EUCAST THE EUCAST DEVELOPMENT LABORATORY

European Society of Clinical Microbiology and Infectious Diseases

Phenotypic Methods Used in Antimicrobial Susceptibility Testing

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Methods in Antimicrobial Susceptibility Testing

- **Phenotypic**
  - Based on MIC and breakpoints
  - Predicts resistance and susceptibility
  - Quantitative
  - Reference method: MIC
  - Surrogate tests: Disk diffusion, gradient (strip) test, semi-automated AST systems, computer-aided microscopy, microcalorimetri, flow cytometri
- **Genotypic**
  - Detection of the resistance determinant (gene)
  - Predicts resistance but can't guarantee susceptibility
  - Not-quantitative
  - Breakpoints not required but validation does
  - No reference method
- **Mechanistic**
  - Detection of a resistance mechanism indicates resistance
  - Predicts resistance but can't guarantee susceptibility
  - Not-quantitative
  - Breakpoints not required but validation does
- **Expert rules**
  - Intrinsic resistance (or susceptibility)
  - IF-THEN rules
  - Not-quantitative
  - Untrustworthy – prone to change (changes over time)

Rationale behind Antimicrobial Susceptibility Testing

Wild-type organism

Devoid of phenotypically detectable resistance mechanism

Non-wild-type organism

- *De novo* mutation (*e.g.,* chromosomal mutations)
- Acquisition of resistance determinants (*e.g.,* plasmid, transposon, integron) from another organism

Antimicrobial Susceptibility Testing

Aim: Produce results to determine/categorise the susceptibility

S - Susceptible, standard dosing regimen
I - Susceptible, increased exposure
R - Resistant

Qualitative assessments using the categories

- Disk diffusion

Quantitative results (e.g., minimum inhibitory concentration)

- Broth dilution

- Agar dilution

- Gradient strips

- Semi-automated antimicrobial susceptibility testing systems

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is performed with phenotypic or genotypic methods. The basis of phenotypic methods is the minimum inhibitory concentration (MIC). Clinical MIC breakpoints determine whether the organism is categorised as susceptible, intermediate or resistant to the agent in question. Other methods should be calibrated to reference MIC methods.

Users of EUCAST breakpoints should use the EUCAST disk diffusion method or other susceptibility testing systems calibrated to EUCAST breakpoints and terminology in accordance with EUCAST breakpoint tables.

For videos on how to perform disk diffusion testing according to EUCAST - CLICK here!
For more information - CLICK here.

- **Media preparation**
  On how to prepare media for MIC and disk testing
- **MIC determination** of nonfastidious and fastidious organisms
  Broth microdilution methodology according to ISO and EUCAST
- **Disk diffusion methodology**
  Detailed description of the EUCAST disk diffusion test
- **Disk diffusion implementation**
  Guidance documents on how to implement the disk diffusion test
- **Breakpoint tables**
  Current MIC and zone diameter breakpoint tables
- **Quality Control and QC tables**
  Current tables of MIC and zone diameter ranges for quality control strains
- **Strains with well defined susceptibility**
  Strains with resistance mechanisms where MIC-values (and zone diameters) have been well defined using several different broth microdilution panels before and after freeze drying.
- **Calibration and validation**
  Presentation of raw data used in the development and calibration of EUCAST disk diffusion breakpoints against broth microdilution MIC-values.
- **Warnings!**
  EUCAST alerts on malfunctioning susceptibility testing material and procedures.
- **Guidance documents**
  Guidance notes on specific susceptibility testing issues
- **MIC determination services provided by the EUCAST Development Laboratory.**
- **Previous breakpoints and QC tables**
  Earlier versions of breakpoint and QC tables

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)
- **Disk diffusion of anaerobic bacteria - Reading guide v 1.0** (20 September, 2021)

For translations to other languages - see ➔ **Translations**.

Antimicrobial susceptibility testing

EUCAST disk diffusion method

Version 9.0

January 2021

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Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories

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Abstract

With the support of ESCMID and European countries, EUCAST has developed a disk diffusion test with zone diameter breakpoints correlated with the EUCAST clinical MIC breakpoints. The development of the EUCAST disk diffusion method and quality control criteria are described, together with guidance on quality control and implementation of the method in clinical microbiology laboratories. The method includes the use of Mueller–Hinton agar without supplements for non-fastidious organisms and with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD for fastidious organisms, a standardized inoculum resulting in confluent growth, an incubation time of 16–20 h, a reading guide on how to read zone diameters on individual species-agent combinations and zone diameter breakpoints calibrated to the EUCAST clinical MIC breakpoints. EUCAST recommendations are described in detail and updated regularly on the EUCAST website (http://www.eucast.org).

Keywords: Antimicrobial susceptibility testing, disk diffusion, European Committee on Antimicrobial Susceptibility Testing, MIC, Mueller–Hinton agar, zone diameter breakpoints

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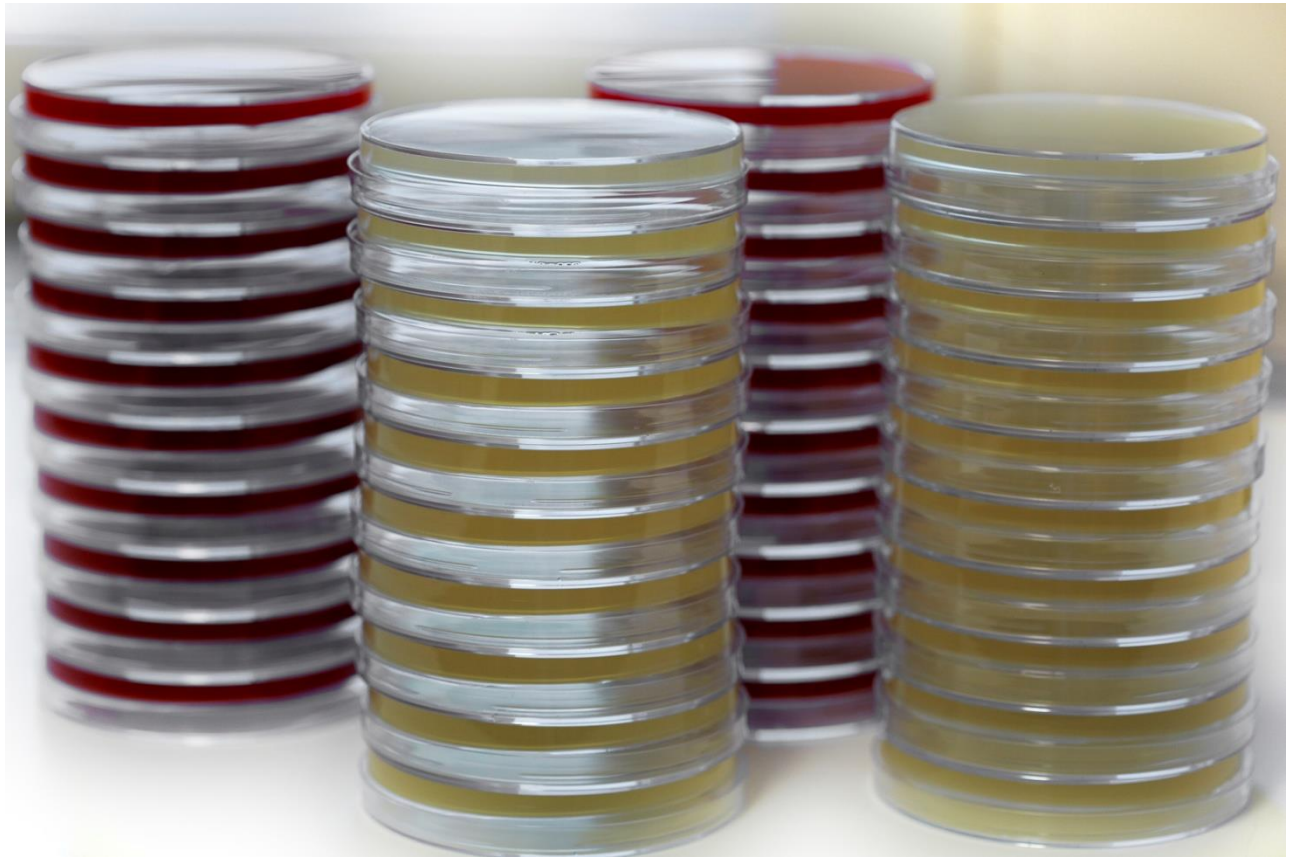
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 9.0
January 2021

Susceptibility testing media



EUCAST 2021 Version 9.0

Susceptibility testing media

- Un-supplemented Mueller-Hinton (MH) agar is used for non-fastidious organisms.
- MH with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD (MH-F, **M**ueller-**H**inton **F**astidious) is used for fastidious organisms.
- Use β-NAD with a purity of ≥ 98%.

Media for non-fastidious organisms

Organisms	Medium
Enterobacterales *Pseudomonas* spp. *Stenotrophomonas maltophilia* *Acinetobacter* spp. *Staphylococcus* spp. *Enterococcus* spp. *Aeromonas* spp. *Achromobacter xylosoxidans* *Bacillus* spp. *Burkholderia pseudomallei*	Mueller-Hinton agar

Media for fastidious organisms

Organisms	Medium
Streptococcus pneumoniae Streptococcus groups A, B, C and G Viridans group streptococci *Haemophilus influenzae* *Moraxella catarrhalis* *Listeria monocytogenes* *Pasteurella multocida* *Campylobacter jejuni* and *coli* *Corynebacterium* spp. *Aerococcus sanguinicola* and *urinae* *Kingella kingae*	Mueller-Hinton agar + 5% mechanically defibrinated horse blood + 20 mg/L β-NAD (MH-F)
Other fastidious organisms	Pending

In-house preparation of media

- Prepare media according to the manufacturer's instructions.
- For MH-F, do not add blood or β-NAD until the medium has cooled to 42-45°C and mix well after the supplements have been added to the cooled medium.
- Pour plates on a level surface to give a uniform depth of 4.0 ± 0.5 mm. Adjust the volume if the agar depth is within the acceptable range but repeatedly above or below 4 mm.

  Approximate volume for 90 mm circular plate: 25 mL, 100 mm circular plate: 31 mL, 150 mm circular plate: 71 mL, 100 mm square plate: 40 mL. Plate dimensions may differ between manufacturers. Ascertain that a correct volume, based on the true dimensions of the Petri dish in use, is calculated.

Agar plates may be prepared in-house from dehydrated media or purchased ready-poured from commercial sources. Dehydrated Mueller-Hinton media should meet the requirements in the ISO Technical specification, ISO/TS 16782, 2016 and the quality control criteria published by EUCAST.

MH and MH-F agar plates are prepared as follows:

1. Reagents	
1.1	MH agar powder from commercial source.
1.2	Mechanically defibrinated horse blood.
1.3	β-Nicotinamide adenine dinucleotide (β-NAD), purity ≥98%.

2. Preparation of β-NAD stock solution	
2.1	Dissolve β-NAD in sterile deionized water to a concentration of 20 mg/mL.
2.2	Sterilize the solution through a 0.2 µm membrane filter.
2.3	The stock solution may be stored at -20°C in aliquots and defrosted as required. Do not refreeze unused solution.

MH and MH-F agar plates

3. Preparation of agar plates	
3.1	Prepare and autoclave MH agar according to the manufacturer's instructions.
3.2	Cool medium to 42-45°C.
3.3	For MH-F, aseptically add 50 mL mechanically defibrinated horse blood and 1 mL β-NAD stock solution per litre medium. Mix well and dispense immediately.
3.4	Dispense medium into sterile Petri dishes to give a level depth of 4 ± 0.5 mm (approximately 25 mL in a 90 mm circular plate, 31 mL in a 100 mm circular plate, 71 mL in a 150 mm circular plate, 40 mL in a 100 mm square plate). Ascertain that a correct volume, based on the true dimensions of the Petri dish in use, is calculated. Plate dimensions may differ between manufacturers.
3.5	Allow the agar to set before moving the plates.
3.6	The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates.

MH and MH-F agar plates

4. Storage of agar plates	
4.1	Store plates prepared in-house at 4-8°C.
4.2	For plates prepared in-house, plate drying, storage conditions and shelf life should be determined as part of the laboratory quality assurance programme.
4.3	Commercially prepared plates should be stored as recommended by the manufacturer and used within the labelled expiry date.
4.4	For agar plates (commercially or in-house prepared) 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.

Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method
Version 6.0, January 2020 www.eucast.org

MH and MH-F agar plates

5. Quality control	
5.1	Use a surface pH electrode to check that the pH is within the range 7.2-7.4.
5.2	Check that the agar depth is 4 ± 0.5 mm.
5.3	Check that the medium supports good growth of control strain(s) of the intended test organisms.
5.4	Perform disk diffusion for quality control strains according to EUCAST recommendations and check that inhibition zones are within acceptable ranges for all bacteria-antimicrobial agent combinations used (EUCAST QC tables).

Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method
Version 6.0, January 2020 **www.eucast.org**

Drying and storage of agar plates

- In-house prepared plates:
  - Store at 4-8°C.
  - Plate drying, storage conditions and shelf life should be determined locally.
- Commercially prepared plates:
  - Store as recommended by the manufacturer.
  - Use within the labelled expiry date.

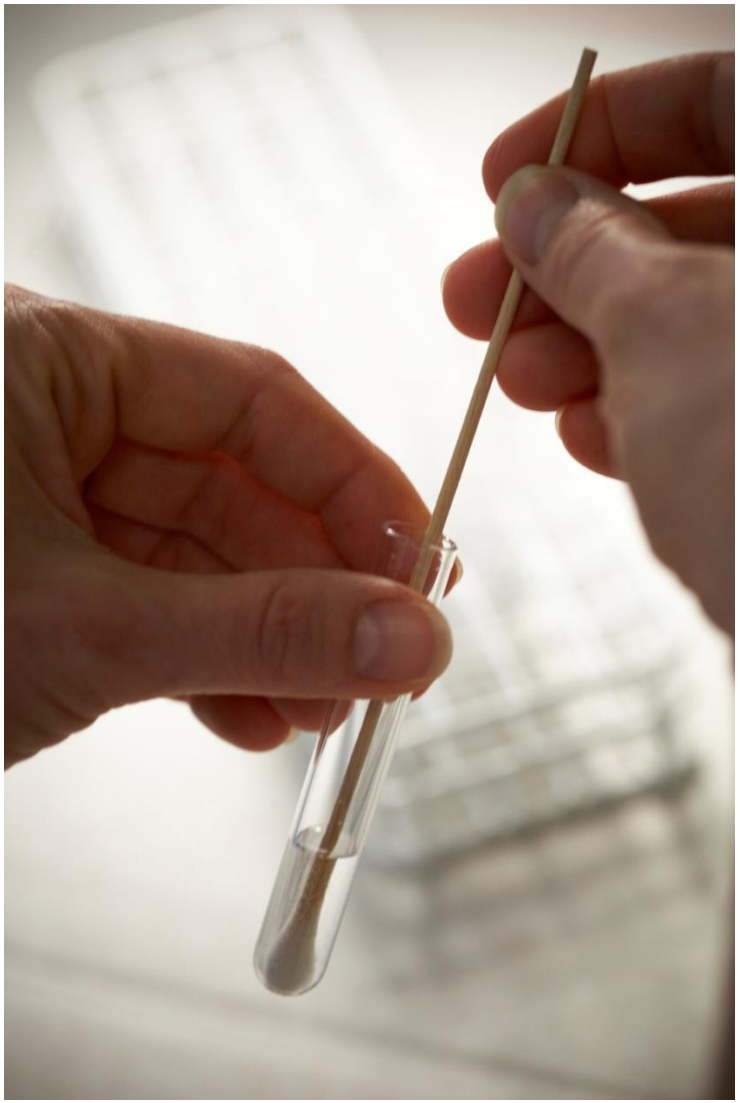
Drying and storage of agar plates

- Make sure that agar plates are at room temperature prior to inoculation.
- The surface of the agar should be dry before use. Excess moisture may cause fuzzy zone edges and/or haze within zones.
  - No drops of water should be visible on the surface of the agar or inside the lid. This is often seen with plates stored in plastic bags or sealed containers.
- If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min.
- Do not over-dry plates.

Inoculum

- The method requires an inoculum suspension with a turbidity equivalent to a 0.5 McFarland standard*.

* Approximately corresponding to 1-2 x10^8 CFU/mL for *E. coli.*



Select well-isolated colonies from overnight growth on non-selective medium

Inoculum preparation

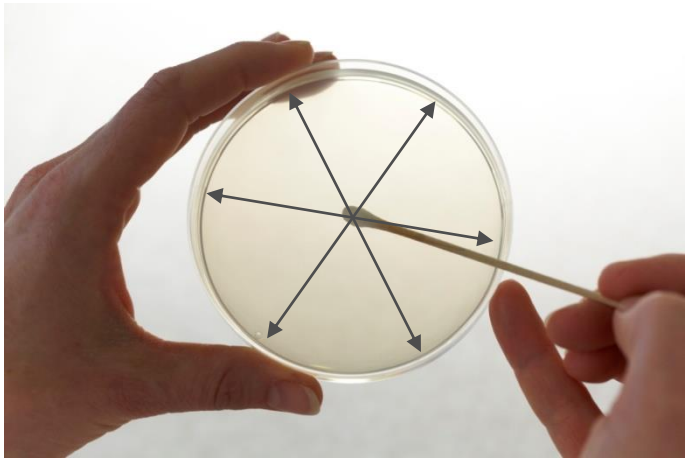
- Use a sterile loop or cotton swab to pick colonies from an overnight culture on non-selective media. If possible, use several morphologically similar colonies to avoid selecting an atypical variant.
- Suspend in saline and mix to an even turbidity.
- Adjust the density of the suspension to 0.5 McFarland by adding saline or more bacteria. Preferably use a photometric device to measure the turbidity.
  - Exception: *Streptococcus pneumoniae* is suspended to 0.5 McFarland from a blood agar plate, but to 1.0 McFarland from a chocolate agar plate.

