Quality control of susceptibility testing

Erika Matuschek, Ph D
EUCAST Development Laboratory (EDL)
Växjö, Sweden

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Internal quality control (QC)

• Routine QC
  – Monitors day-to-day reliability and reproducibility

• Batch controls of media, disks and MIC panels

• QC as part of implementing a method or after making a change in the AST system
Routine quality control (QC)

• Control of materials and equipment
  – Medium
  – Antibiotic disks/concentrations in panels
  – Incubators
  – Etc.

• Control of the procedures
  – Inoculum and inoculation
  – Incubation time
  – Reading of results
  – Etc.

Why is routine QC important?

• QC mirrors testing of clinical isolates

• QC is performed to avoid:
  – Reporting clinical isolates as false susceptible or false resistant
  – Treatment failure and poor outcome for patients due to inaccurate AST results
Quality control of AST products

- Disk diffusion
  - Always on-scale results

- Gradient tests
  - Mainly on-scale results
  - Difficult to control the whole gradient

- Semi-automated systems and commercial BMD products
  - Off-scale results for the standard QC strains due to short MIC ranges

QC responsibility

- Manufacturers:
  - To ensure that products have been appropriately manufactured and validated against reference methodology.

- Laboratories/users:
  - To ensure that products are maintained properly (storage and handling).
  - To ensure that testing is performed according to methodology.
Options for internal QC

- QC strains
  - Individual results are evaluated against EUCAST ranges and target values
  - Evaluation of QC data over time
    - Comparison of mean values (≥10 tests) with EUCAST target values
    - Trends over time

- Comparison of distributions for clinical isolates with reference distributions (www.eucast.org)

EUCAST QC recommendations for disk diffusion

- Perform routine quality control daily, or at least four times a week.

- EUCAST Disk Diffusion Manual, section 9
  www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/

- EUCAST QC Tables
  www.eucast.org/ast_of_bacteria/qc_tables/
### Routine quality control

#### Recommended control strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>ATCC 25922</td>
<td>Susceptible, wild type</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>ATCC 27853</td>
<td>Susceptible, wild type</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ATCC 29213</td>
<td>Weak β-lactamase producer</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>ATCC 29212</td>
<td>Susceptible, wild type</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>ATCC 49619</td>
<td>Reduced susceptibility to benzylpenicillin</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>ATCC 49766</td>
<td>Susceptible, wild type</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>ATCC 33560</td>
<td>Susceptible, wild type</td>
</tr>
</tbody>
</table>

### Routine quality control

#### Recommended control strains to control the inhibitor component in β-lactamase-β-lactamase inhibitor disks

<table>
<thead>
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<th>Organism</th>
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<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>ATCC 35218</td>
<td>TEM-1 β-lactamase</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>ATCC 700603</td>
<td>SHV-18 ESBL</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>ATCC BAA-2814</td>
<td>KPC-3 carbapenemase</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ATCC 29213</td>
<td>β-lactamase</td>
</tr>
</tbody>
</table>
QC ranges and targets

*Escherichia coli* ATCC 25922  
(NCTC 12241, OIP 7634, DSM 1169, CCUG 17620, CECT 434)

See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>Disk content (µg)</th>
<th>Inhibition zone diameter (mm)</th>
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</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>1-2</td>
<td>30</td>
<td>22-23</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>2-8</td>
<td>10</td>
<td>18-19</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>2-8</td>
<td>10</td>
<td>18-19</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>1-4</td>
<td>10</td>
<td>21-22</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>2-8</td>
<td>10</td>
<td>18-19</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>4-16</td>
<td>30</td>
<td>18-21</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.03-0.06</td>
<td>5</td>
<td>21-22</td>
</tr>
<tr>
<td>Cefixime</td>
<td>0.06-0.125</td>
<td>5</td>
<td>21-22</td>
</tr>
</tbody>
</table>

**Range**  
Used to allow for random variation

**Target**  
Mean values from repeated measurements should optimally be on target ± 1 mm (mode MIC on target)

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Product accuracy vs. day-to-day variation

*Example E. coli ATCC 25922 and piperacillin-tazobactam 30-6 µg*

- **Routine QC data**
  - Results randomly distributed within the QC range
  - Mean value close to the target value
  - Day-to-day variation due to small differences in
    - Inoculum preparation and plate inoculation
    - Incubation time and temperature
    - Reading of results

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11

- No of observations
  - Inhibition zone diameter (mm)

12

- No of observations
  - Inhibition zone diameter (mm)

14
Routine QC

- Control tests should be set up and checked daily, or at least four times per week, for antibiotics which are part of routine panels.

- Control tests should always be read and evaluated before reporting results for clinical isolates.

- Each day that tests are set up, examine the results of the last 20 consecutive tests.

