routine testing
  - Daily, or at least 4 times a week (EUCAST recommendation)
  - Each agent should be tested against at least one relevant strain
  - Strains can be divided between departments (e.g. *S. aureus* at wound dept, and *E. coli* at urine dept)
- Projects
  - At each test occasion or every second day (EDL routines)
  - Include relevant QC strains to cover the species of the clinical isolates and relevant strains to control all agents used

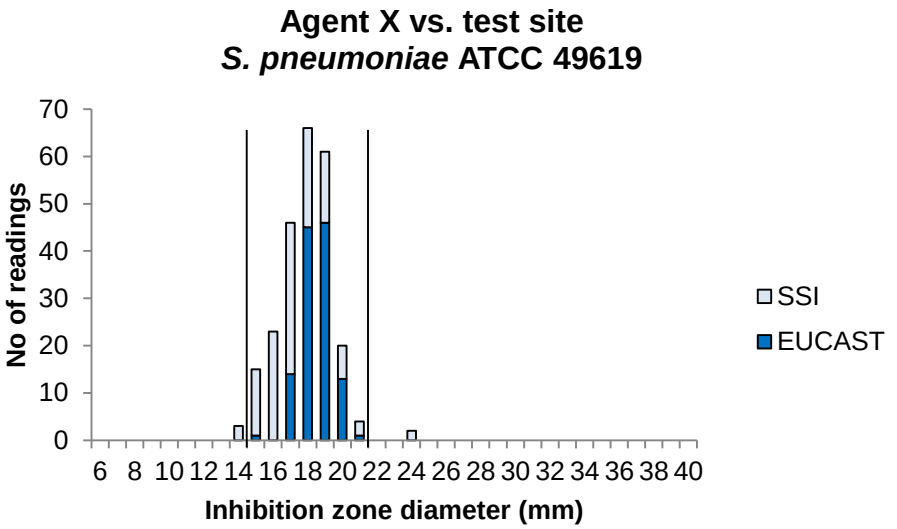
Data used to establish QC ranges

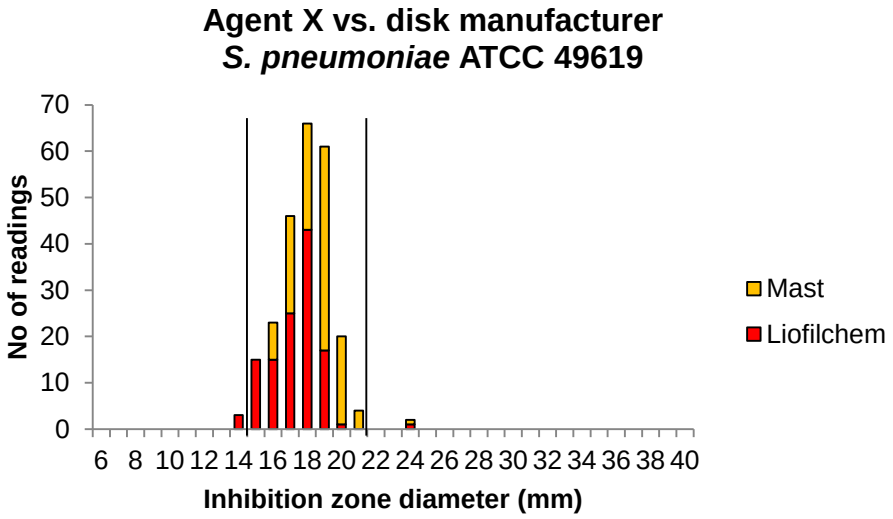
EUCAST disk QC studies

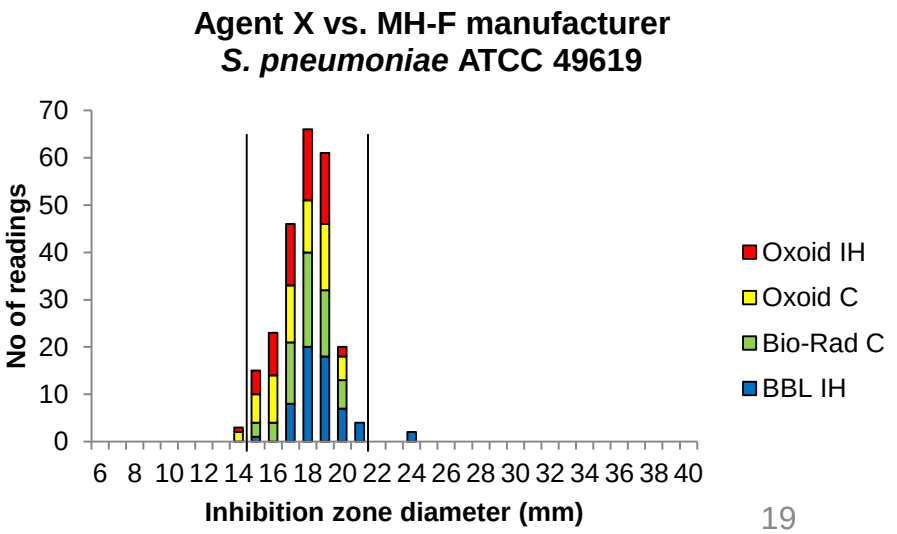
- Study layout (EUCAST SOP 9.2)
  - Initial two-site study: 2 labs x 3-4 media (15 replicates)
  - Validation study: ≥4 sites x local media (10 replicates)
  - Disks from 2-3 disk manufacturers
- Reasonable variation between materials from different manufacturers, test sites and readers are built into the system