Inoculation of plates

- Optimally, use the inoculum suspension within 15 minutes of preparation and always within 60 minutes.
- Make sure that agar plates are at room temperature prior to inoculation.
- Dip a sterile cotton swab into the suspension.
- For Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation.
- For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

Inoculation of plates

- Spread the inoculum evenly over the entire surface by swabbing in three directions or by using a plate rotator.
- For Gram-positive bacteria, take particular care to ensure that there are no gaps between streaks.
- When inoculating several agar plates with the same inoculum, dip the cotton swab into the suspension for each agar plate.



Storage of antimicrobial disks

- Store stocks and working supplies of disks according to the manufacturers' instructions.
  - Some agents are more labile than others and may have specific recommendations.
- Store disks in current use in sealed containers with a moisture-indicating desiccant and protected from light.
- To prevent condensation, allow disks to reach room temperature before opening containers.
  - Rather keep disks at room temperature during the day than transfer repeatedly to and from cold storage.
- Do not use disks beyond the manufacturer's expiry date.

TABLE 2. Differences in disk content between EUCAST and CLSI disk diffusion methods

Antimicrobial agent[a]	EUCAST disk content	CLSI disk content
Benzylpenicillin	1 unit	10 units
Ampicillin	2 and 10 μg[b]	10 μg
Amoxicillin-clavulanate	2–1 and 20–10 μg[c]	20–10 μg
Piperacillin	30 μg	100 μg
Piperacillin-tazobactam	30–6 μg	100–10 μg
Cefotaxime	5 μg	30 μg
Ceftaroline	5 μg	30 μg
Ceftazidime	10 μg	30 μg
Gentamicin (test for HLAR)	30 μg	120 μg
Vancomycin	5 μg	30 μg
Linezolid	10 μg	30 μg
Nitrofurantoin	100 μg	300 μg

HLAR, high-level aminoglycoside resistance.
[a]Ceftriaxone 30 μg and cefepime 30 μg are also under consideration for lower disk contents in the EUCAST disk diffusion test.
[b]2 μg for *Haemophilus influenzae*, *Pasteurella multocida*, *Listeria monocytogenes*, *Staphylococcus saprophyticus* and streptococci.
[c]2–1 μg for *Haemophilus influenzae*, *Moraxella catarrhalis* and *Pasteurella multocida*.

Matuschek et al. *Clin Microbiol Infect* 2014;20:O255-O266
doi: 0.1111/1469-0691.12373

Application of antimicrobial disks

- Apply disks within 15 min of inoculation.
- Disks must be in close and even contact with the agar surface.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured.

Incubation of plates

- Invert agar plates and make sure disks do not fall off the agar surface.
- Incubate plates within 15 min of disk application.
- Stacking plates in the incubator may affect results due to uneven heating. The efficiency of incubators varies, but for most incubators, a maximum of five plates per stack is appropriate.
- Incubate MH plates at 35±1°C in air.
- Incubate MH-F plates at 35±1°C in air with 4-6% CO_2 (except for *Campylobacter*).

Incubation of plates

Organism	Incubation conditions
Enterobacterales	35±1ºC in air for 18±2 h
Pseudomonas spp.	35±1ºC in air for 18±2 h
Stenotrophomonas maltophilia	35±1ºC in air for 18±2 h
Acinetobacter spp.	35±1ºC in air for 18±2 h
Staphylococcus spp.	35±1ºC in air for 18±2 h
Enterococcus spp.	35±1ºC in air for 18±2 h (24 h for glycopeptides)
Aeromonas spp.	35±1ºC in air for 18±2 h
Achromobacter xylosoxidans	35±1ºC in air for 18±2 h
Bacillus spp.	35±1ºC in air for 18±2 h
Burkholderia pseudomallei	35±1ºC in air for 18±2 h

Incubation of plates

Organism	Incubation conditions
Streptococcus groups A, B, C and G	35±1ºC in air with 4-6% CO_2 for 18±2 h
Viridans group streptococci	35±1ºC in air with 4-6% CO_2 for 18±2 h
Streptococcus pneumoniae	35±1ºC in air with 4-6% CO_2 for 18±2 h
Haemophilus influenzae	35±1ºC in air with 4-6% CO_2 for 18±2 h
Moraxella catarrhalis	35±1ºC in air with 4-6% CO_2 for 18±2 h
Listeria monocytogenes	35±1ºC in air with 4-6% CO_2 for 18±2 h
Pasteurella multocida	35±1ºC in air with 4-6% CO_2 for 18±2 h
Campylobacter jejuni and *coli*	41±1ºC in microaerobic environment for 24 h (40-48 h)
Corynebacterium spp.	35±1ºC in air with 4-6% CO_2 for 18±2 h (40-44 h)
Aerococcus sanguinicola and *urinae*	35±1ºC in air with 4-6% CO_2 for 18±2 h (40-44 h)
Kingella kingae	35±1ºC in air with 4-6% CO_2 for 18±2 h (40-44 h)
Other fastidious organisms	Pending

The 15-15-15 minute rule

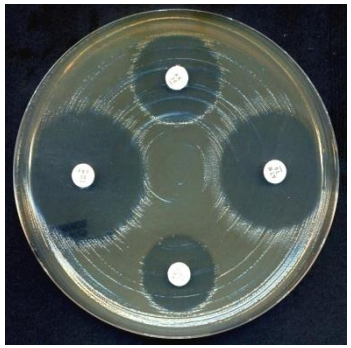
Follow these instructions for disk diffusion:

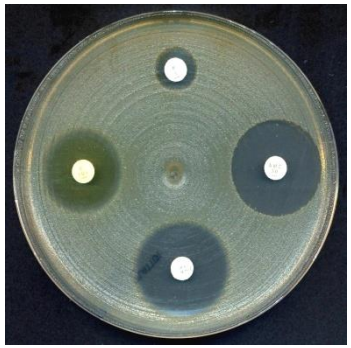
- Use the inoculum suspension optimally within **15 minutes** of preparation, and always within 60 minutes.
- Apply disks within **15 minutes** of inoculation.
- Incubate plates within **15 minutes** of disk application.

Examination of plates after incubation

- A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth.
- The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones (see next slide).
- If individual colonies can be seen, the inoculum is too light and the test must be repeated.

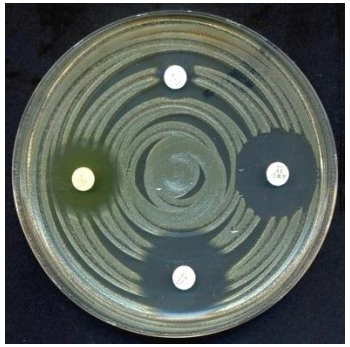
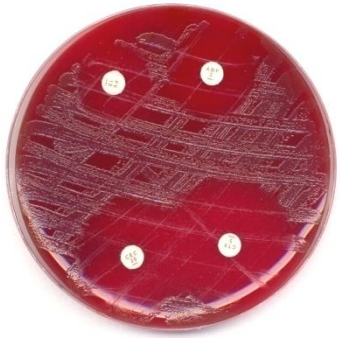
The growth should be confluent and evenly spread over the plate






Plates should look like this..



..and NOT like this!

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European Society of Clinical Microbiology and Infectious Diseases

Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 8.0

January 2021

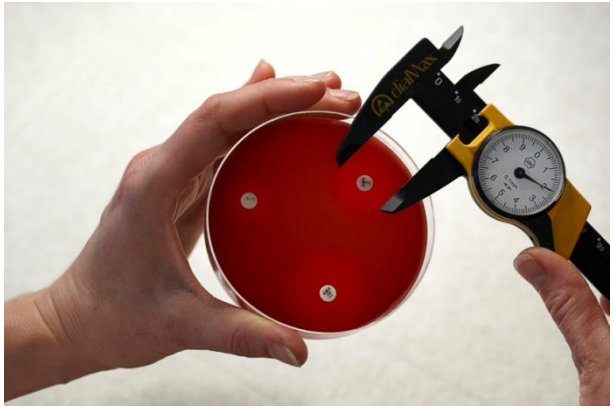
Reading zones

- The following instructions for reading inhibition zone diameters are part of the EUCAST disk diffusion method.
- Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye (for exceptions and specific reading instructions, see slides 15-26).
- Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
- Measure zone diameters to the nearest millimetre with a ruler or a calliper. If an automated zone reader is used, it must be calibrated to manual reading.

Reading zones

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

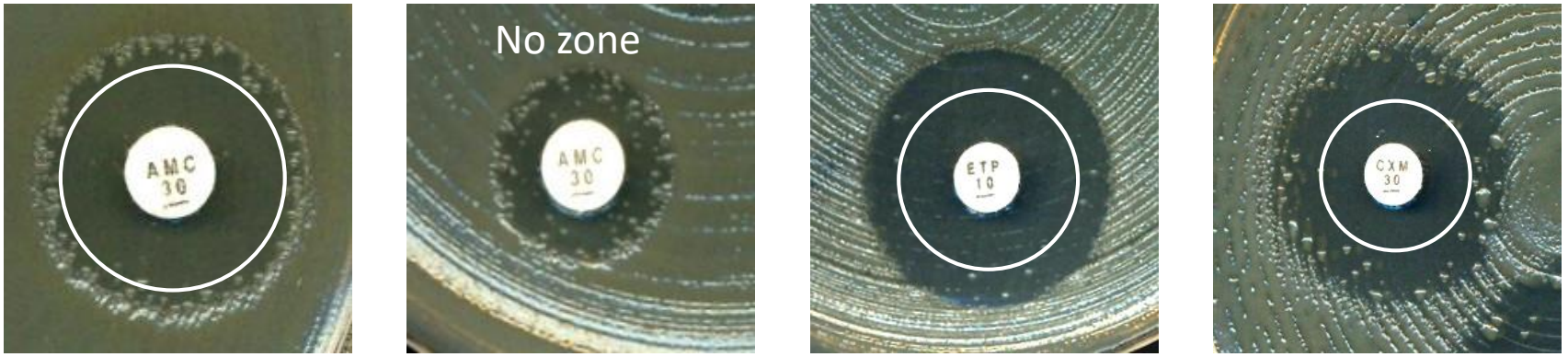
- Read **MH-F** plates from the front with the lid removed illuminated with reflected light.



Colonies within zone

- In case of distinct colonies within zones, check for purity and repeat the test if necessary.

- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.



Reading of zones with colonies within the zone.

Colonies within zone

- In case of distinct colonies within zones, check for purity and repeat the test if necessary.

- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.

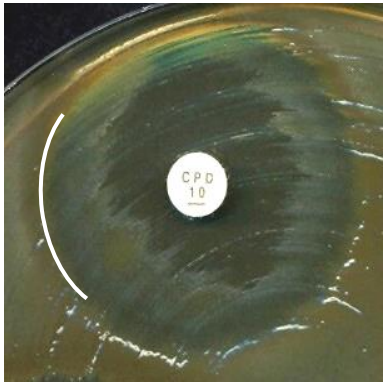
E. coli with ESBL

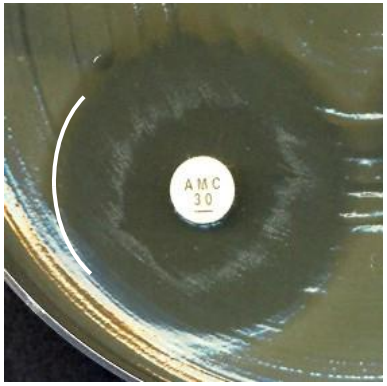
H. influenzae with PBP mutations

Reading of zones with colonies within the zone.

Swarming

- For *Proteus* spp., ignore swarming and read inhibition of growth.

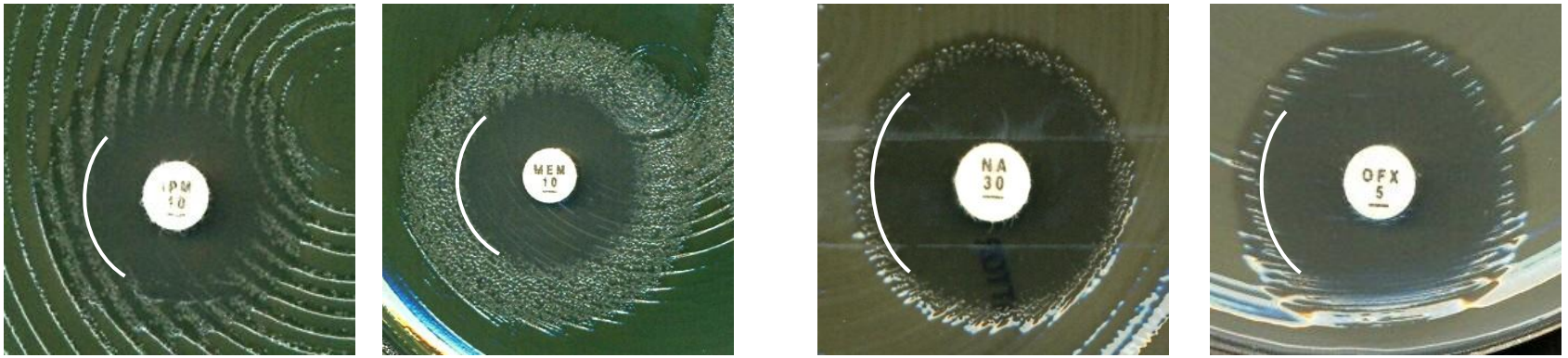




Double zones

- In case of double zones, check for purity and repeat the test if necessary.

- If cultures are pure, read the inner zone.



Reading of double zones.

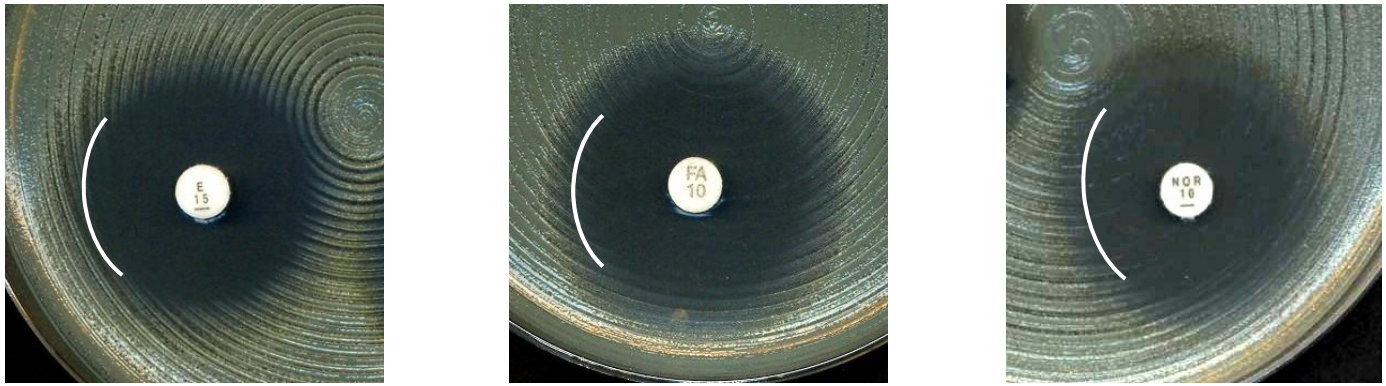
Fuzzy zone edges
Enterobacterales

- 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.

Fuzzy zone edges
Staphylococci

- 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 staphylococci.

Fuzzy zone edges
S. pneumoniae

- Small colonies that are visible when the plate is hold about 30 cm from the naked eye should be taken into account when reading zones.
- The presence of small colonies close to the zone edge may be related to excess humidity in the MH-F media, and may be reduced by drying the plates prior to use.

Reading of zones with fuzzy zone edges for *S. pneumoniae*.

Growth or haemolysis?

- Read inhibition of growth and not inhibition of haemolysis.

- It is sometimes difficult to distinguish between haemolysis and growth.
  - β-Haemolysins diffuse in agar. β-haemolysis is therefore usually free from growth.
  - α-Haemolysins do not diffuse. There is often growth within areas of α-haemolysis.
  - Zone edges accompanied with α-haemolysis is most common with *S. pneumoniae* and β-lactam antibiotics.

β-haemolysis

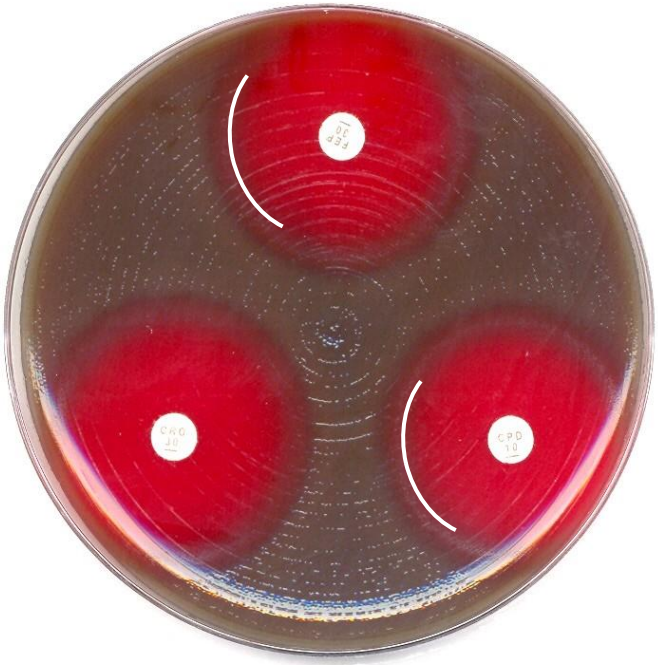
- Tilt the plate back and forth to better differentiate between haemolysis and growth.
- β-haemolysis is usually free from growth.

