EUCAST disk diffusion method for antimicrobial susceptibility testing

Reading guide
Version 5.0
January 2017
Changes from previous version (v 4.0)

<table>
<thead>
<tr>
<th>Slide</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Clarification on reading of zones and the use of automated zone readers.</td>
</tr>
<tr>
<td>17</td>
<td>Clarification on reading of trimethoprim-sulfamethoxazole zones for <em>Stenotrophomonas maltophilia</em>.</td>
</tr>
<tr>
<td>18</td>
<td>Ampicillin-sulbactam and amoxicillin-clavulanic acid added.</td>
</tr>
<tr>
<td>20</td>
<td>Instructions for reading of fosfomycin zones for <em>Escherichia coli</em> added.</td>
</tr>
<tr>
<td>21</td>
<td>Information added on reading of vancomycin and enterococci.</td>
</tr>
</tbody>
</table>
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 15-24).

• 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.

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
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 of 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.
Fuzzy zone edges

*S. pneumoniae*

- Small colonies that are visible when the plate is held about 30 cm from the naked eye 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

• Trimethoprim and trimethoprim-sulfamethoxazole in general
• *Stenotrophomonas maltophilia* and trimethoprim-sulfamethoxazole
• Enterobacteriaceae with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid
• *E. coli* and mecillinam
• *E. coli* and fosfomycin
• Enterococci and vancomycin
• *S. aureus* and benzylpenicillin
• Detection of inducible clindamycin resistance in staphylococci and streptococci
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.

\[ E. \text{coli} \quad \text{CoNS} \quad \text{Moraxella} \quad \text{Haemophilus} \]
**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
Enterobacteriaceae 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.
E. coli and mecillinam

- Ignore isolated colonies within the inhibition zone.
**E. coli** and fosfomycin

- Ignore isolated colonies within the inhibition zone and read the outer zone edge.
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 $\geq 12$ mm and the zone edge is fuzzy, investigate further.
  - Isolates must not be reported susceptible before 24 h incubation.

![E. faecalis non-VRE](image1)

![E. faecium VRE](image2)
**S. aureus** 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.

<table>
<thead>
<tr>
<th>S. aureus with sharp zone edge and zone diameter ≥ 26 mm</th>
<th>S. aureus with fuzzy zone edge and zone diameter ≥ 26 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>= Resistant</td>
<td>= Susceptible</td>
</tr>
</tbody>
</table>
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.