1: Initial two-site study

Agent X		*S. pneumoniae* ATCC 49619
Initial two-site QC study (n=240)	Mean (mm)	17.9
	Median (mm)	18
	Range (mm)	14-24
QC validation study (n=100)	Mean (mm)	17.9
	Median (mm)	18
	Range (mm)	15-22
Suggested range (target)		**15-21 (18)**
Readings within range (%)		**98.2**



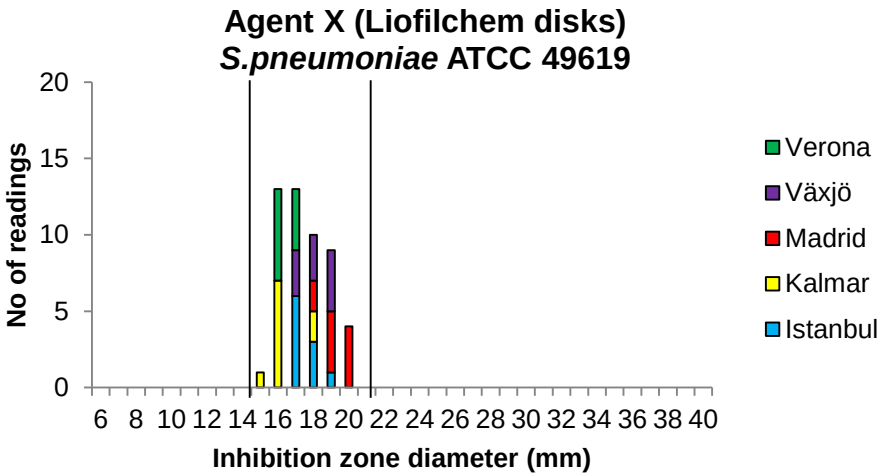


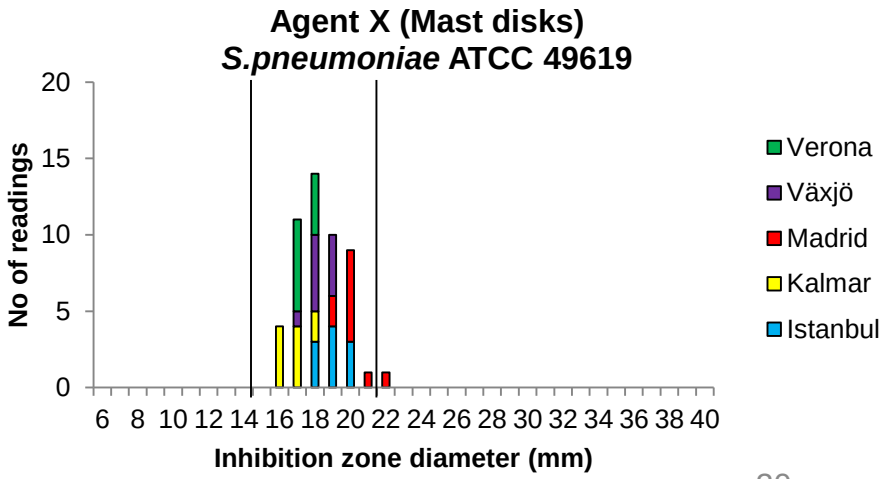


2: Validation study

Agent X		*S. pneumoniae* ATCC 49619
Initial two-site QC study (n=240)	Mean (mm)	17.9
	Median (mm)	18
	Range (mm)	14-24
QC validation study (n=100)	Mean (mm)	17.9
	Median (mm)	18
	Range (mm)	15-22
Suggested range (target)		**15-21 (18)**
Readings within range (%)		**98.2**

Laboratory	**Medium**
Istanbul	bioMérieux commercial
Kalmar	Oxoid in-house
Madrid	Oxoid commercial
Växjö	Bio-Rad in-house
Verona	Oxoid in-house





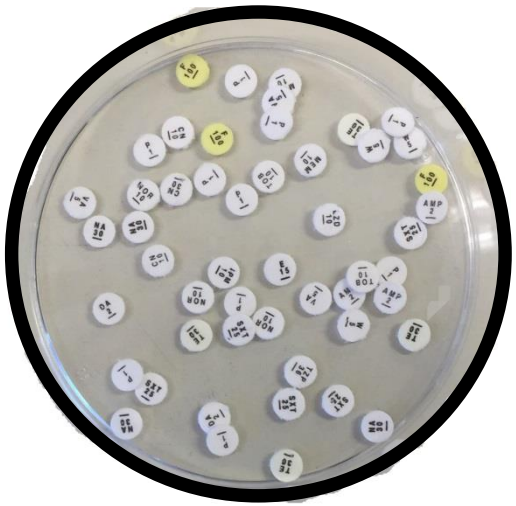
Cases

Examples of QC problems and suggestions for troubleshooting and solutions

Example 1

- **Problem:** Amoxicillin-clavulanic acid zone diameters above QC range for *H. influenzae* ATCC 49766.
  - Correct disk potency?
    - No, 20-10 µg-disk used!

- **Solution:** Change to correct disk potency



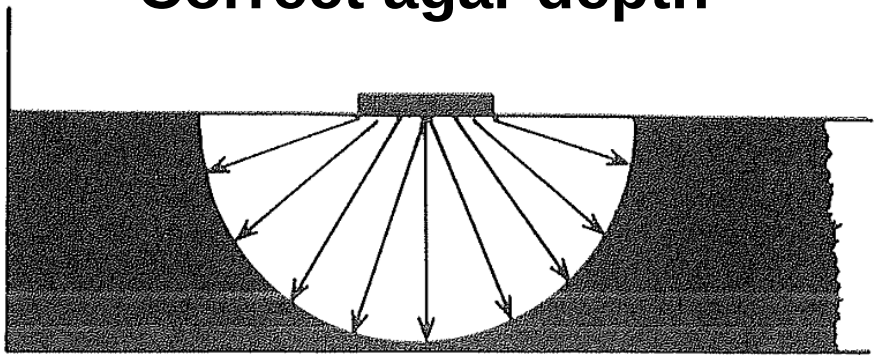
Example 2

- **Problem:** Amoxicillin-clavulanic acid zone diameters above QC range for *H. influenzae* ATCC 49766.
  - Correct disk potency?
    - Yes, 2-1 µg-disk used.
  - Other agents within range?
    - Yes, but some are in the upper part of the QC ranges.
  - Amoxicillin-clavulanic acid zones for *S. pneumoniae* ATCC 49619 within range?
    - Yes, but in the upper part of the QC range.

