Implementation of the EUCAST disk diffusion method

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EUCAST Scientific Secretary
EUCAST disk diffusion method

• Why develop a EUCAST disk diffusion test?
• Development of the method
• Calibration of the method
• Implementation in individual laboratories
EUCAST early 2007

- No plans for EUCAST disk diffusion test
- MICs can be interpreted with EUCAST breakpoints
- Automated systems can be calibrated to EUCAST breakpoints
- Disk diffusion methods calibrated to EUCAST breakpoints are available in France, Sweden and the UK.

......little enthusiasm for a new “EUCAST disk diffusion method”
EUCAST consultation and questionnaire 2007-8

- France and UK likely to maintain their national methods, calibrated to EUCAST breakpoints, for the immediate future.
- Other countries were not keen to take on other national methods with unfamiliar technique.
- Most countries were currently using Kirby-Bauer type methods (particularly “CLSI”) and, if they adopted EUCAST breakpoints, would prefer to retain the same methodology calibrated to EUCAST breakpoints (…..but get rid of HTM)
Benefits of a EUCAST disk diffusion method (based on the Kirby-Bauer approach)

- Identified as EUCAST method calibrated to EUCAST MIC breakpoints
- Wide base of expertise throughout Europe (and worldwide)
- KB technique familiar to most laboratories in countries without their own method
- Extensive database of MIC v Zone diameters available for KB method?
EUCAST decision to develop a disk diffusion method June 2008

Based on KB approach

- MH medium
- MH-F for fastidious organisms
- 0.5 MF inoculum
- 16-20h incubation
- Most disk contents same
- Most control strains same
- Calibrated to EUCAST MIC breakpoints
Mueller-Hinton-fastidious (MH-F)
Mueller-Hinton agar + 5% defibrinated horse blood
and 20 mg/L β-NAD

*S. pneumoniae* ATCC 49619  *H. influenzae* NCTC 8468
EUCAST disk diffusion test for routine antimicrobial susceptibility testing

During 2009 - 2010 EUCAST is developing a disk diffusion test for routine antimicrobial susceptibility testing. The method is derived from the Kirby-Bauer method, variants of which are currently widely used in Europe, but is calibrated to EUCAST MIC breakpoints.

The method is based on two media, Mueller-Hinton agar without supplements (MH) for non-fastidious organisms, including enterococci, and MH with 5% horse blood and 20 mg 3-NADL (MH-F) for Streptococcus spp. including Streptococcus pneumoniae, Haemophilus spp. and other fastidious organisms.

The plates are incubated at 35 ± 1 °C for 18 ± 2 h within 15 minutes from application of the disks. MH plates are incubated in air and MH-F plates in 5% CO2.

Breakpoint tables with tentative zone diameter breakpoints were published December 21, 2009.

EUCAST breakpoints and commercially available material and systems for AST - important information to laboratories - click here.
<table>
<thead>
<tr>
<th>Carboxylic acids</th>
<th>MIC breakpoint</th>
<th>Disk content</th>
<th>Zone diameter</th>
<th>Notes</th>
</tr>
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<tbody>
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<td>A</td>
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</table>

**Notes**
- Numbers for comments on MIC breakpoints
- A. The aminoglycoside breakpoints for Enterobacteriaceae are based on once-daily administration of high aminoglycoside dosages. Aminoglycosides are given in combination with beta-lactam agents.
- B. Macrolide content may be used to screen for fluoroquinolone resistance in Enterobacteriaceae. A breakpoint corresponds to an MIC value of 15 μg/mL in most Enterobacteriaceae. If Salmonella spp. resistant to all fluoroquinolones, if other Enterobacteriaceae are resistant, then test the agent in combination with a beta-lactam agent.
# EUCAST QC tables

## MIC and Zone Diameter Limits for Quality Control Strains

### Escherichia coli ATCC 25922

Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2 h. Read complete inhibition from the back of the plates against a black background illuminated with reflected light.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>Disk content (µg)</th>
<th>Inhibition zone size (mm)</th>
<th>Comments</th>
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<tbody>
<tr>
<td></td>
<td>Target</td>
<td>Range¹</td>
<td>Target</td>
<td>Range²</td>
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<td>Amikacin</td>
<td>1-2</td>
<td>0-5-4</td>
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<td>23</td>
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<tr>
<td>Amoxicillin-clavulanic acid</td>
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<td>2/1.8/4</td>
<td>20/10</td>
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<td>Ampicillin</td>
<td>4</td>
<td>2-6</td>
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<td>0.12</td>
<td>0.06-0.25</td>
<td>30</td>
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<tr>
<td>Cefadroxil</td>
<td>-</td>
<td>-</td>
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<td>18</td>
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<tr>
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<td>0.015-0.12</td>
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<td>0.03-0.12</td>
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<td>32</td>
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<td>2-6</td>
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<tr>
<td>Cefpodoxime</td>
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<td>0.25-1</td>
<td>10</td>
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<td>0.06-0.5</td>
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<td>Chloramphenicol</td>
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<td>1/4/4/4</td>
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<td>4-16</td>
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<td>0.5-2</td>
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</table>

