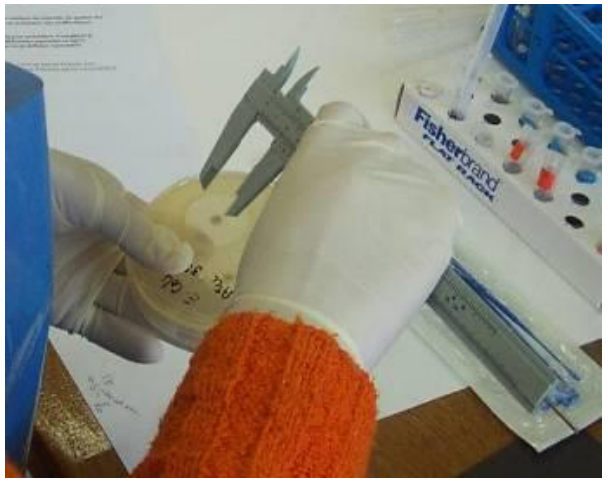
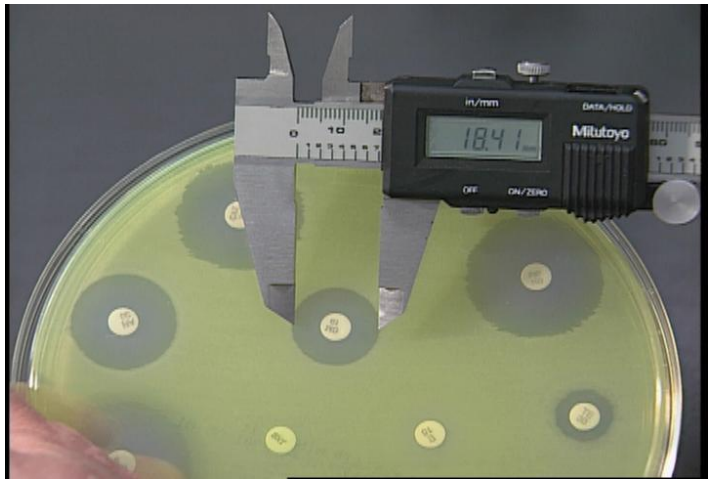
Reading guide

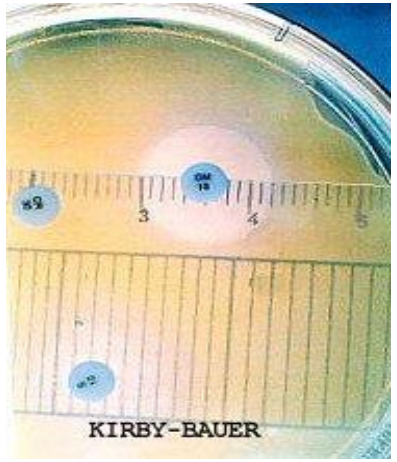
EUCAST disk diffusion method for antimicrobial susceptibility testing