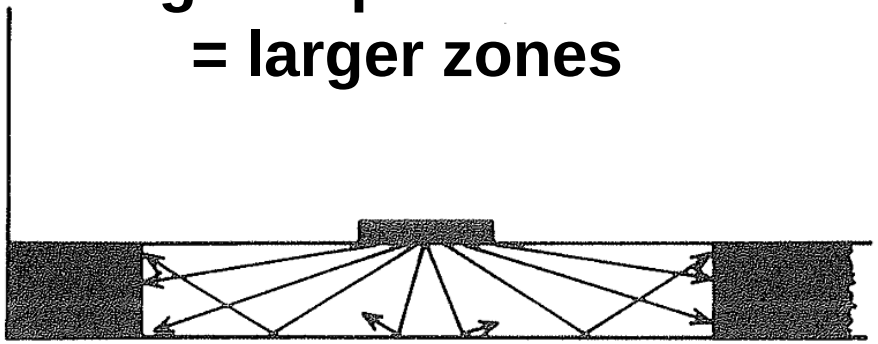
Example 2

- **Problem:** Amoxicillin-clavulanic acid 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

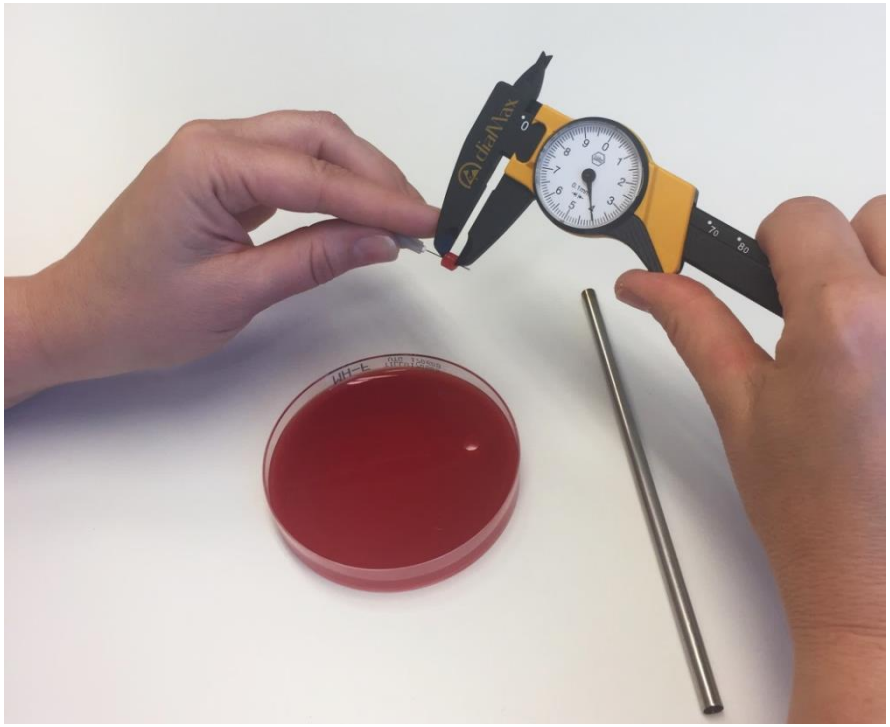


Agar depth too thin = larger zones



Measuring agar depth

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



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

Example 3

- **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 3

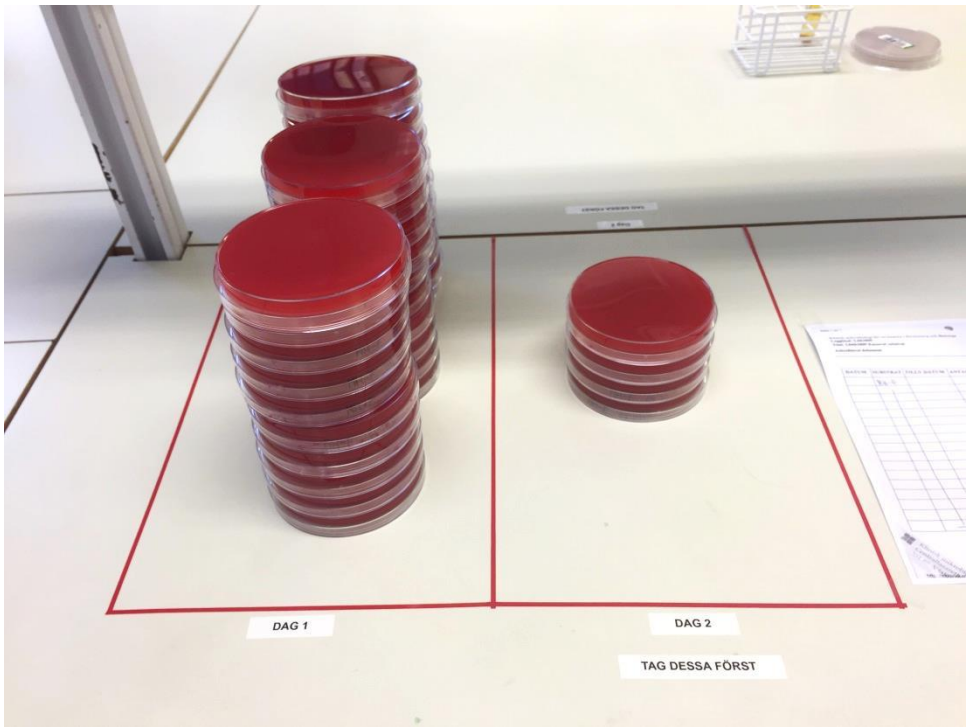
- **Problem:** Meropenem zone diameters below QC range for *H. influenzae* ATCC 49766.
  - MH-F plates within the expiry date?
    - Yes!
  - MH-F plates sufficiently dry?
    - No, condensation inside the lid!