Targets NOT in italics are from ISO and/or CLSI recommendations.
Calibration of EUCAST disk diffusion method

- Existing distributions of MIC v zone diameter with the same technique
- New distributions of MIC v zone diameter
- Zone diameter distributions for routine isolates
- Targeting critical areas of the MIC and zone diameter distributions
- Targeting specific resistances
Figure 7: Ceftriaxone MIC v zone diameter for ceftriaxone with Enterobacteriaceae
Ceftriaxone / Escherichia coli
EUCAST zone diameter distribution - Reference database
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

Disk content: 30
Epidemiological cut-off: -
Clinical breakpoints: S ≥ 23 mm, R < 20 mm
Mecillinam / Escherichia coli
EUCAST zone diameter distribution - Reference database
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

Zone diameter (mm)

Disk content: 10
Epidemiological cut-off: -

5504 observations (2 data sources)
Clinical breakpoints: S ≥ 15 mm, R < 15 mm
Gentamicin / Enterococcus faecalis
EUCAST zone diameter distribution - Reference database
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

Disk content: 30
Epidemiological cut-off: WT ≥ 8 mm (MIC: ≤ 32 mg/L)

817 observations
Clinical breakpoints: Inappropriate
Development of EUCAST disk diffusion method

• Tentative breakpoints have been published for all agents with MIC breakpoints

• Continuing validation through
  – Existing and new distributions of MIC v zone diameter
  – Targeting critical areas of MIC v zone diameter distributions and specific resistances
  – Zone diameter distributions for routine isolates

• Ongoing maintenance
Implementation – EUCAST action

• Documentation
  – Method description
  – Breakpoints
  – QC limits

• Education
  – Teaching material (slideshow)
  – Meetings
  – Practical workshops
  – Laboratory visits
    • The laboratory in Vaxjo is an ESCMID Collaborative Centre – the ESCMID Observership program could be used for visit.
  – Publications/website

• Inform media, disk, zone reader manufacturers
Implementation at national level
"National antimicrobial committees"

• Strategy at national level for implementation of breakpoints and methods
• Inform people of implications for their laboratory/country
• Education through meetings, publications, websites, practical workshops
• Liaison with media, disk, zone-reader manufacturers
• Liaison and consultation with EUCAST.
• Liaison with groups involved in AMR-surveillance
• External Quality assessment
• Staged introduction may be appropriate – eg. large labs first
Implementation at local level
Before routine use

• Ensure all stakeholders are informed of implications for their laboratory/hospital
• Appoint a "champion" to implement the method
• Visit laboratories using the method
• Plan well in advance
  – media, disk, supplies
  – templates, zone-reader setup
  – computing (breakpoints/QC ranges)
  – documentation
• Train staff in advance – demonstrations, practical experience, ensure that QC requirements are met
• Use contacts in other laboratories and at EUCAST directly or through NACs or national EUCAST GC rep
EUCAST contacts for the disk diffusion method

- Erika Matuschek  
  (Swedish External Reference Laboratory for Antimicrobial Susceptibility Testing; "SERLAST")  
  erika.matuschek@ltkronoberg.se

- Gunnar Kahlmeter  
  (EUCAST and SERLAST)  
  gunnar.kahlmeter@ltkronoberg.se

- Derek Brown  
  (EUCAST)  
  derek.brown222@btinternet.com
Implementation at local level
During introduction

• Ensure that adequate staffing is available – will take more time at first

• Ensure that senior staff are available to answer questions and deal with problems

• Use national contacts
  – National Antibiotic Committees
  – Use contacts in laboratories already using the method

• Use EUCAST contacts
Implementation at local level

After implementation

• Keep the method up-to-date

• Continue to educate staff

• Report problems to EUCAST
  – Erika Matuschek (SERLAST)

• Be prepared to help other laboratories
Acknowledgements

• Gunnar Kahlmeter
• Erika Matuschek and Jenny Åhman

• EUCAST Steering Committee
• National Committees
• Collaborating laboratories
• Individual experts