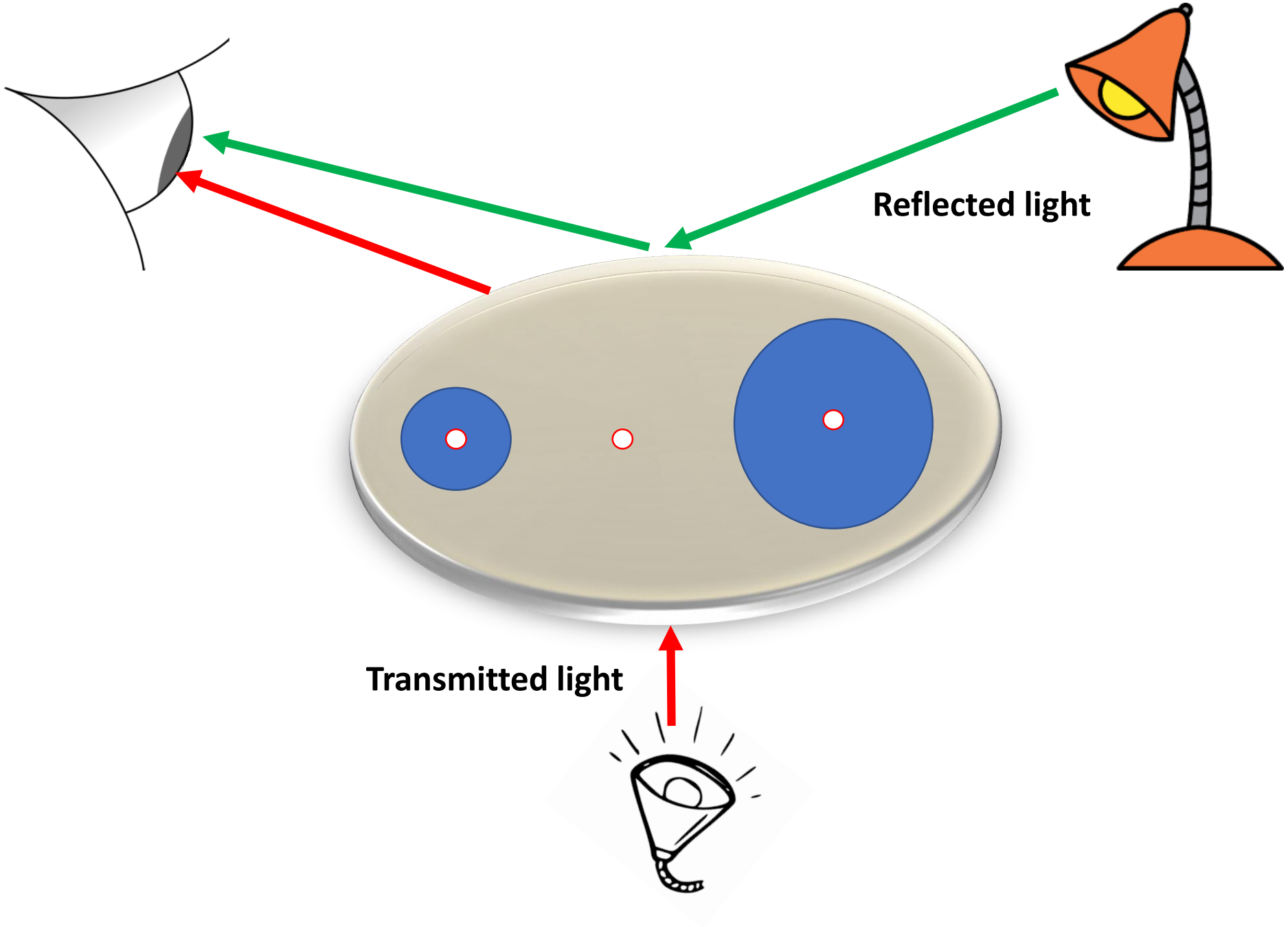
Version 10

January 2023



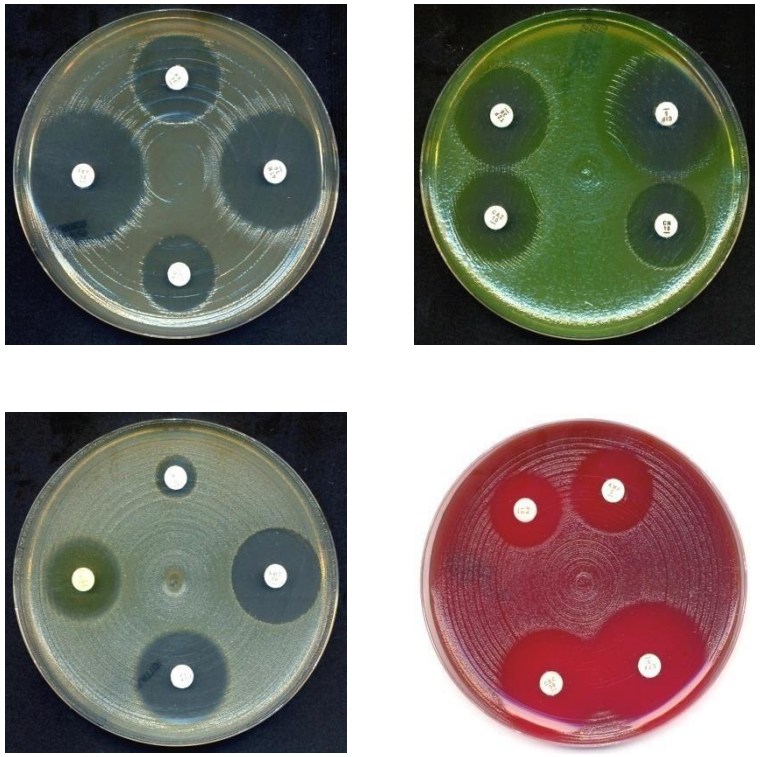




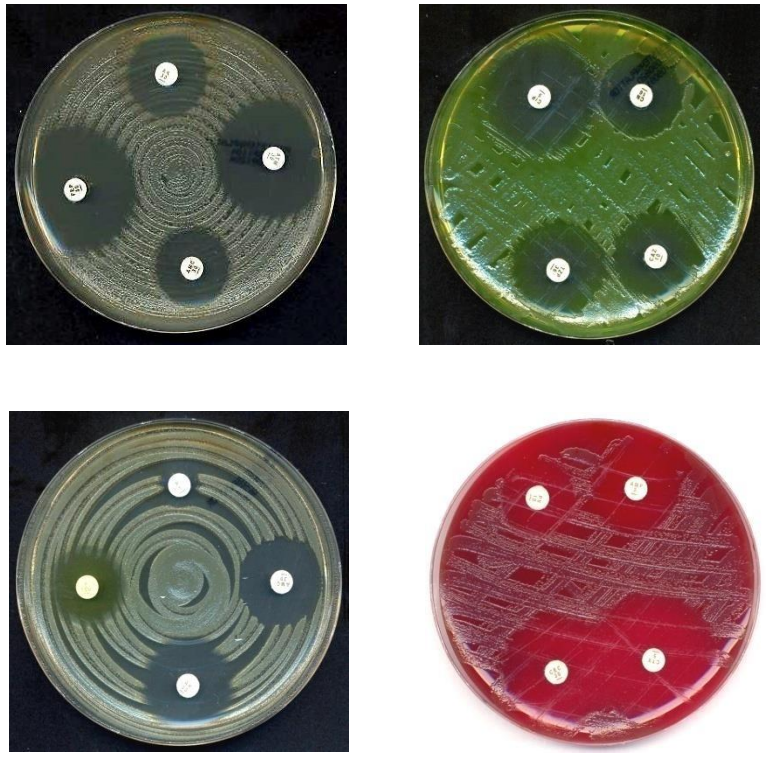

Reflected light
Transmitted light

Examining purity of plate
Select the Colonies from Pure Isolates

The growth should be confluent and evenly spread over the plate



Plates should look like this..



..and NOT like this!

Reading Plates and Interpreting Results

* **16 to 18 hours** of incubation

* If the plate was satisfactorily streaked, and the inoculum was correct, the **zones of inhibition will be uniformly circular** The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc.

* Zones are measured using sliding calipers or a ruler, which is held on the **back of the inverted petri plate**.

* The petri plate is held a few inches above a **black, nonreflecting background** and illuminated with **reflected light**.

* If **blood** was added to the agar base (as with streptococci), the zones are measured from the **upper surface of the agar** illuminated with reflected light, with the **cover removed**.

* If the test organism is a ***Staphylococcus* or *Enterococcus* spp** , **24 hours of incubation** are required for **vancomycin and oxacillin**,

* **Transmitted light (plate held up to light)** is used to examine the **oxacillin and vancomycin** zones for light growth of methicillin- or vancomycin- resistant colonies, Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

If cefoxitin is tested against Staphylococcus spp., read the zone diameters with reflected, not transmitted, light.

