EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Reading guide

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 10.0
January 2023

Changes from previous version (v 9.0)

Slide	Change
25	Clarification on that zone edges for enterococci and vancomycin only have to be examined for zones ≥12 mm.
26	Clarification on that zone edges for *S. aureus* and benzylpenicillin only have to be examined for zones ≥26 mm.

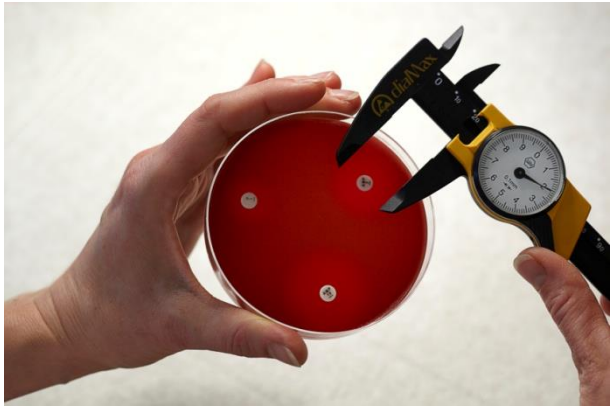
Reading zones

- 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 15-29).
- 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.

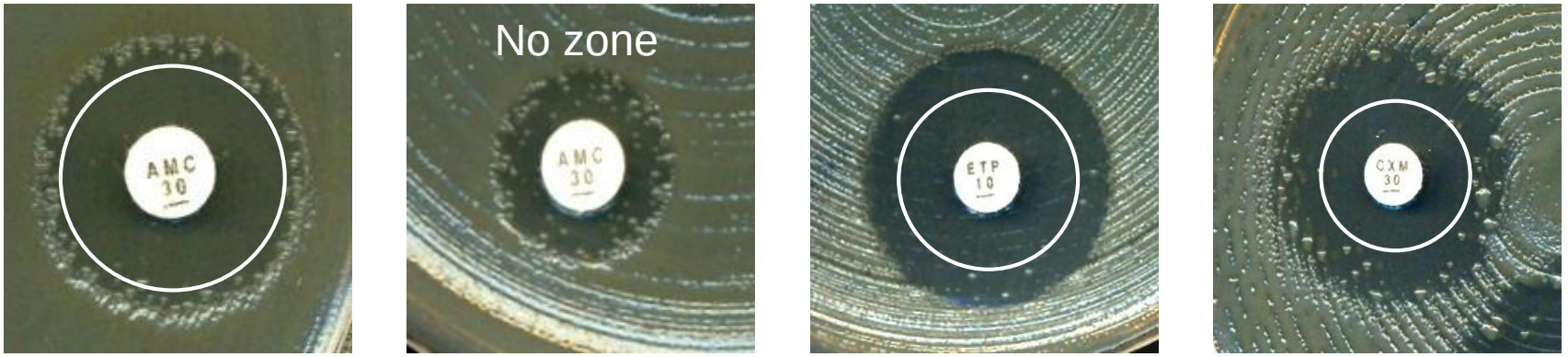
- Read **MH-F** plates from the front with the lid removed illuminated with reflected light.



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.

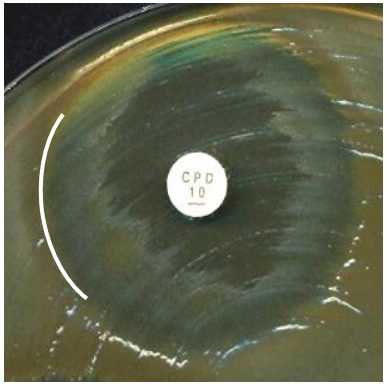
E. coli with ESBL

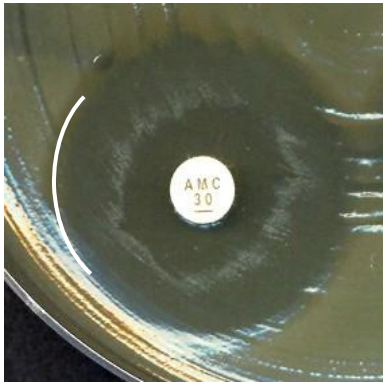
H. influenzae with PBP mutations

Reading of zones with colonies within the zone.