S. pyogenes

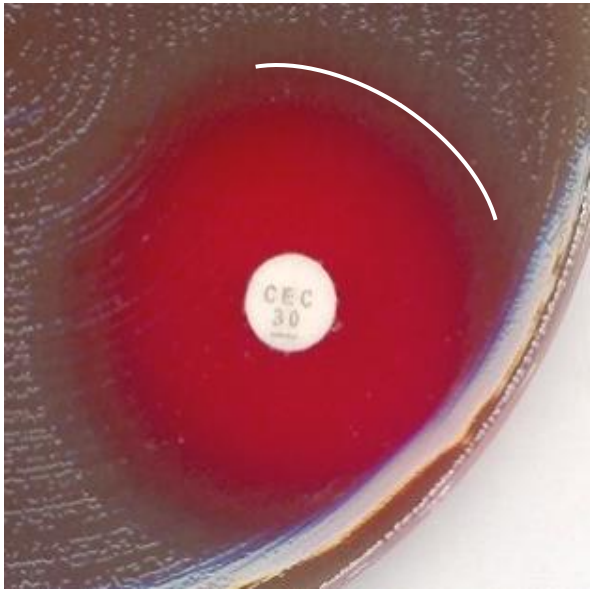
Streptococcus group C

α-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.



There is usually growth in the whole area of α-haemolysis.



For some organisms, there is additional α-haemolysis without growth. Tilt the plate to differentiate between haemolysis and growth.

Reading zones – exceptions (1)

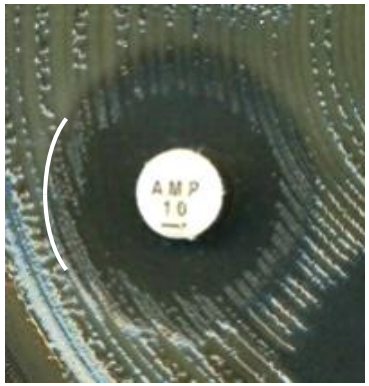
Organism	Antimicrobial agent	Reading inhibition zones
Enterobacterales	Ampicillin Ampicillin-sulbactam Amoxicillin-clavulanic acid	Ignore fine growth that may appear as an inner zone on some batches of MH agar.
Enterobacterales	Temocillin	Ignore isolated colonies within the inhibition zone.
Enterobacterales	Mecillinam	Ignore isolated colonies within the inhibition zone.
E. coli	Fosfomycin	Ignore isolated colonies within the inhibition zone and read the outer zone edge.
Proteus spp.	Any	Ignore swarming.
S. maltophilia, *A. xylosoxidans* and *B. pseudomallei*	Trimethoprim-sulfamethoxazole	Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
S. aureus	Benzylpenicillin	Examine zone edge from the front of the plate with transmitted light (plate held up to light).

Reading zones – exceptions (2)

Organism	Antimicrobial agent	Reading inhibition zones
Staphylococci	Cefoxitin	Examine zones carefully to detect colonies within the inhibition zone.
Enterococcus spp.	Vancomycin	Examine zone edge from the front of the plate with transmitted light (plate held up to light).
Streptococcus spp.	Any	Read inhibition of growth and not the inhibition of haemolysis.
H. influenzae	Beta-lactam agents	Read the outer edge of zones where an otherwise clear inhibition zone contains an area of growth around the disk.
Aeromonas spp.	Trimethoprim-sulfamethoxazole	Read the obvious zone edge and disregard haze or growth within the inhibition zone
Any	Trimethoprim Trimethoprim-sulfamethoxazole	Ignore faint growth up to the disk and measure at the more obvious zone edge.

Enterobacterales with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid

- Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars. The inner zone is not seen with some batches of agar and when the outer zone is read there is no difference between batches.

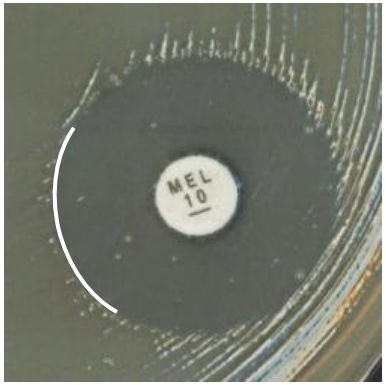


Enterobacterales and temocillin

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

Enterobacterales and mecillinam

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



E. coli and fosfomycin

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

Trimethoprim and trimethoprim-sulfamethoxazole

- Follow the instructions for reading and read the inner zone when double zones appear (see examples below).
- Ignore haze or faint growth up to the disk within a zone with otherwise clear zone edge.

E. coli

CoNS

Moraxella

Haemophilus

S. maltophilia with trimethoprim-sulfamethoxazole

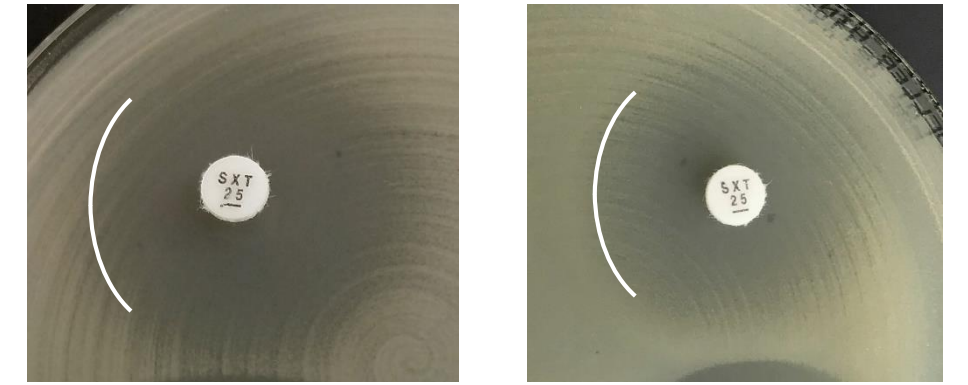
- Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to the disk and no sign of inhibition zone, report resistant.

An outer zone can be seen

Growth up to the disk

B. pseudomallei with trimethoprim-sulfamethoxazole

- Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to the disk and no sign of inhibition zone, report resistant.

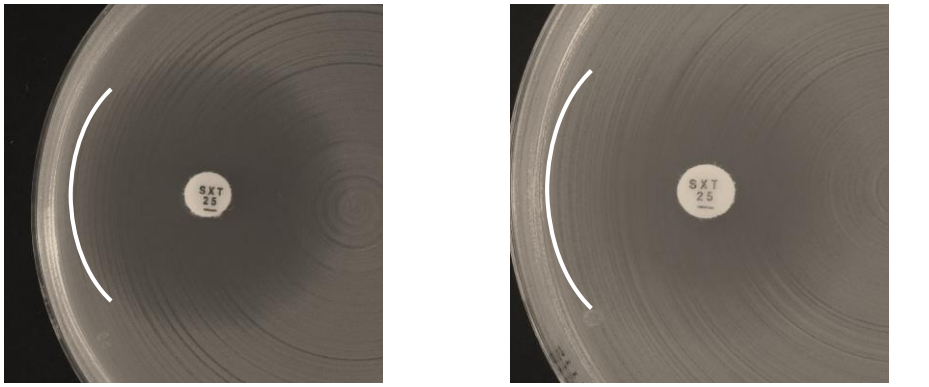


An outer zone can be seen

Growth up to the disk

A. xylosoxidans with trimethoprim-sulfamethoxazole

- Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to the disk and no sign of inhibition zone, report resistant.

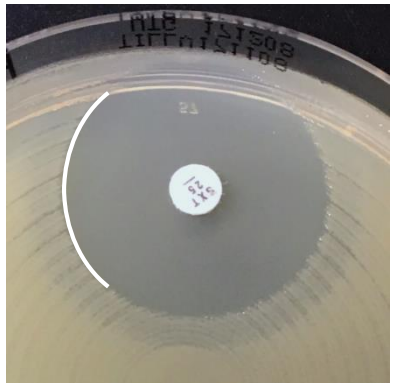


An outer zone can be seen

Growth up to the disk

Aeromonas spp. and trimethoprim-sulfamethoxazole

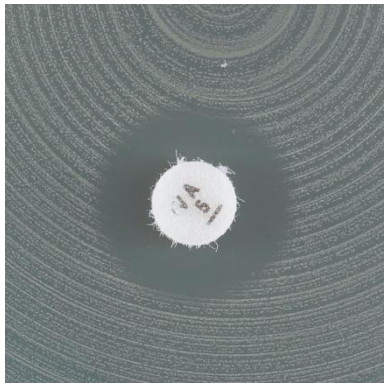
- Read the obvious zone edge and disregard haze or growth within the inhibition zone.
- If there is an obvious inner zone edge, read the inhibition zone as the inner zone.



Enterococci and vancomycin

- Examine zone edge from the front of the plate with transmitted light (plate held up to light).
  - If the zone edge is sharp, report susceptible.
  - If the zone edge is fuzzy, colonies grow within the zone or if you are uncertain, suspect VRE and perform confirmatory testing, even if the zone diameter is ≥ 12 mm.
  - Isolates must not be reported susceptible before 24 h incubation.

non-VRE



VRE

S. aureus and benzylpenicillin

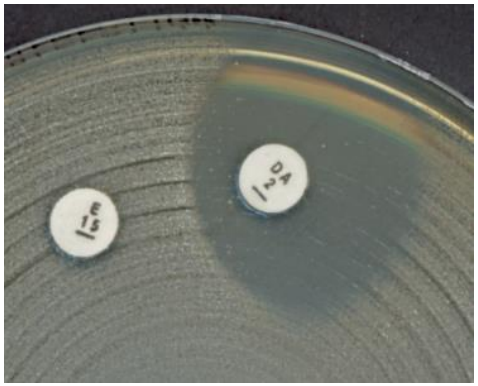
- Examine zone edge from the front of the plate with transmitted light (plate held up to light).
  - To detect penicillinase production, read the zone diameter AND examine the zone edge closely.
  - If the zone is ≥ 26 mm and the zone edge is sharp, the isolate is a pencillinase producer, report resistant.
  - If the zone is ≥ 26 mm and the zone edge is fuzzy, report susceptible.

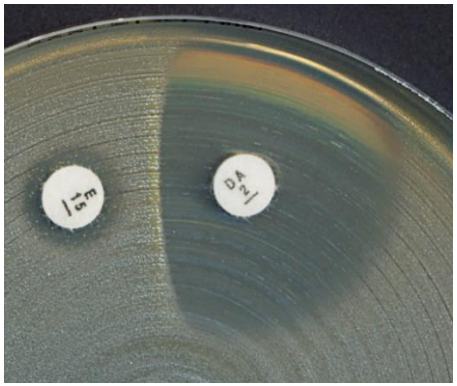
Zone ≥ 26 mm and
sharp zone edge= Resistant

Zone ≥ 26 mm and
fuzzy zone edge = Susceptible

Detection of inducible clindamycin resistance in staphylococci

- Inducible clindamycin resistance can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place the erythromycin and clindamycin disks **12-20 mm apart** (edge to edge) and look for antagonism (the D phenomenon).

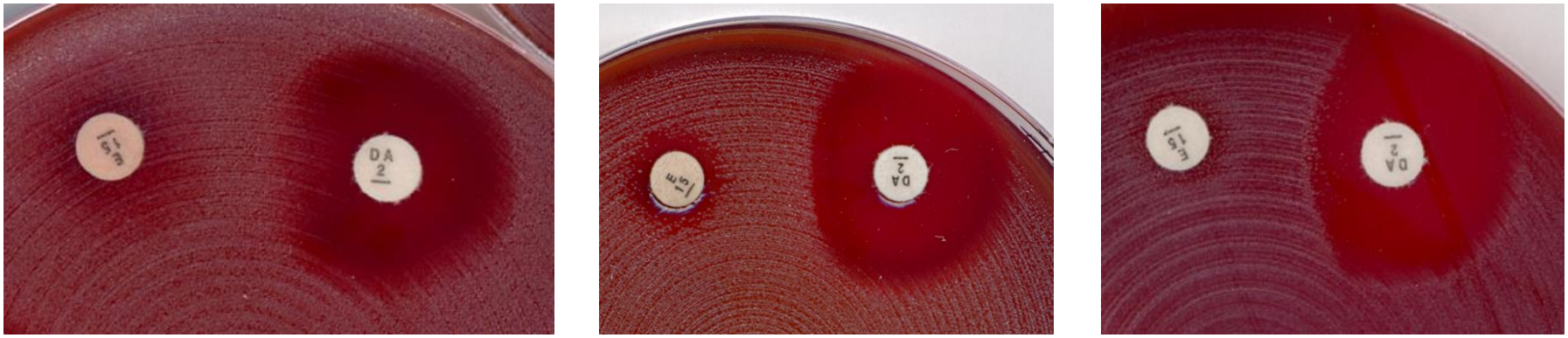




Examples of D phenomenon for staphylococci.

Detection of inducible clindamycin resistance in streptococci

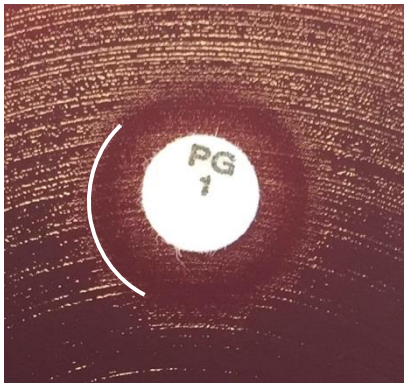
- Inducible clindamycin resistance can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place the erythromycin and clindamycin disks **12-16 mm apart** (edge to edge) and look for antagonism (the D phenomenon).



Examples of D phenomenon for streptococci.

H. influenzae and beta-lactam agents

- Read the outer edge of zones where an otherwise clear inhibition zone contains an area of growth around the disk.



Disk diffusion method

Limitations:

Organisms:

Not suitable for some fastidious, slow growing organisms (e.g., *Neisseria gonorrhoeae, N. meningitidis, H. pylori* – although work is ongoing also for these organisms)

Organism/antibiotic combination:

***Staphylococcus aureus* vs. vancomycin**
***Enterobacterales, Acinetobacter* spp., and *Pseudomonas aeruginosa* vs. colistin**

Antibiotic:

Oxacillin vs. *Streptococcus pneumoniae* (penicillin MIC testing required if oxacillin zone diameter < 20 mm)

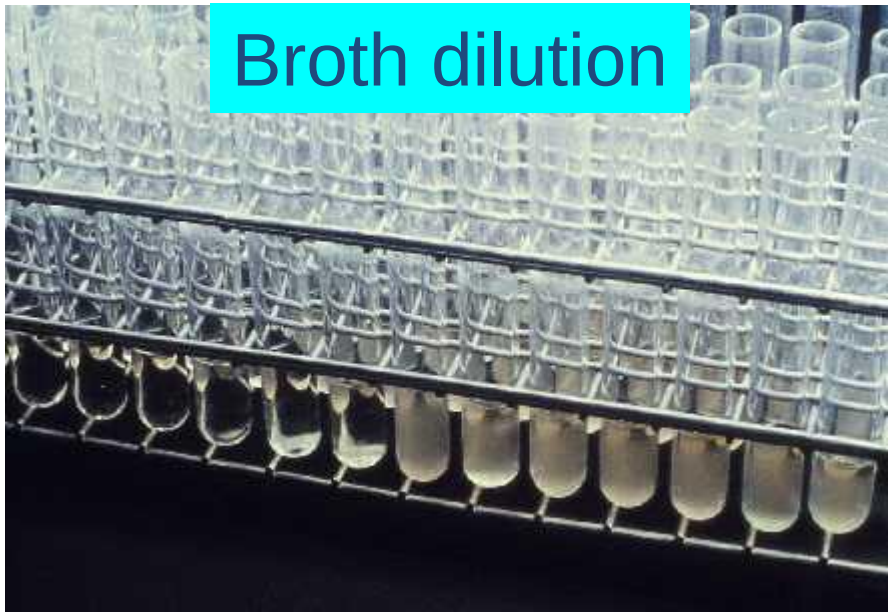
Resistance mechanism:

Inducible beta lactamases
VISA/h-VISA

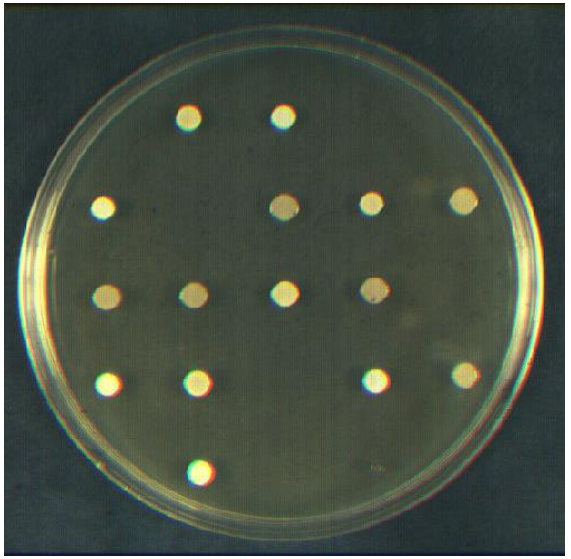
MIC

- MIC – minimum inhibitory concentration (mg/L)
- Lowest concentration of the agent that completely inhibits visible growth.
- MIC dilution series are based on twofold dilutions up and down from 1 mg/L
- 0.002, 0.004, 0.008, 0.016, 0.03, 0.06, 0.125, 0.25, 0.5, **1**, 2, 4, 8, 16, 32, 64, 128, 256, 512

