



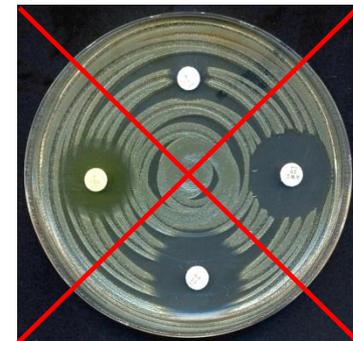
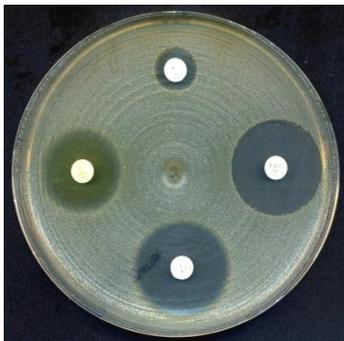
The EUCAST disk diffusion test

Reading of zones

Jenny Åhman – EUCAST Development Laboratory, Sweden
Ingvild Haugan – St. Olavs Hospital, Norway
Nathalie Friberg – HUSLAB, Finland

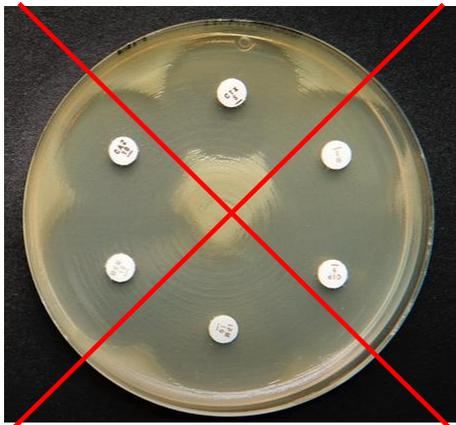
Inoculum

- Examine plates after incubation
- Growth should be confluent and evenly spread over the agar plate.
 - If the inoculum is too light the test should be repeated!
- Inhibition zones should be uniformly circular.
 - The streaking of plates affect the end result!



Application of disks

- Limit the number of disks per plate to avoid overlapping of zones and interference between agents.
 - It is important that zone diameters can be reliably measured.
- The number of disks per plate depends on the organism and agents: maximum 6 disks, optimally 3-4 disks.
 - Arrange disk dispensers to avoid overlapping!



Reading devices

- Calliper

- Analog or digital

- Ruler



- Templates

- Make sure templates are in accordance with the latest version of the EUCAST Breakpoint Tables.

- Automated zone readers

- Must be calibrated to manual reading!



Light source

- Plates should be illuminated with reflected light.
 - Use a desk light in addition to ceiling lamps!
- Do not use transmitted light (plate held up to light), unless otherwise stated.
- Do not use magnifying glass.



General reading instructions

- Hold the plate about 30 cm from the eye.
- Read zone edges at the point of complete inhibition as judged by the naked.
- Read bactericidal and bacteriostatic drugs the same way.
- Report zone diameters to the nearest millimetre.



Reading zones

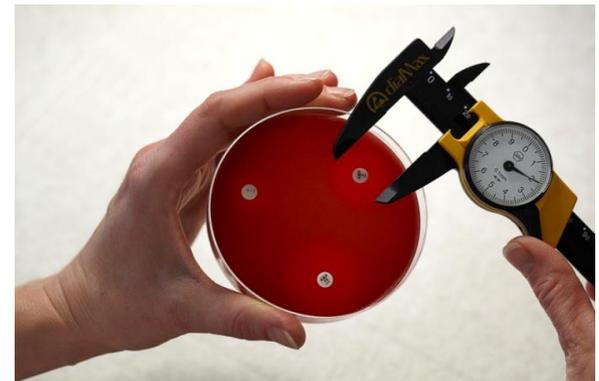
Mueller-Hinton (MH) plates

Read from the back of the plate against a dark background.



MH-F and FAA-HB plates

Read from the front of the plate with the lid removed.



How to deal with:

- fuzzy zone edges
- colonies within zone
 - double zones
 - swarming
 - haemolysis

Fuzzy zone edges – MH plates

- Hold the plate against a dark background about 30 cm from the eye and estimate the zone edge.
- Do not hold the plate up to light (transmitted light) or use a magnifying glass.
- In case of double zones, read the inner zone.