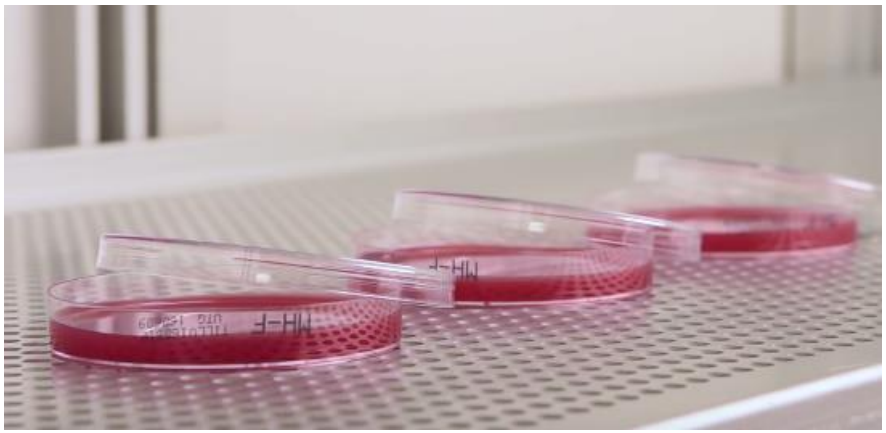
- **Solution:** Check storage conditions and/or dry plates prior to inoculation.

Drying and storage of MH-F

Drying and storage of MH-F

- 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.





Example 4

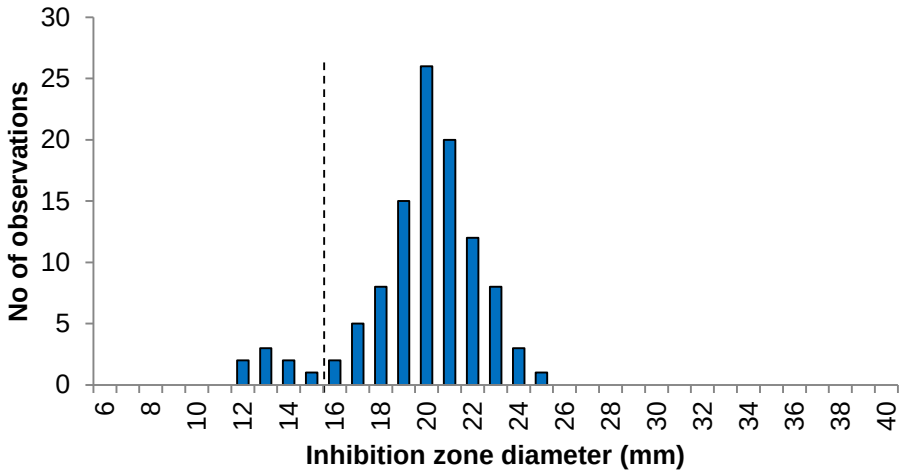
- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
  - Methodology adhered to?
    - Yes!
  - Disks and media stored and handled according to instructions?
    - Yes!
  - Reproducible between batches of disks?
    - Yes!
  - Reproducible between batches of media?
    - Yes!

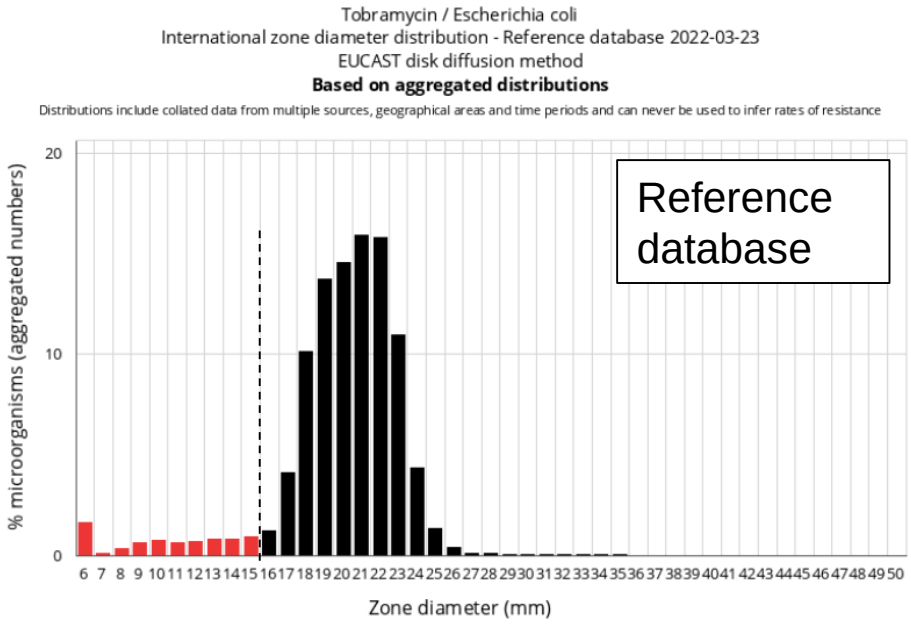
Example 4

- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
  - Are results related to the MH media used?
    - Yes, zones are larger when tested using the same strain and disk on agar from another manufacturer.