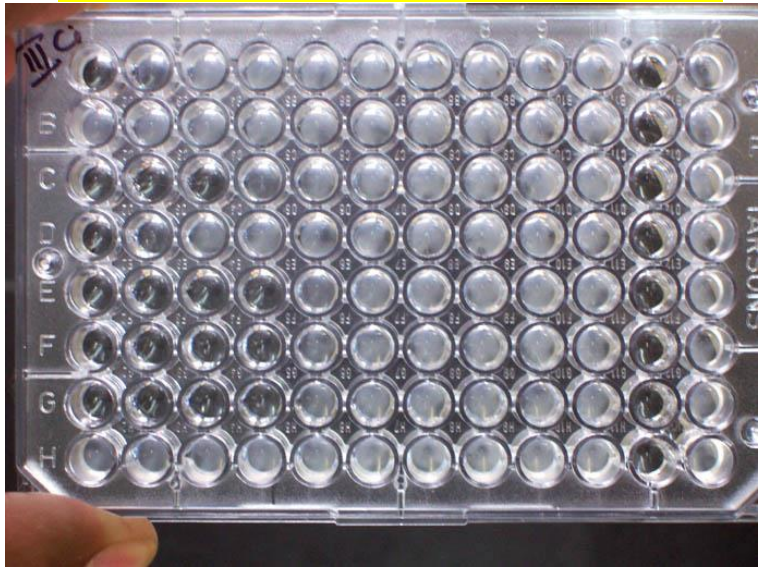
Broth dilution



Agar dilution



Broth microdilution



Gradient MIC tests

Various manufacturers:
bioMérieux (Etest)
Liofilchem (MTS)

Broth dilution tests

One of the earliest AST methods; macrobroth or tube-dilution

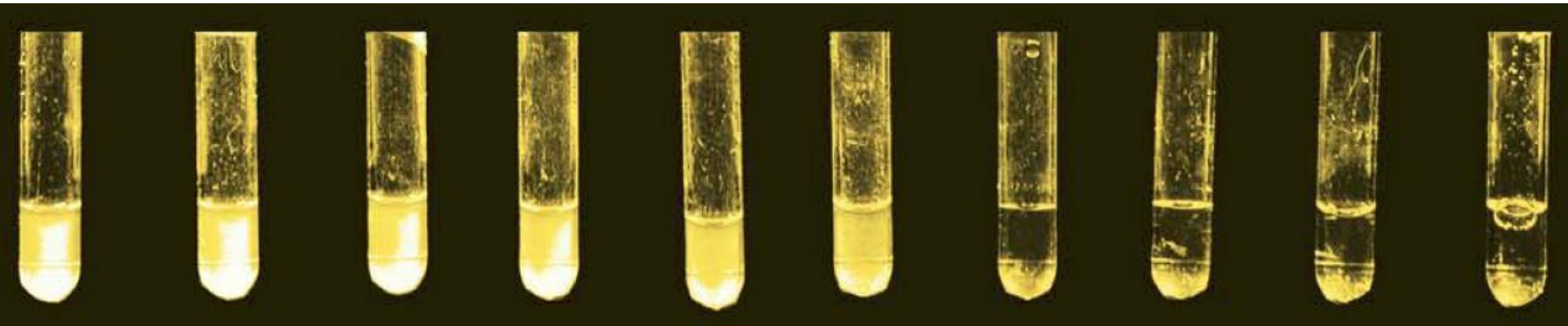
Two-fold dilutions of antibiotics (e.g., 0.125, 0.25, 0.5, **1**, 2, 4, 8, 16, 32 mg/L) in a liquid growth medium dispensed in test tubes

The antibiotic containing tubes are inoculated with a standardized bacterial suspension of 1–5 x 10^5 CFU/mL.

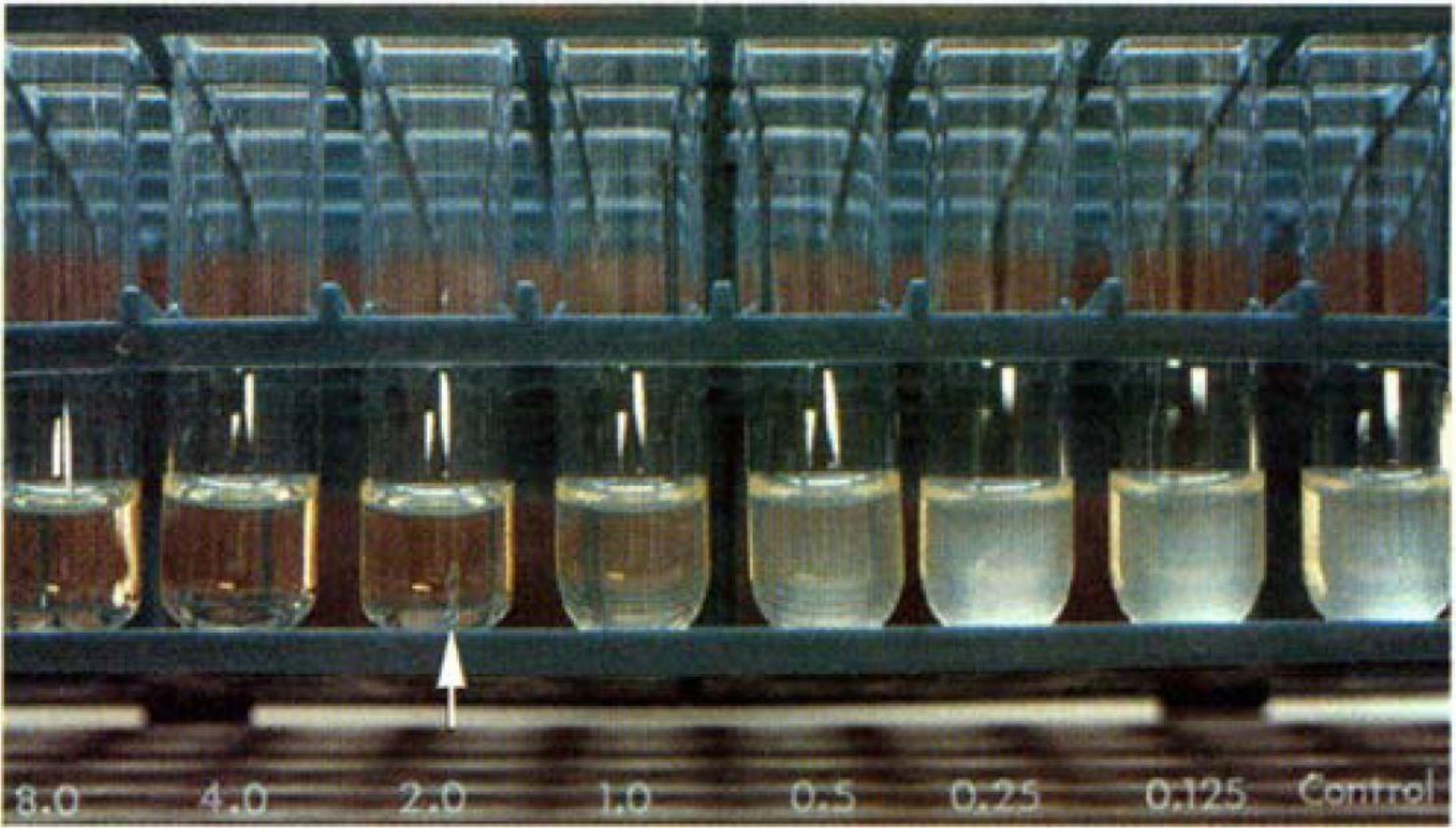
Following overnight incubation at 35°C, the tubes are examined for visible bacterial growth as evidenced by turbidity.

The lowest concentration of antibiotic that prevents growth represents the minimal inhibitory concentration (MIC).

Precision: Plus or minus 1 two-fold concentration



Broth dilution tests



Macrobroth dilution test

Broth dilution tests

Advantages

Generation of a quantitative result (i.e., the MIC).

For serious infections, and those in sites where antibiotics penetrate poorly, and among immune-suppressed patients, categorical results have limited predictive value

Disadvantages

Tedious, manual task of preparing the antibiotic solutions for each test.

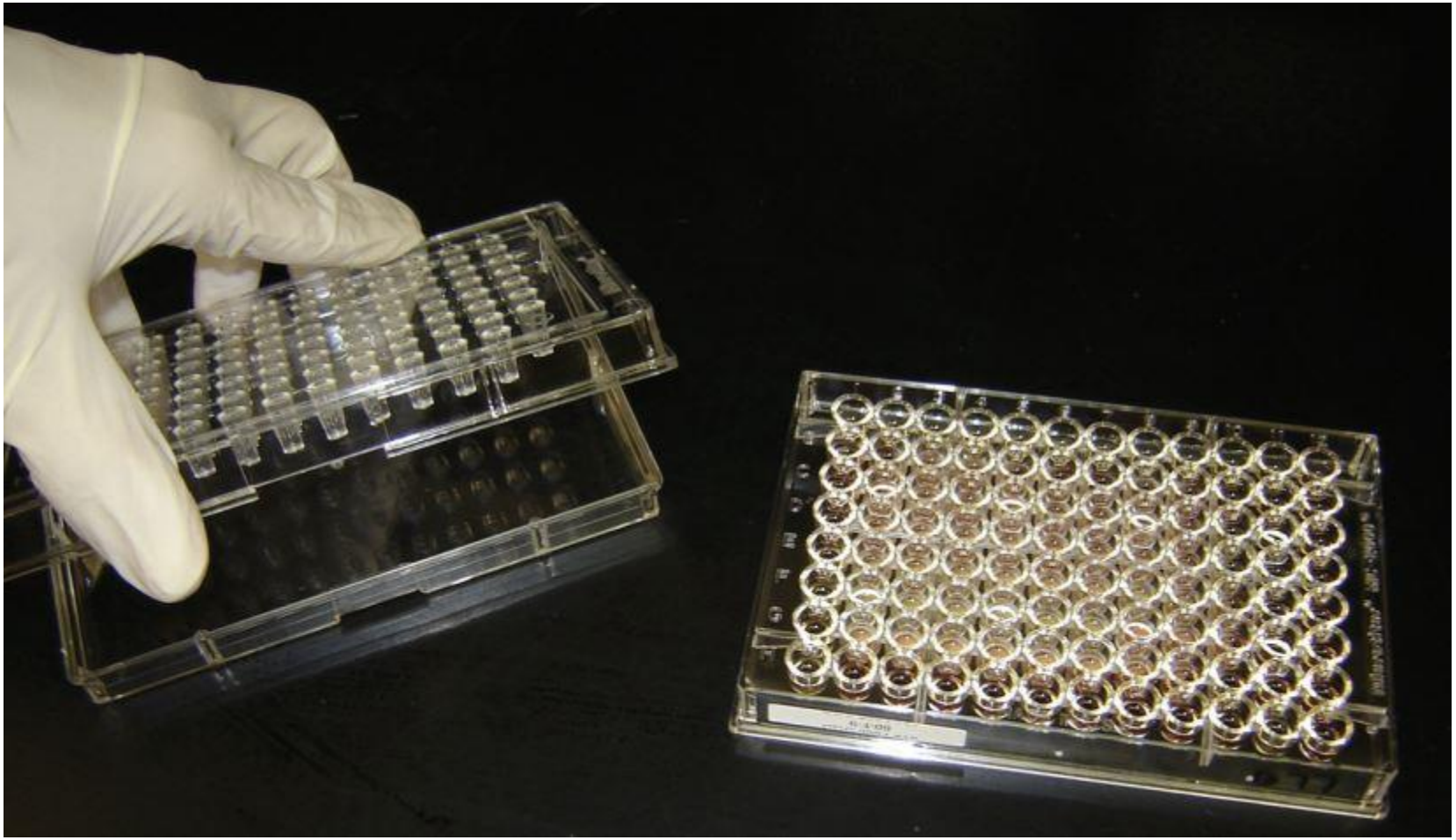
The possibility of errors in preparation of the antibiotic solutions.

The relatively large amount of reagents and space required for each test.

$\Rightarrow$ Macrodilution $\rightarrow$ Microdilution

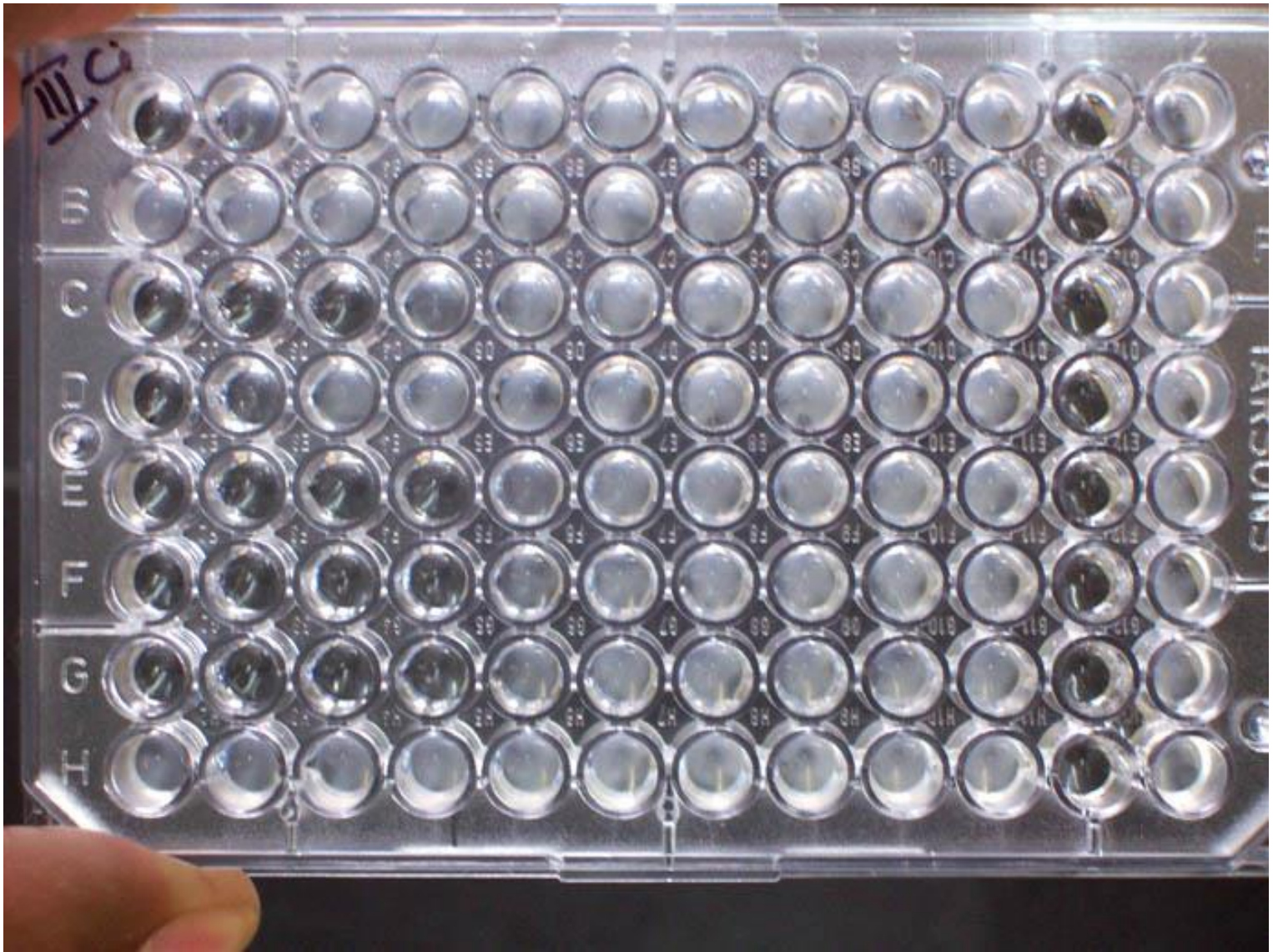
Broth dilution tests

Broth microdilution (BMD) test

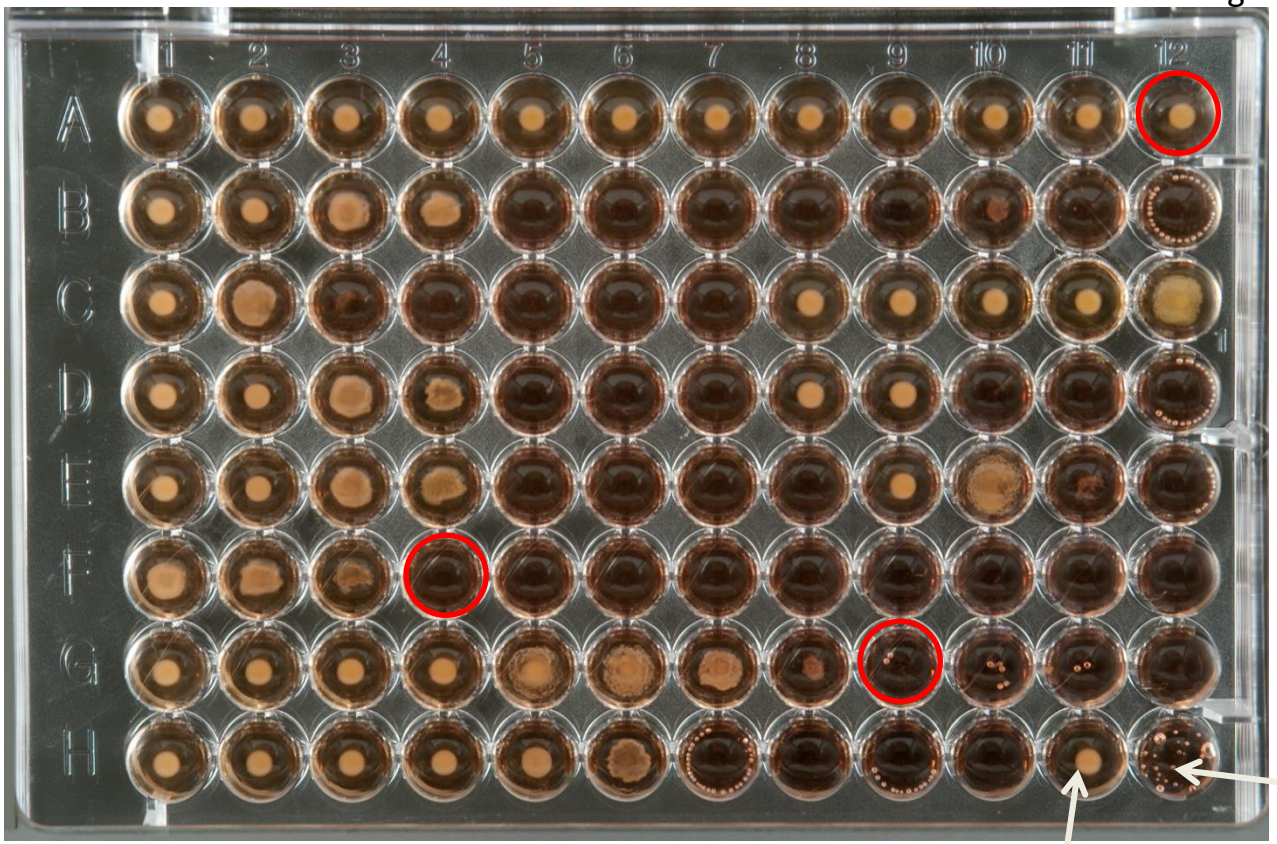


Broth dilution tests

Broth microdilution (BMD) test



Antimicrobial Susceptibility Test
Broth Microdilution Test



Clindamycin

MIC >32 µg/ml

Penicillin

MIC 0.06 µg/ml

Erythromycin

MIC 8 µg/ml

Sterile control

Growth Control

(MIC) Minimum Inhibitory Concentration

Photo courtesy of Dr. Lesley McGee, CDC

Broth dilution tests

$\Rightarrow$ Macrodilution $\rightarrow$ Microdilution

The miniaturization and mechanization of the test by use of small, disposable, plastic “microdilution” trays

$\rightarrow$ Broth dilution testing became practical and popular

Standard trays contain 96 wells, each containing a volume of 0.1 mL that allows approx. 12 antibiotics to be tested in a range of 8 two-fold dilutions in a single tray.