Swarming

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

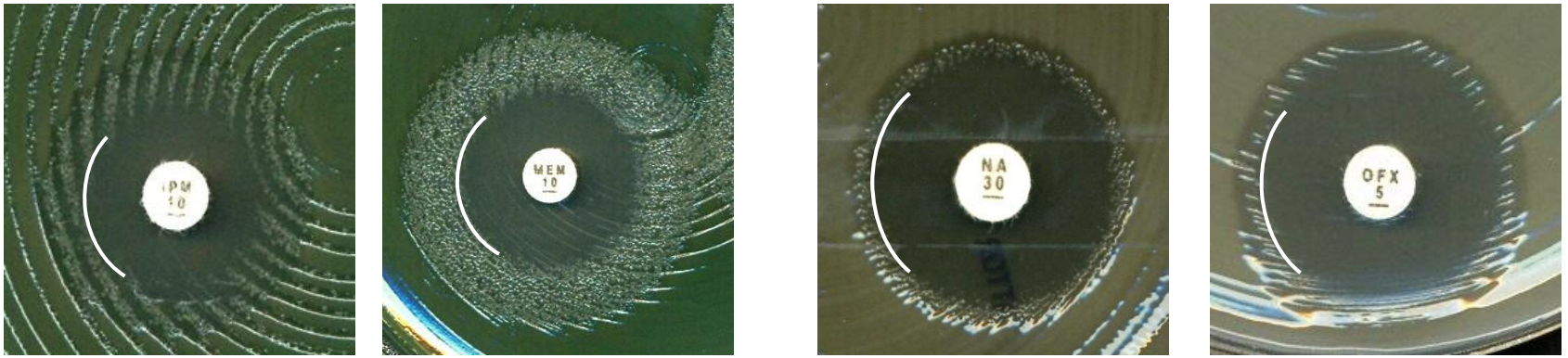




Double zones

- 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.

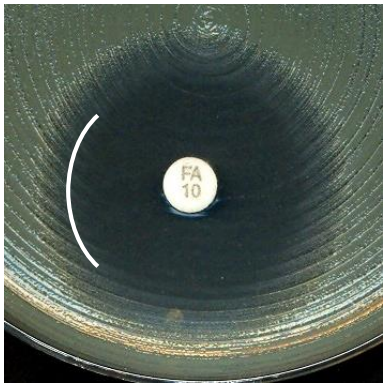
Fuzzy zone edges
Enterobacterales

- 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 held about 30 cm from the naked eye at a 45-degree angle to the work bench 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.

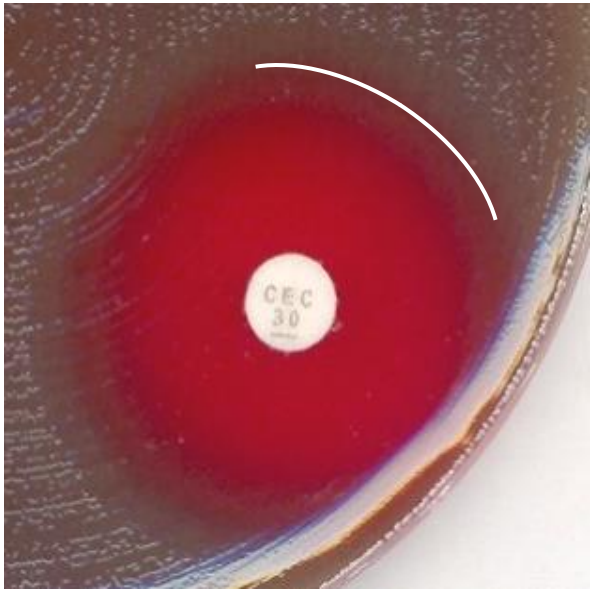
S. pyogenes

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.



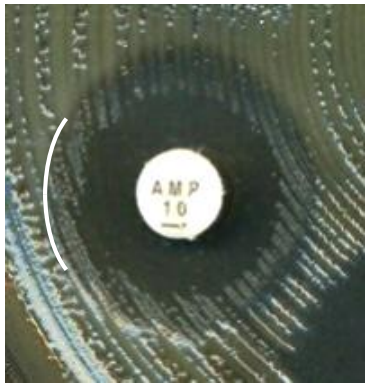
For some organisms, there is additional α-haemolysis without growth. Tilt the plate to differentiate between haemolysis and growth.

Specific reading instructions

- *Enterobacterales* with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid
- *Enterobacterales* and temocillin
- *Enterobacterales* and mecillinam
- *E. coli* and fosfomycin
- Trimethoprim and trimethoprim-sulfamethoxazole in general
- *Stenotrophomonas maltophilia, Achromobacter xylosoxidans* and *Burkholderia pseudomallei* with trimethoprim-sulfamethoxazole
- *Aeromonas* spp. and trimethoprim-sulfamethoxazole
- Enterococci and vancomycin
- *S. aureus* and benzylpenicillin
- Detection of inducible clindamycin resistance in staphylococci and streptococci
- *H. influenzae* and beta-lactam agents

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.

