

# The EUCAST disk diffusion test

## Reading of zones

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# General reading instructions

- 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.
  - Both for bactericidal and bacteriostatic agents.
- 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 of zones

- Hold the plate at a 45-degree angle to the work bench to facilitate reading when zone edges are fuzzy or difficult to define.
- Specifically recommended for *Campylobacter* and anaerobic bacteria.



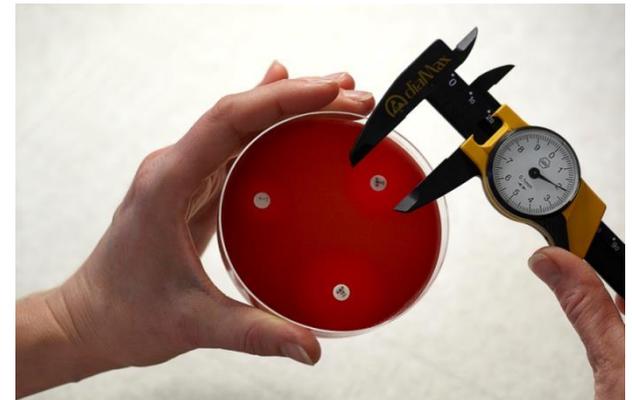
# Reading of zones

- 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

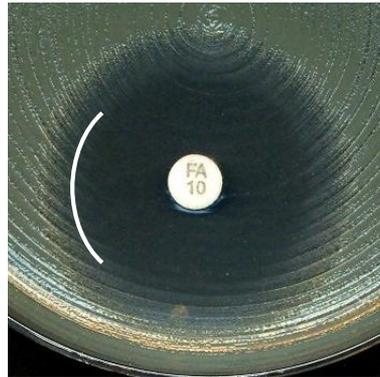
- **MH plates:**  
Read zones from the back against a dark background.
- **MH-F and FAA-HB plates:**  
Read zones from the front with the lid removed.



# Fuzzy zone edges

## Example staphylococci

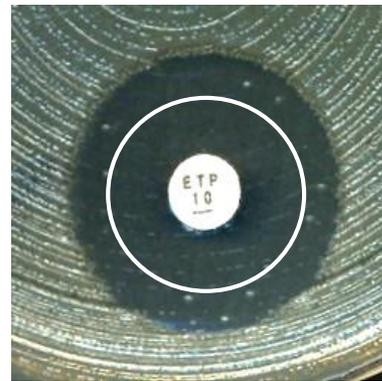
- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is.
  - Do not hold the plate up to light (transmitted light) or use a magnifying glass.



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.

# Colonies within zone

- 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.

# Double zones

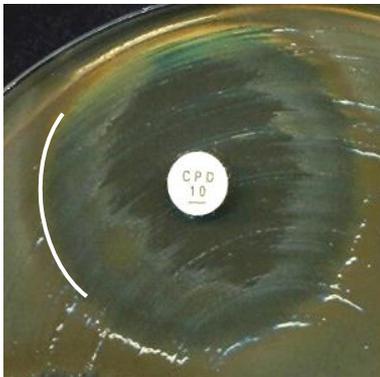
- In case of double zones, check for purity and repeat the test if necessary.
- If cultures are pure, read the inner zone.



Reading of double zones.

# Swarming

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



# $\beta$ -haemolysis

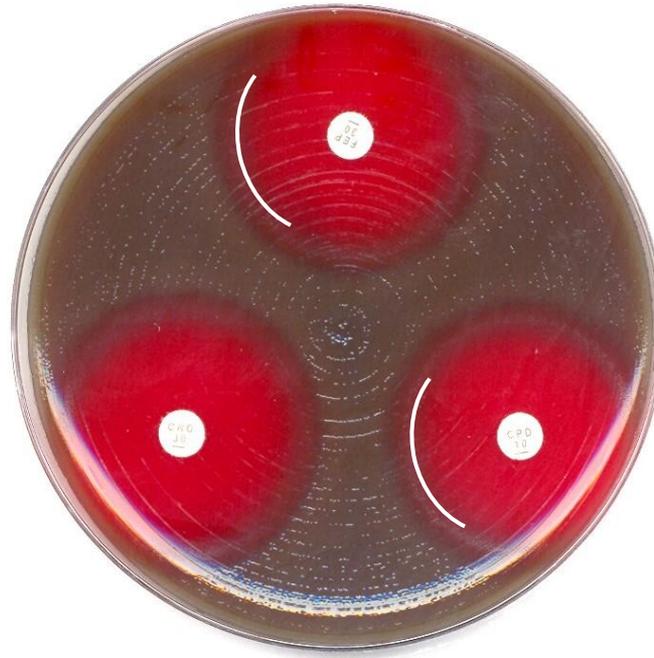
- $\beta$ -haemolysis is usually free from growth.
  - $\beta$ -haemolysins diffuse in agar.
- Read inhibition of growth (and not haemolysis).
  - Tilt the plate to differentiate between growth and haemolysis.



