Quality Assurance of antimicrobial susceptibility testing

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Quality Assurance

The total process by which the quality of laboratory reports can be guaranteed
Components of quality assurance

- Routine Quality Control
- External Quality Assessment (proficiency testing)
- Internal Quality Assessment (specimen reprocessing)
- Standardisation & documentation
- Audit
- Accreditation
- Education
- Validation
Components of quality assurance

Quality Assurance

- Routine quality control
  - External Quality Assessment (proficiency testing)
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Routine quality control

Repeated testing of controls in parallel with tests to ensure that the test system is performing reproducibly within defined limits
Quality control of disk diffusion antimicrobial susceptibility tests

Specified routine quality control strains are used to monitor test performance

• Quality control strains may be purchased from culture collections or from commercial sources
• See EUCAST website for guidance on storage of control strains
## EUCAST routine quality control strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Culture collection numbers</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620, CECT 434</td>
<td>Susceptible, wild-type</td>
</tr>
<tr>
<td></td>
<td>ATCC 35218; NCTC 11954; CIP 102181; DSM 5564; CCUG 30600; CECT 943</td>
<td>TEM-1 β-lactamase producer</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619; CECT 108</td>
<td>Susceptible, wild-type</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915; CECT 794</td>
<td>Weak β-lactamase producer</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997; CECT 795</td>
<td>Susceptible, wild-type</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638</td>
<td>Penicillin intermediate</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>ATCC 49766; NCTC 12975 CIP 103570; DSM 11970 CCUG 29539</td>
<td>Susceptible, wild-type</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>ATCC 33560; NCTC 11351; CIP 702 DSM 4688; CCUG 11284</td>
<td>Susceptible, wild-type</td>
</tr>
</tbody>
</table>
Staphylococcus aureus ATCC 29213*
(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

* β-lactamase-producing strain (weak)

Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth from the back of the plate 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target¹</td>
<td>Range²</td>
<td>Target¹</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2</td>
<td>1-4</td>
<td>30</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1</td>
<td>0.5-2</td>
<td>-</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>0.5-1</td>
<td>0.25-2</td>
<td>1 unit</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2</td>
<td>1-4</td>
<td>30</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>0.25</td>
<td>0.125-0.5</td>
<td>5</td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>0.25-0.5</td>
<td>0.125-1</td>
<td>5</td>
</tr>
</tbody>
</table>
Monitoring disk diffusion test performance

Single results outside control limits

Upper limit of range
Target
Lower limit of range

Consecutive results outside limits on same side of the mean
Response to disk diffusion QC results out of range

• Single test out of range – report susceptibility if no obvious problem.

• If two non-consecutive control zone diameters of 20 consecutive tests are out of range – then report results if no obvious problem but investigate.

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

• If multiple agents (>2) are out of range on one day – then investigate before reporting results. The tests may have to be repeated.
EUCAST strains for detection of resistance mechanisms

Quality control strains with defined resistance mechanisms may be used to confirm the ability to detect resistance. If resistance in a resistant control strain is not recognised suppress test results, retest and investigate.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Culture collection numbers</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em></td>
<td>ATCC 700603; NCTC 13368; CCUG 45421; CECT 7787</td>
<td>ESBL producer (SHV-18)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>NCTC 12493</td>
<td>Oxacillin hetero-resistant, <em>mecA</em> positive</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>ATCC 51922; NCTC 13379; CIP 104676; DSM 12956; CCUG 34289</td>
<td>High-level aminoglycoside resistant (HLAR) and vancomycin resistant (<em>vanB</em> positive)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>ATCC 49247; NCTC 12699; CIP 104604; DSM 9999; CCUG 26214</td>
<td>β-lactamase negative, ampicillin-resistant (BLNAR)</td>
</tr>
</tbody>
</table>
EUCAST strains for detection of resistance mechanisms (possible additional strains)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em></td>
<td>Penicillin resistant (MIC 4 mg/L)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Different ESBL phenotypes</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Plasmid AmpC</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Carbapenemase producers</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>KPC enzyme</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>mcr-1</em></td>
</tr>
</tbody>
</table>
Quality control by comparison of wild type with reference distributions from EUCAST website

Ampicillin / Escherichia coli

International wild type zone diameter distribution - Reference database 2016-09-10
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: 10
Epidemiological cut-off (ECOFF): ≥ 14 mm (MIC = 8 mg/L)
Wildtype (WT) organisms: ≤ 14 mm (MIC = 8 mg/L)

