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 2.0
May 2012

Modifications to EUCAST reading guide slide show

Version	Modifications
Version 2.0 May 2012	• Clarification regarding reading, slides 3, 9, 10 and 22 • Additional examples with colonies within zone, slide 6 • Additional example with fuzzy zone edges for staphylococci, slide 10 • Example with β-haemolysis, slide 13 • Specific instructions for *S. maltophilia*, slide17 • Specific instructions for *E. coli* and mecillinam, slide 19 • Additional example with enterococci and vancomycin, slide 20 • Specific instructions for staphylococci and benzylpenicillin, slide 21
Version 1.1 December 2010	• Clarification regarding Enterobacteriaceae and ampicillin on slide 3 and 17
Version 1.0 April 2010	• EUCAST reading guide slide show first published on EUCAST website

Reading zones

- The following instructions for reading inhibition zone diameters are part of the EUCAST disk diffusion method.

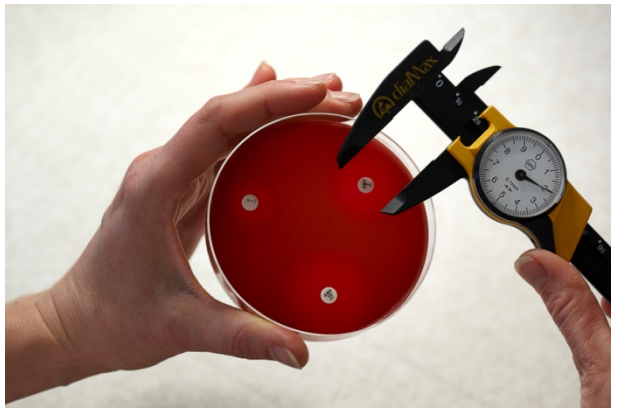
- Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye (for exceptions and specific reading instructions, see slides 15-21).

- Measure zone diameters with a ruler, a calliper or an automated zone reader.

Reading zones

- MH plates
  - Read zones from the back of the plate against a dark background and illuminated with reflected light.

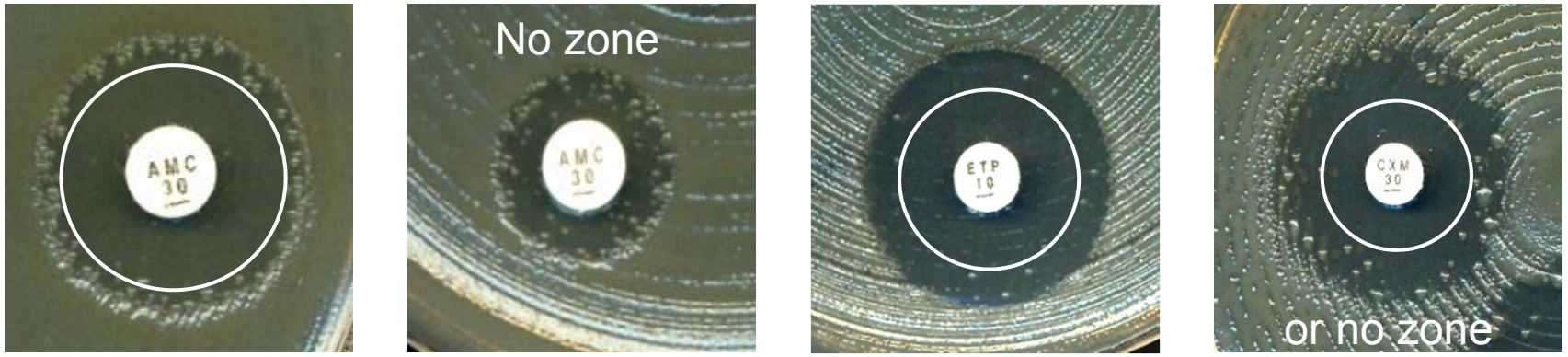
- MH-F plates
  - Read zones from the front with the lid removed and illuminated with reflected light.



Colonies within zone

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

- Colonies that are not contaminations should be taken into account when reading zones.



Reading of zones with colonies within the zone.

Colonies within zone

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

- Colonies that are not contaminations should be taken into account when reading zones.

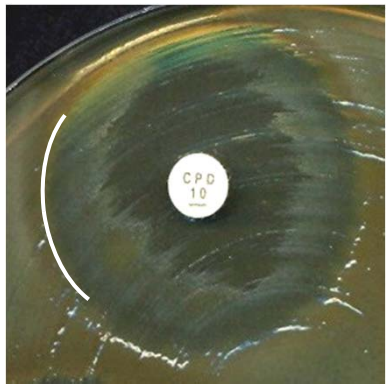
E. coli with ESBL

H. influenzae with PBP mutations

Reading of zones with colonies within the zone.