Broth dilution tests

Broth microdilution (BMD) test

Few clinical microbiology laboratories prepare their own panels; instead frozen or dried microdilution panels are purchased from one of several commercial suppliers

Inoculation of panels with the standard 5×10^5 CFU/mL is accomplished using a disposable device that transfers 0.01 to 0.05 mL of standardized bacterial suspension into each well of the microdilution tray or by use of a mechanized dispenser.

MICs are determined using a manual or automated viewing device for inspection of each of the panel wells for growth.

Broth dilution tests

Broth microdilution (BMD) test

Advantages:
generation of MICs, the reproducibility and convenience of having preprepared panels, and the economy of reagents and space that occurs due to the miniaturization of the test.

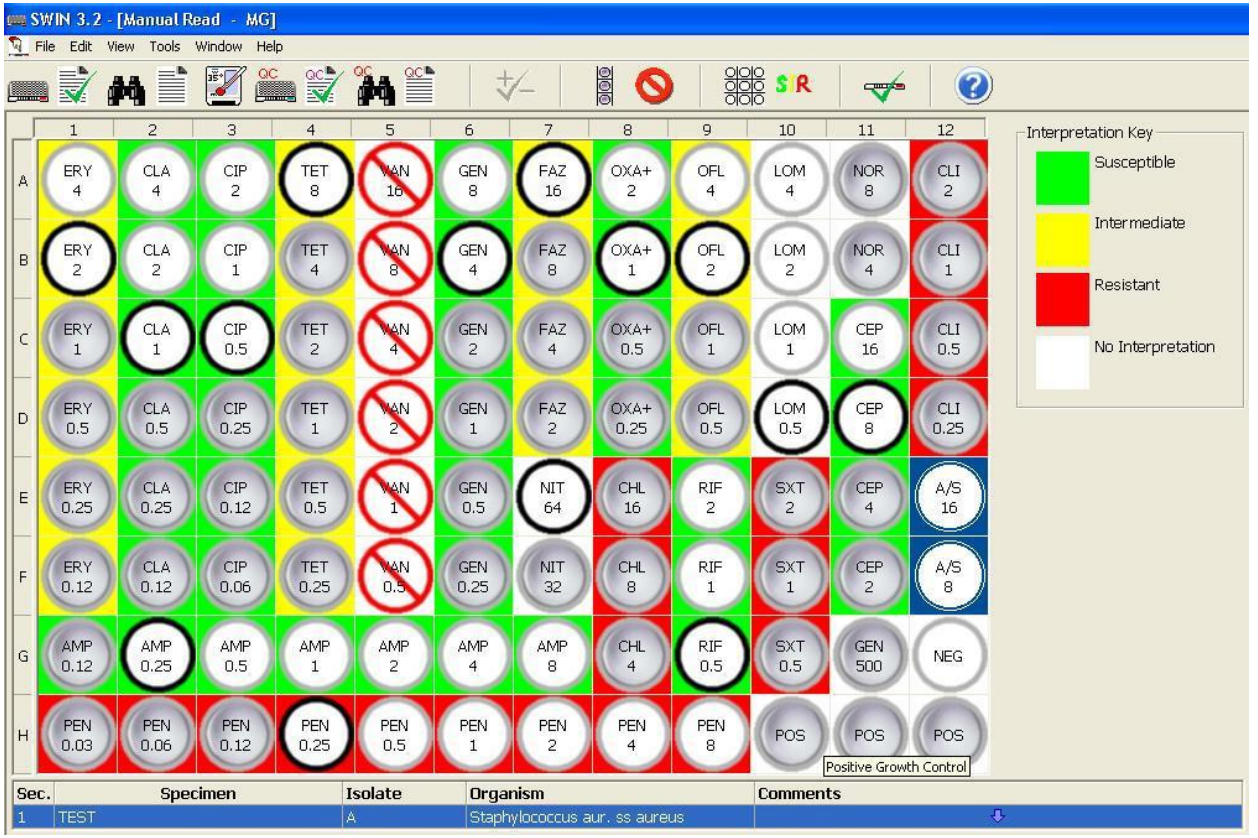
Disadvantages:
inflexibility of drug selections available in standard commercial panels.

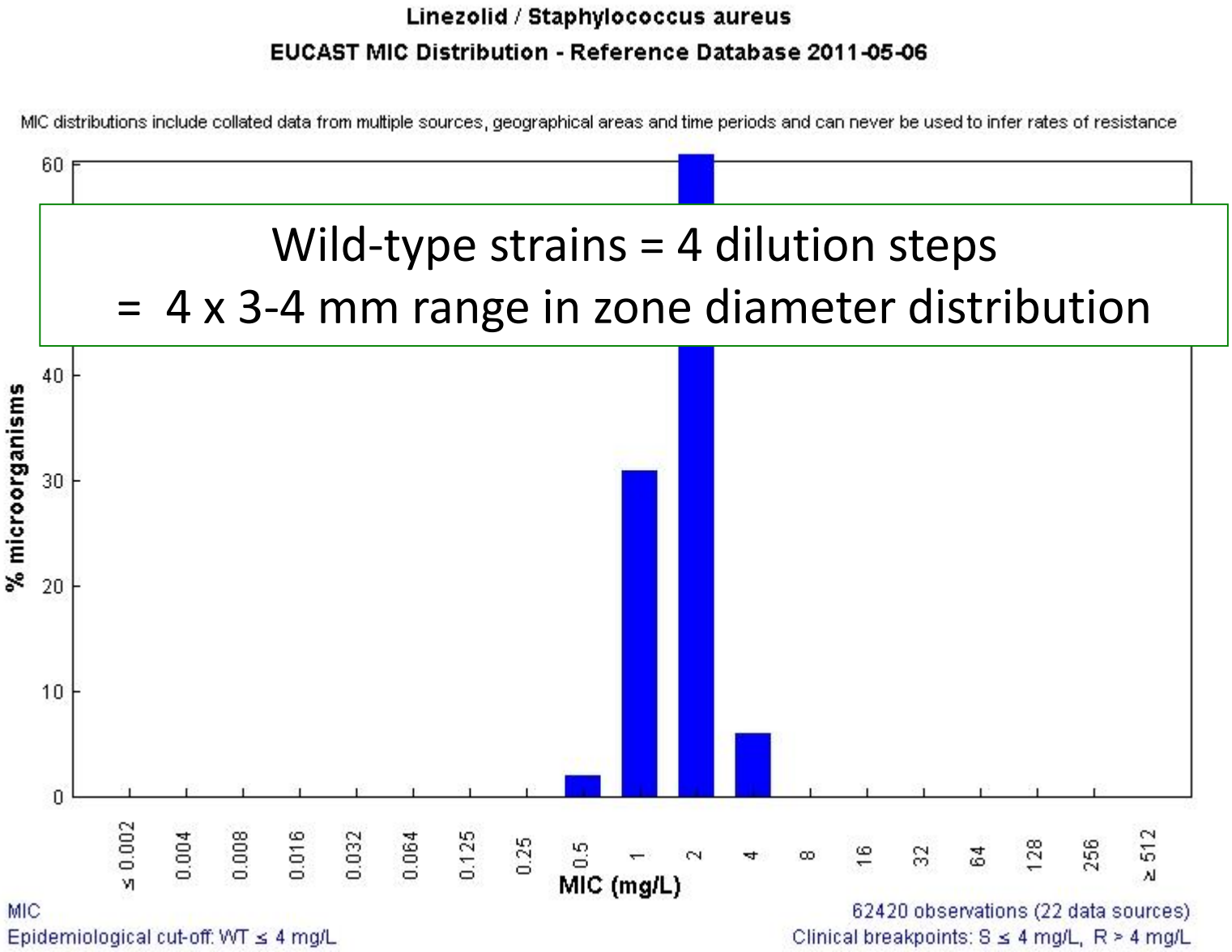
Broth dilution tests

Broth microdilution (BMD) test

Broth dilution tests

Broth microdilution (BMD) test



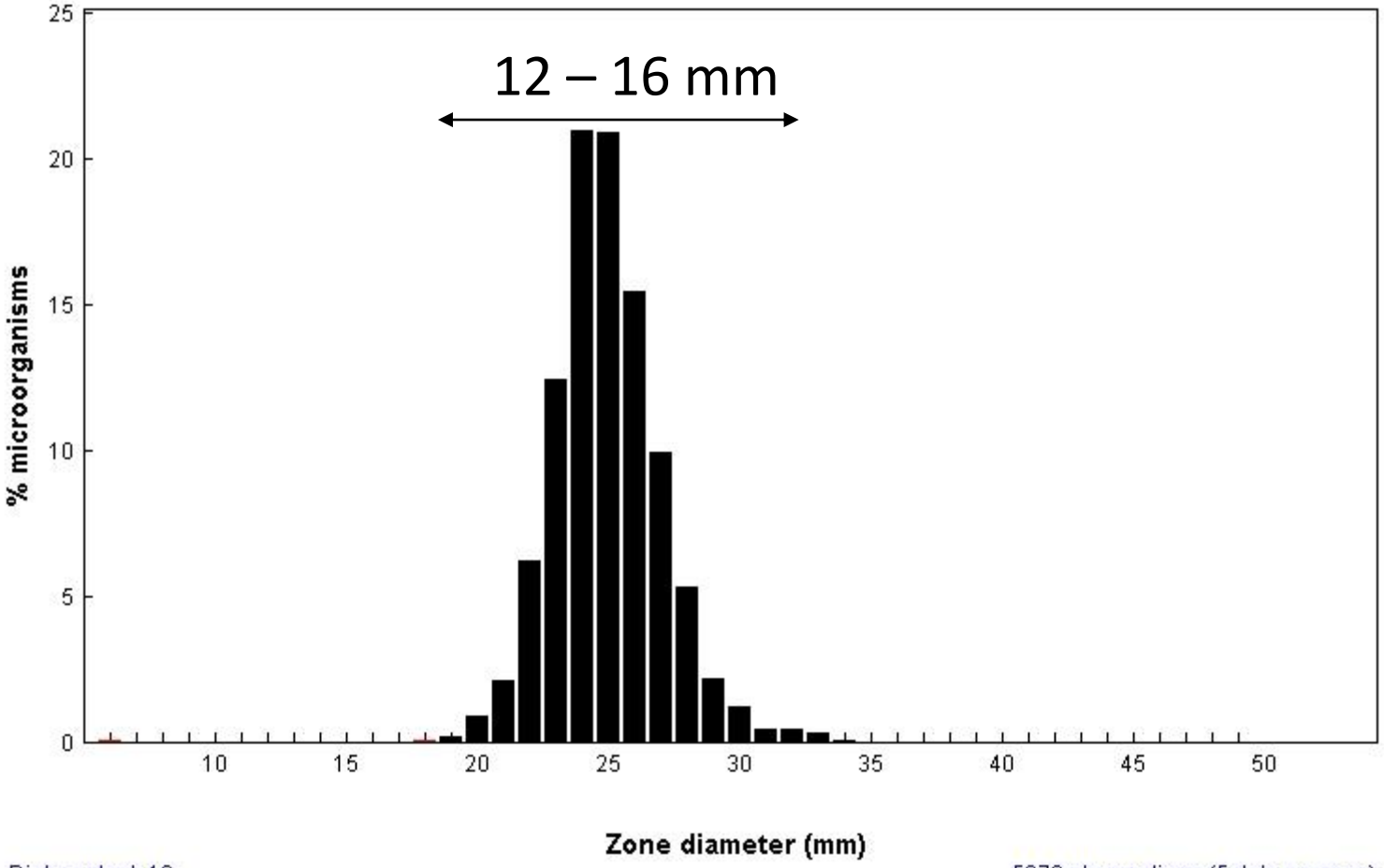

Linezolid / Staphylococcus aureus
EUCAST MIC Distribution - Reference Database 2011-05-06
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance
Wild-type strains = 4 dilution steps
= 4 x 3-4 mm range in zone diameter distribution
% microorganisms
0
10
20
30
40
60
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
≥ 512
MIC (mg/L)
MIC
Epidemiological cut-off: WT ≤ 4 mg/L
62420 observations (22 data sources)
Clinical breakpoints: S ≤ 4 mg/L, R > 4 mg/L

Linezolid / Staphylococcus aureus

EUCAST zone diameter distribution - Reference database 2011-05-06

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



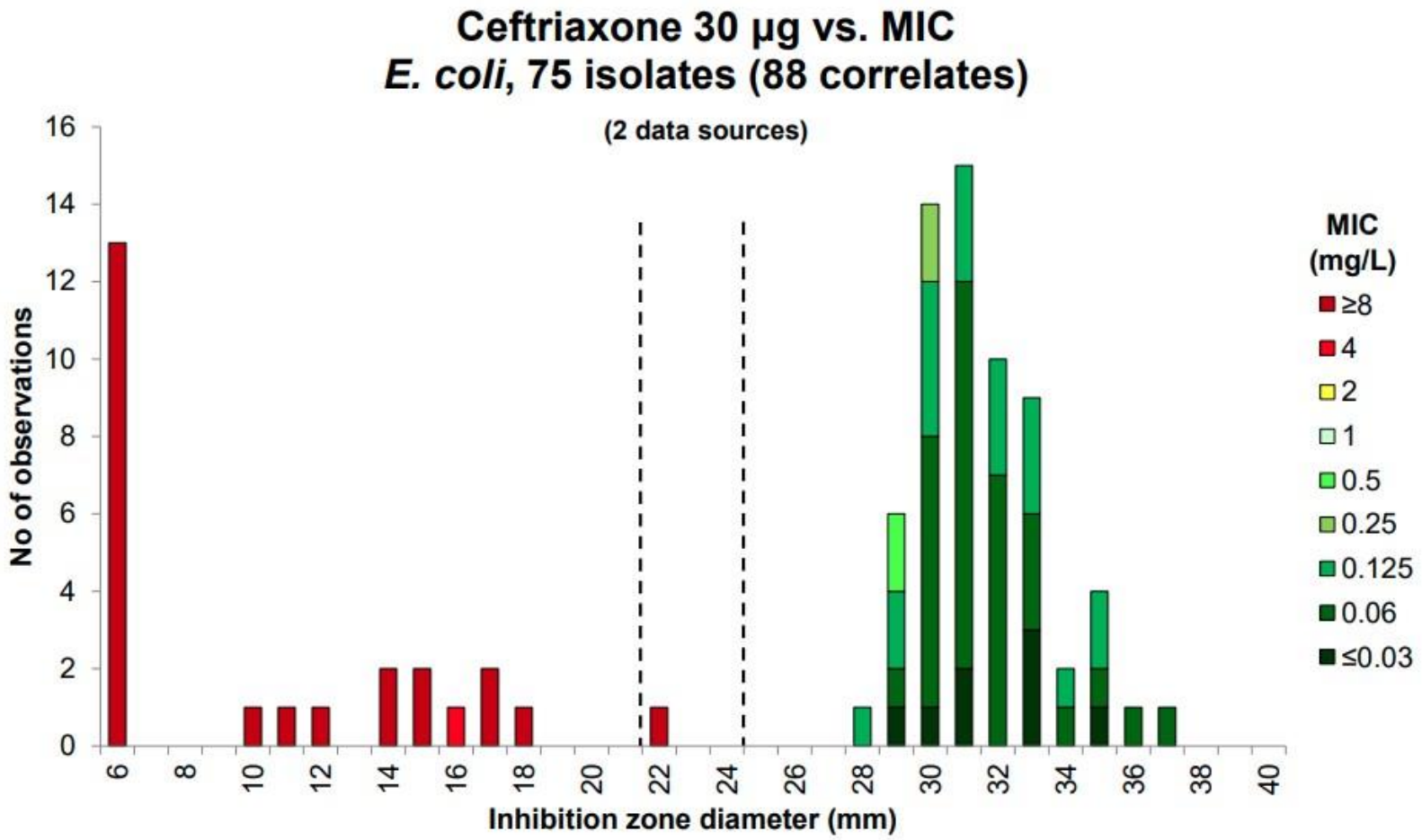
Disk content: 10

Epidemiological cut-off: WT ≥ 19 mm (MIC: ≤ 4 mg/L)

5273 observations (5 data sources)

Clinical breakpoints: S ≥ 19 mm, R < 19 mm

MIC and zone diameter correlates



Breakpoints (non-meningitis)	
MIC	S≤1, R>2 mg/L
Zone diameter	S≥25, R<22 mm

Calibration of zone diameter breakpoints to MIC values

MH and MH-F broth

B. Media for MIC determination by the broth microdilution method

Cation-adjusted Mueller-Hinton broth (MHB) and MHB supplemented with lysed horse blood and β-NAD (MH-F broth)

MH broth, un-supplemented cation-adjusted Mueller-Hinton broth, is used for testing of non-fastidious organisms according to the ISO standard 20776-1, 2019.