Fuzzy zone edges - staphylococci

Fuzzy zone edges – MH-F plates

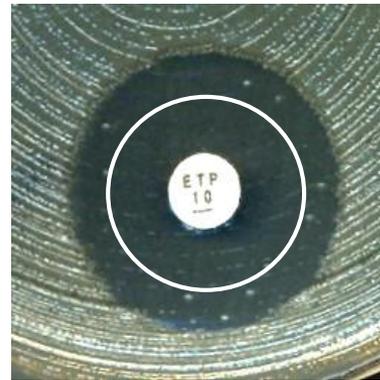
- Hold the plate at a 45-degree angle to the work bench to facilitate reading when zone edges are difficult to define.
- Problems with fuzzy zone edges can be related to excess humidity and may be reduced by drying MH-F plates.



Fuzzy zone edges - streptococci

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.



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

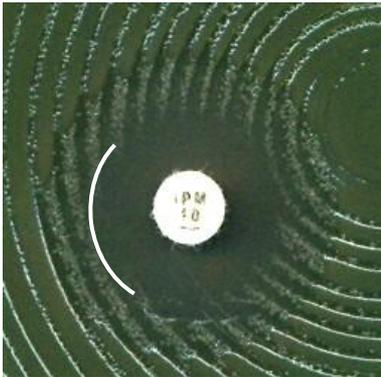


H. influenzae with
PBP mutations



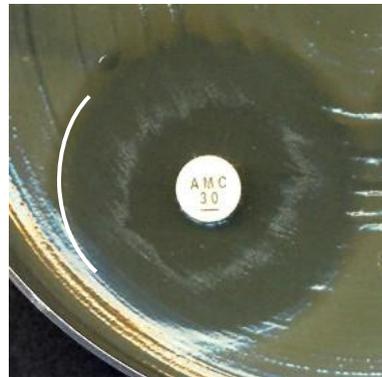
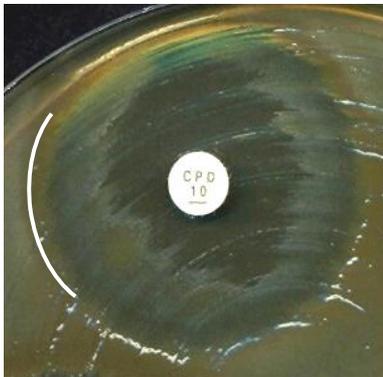
Double zones

- In case of double zones, check for purity and repeat the test if necessary.
- If cultures are pure, read the inner zone.



Swarming

- For *Proteus* spp., ignore swarming and read inhibition of growth.
- Swarming usually appears like a non-circular pattern.



Haemolysis

- Read inhibition of growth and not inhibition of haemolysis.
- β -haemolysis is usually free from growth
 - β -Haemolysins diffuse in agar
- There is usually growth within areas of α -haemolysis.
 - α -Haemolysins do not diffuse in agar