- Examine results for trends and for zones falling consistently above or below the target.

- If two or more of 20 tests are out of range investigation is required.

Response to QC results out of range

- If two non-consecutive control zone diameters of 20 tests are outside the acceptable range – then report susceptibility test results and investigate.

- If two consecutive control zone diameters of 20 tests are outside the acceptable range – then investigate before reporting susceptibility test results. The tests may have to be repeated.

- If multiple disks (>2) are out of range on one day – then investigate before reporting susceptibility test results. The tests may have to be repeated.

- If resistance in a resistant control strain is not recognised – then suppress susceptibility test results, investigate and retest.
Monitoring Laboratory QC results

- **Target**
- **Upper limit**
- **Lower limit**

- **Single results out of range**
- **All results within range but on one side of the target**
- **Consecutive results out of range on same side of the target**

**Daily vs. weekly QC**

- **EUCAST recommends daily QC or at least four times a week!**
Example routine data Växjö
MH from 3 manufacturers, 10-15 technicians
S. aureus ATCC 29213 with cefoxitin 30 µg

Questions?
Comparison with reference distributions

- MIC and zone diameter distributions are available in the EUCAST database (www.eucast.org).

- Comparison with reference distributions can help detect systematic deviations not detected by regular QC testing.

- Compare the median for the wild-type distribution of clinical isolates with the median in the reference distribution.
Comparison with reference distributions – MIC

- **S. aureus with gentamicin**
  - 2666 observations
  - 21 data sources
  - Median 0.25-0.5 mg/L

- **S. pneumoniae with levofloxacin**
  - 85662 observations
  - 18 data sources
  - Median 1 mg/L

Comparison with reference distributions – Disk diffusion

**Example E. coli and cefotaxime 5 µg**

- **Reference distribution**
  - [www.eucast.org](http://www.eucast.org)
  - Median: 28 mm
  - Range: 23-34 mm

- **Lab A**
  - Median: 28-29 mm
  - Range: 25-32 mm

- **Lab B**
  - Median: 30 mm
  - Range: 26-35 mm

Median of wild-type distribution should be at median for reference ± 1 mm.
Evaluation of disk diffusion QC data

• Are zones within range?
• Is the mean value on target ± 1 mm?
• Are there systematic differences from the target value towards the lower or upper limit?
• (No of readings on target ± 1 mm)

Example of registration of disk diffusion QC data

<table>
<thead>
<tr>
<th>Strain</th>
<th>Strain</th>
<th>CTX5</th>
<th>CAZ10</th>
<th>MER10</th>
<th>TZP10</th>
<th>SXT25</th>
<th>CIPS</th>
<th>LEVS</th>
<th>PEFS</th>
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</thead>
<tbody>
<tr>
<td>E. coli ATCC 25022</td>
<td></td>
<td>26</td>
<td>27</td>
<td>31</td>
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<td>23</td>
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</table>

| Mean          |       | 27   | 27    | 31    | 23    | 26    | 34   | 35   | 30   |

Target        |       | 28   | 26    | 31-32 | 24    | 25    | 33   | 33   | 29   |


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<td>25</td>
<td>26</td>
<td>32</td>
<td>31</td>
<td>27</td>
</tr>
</tbody>
</table>

| Mean          |       | 30   | 29    | 34    | 25    | 26    | 32   | 35   | 28   |

Target        |       | 28   | 26    | 31-32 | 24    | 25    | 33   | 33   | 29   |

Batch control of Mueller-Hinton agar

Test each new batch of MH agar to ensure that all zones are within EUCAST QC ranges.

Particular problems:
- High or low concentrations of divalent cations (Ca$^{2+}$, Mg$^{2+}$) may be indicated by inhibition zones for aminoglycosides with *P. aeruginosa* ATCC 27853 below/above quality control limits, respectively.
- Excess thymine and thymidine may be indicated by inhibition zones for trimethoprim-sulfamethoxazole and *E. faecalis* ATCC 29212 below quality control limits.

Quality control of BMD

- Check that MICs are within QC ranges (EUCAST website) for relevant QC strains:
  - New lot Mueller Hinton / MH-F broth
  - New lot Microtiter plate
- Frequency to be determined locally
- Use several QC strains to cover all agents
  - Commercial panels often have few concentrations for each agent
- Include resistant QC strains to test β-lactam β-lactamase inhibitor combinations.
- Variation within ± one doubling dilution is acceptable, but deviations should not be systematic.
Example of registration of broth microdilution QC data