*S. pyogenes*

# $\alpha$ -haemolysis

- There is usually growth within the area of  $\alpha$ -haemolysis. This area should be taken into account when reading.
  - $\alpha$ -haemolysins do not diffuse in agar.

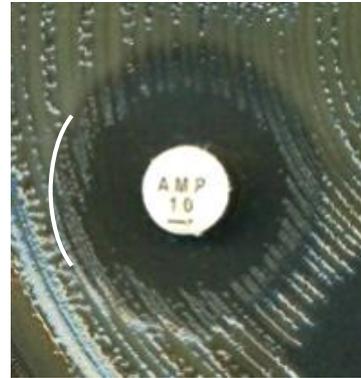


Often seen with beta-lactam agents and *S. pneumoniae*.

**Specific reading instructions**

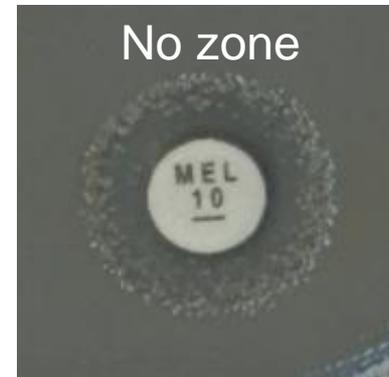
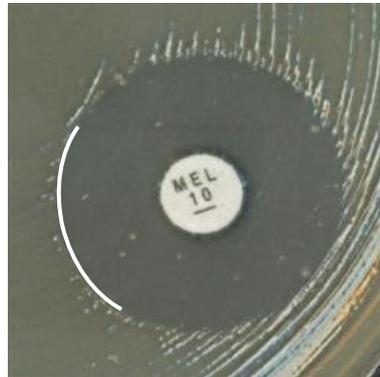
# *Enterobacterales* with ampicillin and amoxicillin-clavulanic acid

- Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agar.
- 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 if growth is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to 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, *i.e.* holding the plate up to light.

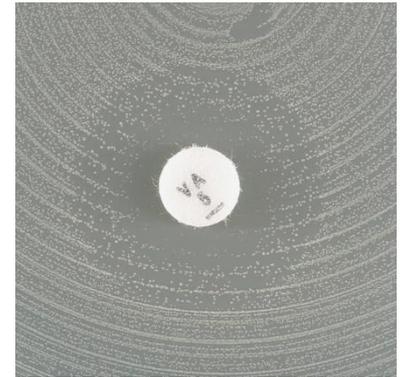
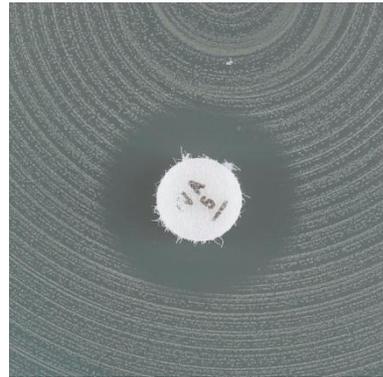


# Enterococci and vancomycin

- **For isolates with zone diameters  $\geq 12$  mm:**
  - Sharp zone edge: Report susceptible.
  - Fuzzy zone edge or colonies within the zone or if uncertain: Suspect VRE and perform confirmatory testing, even if the zone diameter is  $\geq 12$  mm.



non-VRE



VRE

# *S. aureus* and benzylpenicillin

- **For isolates with zone diameters  $\geq 26$  mm:**
  - Sharp zone edge (no reduction of growth towards zone edge, like a “cliff”): Report resistant even if the zone diameter is  $\geq 26$  mm.
  - Fuzzy zone edge (reduction of growth towards zone edge, like a “beach”): Report susceptible.



Sharp zone edge (“cliff”)  
= Resistant



Fuzzy zone edge (“beach”)  
= Susceptible

# *H. influenzae* and beta-lactam agents

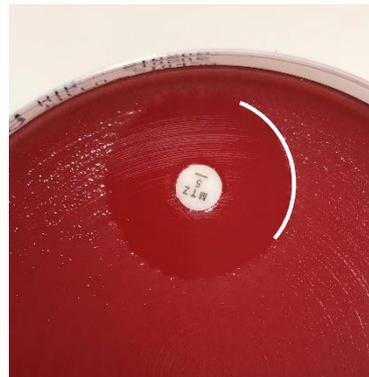
- In the case of a clear inhibition zone with regrowth around the disk, read the outer zone edge.



# Anaerobic bacteria

## General instructions

- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- If faint haze within the zone occurs, disregard haze and read the most obvious zone edge.
  - Tilt the plate towards you to better define the obvious zone edge.



# Anaerobic bacteria

## Specific instructions



- 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 Guides



## Reading guide

EUCAST disk diffusion  
method for antimicrobial  
susceptibility testing

Version 11.0  
January 2025

Aerobic 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

Anaerobic bacteria

[www.eucast.org/ast\\_of\\_bacteria/disk\\_diffusion\\_methodology](http://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology)



**EUCAST**

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