MH-F broth, cation-adjusted MH broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD, is used for testing *Streptococcus* spp. (including *S. pneumoniae*), *Haemophilus influenzae*, *Moraxella catarrhalis, Listeria monocytogenes*, *Campylobacter jejuni* and *coli*, *Pasteurella multocida*, *Corynebacterium* spp., *Aerococcus sanguinicola and urinae, Kingella kingae* and several other fastidious organisms.

Un-supplemented MH broth may be purchased from commercial sources or prepared locally according to the manufacturers' instructions. MH broth should meet the requirements in the ISO Technical specification, ISO/TS 16782, 2016 and the quality control criteria published by EUCAST.

MH-F broth

MH-F broth is prepared as follows:

1. Reagents	
1.1	Cation-adjusted MHB from commercial source.
1.2	50% lysed horse blood.
1.3	β-Nicotinamide adenine dinucleotide (β-NAD), purity ≥98%.

2. Preparation of 50% lysed horse blood stock solution	
2.1	Aseptically dilute mechanically defibrinated horse blood with an equal amount of sterile deionized water.
2.2	Freeze the blood at -20°C overnight and thaw. Repeat the cycle until the cells are completely lysed (three cycles is usually sufficient but the ISO standard 20776-1 suggests that up to seven cycles may be required).
2.3	Clarify the 50% lysed horse blood by centrifugation and discard the pellet. A clear solution is essential for reading. Failure to clarify the solution may be due to inadequate lysis or centrifugation. Repeating the centrifugation may improve the clarity of the solution.
2.4	The stock solution may be stored at -20°C in aliquots and defrosted as required. Do not refreeze unused solution.

MH-F broth

3. Preparation of β-NAD stock solution	
3.1	Dissolve β-NAD in sterile deionized water to a concentration of 20 mg/mL.
3.2	Sterilize the solution through a 0.2 µm membrane filter.
3.3	The stock solution may be stored at -20°C in aliquots and defrosted as required. Do not refreeze unused solution.

4. Preparation MH-F broth	
4.1	Prepare and autoclave cation-adjusted MHB according to the manufacturer's instructions, but with 100 mL less deionized water per litre to allow for the addition of lysed horse blood.
4.2	Cool medium to 42-45°C.
4.3	Aseptically add 100 mL 50% lysed horse blood and 1 mL β-NAD stock solution per litre medium and mix well.
4.4	Dispense MH-F broth in sterile containers with screw caps.

Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method

Version 6.0, January 2020 www.eucast.org

MH and MH-F broth

5. Storage of MH-F broth	
5.1	Store MH-F broth at 4-8°C.
5.2	Storage conditions and shelf life should be determined as part of the laboratory quality assurance programme. A shelf life of 3 months can be expected.

6. Quality control	
6.1	Check that the pH is within the range 7.2-7.4.
6.2	Check that the medium supports good growth of control strain(s) of the intended test organisms.
6.3	Check that MICs are within control limits for all bacteria-antimicrobial agent combinations used (EUCAST QC tables).

Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method
Version 6.0, January 2020 www.eucast.org

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

EUCAST reading guide for broth microdilution

Version 3.0

January 2021

Broth microdilution

- Broth microdilution is the reference method for antimicrobial susceptibility testing of rapidly growing aerobic bacteria, except for mecillinam and fosfomycin, where agar dilution is the reference method.

- EUCAST recommends testing according to the International Standard ISO 20776-1, but with the use of MH-F broth (Mueller- Hinton broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD, see instructions for preparation at www.eucast.org) for fastidious organisms.

- Results are recorded as the lowest concentration of antimicrobial agent that inhibits visible growth of a microorganism, the Minimum Inhibitory Concentration (MIC), expressed in mg/L or µg/mL.

Reading broth microdilution

Results are only valid when the following criteria are met:

- Sufficient growth, *i.e.* obvious button or definite turbidity, in the positive growth control.

- Pure culture
  - Check for purity by subculturing from the growth-control well immediately after inoculation onto a non-selective agar plate for simultaneous incubation.

- Correct inoculum 5 x 10^5 CFU/mL
  - Viable colony counts can be performed by removing 10 µL from the growth-control well or tube immediately after inoculation and diluting in 10 mL of saline. Mix and spread 100 µL onto a non-selective agar plate. After incubation, the number of colonies should be approximately 20-80.

Growth appearance

- Growth appears as turbidity or as a deposit of cells at the bottom of the well. The appearance of growth differs depending on the microorganism and the antimicrobial agent tested.

- For round-bottom wells, growth will most often appear as a button/pellet centered in the middle. For flat-bottom wells, growth may be scattered.

- Growth in antibiotic-containing wells may differ from growth seen in the positive growth control, even for pure cultures.

Reading MIC endpoints

- Results should be read manually. The use of a mirror may facilitate reading.

- If an automated reader or camera system is used, it must be calibrated to manual reading.

- Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.

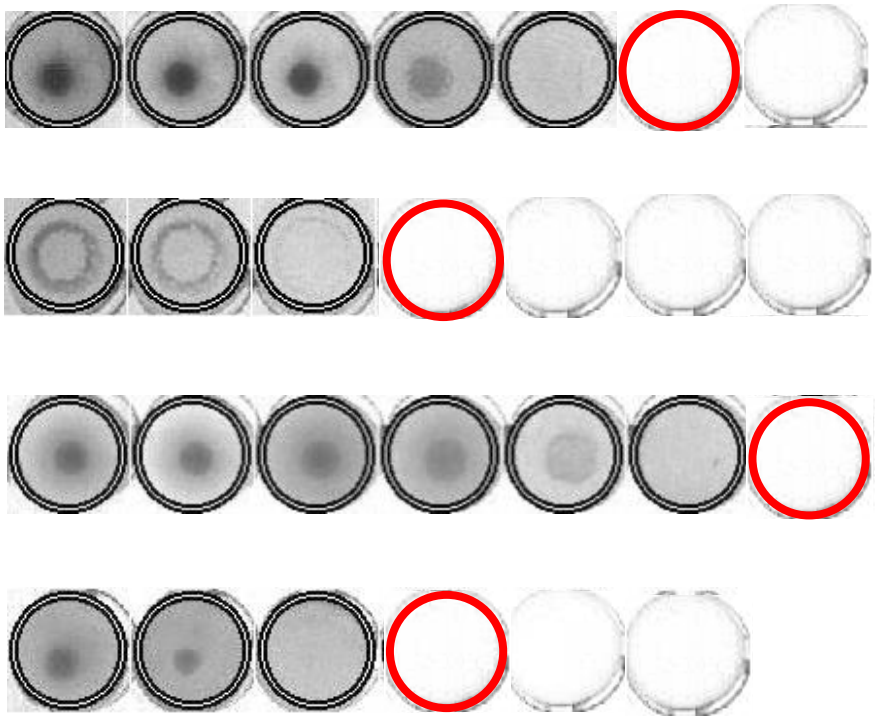
  For exceptions, see slides 12-16.

Trailing endpoints

- Most antimicrobial agent-organism combinations give distinct endpoints.
- Some agent-organism combinations may give trailing endpoints with a gradual fading of growth over 2 to 3 wells.
- Unless otherwise stated, endpoints should be read at complete inhibition of growth (for exceptions, see slides 12-16).

Turbidity without pellet

- Haze or turbidity without a pellet is often seen for *Pseudomonas* spp. and *Acinetobacter* spp. This should be regarded as growth and the endpoint read at the first well with complete inhibition (clear broth).



Haemolysis

- For fastidious organisms tested in MH-F broth, haemolysis of the blood can be seen. This is often accompanied by turbidity or a deposit of growth (pellet).

- Haemolysis with turbidity or pellet should be regarded as growth when determining endpoints.

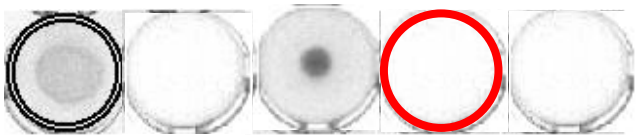
Skipped wells

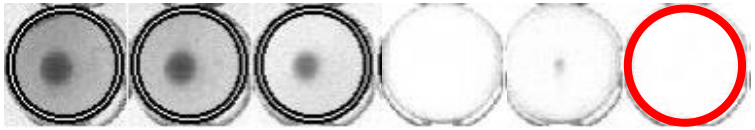
- Occasionally a skip may be seen, *i.e.* a well showing no growth bordered by wells showing growth. There are several possible explanations including incorrect inoculation, contaminations, heterogenous resistance etc.

- When a single skipped well occurs, retest the isolate or read the highest MIC value to avoid reporting isolates as false susceptible.

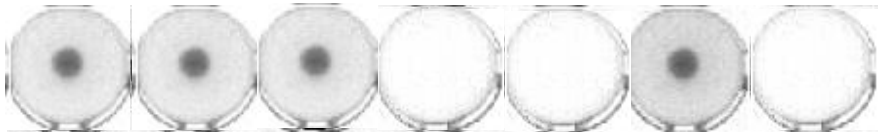
- Do not report results for antimicrobial agents for which there is more than one skipped well.

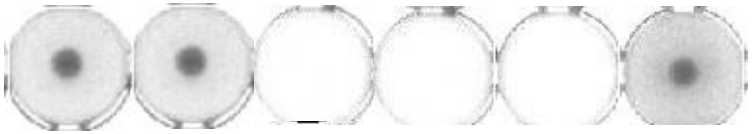
Examples skipped wells





Retest or read the highest MIC value!





Results invalid!

Specific reading instructions

- The following antimicrobial agents require specific reading instructions:
  - Bacteriostatic antimicrobial agents, both with Gram-positive and Gram-negative organisms
  - Trimethoprim and trimethoprim-sulfamethoxazole
  - Cefiderocol

Gram-positive cocci with bacteriostatic antimicrobial agents

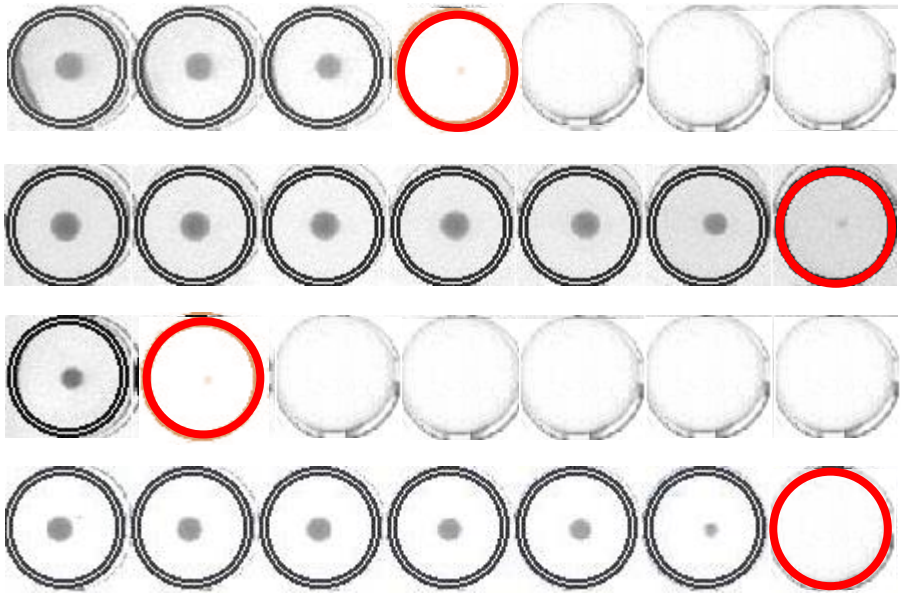
- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

Doxycyline

Fusidic acid

Tetracycline

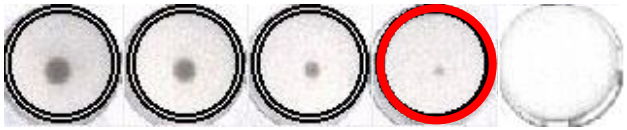
Doxycyline



Gram-positive cocci with bacteriostatic antimicrobial agents

- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

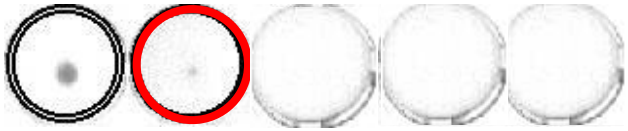
Linezolid



Linezolid

Tedizolid

Tedizolid

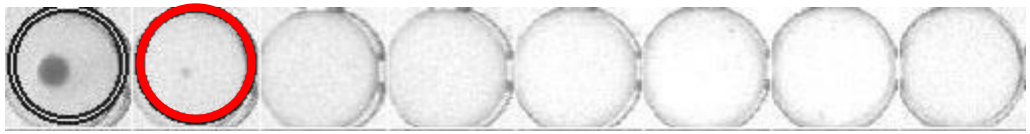


Gram-negative organisms with tigecycline and eravacycline

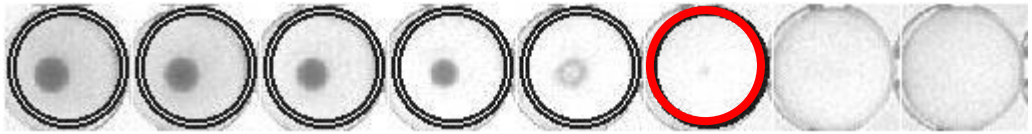
- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

Tigecycline

Tigecycline



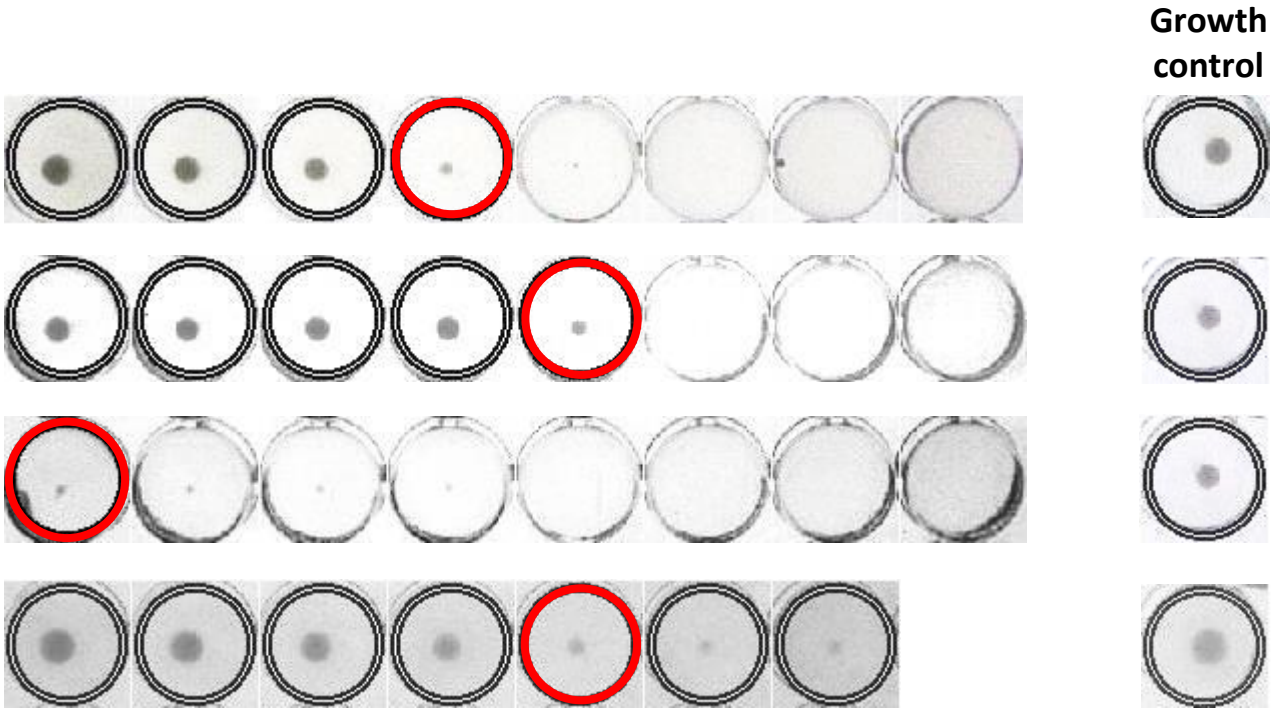
Eravacycline



Eravacycline

Trimethoprim and trimethoprim-sulfamethoxazole

Read the MIC at the lowest concentration that inhibits ≥80 % of growth as compared to the growth control.



