



# Reading of disk diffusion inhibition zones

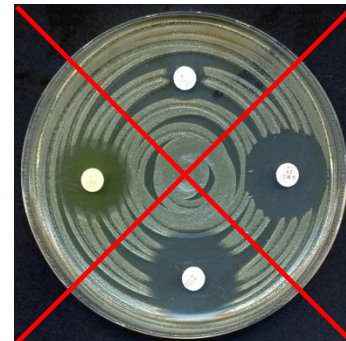
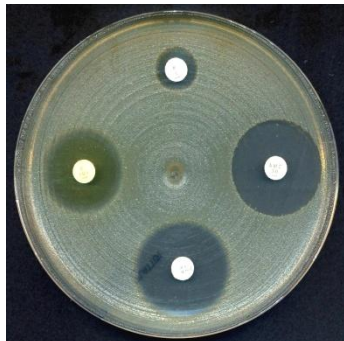
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Nathalie Friberg, HUS Diagnostic center, Finland

# Inoculum

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



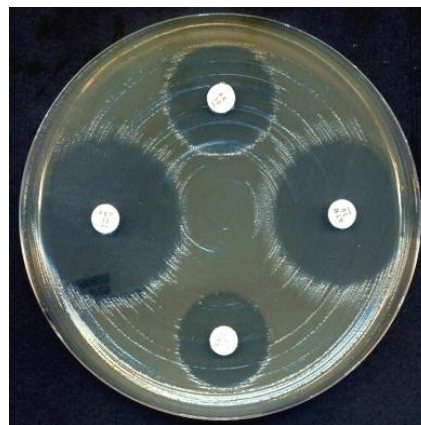
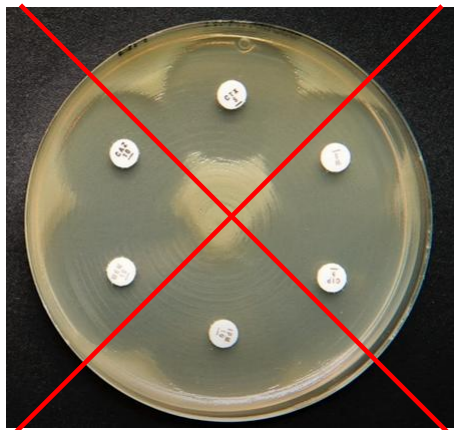
# Drying of agar plates

- Plates should be at room temperature prior to testing.
- The surface of the agar should be dry and no drops of water on the surface of the agar or inside the lid.
  - Excess moisture may cause fuzzy zone edges and/or haze within zones.
- If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min.



# Application of disks

- Limit the number of disks per plate to avoid overlapping of zones and interference between agents.
- 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 of zones!



# Reading devices

- Calliper - Analogue 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 eye.
- Read bactericidal and bacteriostatic agents the same way.
- Report zone diameters to the nearest millimetre.



# Reading zone diameters

## **Mueller-Hinton (MH) plates:**

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



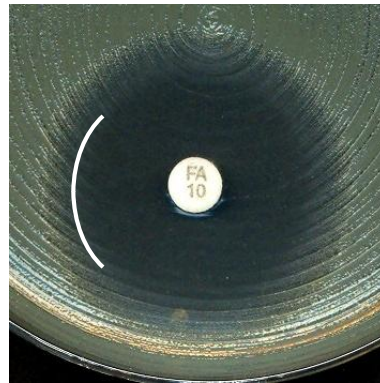
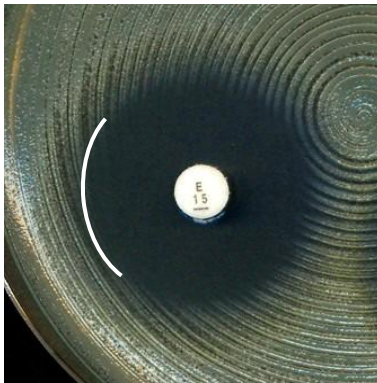
## **MH-F and FAA-HB plates**