E. coli and tobramycin

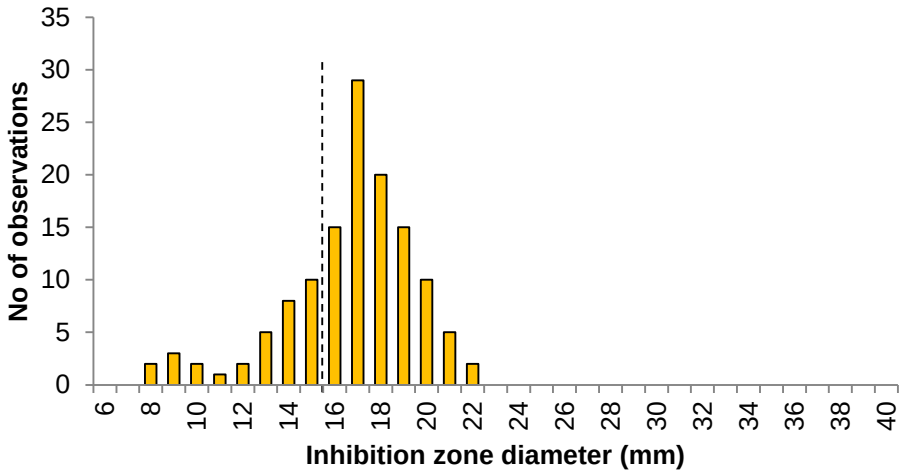
- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
  - Do results for clinical isolates comply with reference data in the EUCAST database?
    - **Yes!**
      - **Solution:** Monitor data over time

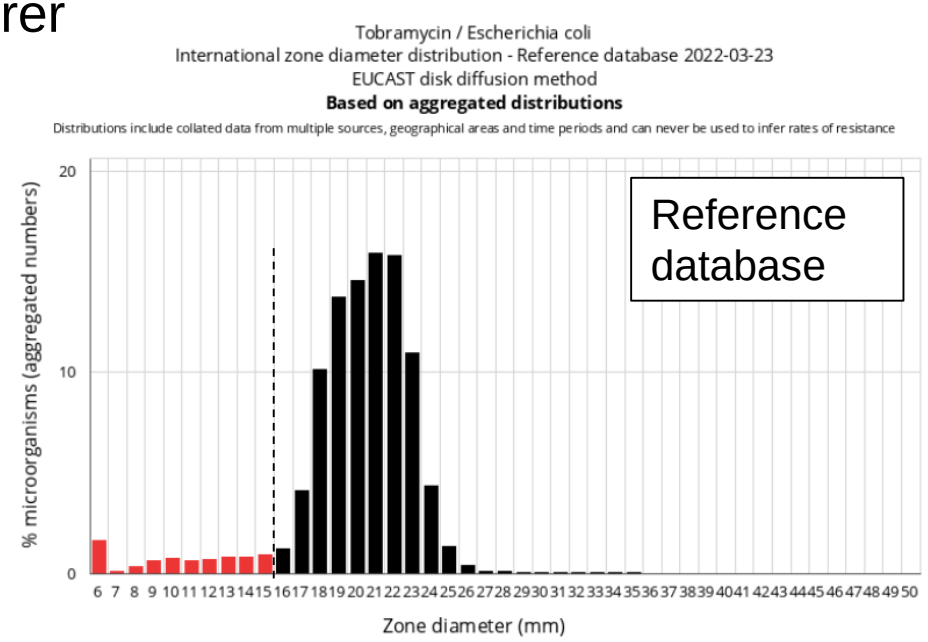




E. coli and tobramycin

- **Problem:** Tobramycin zones in the lower part of the range, and sometimes out of range for *E. coli* ATCC 25922.
  - Do results for clinical isolates comply with reference data in the EUCAST database?
    - **No!**
      - **Solution:** Contact manufacturer and consider changing to another manufacturer





Example 5

- **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 4

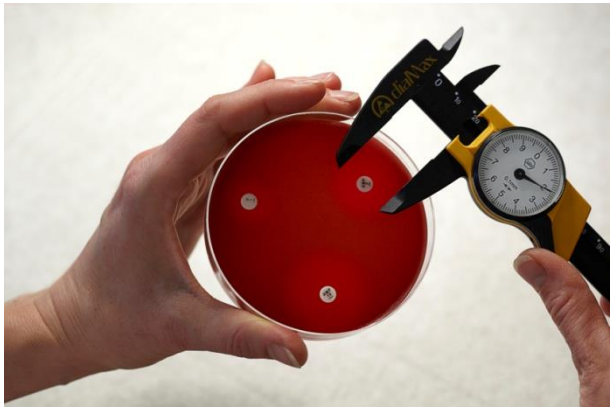
- **Problem:** Ciprofloxacin zone diameters below QC range for *E. coli* ATCC 25922.
  - Agar plates correctly inoculated?
    - Yes!
  - Ciprofloxacin zones edges?
    - Fuzzy zone edges!

- **Solution:** Follow reading instructions and perform reading exercises to educate staff.

Reading zones

- Read **MH** plates from the back against a dark background illuminated with reflected light.

- Read **MH-F** plates from the front with the lid removed illuminated with reflected light.



Fuzzy zone edges
Enterobacteriaceae

- 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.