β -haemolysis



β -haemolysis



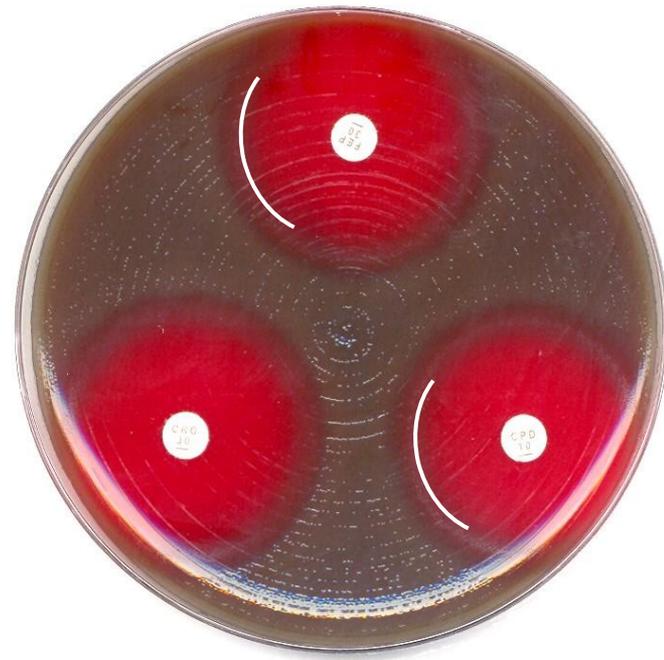
α -haemolysis

Haemolysis

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



β -haemolysis is usually free from growth



There is usually growth in areas of α -haemolysis

Specific reading instructions

Exceptions from the general
reading instructions

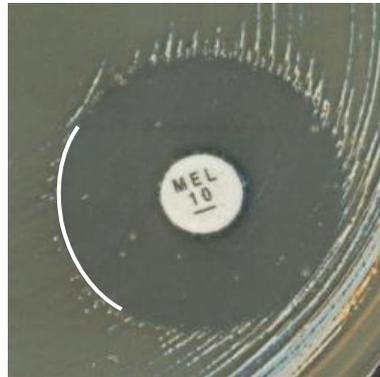
Enterobacterales with aminopenicillins

- 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.



Enterobacteriales with mecillinam

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



E. coli with fosfomicin

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



Stenotrophomonas 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

Enterococci and vancomycin

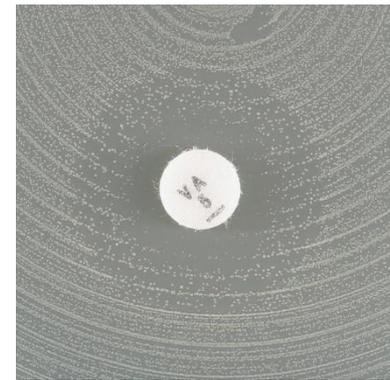
- **If vancomycin zone ≥ 12 mm:** Examine the zone edge from the front with transmitted light (plate held up to light).
 - Sharp zone edge indicate vancomycin susceptibility.
 - Fuzzy zone edge or colonies within the zone indicate vancomycin resistance (VRE) → Perform confirmatory testing.



non-VRE



VRE



VRE

S. aureus and benzylpenicillin

- **If benzylpenicillin zone ≥ 26 mm:** Examine the zone edge from the front with transmitted light (plate held up to light).
 - Fuzzy zone edge (reduction of growth towards zone edge, like a “beach”), report susceptible.
 - Sharp zone edge (no reduction of growth towards zone edge, like a “cliff”) indicate penicillinase production, report resistant.



Fuzzy zone edge (“beach”)
= Susceptible

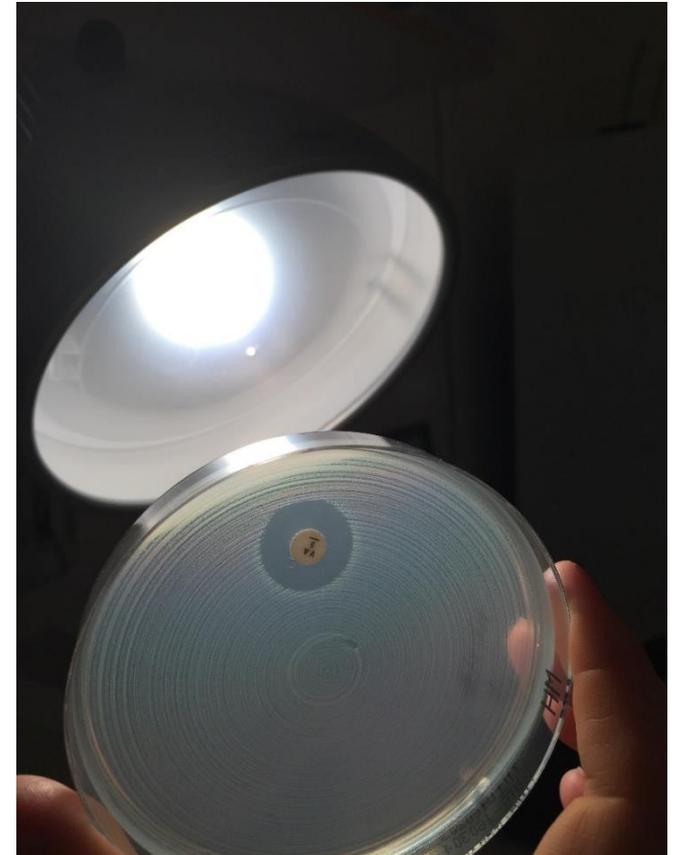


Sharp zone edge (“cliff”)
= Resistant

Enterococci and vancomycin *S. aureus* and benzylpenicillin

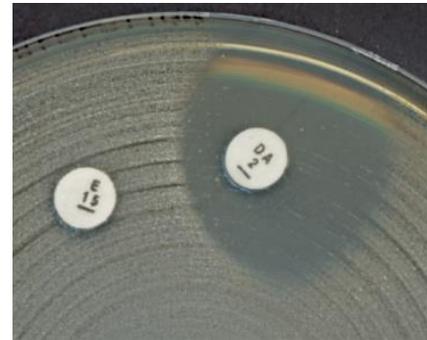
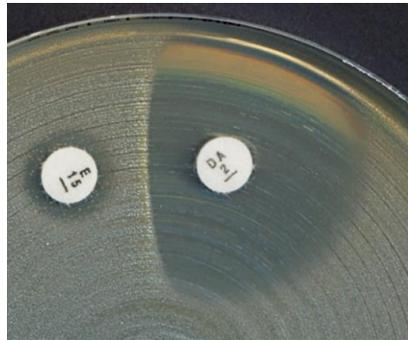
For isolates with zone diameters above the susceptible breakpoint:

- Remove the lid.
- Hold the plate up to light.
- Examine the zone edge closely from the front of the plate (transmitted light).



Inducible clindamycin resistance in staphylococci

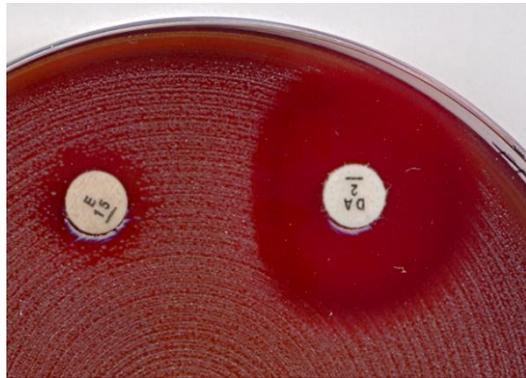
- Can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place erythromycin and clindamycin disks **12-20 mm apart** (edge to edge) and look for antagonism, “D phenomenon”.



D phenomenon for staphylococci

Inducible clindamycin resistance in streptococci

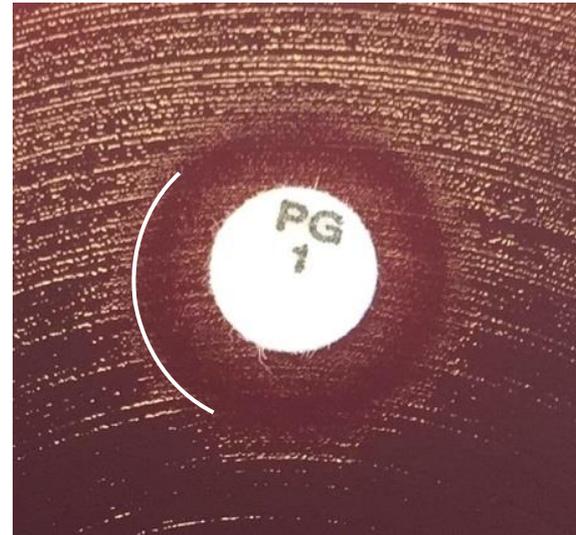
- Can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place erythromycin and clindamycin disks **12-16 mm apart** (edge to edge) and look for antagonism, “D phenomenon”.



D phenomenon for streptococci

H. influenzae and beta-lactam agents

- If there is an area of growth around the disk in an otherwise clear inhibition zone, read the outer zone edge.



Reading instructions for anaerobic bacteria

General reading instructions

- Read FAA-HB plates from the front with the lid removed and illuminated with reflected light.
- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- Note that the disk diffusion method is valid only for:
 - *Bacteroides* spp. (including *Parabacteroides* spp.)
 - *Prevotella* spp.
 - *Fusobacterium necrophorum*
 - *Clostridium perfringens*
 - *Cutibacterium acne*.

Specific reading instructions

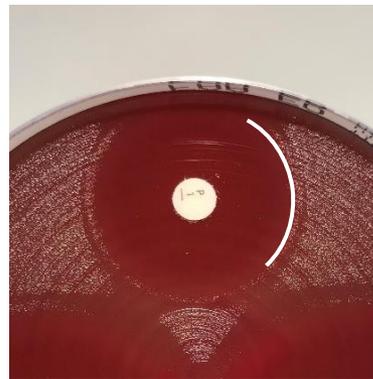
- Ignore any faint haze within the inhibition zone and read the most obvious zone. Tilt the plate towards you to better define the obvious zone edge.
- Problems with haze within zone can be related to excess humidity and may be reduced by drying FAA-HB plates.



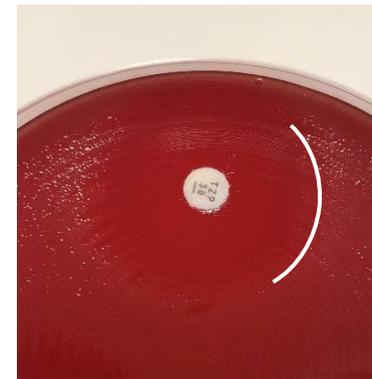
Bacteroides spp.
Meropenem



Bacteroides spp.
Piperacillin-tazobactam



F. necrophorum
Benzylpenicillin



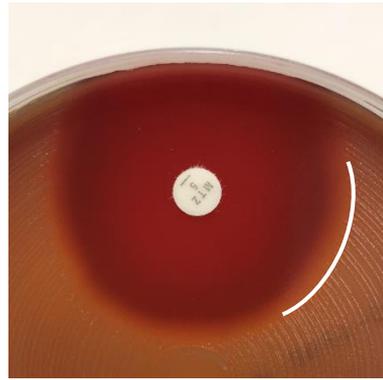
C. perfringens
Piperacillin-tazobactam

Specific reading instructions

- Ignore haemolysis and swarming and read inhibition of growth.



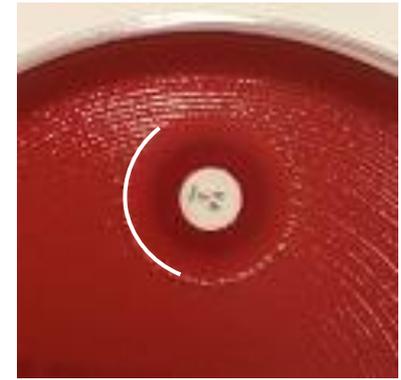
Bacteroides spp.
Piperacillin-tazobactam



F. necrophorum
Metronidazole



C. perfringens
Benzylpenicillin



C. perfringens
Vancomycin

Specific reading instructions

- Isolated colonies within the inhibition zone should be taken into account.
- For clindamycin, it is particularly important to examine zones carefully for colonies growing within the zone.



Bacteroides spp.
Clindamycin



C. perfringens
Clindamycin

EUCAST Reading Guides

Aerobic bacteria



Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 10.0
January 2023

Anaerobic bacteria



Reading guide

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

Version 2.0
January 2023

www.eucast.org/ast_of_bacteria/disk_diffusion_methodology

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](#)
[Warnings!](#)
[MIC testing services from EUCAST](#)
[Previous versions of documents](#)
[AST of mycobacteria](#)




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.

The EUCAST Development Laboratory has systematically investigated the quality of MH-powders, antibiotic disks and of pre-poured MH plates for disk diffusion. The publications from these investigations are available from the EUCAST website ([antibiotic disk quality](#), [MH powder quality](#), the [quality of pre-poured MH plates](#)).

See also [EUCAST instruction videos](#) with subtitles in many languages.

- [Disk diffusion manual v 11.0](#) (2 January, 2023)
- [Disk diffusion - Slide show v 11.0](#) (2 January, 2023)
- [Disk diffusion - Reading guide v 10.0](#) (2 January, 2023)
- [Anaerobic bacteria - disk diffusion methodology v 2.0](#) (2 January 2023). QC recommendations are now included in the general [QC document](#). Disk diffusion breakpoints for anaerobic bacteria are valid for FAA with 5% mechanically defibrinated horse blood as the only additive.
- [Anaerobic bacteria - disk diffusion reading guide v 2.0](#) (2 January 2023). Disk diffusion breakpoints for anaerobic bacteria are valid for FAA with 5% mechanically defibrinated horse blood as the only additive.

For translations to other languages - contact National AST committees (NAC).