With glycopeptide susceptibility tests on *Enterococcus spp. resistant colonies* may not be visible until plates have been incubated for 24 h. However, plates may be examined after 16-20 h and any resistance reported, but plates of isolates appearing susceptible must be re-incubated and reread at 24 h.

Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored.

When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis.

With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

In case of double zones, or distinct colonies within zones, check for purity and repeat the test if necessary.

For *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole, an isolate showing any sign of inhibition zone = the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones. Read as no zone only if there is growth up to the disk and no sign of an inhibition zone.

For Enterobacterales with ampicillin, ampicillin-sulbactam and amoxicillin clavulanic acid, Ignore growth that may appear as a thin film producing an inner zone on some batches of Mueller-Hinton agar.

For *Staphylococcus aureus with benzylpenicillin, examine the zone edge* closely from the front of the plate with the plate held up to light (transmitted light). Isolates with inhibition zone diameters = the susceptible breakpoint, but with sharp zone edges should be reported resistant.

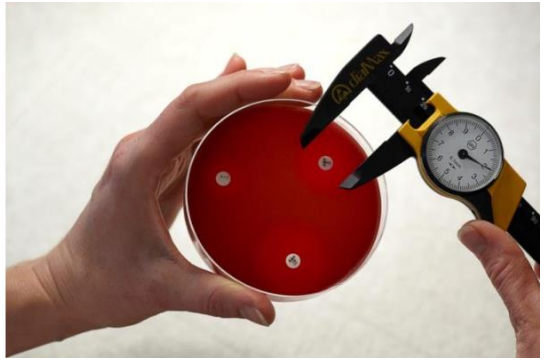
When using cefoxitin for the detection of methicillin resistance in *Staphylococcus aureus, measure the obvious zone, and examine zones* carefully in good light to detect colonies within the zone of inhibition. These may be either a contaminating species or the expression of heterogeneous methicillin resistance.

For enterococci with vancomycin, examine the zone edge closely from the front of the plate with the plate held up to light (transmitted light). Fuzzy zone edges and colonies within zone indicate vancomycin resistance and should be investigated further. Isolates must not be reported susceptible before 24 h incubation.

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.



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.

Examples:

E. coli
Ciprofloxacin

S. aureus
Erythromycin

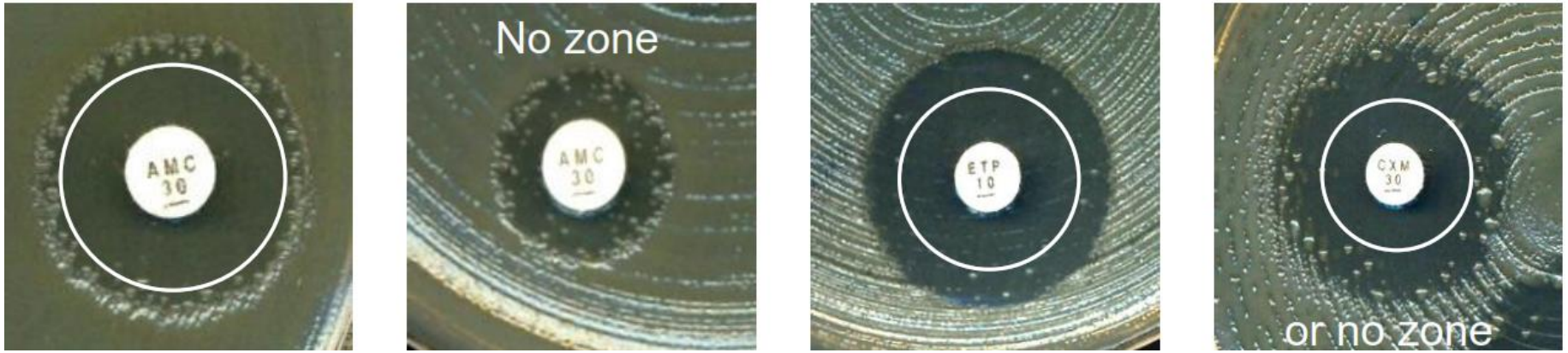
CoNS
Trimethoprim

S. pneumoniae
Rifampicin

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.