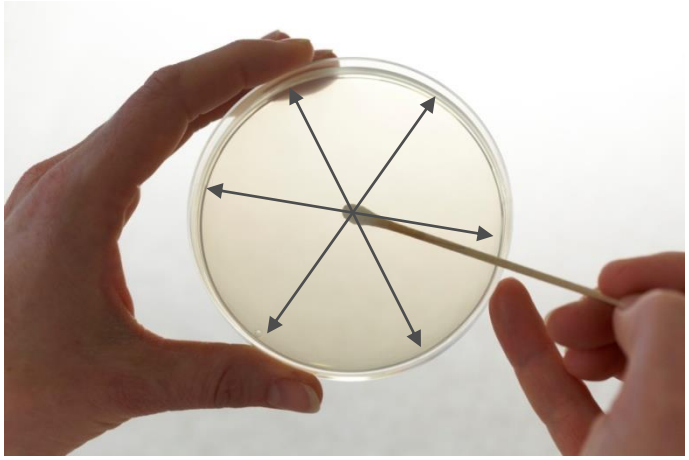
Reading of zones with fuzzy zone edges for Enterobacteriaceae.

Example 6

- **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.



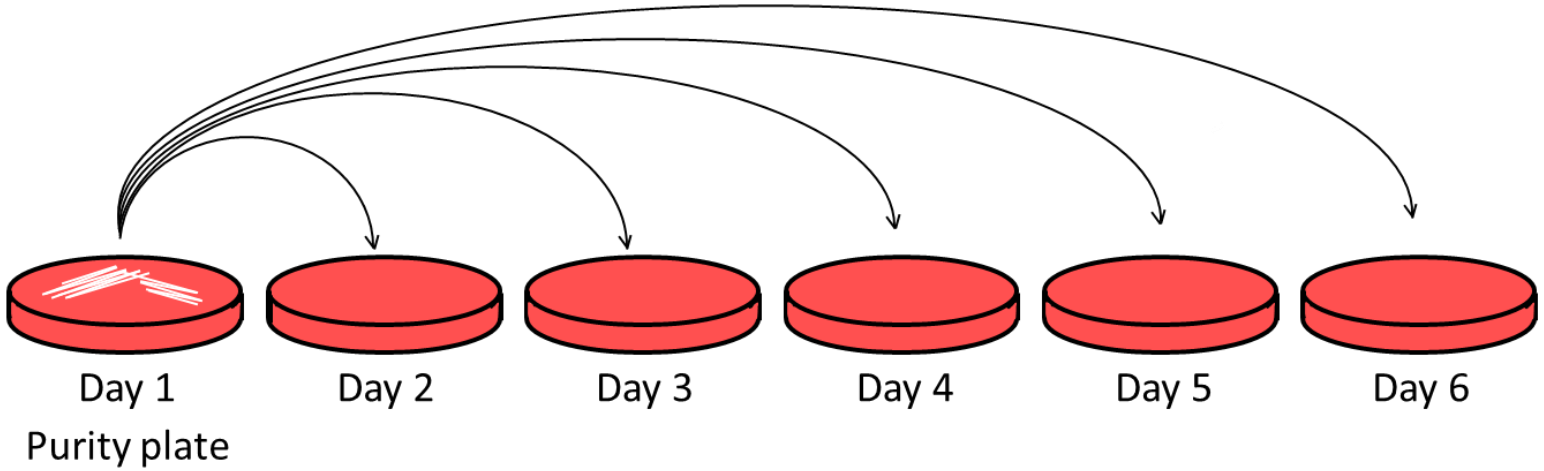
Example 7

- **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 component ok!
  - ATCC 700603 lost resistance!

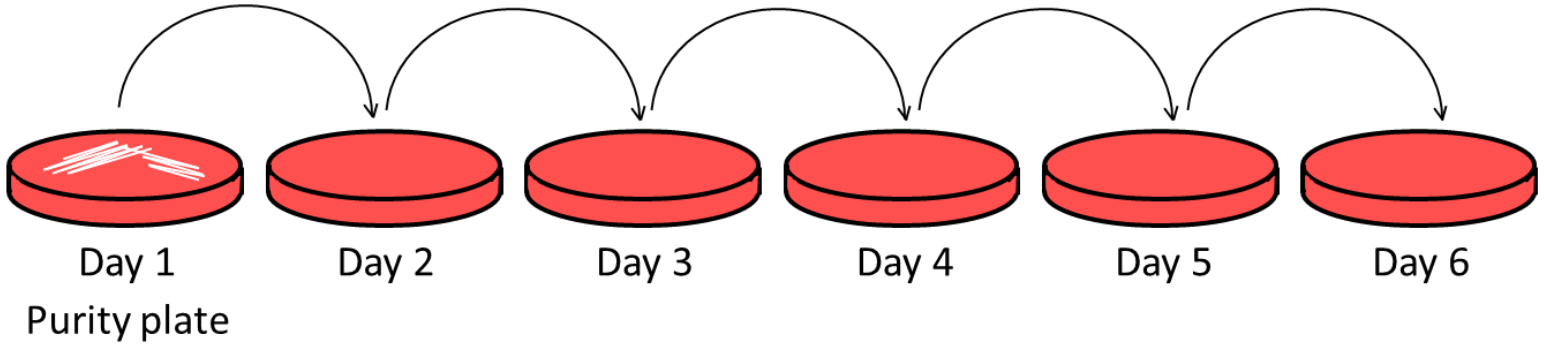
- **Solution:** Prepare a new subculture of the QC strain from frozen stock.