Pseudomonas panel

<table>
<thead>
<tr>
<th>QC strain</th>
<th>AMI</th>
<th>AZT</th>
<th>CIP</th>
<th>CAZ</th>
<th>OFL</th>
<th>COL</th>
<th>IMI</th>
<th>LEV</th>
<th>MER</th>
<th>PTZ</th>
<th>TOB</th>
<th>TGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>8</td>
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<td>4</td>
<td>2</td>
<td>1</td>
<td>0.25</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>16</td>
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<td>0.5</td>
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<td>P. aeruginosa ATCC 27853</td>
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<td>8</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>64</td>
<td>8</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

On target

Red text = EUCAST range
Blue text = CLSI range

Example of registration of broth microdilution QC data

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<thead>
<tr>
<th>QC strain</th>
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<th>PTZ</th>
<th>TOB</th>
<th>TGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae ATCC 700603</td>
<td>≤0.5</td>
<td>32</td>
<td>1</td>
<td>32</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.03</td>
<td>≤0.12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 700603</td>
<td>≤0.5</td>
<td>64</td>
<td>2</td>
<td>32</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.03</td>
<td>≤0.12</td>
<td>4</td>
<td>8</td>
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<td>K. pneumoniae ATCC 700603</td>
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<td>0.03</td>
<td>≤0.12</td>
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<td>K. pneumoniae ATCC 700603</td>
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<td>8</td>
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</tbody>
</table>

On target

Red text = EUCAST range
Blue text = CLSI range
Green text = Expected values based on previous tests
Example of registration of broth microdilution QC data

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<table>
<thead>
<tr>
<th>QC strain</th>
<th>AMI</th>
<th>AZT</th>
<th>PEF</th>
<th>CAM</th>
<th>CHF</th>
<th>COL</th>
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<th>PTZ</th>
<th>TOB</th>
<th>TSU</th>
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<tbody>
<tr>
<td>E. coli NCTC 13846</td>
<td>1</td>
<td>≤1</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>&gt;8</td>
<td>4</td>
<td>0.25</td>
<td>&gt;8</td>
<td>8</td>
<td>&gt;16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli NCTC 13846</td>
<td>2</td>
<td>≤1</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>&gt;8</td>
<td>4</td>
<td>0.25</td>
<td>&gt;8</td>
<td>8</td>
<td>&gt;16</td>
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<td></td>
</tr>
<tr>
<td>E. coli NCTC 13846</td>
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<td>≤1</td>
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<td>&gt;8</td>
<td>4</td>
<td>0.25</td>
<td>&gt;8</td>
<td>8</td>
<td>&gt;16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli NCTC 13846</td>
<td>4</td>
<td>≤1</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>&gt;8</td>
<td>4</td>
<td>0.25</td>
<td>&gt;8</td>
<td>8</td>
<td>&gt;16</td>
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<td></td>
</tr>
<tr>
<td>E. coli NCTC 13846</td>
<td>5</td>
<td>≤1</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>&gt;8</td>
<td>4</td>
<td>0.25</td>
<td>&gt;8</td>
<td>8</td>
<td>&gt;16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On target

Upper limit

Lower limit

Out of range

Red text = EUCAST range

Blue text = CLSI range

Green text = Expected values based on previous tests

Break
Potential sources of errors (Disk diffusion as example)

- Disks
- Media
- Not adhering to methodology
- Equipment
- QC strain

Potential sources of error (I)

- Antimicrobial disks
  - Decreased disk potency
    - Loss of potency during handling and storage
    - Disks passed expiry date
  - Wrong agent
  - Wrong disk potency
  - Poor disk quality
- Change to a new cartridge or a new disk lot
Potential sources of error (II)

- Media
  - Divalent cations, thymidine, pH
  - Supplements: Horse blood and β-NAD
  - Agar depth (4 mm ± 0.5 mm)
  - Preparation and storage
    - Shelf life?
    - Humid agar plates?
  - Media of poor quality

- Batch control for each new (powder) batch
- Check agar depth for each batch
- Check routines for preparation, storage and drying of plates

Why is the agar depth important?

- Antimicrobial agents diffuse into the agar
- Diffusion will be affected by the agar depth

- Agar depth <4 mm: Larger zone diameters
- Agar depth >4 mm: Smaller zone diameters

The method is calibrated to an agar depth of 4 mm!
Measuring agar depth

Adjust the volume if the agar depth is repeatedly above or below 4.0 mm!

Drying and storage of agar plates

• Store plates at 4-8°C
• Make sure agar plates are at room temperature prior to inoculation.
• Excess moisture may cause fuzzy zone edges and/or haze within zones.

• If necessary, dry plates
  • 20-25°C overnight or
  • 35°C for 15 min with the lid removed
MH-F agar plates
Excess moisture and fuzzy zone edges

In-house produced plate stored in ventilated rack
Commercial plate stored in plastic bag

Excess moisture may cause fuzzy zone edges and/or haze within zones. If necessary, dry plates before use.

Potential sources of error (III)

• Not adhering to methodology
  – 15-15-15 minutes rule
  – Wrong inoculum
  – Not using an overnight culture
  – Incubation temperature
  – Incubation atmosphere
  – Incubation time (16-20 h)

• Review AST routines
• Educate staff
Potential sources of error (IV)

• QC strains
  – Wrong strain
  – Old culture
  – Contamination
  – Mutation

• Review routines for QC strains
• If you suspect a problem, prepare a new subculture from freezer

Summary of QC

• Perform frequent QC to detect problems with the materials and methods used.
  – QC for disk diffusion has to be performed more frequent than for BMD.

• Analyse QC data against EUCAST published ranges and target values
  – Aim for the target!

• Compare local data with EUCAST reference distributions to investigate if there are systematic deviations.

• When materials don’t perform according to the standards
  – The EDL can help with troubleshooting
  – Contact the manufacturers and complain!
<table>
<thead>
<tr>
<th><strong>AST of bacteria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organization</strong></td>
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<tr>
<td><strong>Consultations</strong></td>
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<td><strong>EUCAST News</strong></td>
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<td><strong>New definition of S. and R</strong></td>
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<td><strong>Clinical breakpoints and dosing</strong></td>
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<td><strong>Rapid AST in blood cultures</strong></td>
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<td><strong>Expert rules and intrinsic resistance</strong></td>
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<td><strong>Resistance mechanisms</strong></td>
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<td><strong>Guidance documents</strong></td>
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<td><strong>SOP</strong></td>
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<tr>
<td><strong>MIC and zone distributions and ECOFFs</strong></td>
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</table>

**EUCAST Disk Diffusion Test Methodology**

The EUCAST disk diffusion test is based on the inoculum and discs of a good quality. It is calibrated to EUCAST clinical breakpoints using both microdilution for MIC determination. Updates are published regularly.

See also EUCAST instruction videos.

| Disk diffusion - Manual v 9.0 (1 January, 2021) |
| Disk diffusion - Whole show v 9.0.1 (January, 2021) |
| Disk diffusion - Reading guide v 9.0.1 (January, 2021) |

EUCAST disk diffusion of enterococci bacteria is under development 2021. Reviewed critical breakpoints and disk diffusion cut-offs will be published with breakpoint tables v 12.0.0 (1 January, 2022). The method will be valid for 5 species (Enterococci spp., Pseudotabula spp., Proteus mirabilis, Citrobacter freundii and Providencia spp.) and for anaerobic incubation for 48 - 72h (continued incubation not allowed). For anyone who wants to prepare and perform, EUCAST already now publish the methodology, testing guide and QC methods.

MIC determination of non-fastidious and fastidious organisms

EUCAST recommendations for MIC determination for non-fastidious organisms are in complete agreement with the recommendations from the international standards (2004). For fastidious organisms (staphylococcus including S. pneumoniae, A. influenzae, klebsielie carbaminase, E. coli, neumopheumiae, Pasteurella spp., Klebsiella spp. Enterobacteriaceae spp., Campylobacter spp., and others), EUCAST recommends the same methodology but with the use of mini-disc (SAT broth with tryptone base and beta-haemol) instead.

MIC determination of non-fastidious and fastidious organisms (v 8.0, 1 January, 2020)

| Broth microdilution - EUCAST testing guide v 9.0 (1 January, 2021) |
| Guidance on confirmatory MIC testing - see guidance document |

There are a number of commercially available semiautomatic MIC determination methods, such as commercial broth microdilution methods, gradient tests, semi-automated systems, etc.
Questions?
Implementation of disk diffusion

• Education
  – EUCAST DD manual and slide show
  – Reading Guide
  – EUCAST videos

• Training using QC strains
  – Inoculum, inoculation
  – Reading of zones
    • Reading exercises where all staff read zones from the same plate
  – Repeat testing of QC strains
    • Analysis of data

• Training using clinical isolates

Reading exercise, Växjö laboratory

S. aureus

First occasion

<table>
<thead>
<tr>
<th></th>
<th>Cefoxitin</th>
<th>Erythromycin</th>
<th>Clindamycin</th>
<th>Fusidic acid</th>
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First occasion
Discussion

S. aureus and fuzzy zone edges

- 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 exercise, Växjö laboratory

S. aureus

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Mean: 26 27 27 29 22 31

SD: 1.2 1.1 1.2 0.9 2.0 1.0

> 1 mm above mean

> 1 mm below mean
Discussion

*S. aureus* and norfloxacin

- If cultures are pure, read the inner zone.

- There is almost always a double zone for *S. aureus* and norfloxacin.

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Reading exercise, Växjö laboratory

*S. aureus*

Following analysis and discussion

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</table>

> 1 mm above mean

> 1 mm below mean
Problems and/or questions?

Please contact us!

erika.matuschek@eucast.org