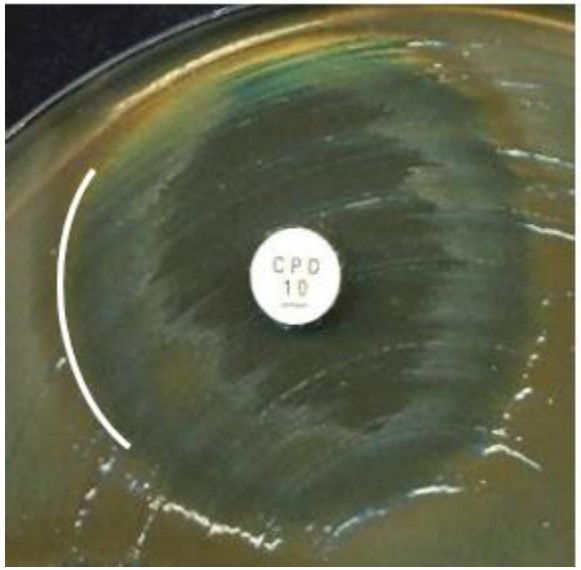
E. coli with ESBL

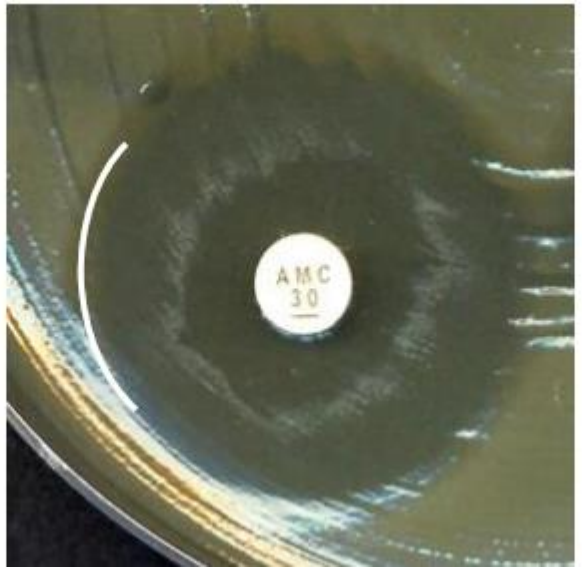
H. influenzae with PBP mutations

Reading of zones with colonies within the zone.

Swarming

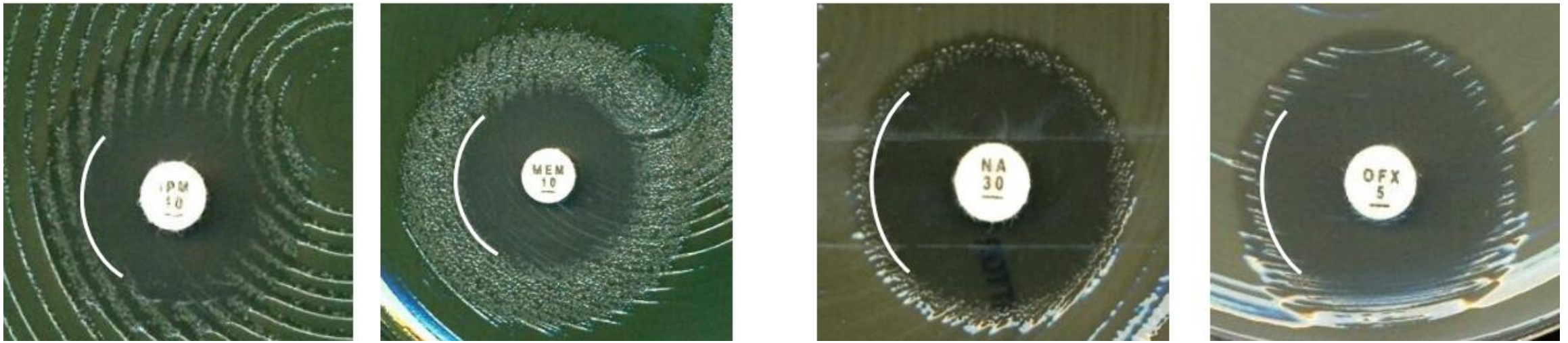
- For *Proteus spp., ignore swarming and read* inhibition of growth.