Handling of QC strains

Non-fastidious QC strains



Fastidious QC strains



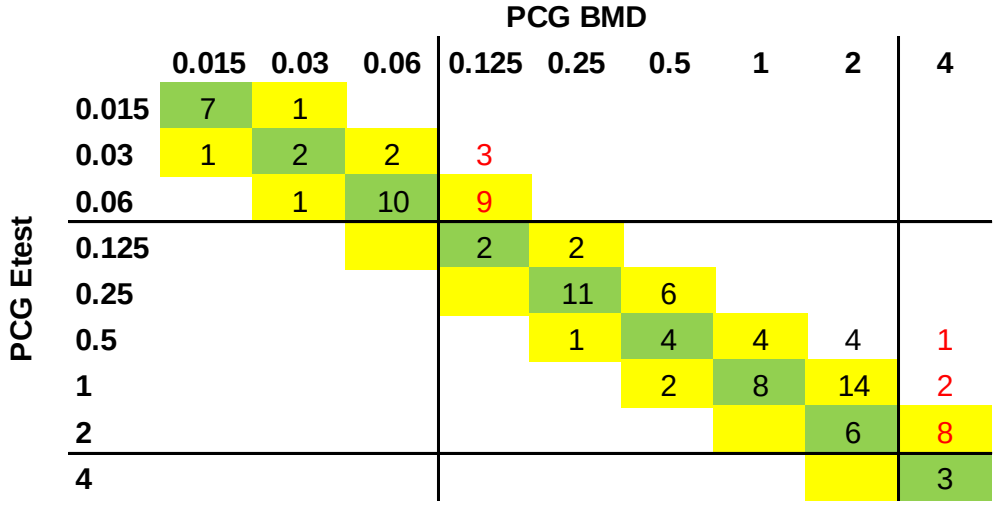
Example 8

- **Problem:** Clinical isolate of *S. pneumoniae* with oxacillin zone 18 mm and PCG gradient test MIC 0.032 mg/L.
  - QC data for *S. pneumoniae* ATCC 49619 ok?
    - Oxacillin disk diffusion: Yes, the mean value is on target ±1 mm.
    - PCG gradient test: Within range, but in the lower part.

- **Solution:** Perform BMD (or send to reference laboratory)!

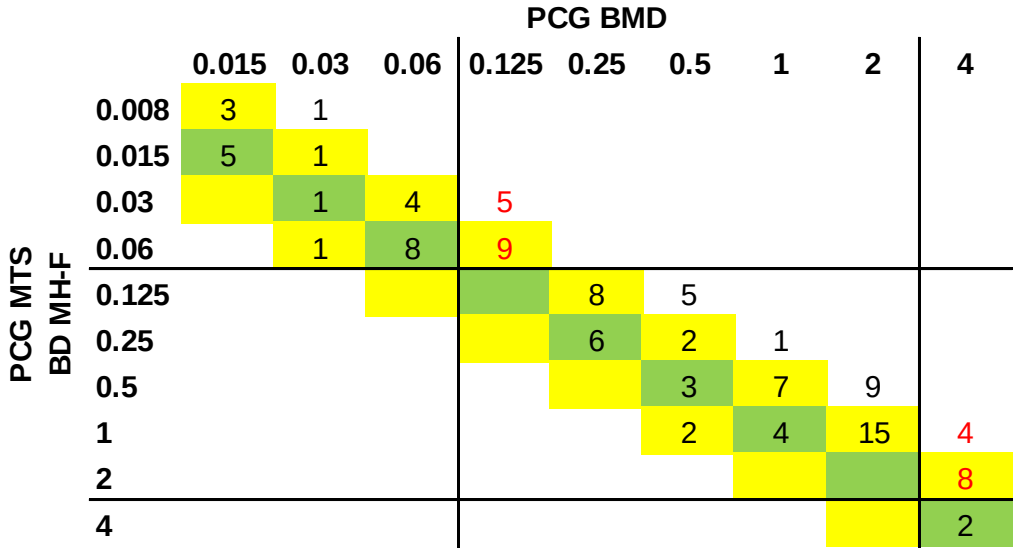
Correlation between gradient test MICs and BMD
S. pneumoniae and benzylpenicillin