Cefiderocol

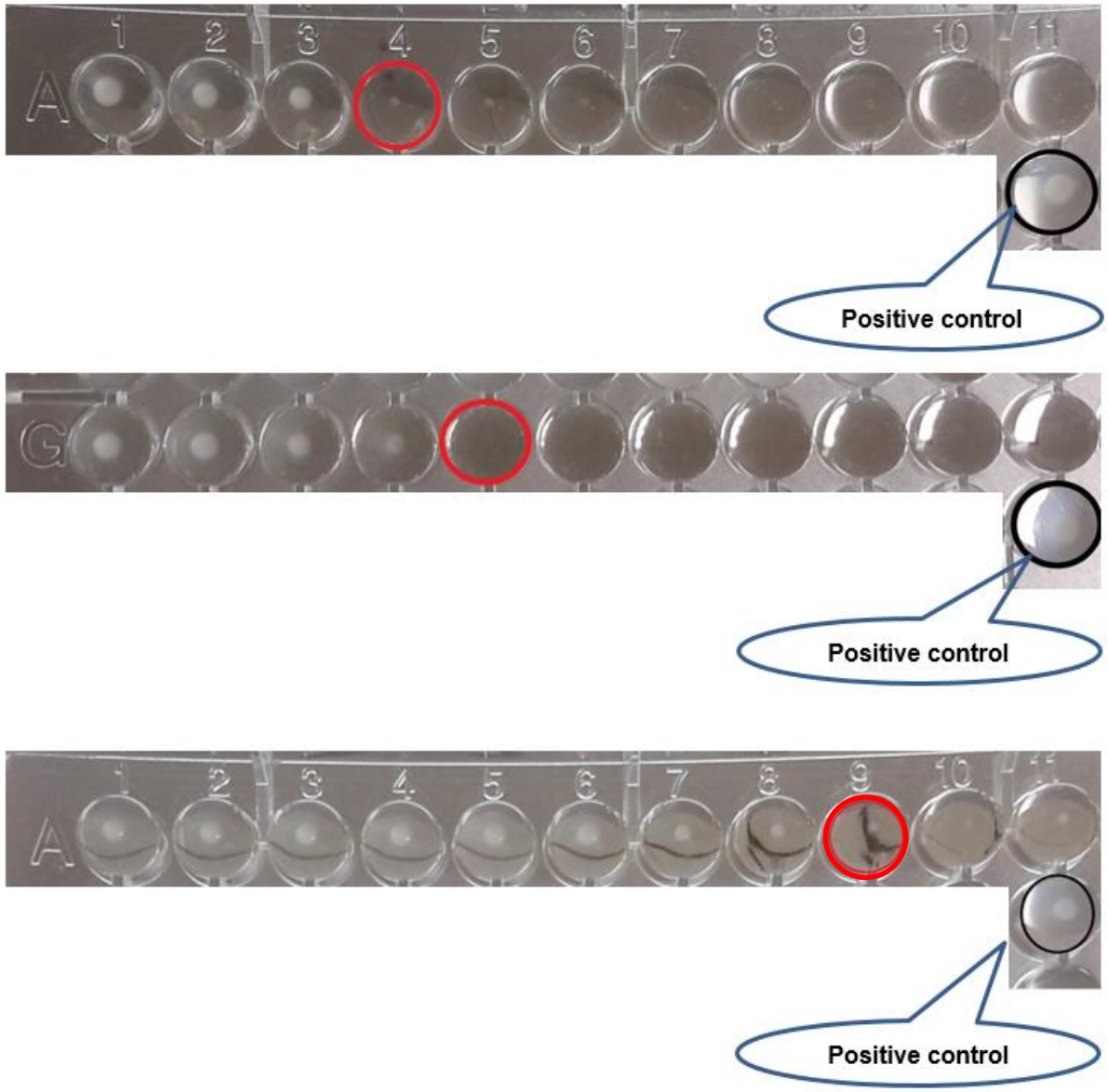
- Broth microdilution MIC determination must be performed in iron-depleted Mueller-Hinton broth and specific reading instructions must be followed. For testing conditions, see http://www.eucast.org/guidance_documents/.

- The MIC is read as the first well in which the reduction of growth corresponds to a button of <1 mm or is replaced by the presence of light haze/faint turbidity.

- The positive control should show strong growth in the form of a button of >2 mm or heavy turbidity.

- See next slide for pictures with reading examples.

Cefiderocol



Interpretation of results

- Make sure that MIC values for relevant Quality Control strains are within acceptable ranges before reporting results for clinical isolates.
  - See quality control criteria in EUCAST QC Tables (www.eucast.org).

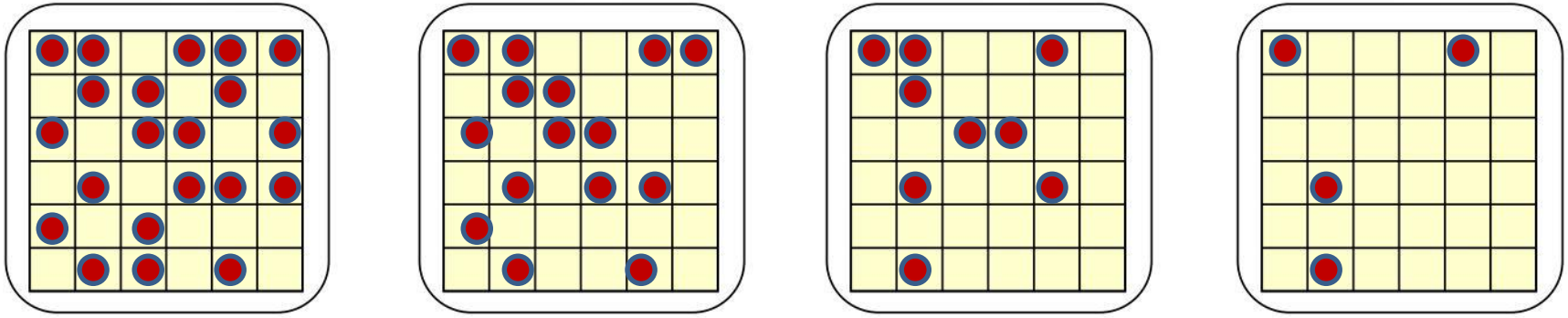
- Interpret MIC values into susceptibility categories (S, I and R) according to the current EUCAST Breakpoint Tables (www.eucast.org).

Agar dilution

Making dilutions of antimicrobial agent in melted media and pouring plates

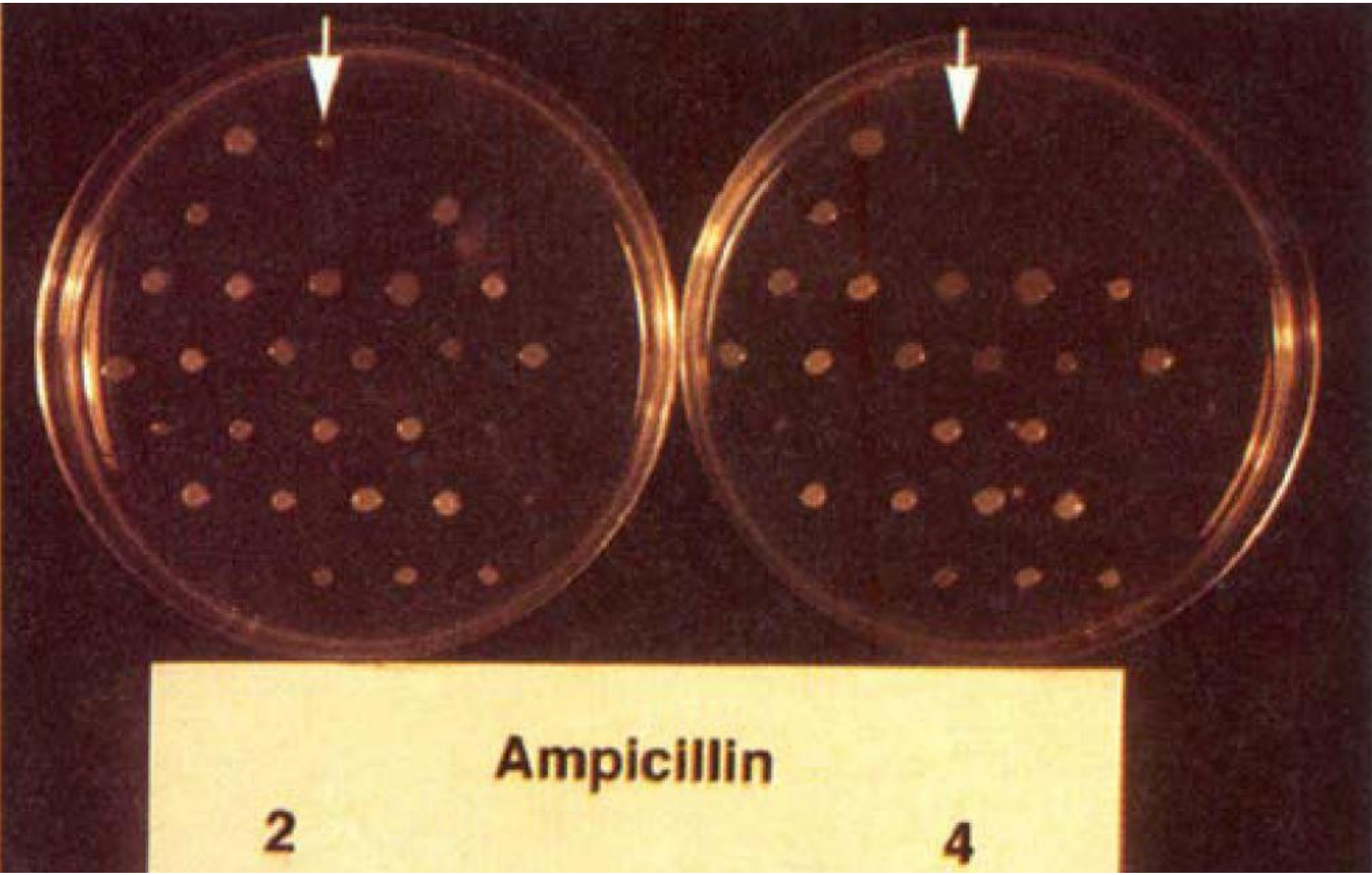
One concentration of antibiotic per plate
Possible for several different strains/plate

Ampicillin



1 mg/L 2 mg/L 4 mg/L 8 mg/L

Agar dilution



Gradient strips

Gradient strip (= Disk diffusion + Agar dilution)

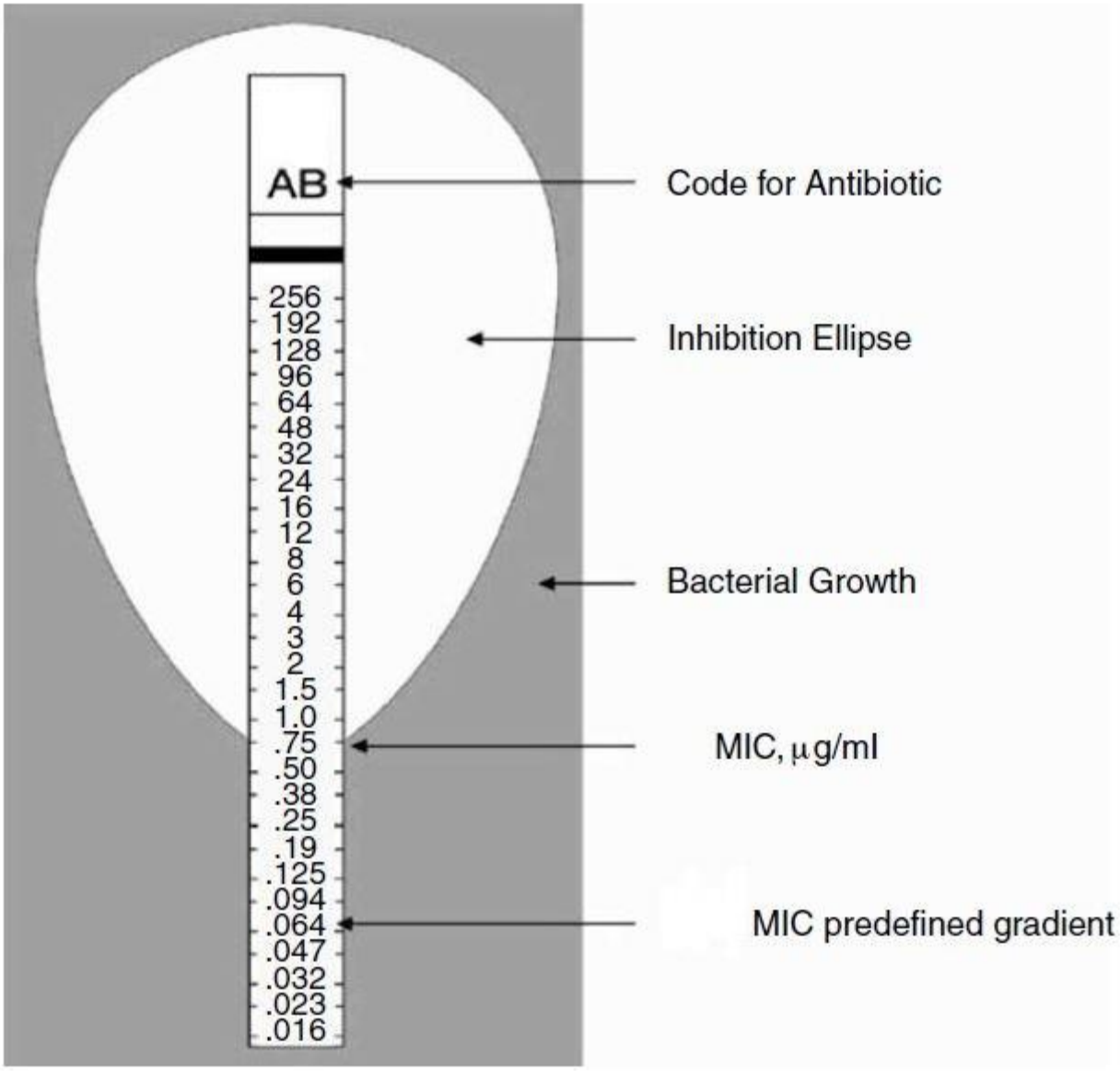
A preformed and predefined gradient of antibiotic concentrations is immobilized in a dry format onto the surface of a strip.

The continuous concentration gradient is calibrated across a corresponding MIC range covering 15 two-fold dilutions.

The MIC is determined by the intersection of the lower part of the ellipse shaped growth inhibition area with the test strip.

Provides flexibility by being able to test the drugs the laboratory chooses. This method is best suited to situations in which an MIC for only 1 or 2 drugs is needed or when a fastidious organism requiring enriched medium or special incubation atmosphere is to be tested (eg, penicillin and ceftriaxone with pneumococci).

Gradient strips



Schematic diagram of the gradient strip inhibition ellipse showing the MIC at the intersection of the growth of the organism and the calibrated strip.

Gradient strips

Gradient strips

Interpreting 1/2 dilutions

Example 1.

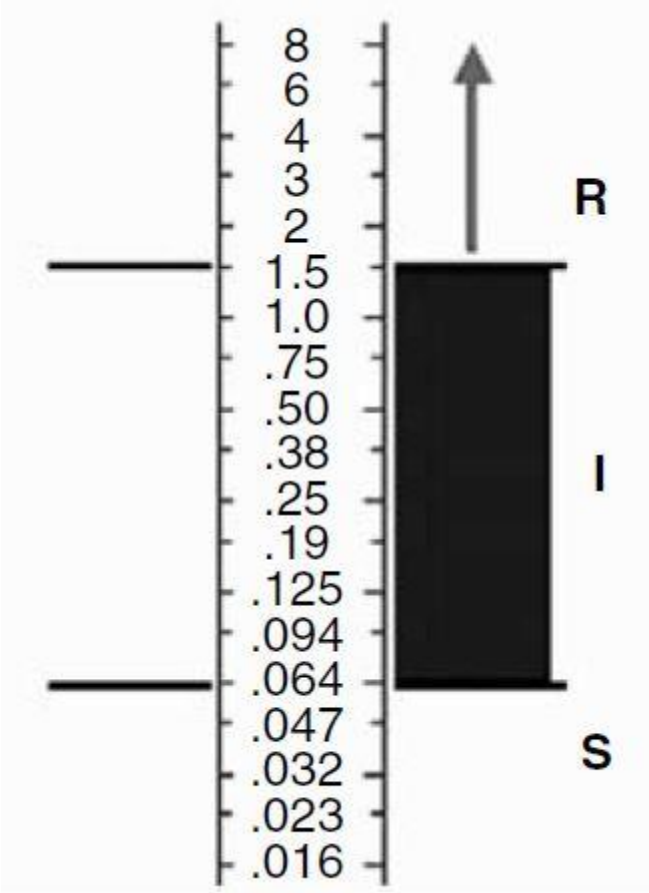
S ≤ 0.5 mg/L
I 1 mg/L
R ≥ 2 mg/L

MIC = 1.5 mg/L Category: R

Example 2.

S ≤ 0.06 mg/L
R > 0.06 mg/L

MIC = 0.06 mg/L Category: S



Because of the continuous concentration gradient, MIC values can fall in between conventional two-fold dilution values. The MIC should be rounded up to the next higher 2-fold dilution value before using EUCAST interpretive criteria for susceptibility categorisation.