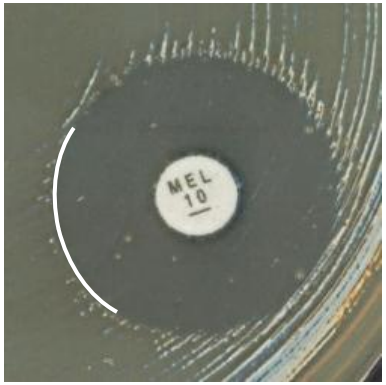


Enterobacterales and temocillin

- 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

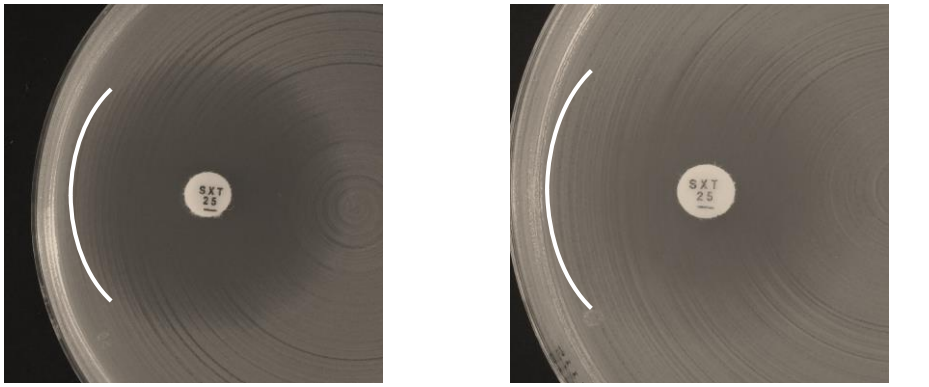
- Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to the disk and no sign of inhibition zone, report resistant.

An outer zone can be seen

Growth up to the disk

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.

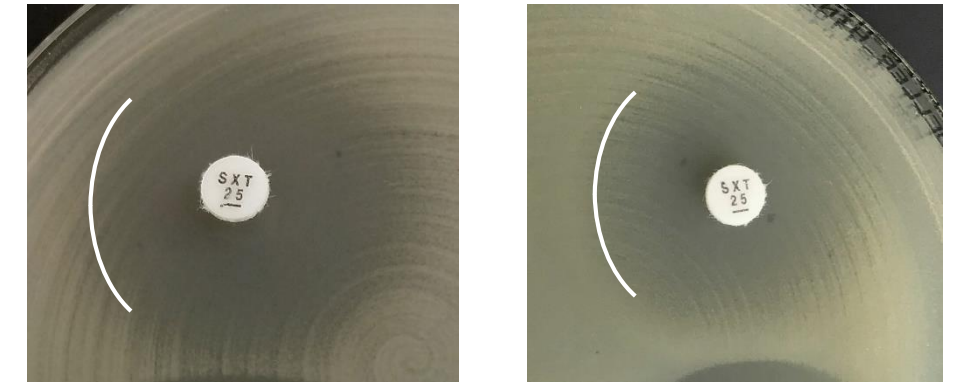


An outer zone can be seen

Growth up to the disk

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.

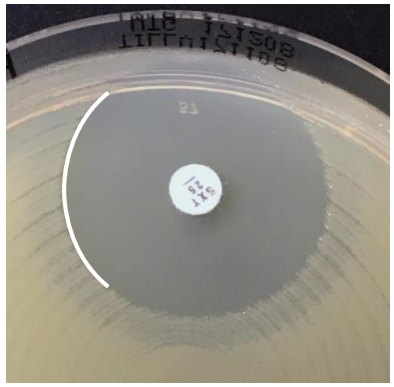


An outer zone can be seen

Growth up to the disk

Aeromonas spp. and trimethoprim-sulfamethoxazole

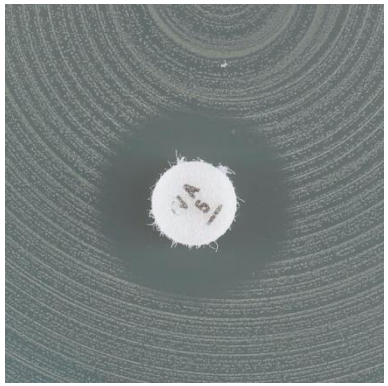
- Read the obvious zone edge and disregard haze or growth within the inhibition zone.
- If there is an obvious inner zone edge, read the inhibition zone as the inner zone.