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



# 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

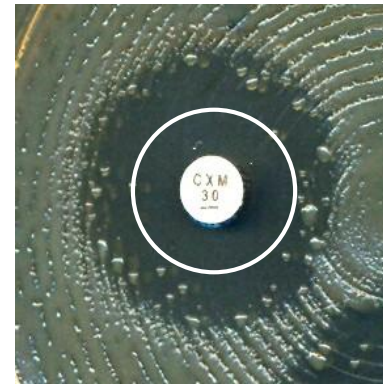
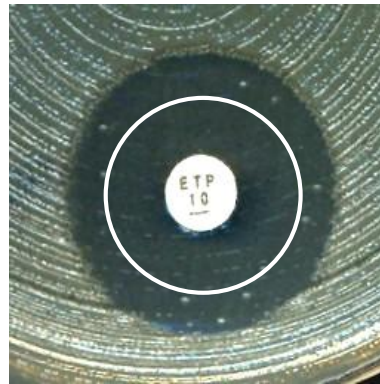
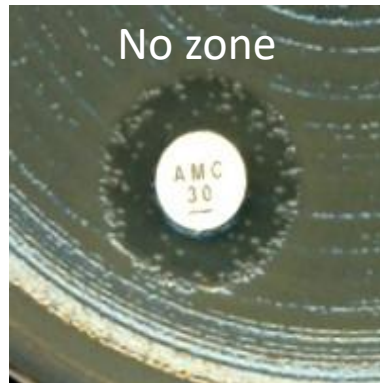
- Hold the plate at a 45-degree angle to the work bench to facilitate reading when zone edges are difficult to define.
- Problems with fuzzy zone edges can be related to excess humidity and may be reduced by drying MH-F plates.



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.



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*E. coli* with  
ESBL



*H. influenzae* with  
PBP mutations



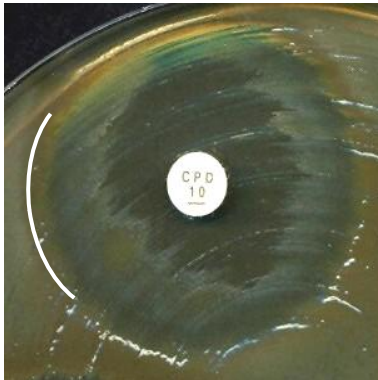
# Double zones

- 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.
- $\beta$ -haemolysis is usually free from growth
  - $\beta$ -Haemolysins diffuse in agar
- There is usually growth within areas of  $\alpha$ -haemolysis.
  - $\alpha$ -Haemolysins do not diffuse in agar



$\beta$ -haemolysis



$\beta$ -haemolysis



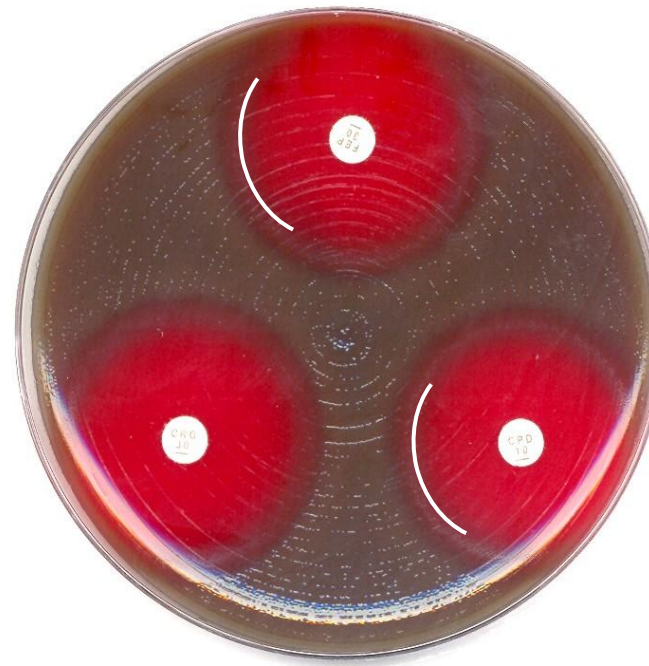
$\alpha$ -haemolysis

# Haemolysis

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



$\beta$ -haemolysis is usually free from growth



There is usually growth in areas of  $\alpha$ -haemolysis

# **Specific reading instructions**

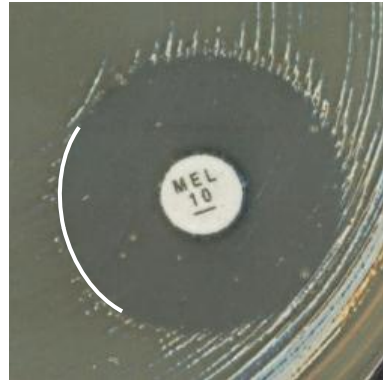
# Enterobacterales with aminopenicillins

- Ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid.
- Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton 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.



# Enterobacterales with mecillinam

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



# *E. coli* with fosfomycin

- 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

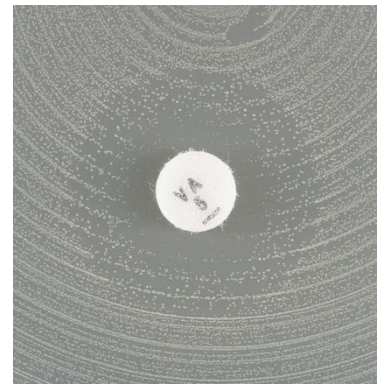
- **If vancomycin zone  $\geq 12$  mm:** Examine the zone edge from the front with transmitted light (plate held up to light).
  - Sharp zone edge indicate vancomycin susceptibility.
  - Fuzzy zone edge or colonies within the zone indicate vancomycin resistance (VRE)  $\rightarrow$  Perform confirmatory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



non-VRE



VRE



VRE

# *S. aureus* and benzylpenicillin

- **If benzylpenicillin zone  $\geq 26$  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

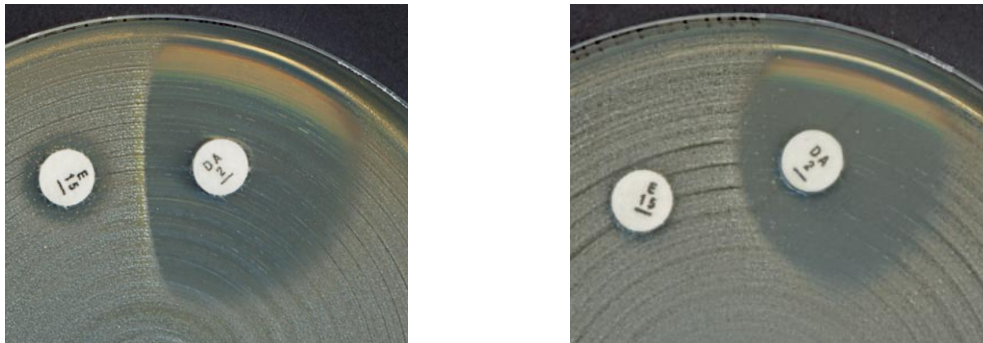
**For isolates with zone diameters above the susceptible breakpoint:**

- 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

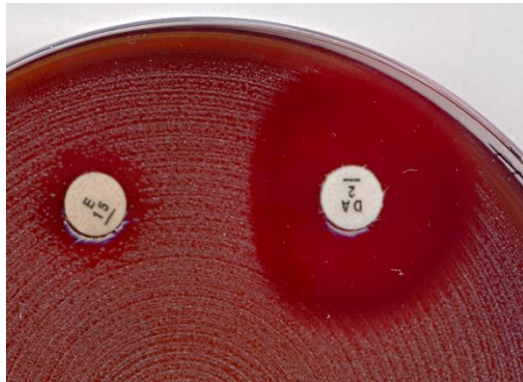
- Can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place erythromycin and clindamycin disks **12-20 mm apart** (edge to edge) and look for antagonism, “D phenomenon”.



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

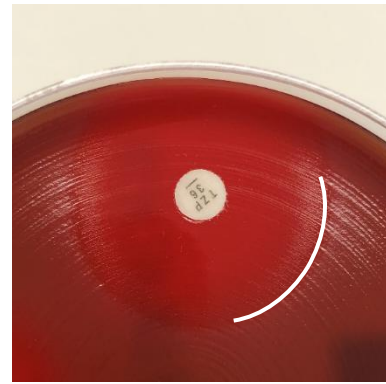
- Read FAA-HB plates from the front with the lid removed and illuminated with reflected light.
- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- Note that the disk diffusion method is valid only for:
  - *Bacteroides* spp.
  - *Prevotella* spp.
  - *Fusobacterium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



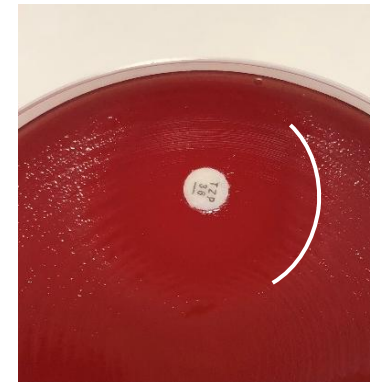
*Bacteroides* spp.  
Meropenem



*Bacteroides* spp.  
Piperacillin-tazobactam



*F. necrophorum*  
Benzylpenicillin



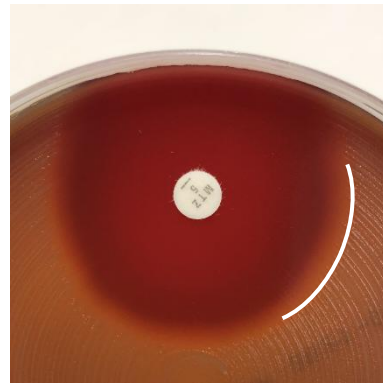
*C. perfringens*  
Piperacillin-tazobactam

# 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 11.0  
January 2025

## 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/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/](http://www.eucast.org/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/)