Gradient strips

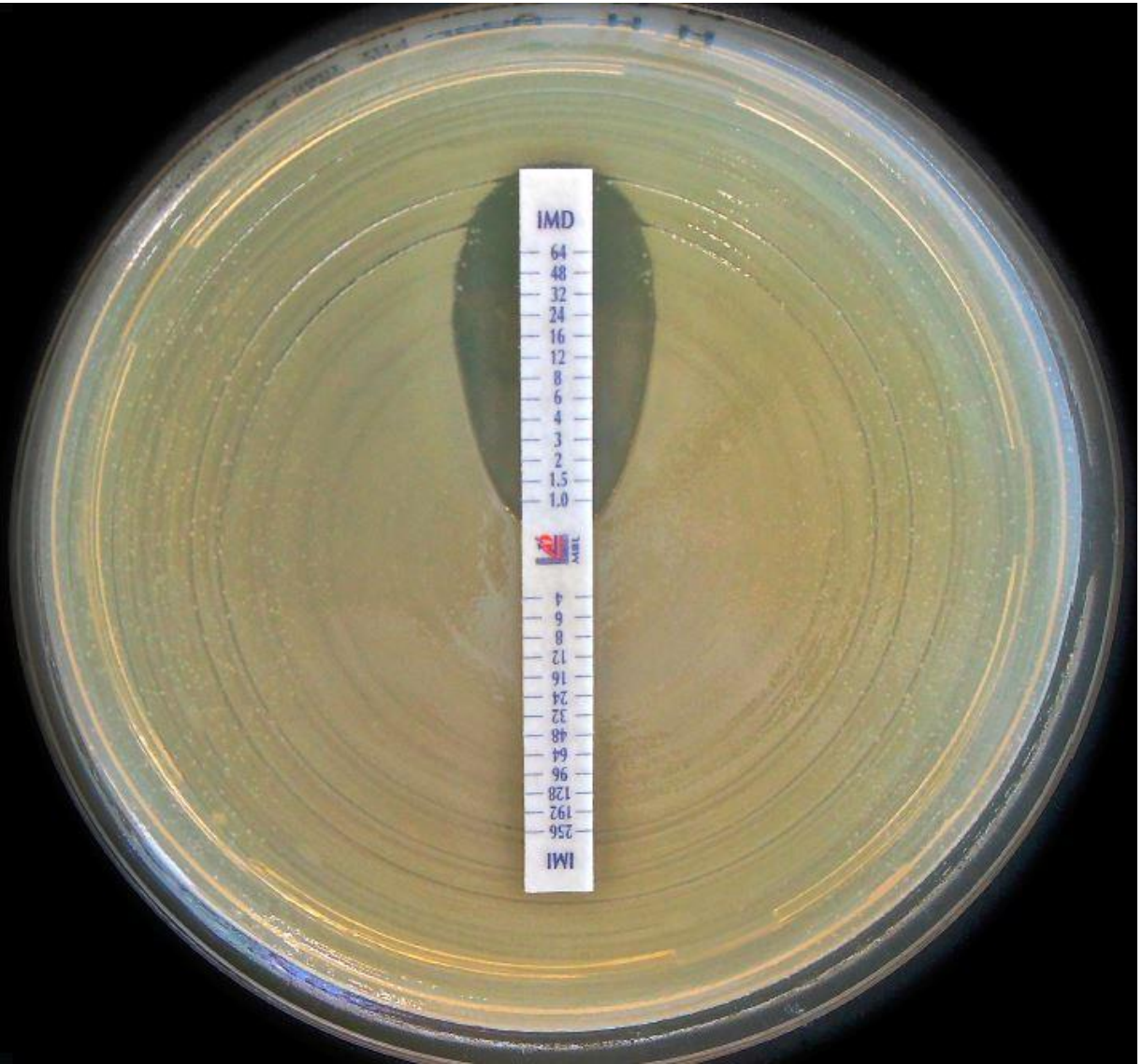
Other uses of gradient strips:

Resistance mechanism detection
(e.g., ESBL, AmpC beta-lactamase, carbapenemase)

Synergy detection
(investigation of interactions between antimicrobials)

Gradient strips

Other uses of gradient strips: Carbapenemase (MBL) detection



Imipenem (IMI):
> 256 mg/L

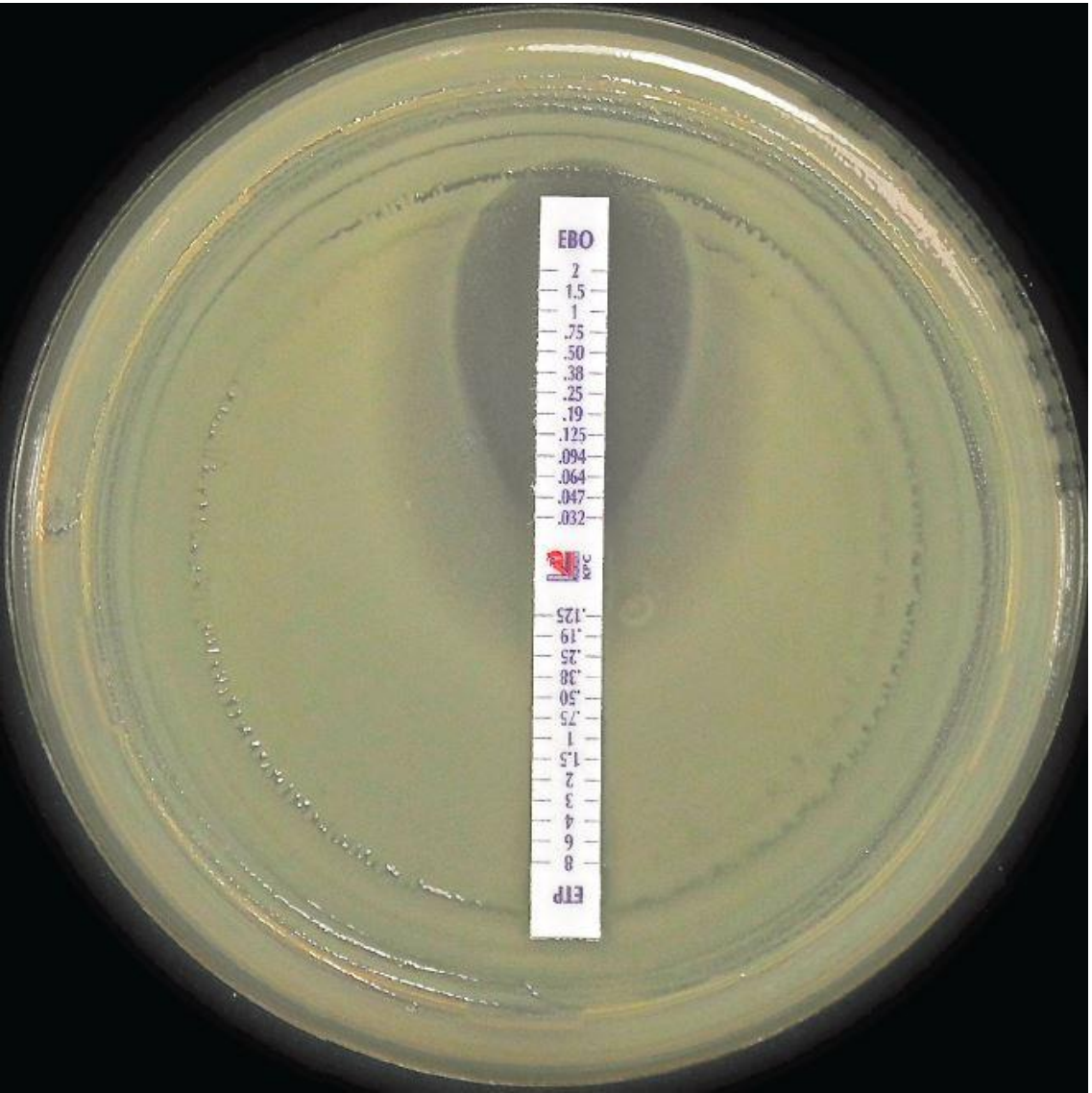
Imipenem+EDTA (IMD):
< 1 mg/L

The test is positive if 8-fold reduction is observed in the MIC of imipenem combined with EDTA (metallo-beta-lactamase inhibitor) compared with the MIC of imipenem alone

⇒ **Metallo-beta-lactamase (+)**

Gradient strips

Other uses of gradient strips: Carbapenemase (KPC) detection



Ertapenem (ETP):
> 8 mg/L

Ertapenem+Boronic acid (EBO):
0.047 mg/L

The test is positive if 8-fold reduction is observed in the MIC of ertapenem combined with boronic acid (KPC carbapenemase inhibitor) compared with the MIC of ertapenem alone

⇒ **KPC (+) ???**

(boronic acid is also an inhibitor of AmpC beta-lactamase)

Automated Systems

Does each laboratory need an automated AST instrument?

Case mix and organism isolation rates

Acute setting, potentially high morbidity, limited infection control, limited isolation rooms, significant numbers of potentially communicable pathogens

→ The cost savings to the patient and the institution on the treatment side may outweigh the expense of automating the laboratory.

Lab primarily performing urine cultures and the predominant organism is a mostly susceptible *E. coli* → no need for automation really.

Automated Systems

Methodology

The instrument should be compatible with the needs of the laboratory and current reporting options.

Will the lab be able to test all of the formulary antibiotics using the panel of the instrument?

How many times the performance of additional tests be required?

Automated Systems

Beckman Coulter MicroScan WalkAway 96 plus

Becton Dickinson Phoenix 100

BioMérieux VITEK 2 Compact

Self-contained incubator & reader

The AST reading is determined by detection of changes in the turbidity of each dilution well of the antibiotic.

Data management software (interpret, store, and report data)

Expert antibiotic resistance pattern alert system

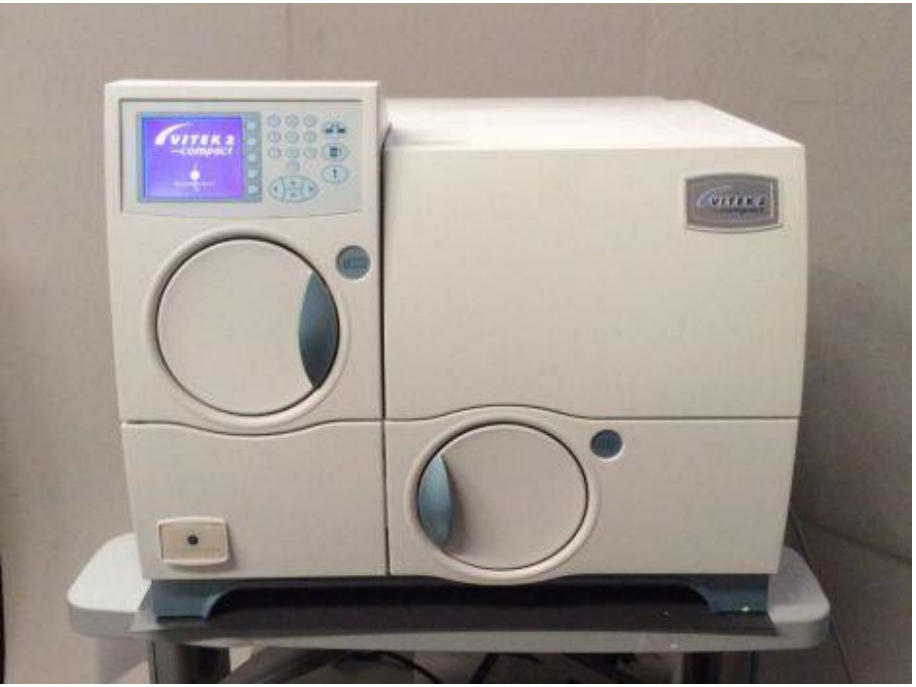
May be interfaced with many current laboratory information systems (LIS)

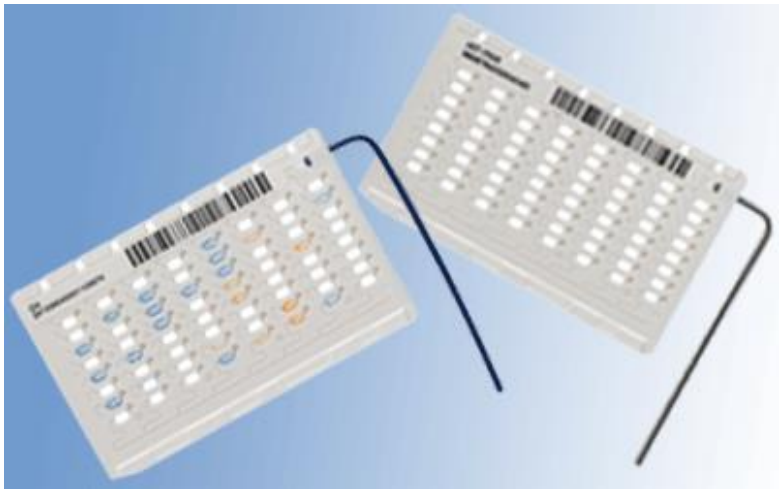
Automated Systems

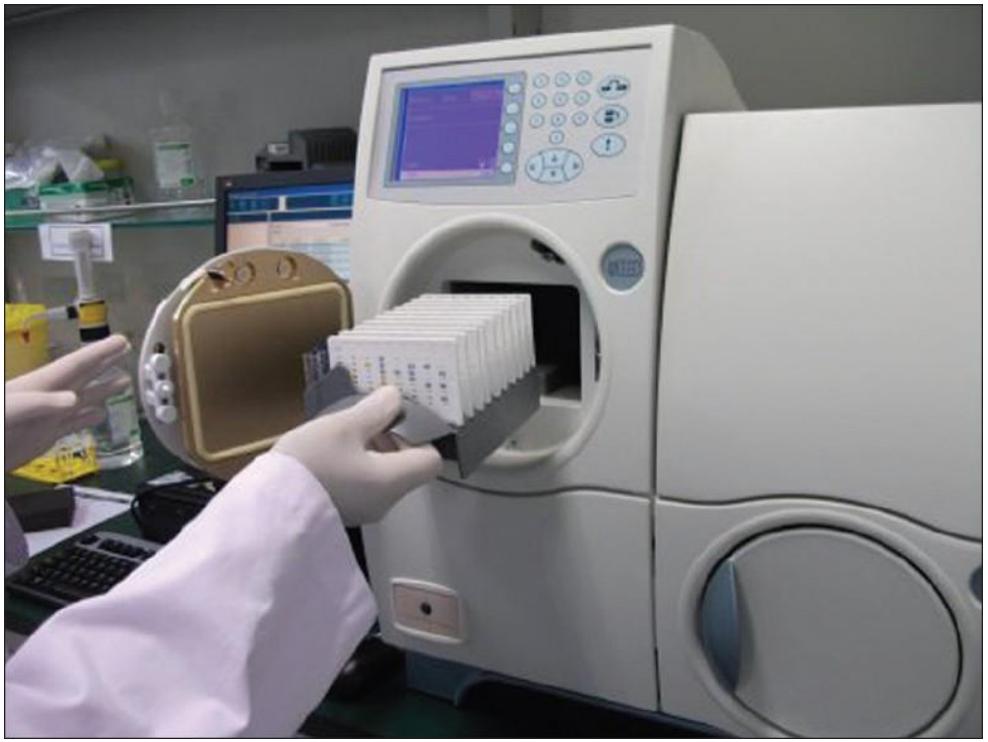
Beckman Coulter MicroScan WalkAway 96 plus

Automated Systems

BioMérieux VITEK 2 Compact

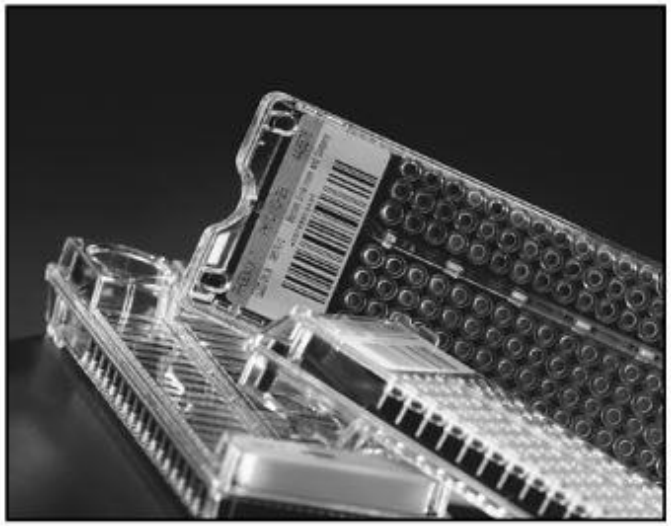






Automated Systems

Becton Dickinson Phoenix 100



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

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

Questions? Please contact
erika.matuschek@escmid.org

EUCAST Frequently Asked Questions

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 β-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 ± 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?

2. EUCAST Disk Diffusion Test - Disks

1. Are EUCAST disk contents all the same as CLSI?

3. EUCAST Disk Diffusion Test - Inoculum preparation

1. Do we have to measure the McFarland value on all suspensions?
2. Can we pick colonies from selective media?
3. Should we pick more than one colony to be sure that we do not miss hetero-resistance?
4. Can we use water or buffer instead of saline for inoculum preparation?
5. In the EUCAST disk diffusion manual it is stated that we have to adjust the inoculum to a density of a McFarland 0.5 turbidity standard. What is the range we can use?
6. Can flooding be used to inoculate plates for antimicrobial susceptibility testing?

4. EUCAST Disk Diffusion Test - Reading zones of inhibition

1. Do we have to measure all inhibition zones?
2. Should inhibition zones on both MH and MH-F be read against a black background?
3. Are all bactericidal and bacteriostatic agents read according to the same recommendations?
4. Why is there sometimes growth within zones of beta-lactams for *Haemophilus influenzae* ATCC 49766?
5. Are isolated colonies within mecillinam inhibition zones significant?
6. Why are there sometimes colonies within the inhibition zones of carbapenems and *Pseudomonas aeruginosa* ATCC 27853?

Videos and online seminars from EUCAST

Instruction videos with subtitles in several languages (below)

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.
- Alternative access to **instruction videos in English with English subtitles.**

Instruction videos - German subtitles.

Instruction videos - Russian subtitles.

Instruction videos - Turkish subtitles.

Instruction videos - French subtitles.

Instruction videos - Spanish subtitles.

Instruction videos - Portuguese subtitles.

Instruction videos - Arabic subtitles.

Instruction videos - Czech subtitles.

Instruction videos - Chinese subtitles.
- Alternative access to instruction videos in english with **chinese subtitles.**

Instruction videos - Romanian subtitles.

- Check the EUCAST website regularly for updates on methodology, QC ranges and breakpoints.

www.eucast.org

- Please send any questions, comments and suggestions to

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erika.matuschek@kronoberg.se
onur.karatuna@kronoberg.se