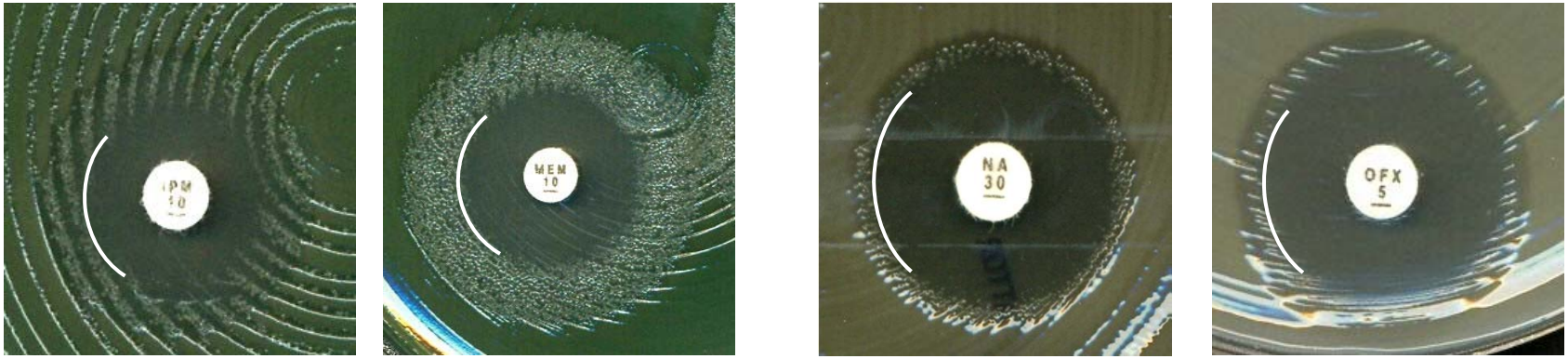
Swarming

- Read inhibition of growth and ignore swarming (most often seen for *Proteus* spp).



Inner zone

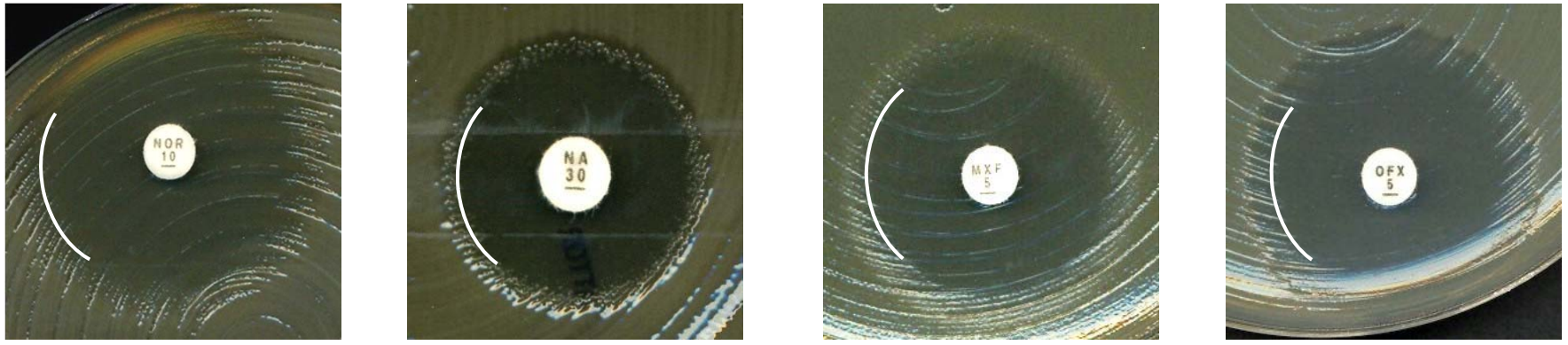
- Check purity and repeat test if necessary.
- Colonies that are not contaminations should be taken into account when reading zones.



Reading of inner zones that are not contaminations.

Fuzzy zone edges
Enterobacteriaceae

- 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 zones with fuzzy zone edges for Enterobacteriaceae.

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 zones with fuzzy zone edges for staphylococci.

Fuzzy zone edges
S. pneumoniae

- Small colonies that are visible to the naked eye should be taken into account when reading zones.

Reading 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 haemolysis from 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

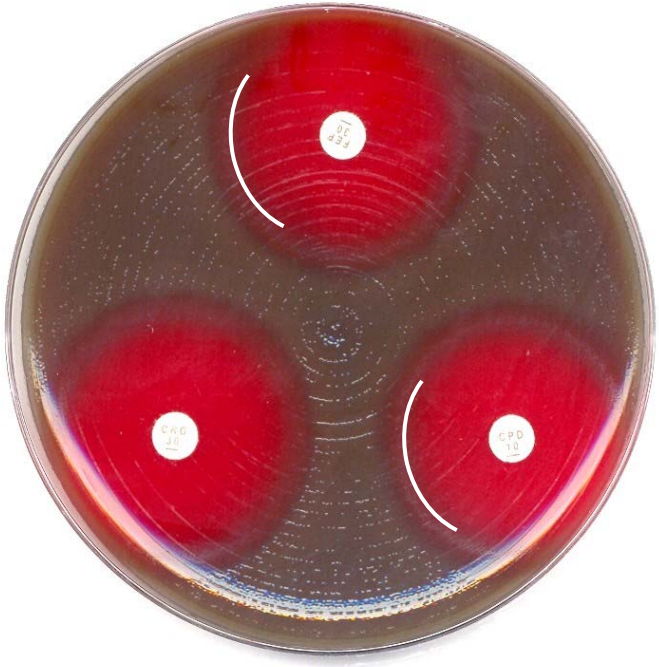
- Tilt the plate to easier differentiate between haemolysis and growth.

S. pyogenes

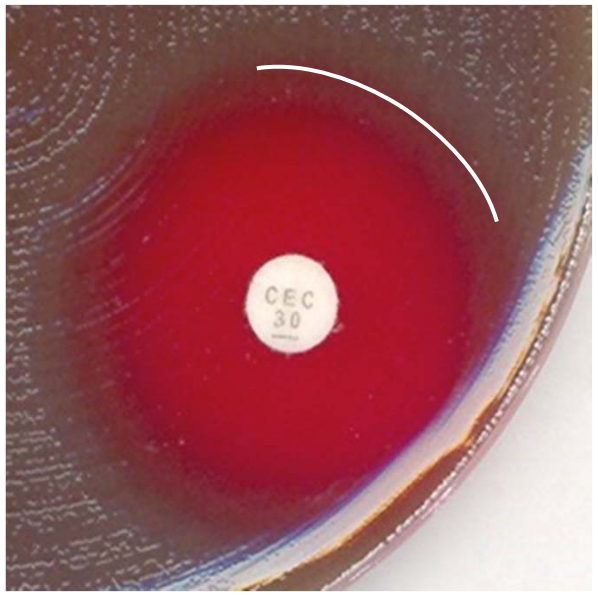
Streptococcus group C

α-haemolysis

- Tilt the plate to easier differentiate between haemolysis and growth.



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



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

Specific reading instructions

- Trimethoprim and trimethoprim-sulfamethoxazole in general
- *Stenotrophomonas maltophilia* and trimethoprim-sulfamethoxazole
- Enterobacteriaceae and ampicillin
- *E. coli* and mecillinam
- Enterococci and vancomycin
- Staphylococci and benzylpenicillin

Trimethoprim and trimethoprim-sulfamethoxazole

- Follow the instructions for reading and read inner zones 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

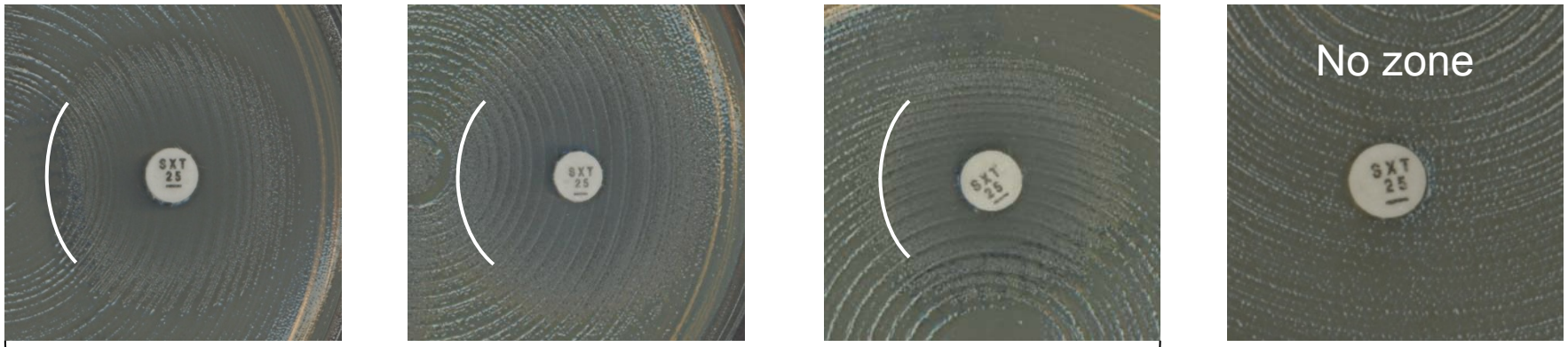
CoNS

Moraxella

Haemophilus

Stenotrophomonas maltophilia and trimethoprim-sulfamethoxazole

- Ignore growth within the inhibition zone, which is common for *Stenotrophomonas maltophilia* and trimethoprim-sulfamethoxazole. The density of growth in the zone may vary from a fine haze to substantial growth.

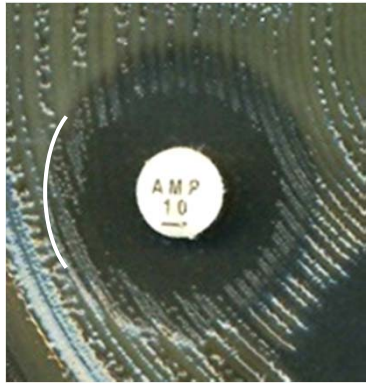


An outer zone can be seen = Susceptible

Heavy growth up to disk =Resistant

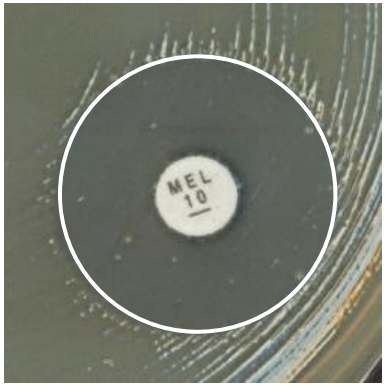
Enterobacteriaceae and ampicillin

- Ignore fine growth that may appear as an 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.



E. coli and mecillinam

- Ignore isolated colonies within the inhibition zone.



Enterococci and vancomycin

- Examine with transmitted light (plate held up to light).
  - Fuzzy zone edges and colonies within zone indicate vancomycin resistance. If the zone diameter is ≥ 12 mm and the zone edge is fuzzy, investigate further.

E. faecalis
non-VRE

E. faecium
VRE

Staphylococci and benzylpenicillin

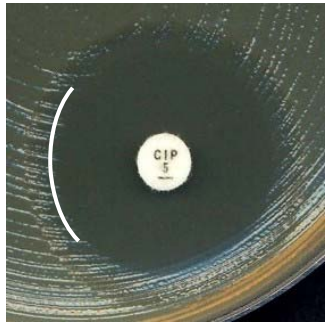
- Examine with transmitted light (plate held up to light).
  - Disk diffusion is more reliable than MIC for detection of penicillinase producers, provided the zone diameter is measured AND the zone edge closely inspected.

S. aureus with
sharp zone edge and
zone diameter ≥ 26 mm
= Resistant

S. aureus with
fuzzy zone edge and
zone diameter ≥ 26 mm
= Susceptible

Reading guide

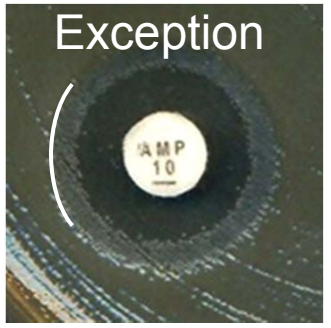
Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.



E. coli
Ciprofloxacin

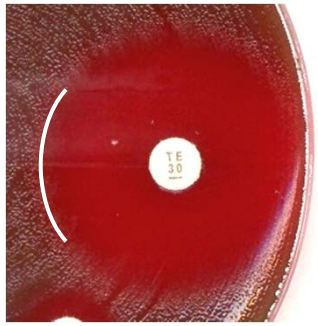
S. aureus
Linezolid

S. aureus
Erythromycin

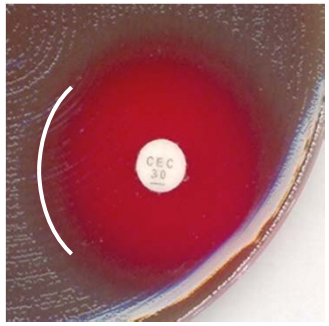


Enterobacteriaceae
Ampicillin

S. pneumoniae
Chloramphenicol



S. pneumoniae
Tetracycline



S. pneumoniae
Cefaclor

There is often growth within areas of α-haemolysis!