Double zones

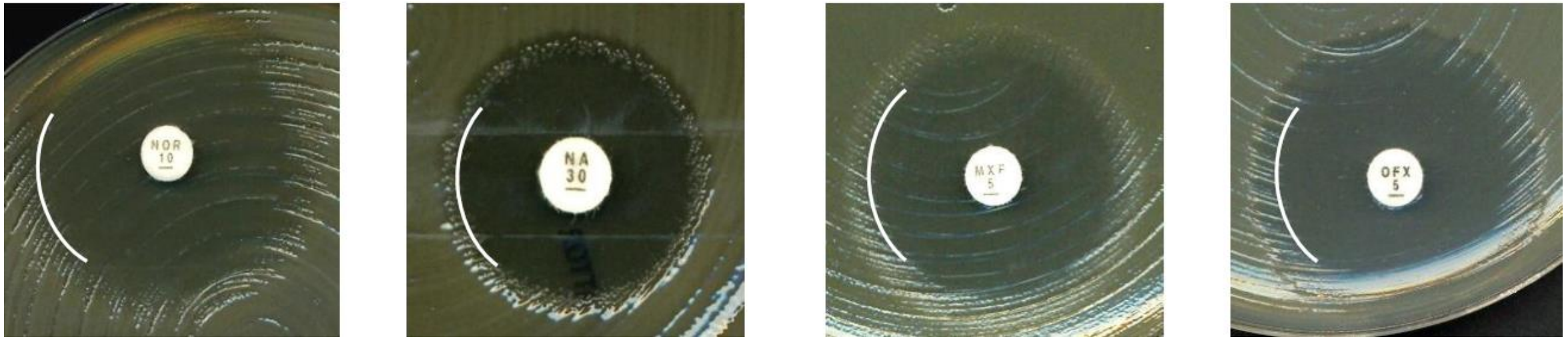
- In case of double zones, check for purity and repeat the test if necessary.
- If cultures are pure, read the inner zone.



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.

ß-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.
- ß-haemolysis is usually free from growth.

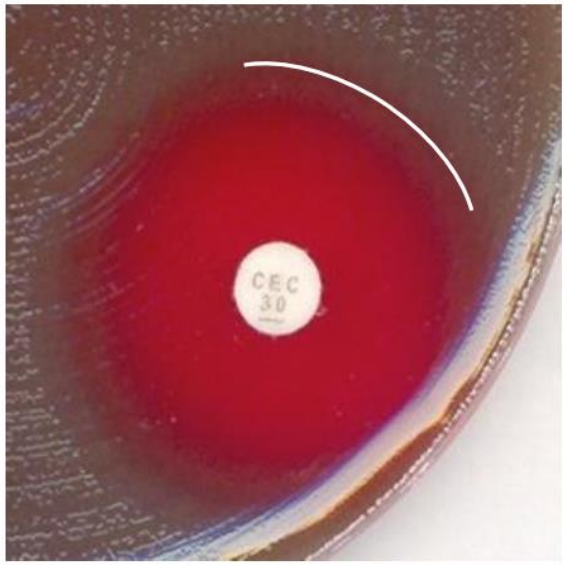
S. pyogenes

Streptococcus group C

a-haemolysis

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

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



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

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.

Trimethoprim and trimethoprim-sulfamethoxazole

- Follow the instructions for reading and read the inner zone when double zones appear
- 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

- An isolate showing any sign of inhibition zone = the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones.

Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone = Resistant

E. coli and fosfomycin

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

Enterococci and vancomycin

• Examine from the front of the plate with transmitted light (plate held up to light).

– Vancomycin-susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone.

– If the zone edge is fuzzy, if colonies grow within the zone or if you are uncertain, investigate further even if the zone diameter is = 12 mm.

– Isolates must not be reported susceptible before 24 h incubation.