Etest



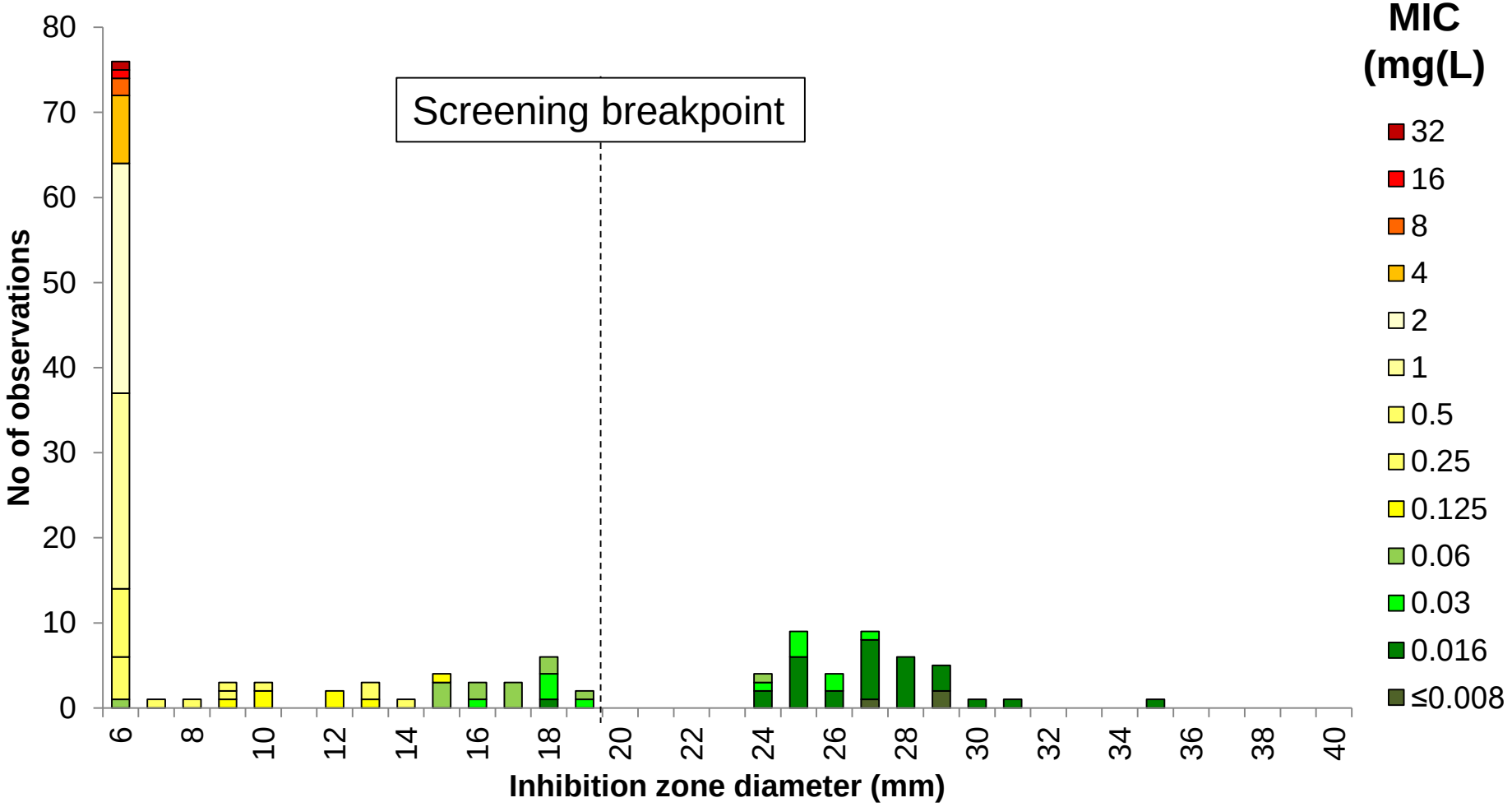
>2 dilutions lower	1
2 dilutions lower	9
1 dilution lower	46
Identical	53
1 dilution higher	5
2 dilutions higher	
>2 dilutions higher	

MTS



>2 dilutions lower	
2 dilutions lower	25
1 dilution lower	57
Identical	29
1 dilution higher	3
2 dilutions higher	
>2 dilutions higher	

Oxacillin 1 µg screening test



EUCAST/CCUG reference panel

- A panel of 10 *S. pneumoniae* with varying beta-lactam susceptibility is available from CCUG for validation of MIC determination of benzylpenicillin in *S. pneumoniae*. Reference MICs are available on the EUCAST website, https://www.eucast.org/ast_of_bacteria/strains_with_defined_susceptibility/.

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”-rule not adhered to (suspension used within 15 min, disks applied within15 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

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

Home Contact Sitemap Newsletter

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

www.eucast.org

search term

AST of bacteria

- Organization
- Consultations
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- SOP
- MIC and zone distributions and ECOFFs
- AST of bacteria
  - Media preparation
  - MIC determination
  - Disk diffusion methodology
  - Disk diffusion implementation
  - Breakpoint tables
  - Quality Control
  - Strains with defined susceptibility
  - Calibration and validation

... Quality Control

EUCAST Quality Control

QC tables v 11.0 (2021-01-01)

Changes between old and new versions are described in the QC table introduction.

QC tables - → previous versions

www.eucast.org

Frequently Asked Questions (FAQ)

- Organization
- Consultations
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- SOP
- MIC and zone distributions and ECOFFs
- AST of bacteria
- AST of mycobacteria
- AST of fungi
- AST of veterinary pathogens
- **Frequently Asked Questions (FAQ)**
- Meetings
- Publications and documents
- Presentations and statistics
- Videos and online seminars
- Warnings!

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 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 2021-06-04

Frequently asked questions concerning EUCAST RAST (Rapid AST directly from blood culture bottles).

Previous versions of FAQ:

- **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

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

www.eucast.org

search term

AST of bacteria

... Disk diffusion methodology

EUCAST Disk Diffusion Test Methodology

The EUCAST disk diffusion test is based on MH media and disks of a good quality. It is calibrated to EUCAST clinical breakpoints using broth microdilution for MIC determination. Updates are published regularly.

See also **EUCAST instruction videos**.

Disk diffusion - Manual v 9.0 (1 January, 2021)

Disk diffusion - Slide show v 9.0 (1 January, 2021)

Disk diffusion - Reading guide v 8.0 (1 January, 2021)

EUCAST disk diffusion of anaerobic bacteria is under development 2021. Reviewed clinical breakpoints and disk diffusion correlates will be published with breakpoint table v 12.0 (1 January, 2022). The method will be valid for 5 species (*Bacteroides* spp, *Prevotella* spp, *Fusobacterium necrophorum*, *Clostridium perfringens* and *Cutibacterium acnes*) and for anaerobic incubation for 16 - 20h (extended incubation not allowed). For anyone who wants to prepare and practice, EUCAST already now publish the methodology, reading guide and QC criteria.

- **Disk diffusion and QC criteria for anaerobic bacteria - Manual v 1.0** (20 September, 2021)

www.eucast.org

Videos and online seminars

Videos and online seminars

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.

The following topics are covered:

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).
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.

Instruction videos on EUCAST susceptibility testing with subtitles in other languages than English:

Instruction videos - English subtitles.

Problems and/or questions?

Please contact us!

erika.matuschek@eucast.org

EUCAST

EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases