

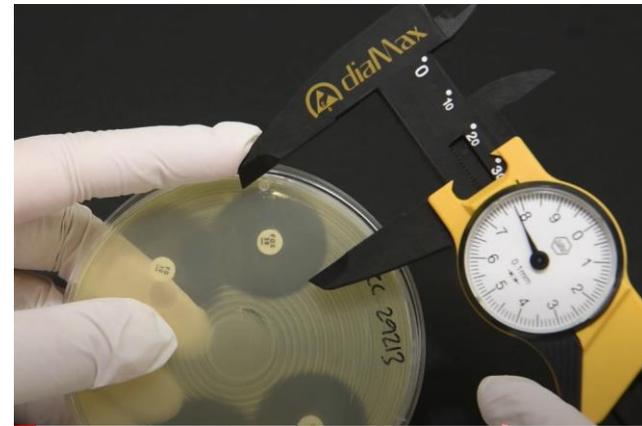
The EUCAST disk diffusion test

Reading of zones

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Reading zones

- 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 (unless otherwise stated).
- Measure zone diameters to the nearest millimetre with a ruler or a calliper.



Reading zones

- Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
 - Recommended specifically for *Campylobacter* and anaerobic bacteria



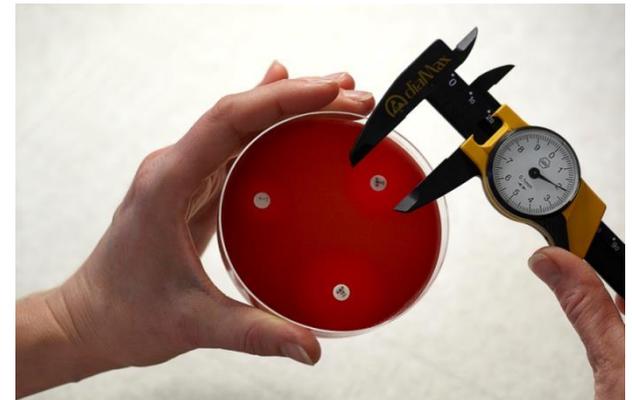
Reading zones

- Plates should be illuminated with reflected light.
- Do not use transmitted light (plate held up to light) or a magnifying glass unless stated.



Reading zones

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



Fuzzy zone edges

Example staphylococci

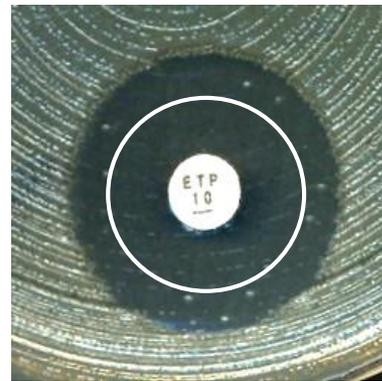
- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is. Do not hold the plate up to light (transmitted light) or use a magnifying glass.



Reading of zones with fuzzy zone edges for staphylococci.

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.

Double zones

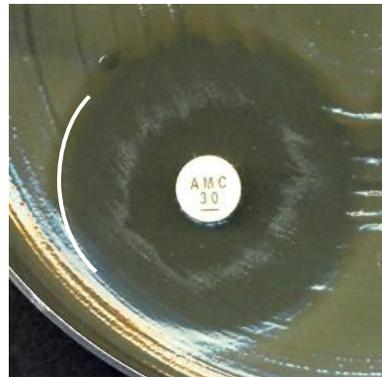
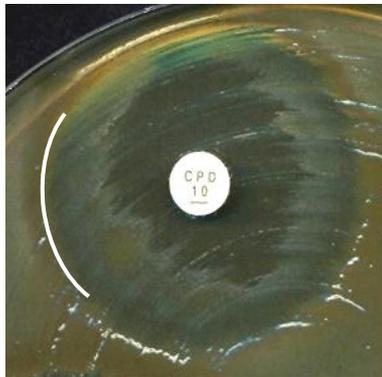
- In case of double zones, check for purity and repeat the test if necessary.
- If cultures are pure, read the inner zone.



Reading of double zones.

Swarming

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



Growth or haemolysis?

- Read inhibition of growth and not inhibition of haemolysis.
- β -Haemolysis is usually free from growth.
- There is often growth in areas with α -haemolysis.

Haemolysis

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



β -haemolysis is usually free from growth

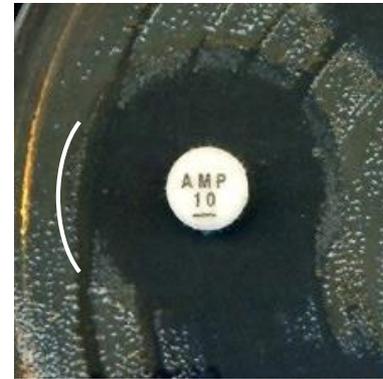
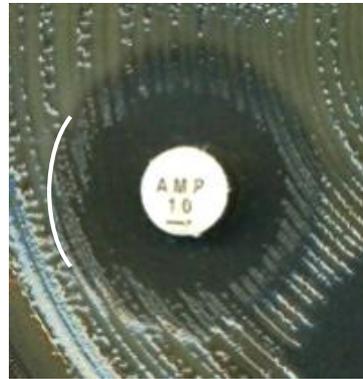


There is usually growth in areas of α -haemolysis

Specific reading instructions

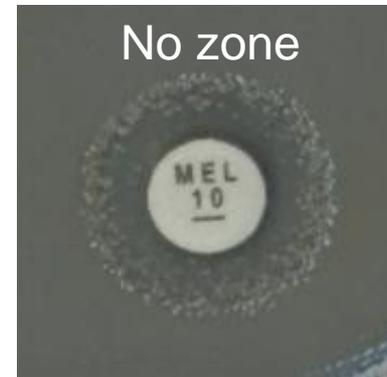
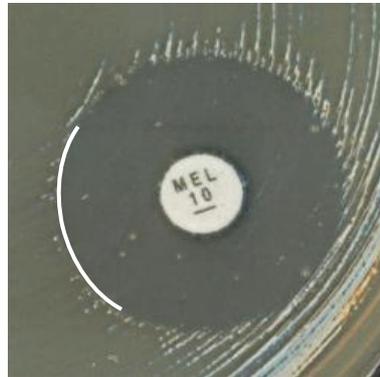
Enterobacterales with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid

- 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.



Enterobacteriales 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.



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

Enterococci and vancomycin *S. aureus* and benzylpenicillin

- For isolates with zone diameters above the susceptible breakpoint: Examine the zone edge from the front of the plate with transmitted light (plate held up to light).

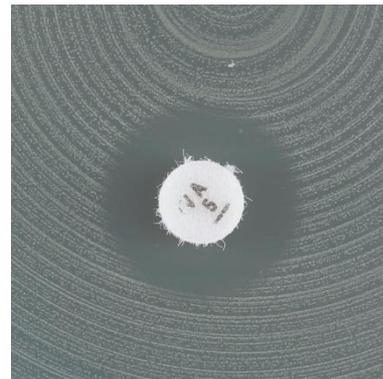


Enterococci and vancomycin

- **For isolates with zone diameters ≥ 12 mm:** Examine the zone edge from the front of the plate with transmitted light (plate held up to light).
 - If the zone edge is sharp, report according to the breakpoints.
 - 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.



non-VRE



VRE

S. aureus and benzylpenicillin

- **For isolates with zone diameters ≥ 26 mm:** Examine the zone edge from the front of the plate with transmitted light (plate held up to light).
 - If the zone edge is sharp (no reduction of growth towards zone edge, like a “cliff”), report resistant even if the zone diameter is ≥ 26 mm.
 - If the zone edge is fuzzy (reduction of growth towards zone edge, like a “beach”), report susceptible.



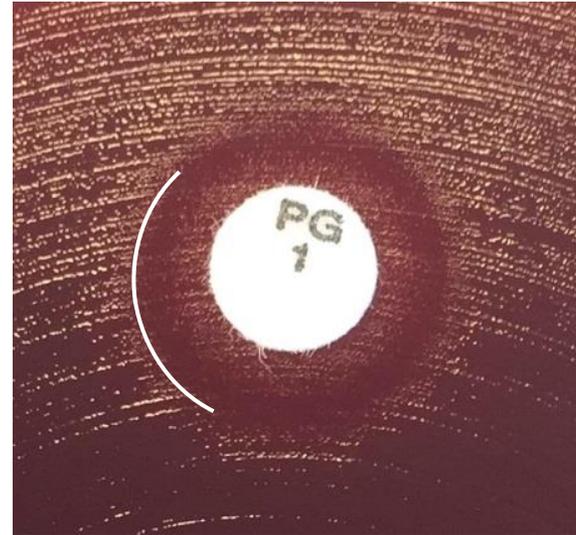
Zone ≥ 26 mm and sharp zone edge (“cliff”) = Resistant



Zone ≥ 26 mm and fuzzy zone edge (“beach”) = Susceptible

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.



Reading instructions for anaerobic bacteria



- Ignore any faint haze within the inhibition zone and read the most obvious zone. **Tilt the plate towards you to better define the obvious zone edge.**
- Ignore haemolysis and swarming and read inhibition of growth.
- 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.**

EUCAST Reading Guide



Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

zones

es from the back background reflected light.

ates from the front moved illuminated ight.



reading instructions

h ampicillin, ampicillin-sulbactam and c acid
d temocillin
d mecillinam
n
methoprim-sulfamethoxazole in general
naltophilia, *Achromobacter xylosoxidans* and
malleri with trimethoprim-sulfamethoxazole
I trimethoprim-sulfamethoxazole
comycin
lpenicillin
e clindamycin resistance in staphylococci and
ta-lactam agents

www.eucast.org/ast_of_bacteria/disk_diffusion_methodology

(Specific Reading Guide for anaerobic bacteria)



EUCAST

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