Enterococci and vancomycin

- **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 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

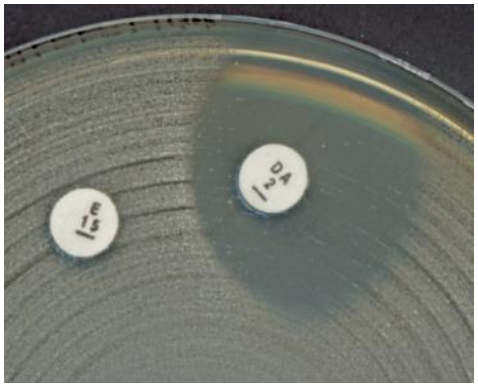
- **For isolates with zone diameters ≥26 mm**: Examine the zone edge from the front of the plate with transmitted light (plate held up to light).
  - If the zone is ≥ 26 mm and the zone edge is sharp (no reduction of growth towards zone edge, like a “cliff”), the isolate is a pencillinase producer, report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

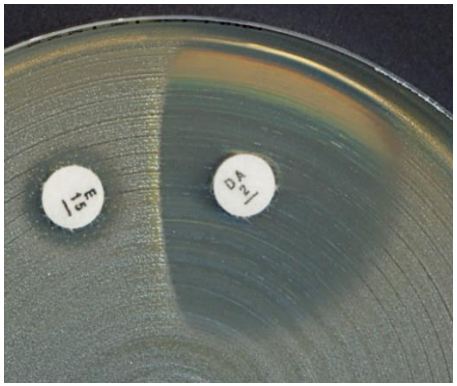
Zone ≥ 26 mm and
sharp zone edge= Resistant

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).

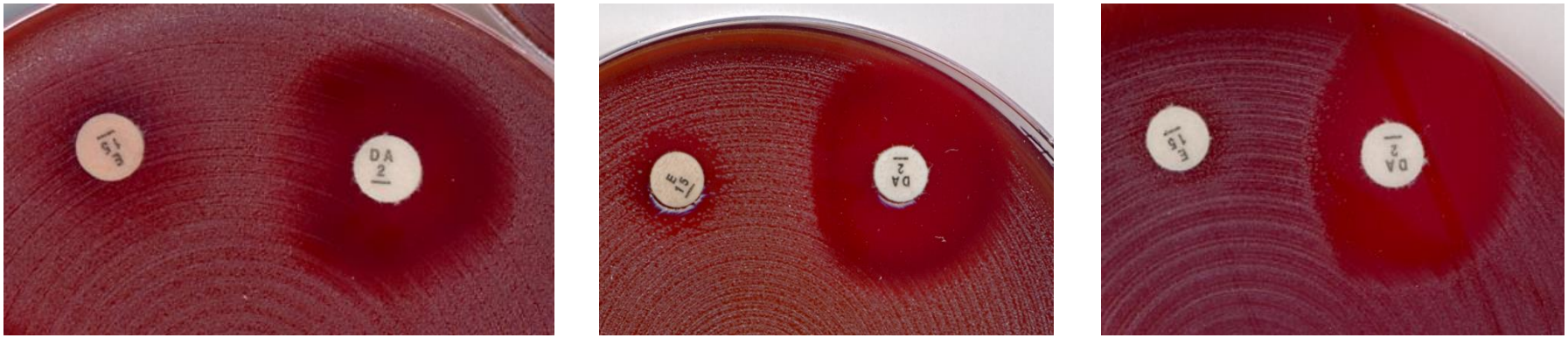




Examples of D phenomenon for staphylococci.

Detection of inducible clindamycin resistance in streptococci

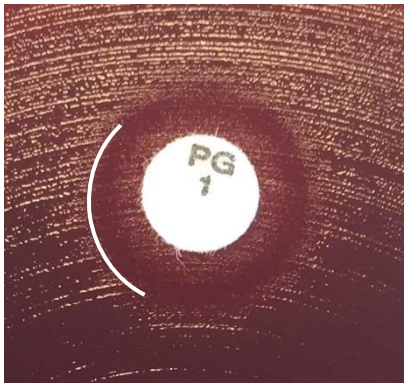
- Inducible clindamycin resistance can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place the erythromycin and clindamycin disks **12-16 mm apart** (edge to edge) and look for antagonism (the D phenomenon).



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.



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