18972 observations (8 data sources)
# Sources of error in disk diffusion

<table>
<thead>
<tr>
<th>Medium</th>
<th>Storage of plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not prepared to instructions</td>
</tr>
<tr>
<td></td>
<td>Batch to batch variation or change of supplier of agar</td>
</tr>
<tr>
<td></td>
<td>Supplements (batch to batch variations, incorrect amount, expired)</td>
</tr>
<tr>
<td></td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>Agar depth/Agar volume</td>
</tr>
<tr>
<td></td>
<td>Expiry date</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test conditions</th>
<th>“15-15-15”-rule not adhered to (suspension used within 15 min, disks applied within 15 min, incubation within 15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation (temperature, atmosphere and time)</td>
</tr>
<tr>
<td></td>
<td>Incorrect inoculation (too light, too heavy or uneven)</td>
</tr>
<tr>
<td></td>
<td>Reading conditions, reading zone edges</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disks</th>
<th>Incorrect disk (wrong agent or wrong disk content)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disk potency (incorrect storage, labile agent, expiry date)</td>
</tr>
<tr>
<td></td>
<td>Disks not at room temperature when containers opened</td>
</tr>
<tr>
<td></td>
<td>Too many disks on plate (interference between agents)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control organisms</th>
<th>Incorrect QC strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutation</td>
</tr>
<tr>
<td></td>
<td>Contamination</td>
</tr>
<tr>
<td></td>
<td>Age of culture</td>
</tr>
</tbody>
</table>
Quality control of MIC determination

- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.
Quality control of MIC testing

Ertapenem / Escherichia coli
International MIC Distribution - Reference Database 2016-09-10

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

Dilution range must include acceptable control range

Control range for *E. coli* ATCC 25922

S R EUCAST breakpoints
Quality control of MIC determination

- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.
- Include a control without antimicrobial agent to ensure that the test strain grows adequately.
- Test the purity of inoculum by culture on solid medium to obtain isolated colonies.
- If MIC of control is out of range the source of error must be sought and the test repeated.
- Check wild type distribution against EUCAST distribution on website.
Quality control by comparison of wild type with reference distributions from EUCAST website
Quality control of automated systems

• Use the recommended routine quality control strains to monitor test performance (see manufacturer’s instructions).

• Restricted range of test concentrations mean that the range may not include the MIC of the control strain.

• Purity of inoculum tested by culture on solid medium to obtain isolated colonies.

• If control is out of range the source of error must be sought and the test repeated.
Components of quality assurance

- Routine quality control
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Quality Assurance
Components of quality assurance

External Quality Assessment (proficiency testing)

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- Education

Quality Assurance
External Quality Assessment (Proficiency testing)

The challenge of laboratory procedures with specimens of known but undisclosed content
The EQA process (UK NEQAS)

- Prepare samples
- Examine samples
- Report results
- Analyse results
- Prepare report
- Evaluate performance
- Participants
  - Examine samples
  - Report results
  - Evaluate performance

Participants
• Reference MIC results
• Your results
• Scores highlighting your performance
• Cumulative score over time and mean for all laboratories
• Detailed results for laboratories using the same method as you
• Details of results with different guidelines
• Comments on particular problems


- Review the results with all staff (include successes and failures)
- Investigate problems
  - How many other participants had problems with the specimen?
  - Are there any comments on technical or interpretive issues?
**Enterococcus faecium (EARS-Net 3082)**

Vancomycin MIC 8 mg/L (VanB)
EUCAST R (S ≤4, R>4 mg/L), CLSI I (S ≤4, R≥32 mg/L)

<table>
<thead>
<tr>
<th>Method</th>
<th>EUCAST</th>
<th>CLSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%) of participants reporting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Disk diffusion</td>
<td>11 (7.4)</td>
<td>4 (2.7)</td>
</tr>
<tr>
<td>Automated</td>
<td>5 (1.8)</td>
<td>0</td>
</tr>
<tr>
<td>MIC</td>
<td>15 (4.9)</td>
<td>8 (2.7)</td>
</tr>
<tr>
<td>Multi/other</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>32 (4.3)</td>
<td>12 (1.6)</td>
</tr>
</tbody>
</table>
Enterococci and vancomycin

- Examine with transmitted light (plate held up to light).
  - Fuzzy zone edges and colonies within zone indicate vancomycin resistance and should be investigated further.

\[ \text{E. faecalis} \quad \text{non-VRE} \]
\[ \text{E. faecium} \quad \text{VRE} \]
Benefits of EQA in antimicrobial susceptibility testing

• Independent assessment of performance
• Assessment of performance over time
• Comparison with other laboratories
• Performance indicator for accreditation
• Highlights problem areas
• Performance related to methods
• Differences in guidelines highlighted
• Education
"Limitations" of EQA in antimicrobial susceptibility testing

- Number of specimens distributed is small
- May be considered inappropriate to send some organisms
- Specimens do not reflect routine isolates
- Laboratories may not treat specimens as routine
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Components of quality assurance

Internal Quality Assessment (specimen reprocessing)

- Standardisation & documentation
- Audit
- Validation
- Accreditation
- Education
- Quality Assurance

External Quality Assessment (proficiency testing)
Internal Quality Assessment (specimen reprocessing)

The challenge of laboratory procedures by repeat testing of specimens of unknown content
Internal quality assessment (IQA) process

- Specimens split and both processed on same day, or same specimen processed twice on the same day, with identification of repeat test blinded
- For susceptibility testing the same organism could be processed twice on same day or repeated on different days
- Reports compared and discrepancies investigated
- Feedback
  - Rapid feedback of discrepancy reports
  - Frequent discussion and action in laboratory meetings
Antimicrobial susceptibility testing problems highlighted by IQA

- Variable susceptibility because different organisms picked from mixture on primary plates
- Wrong disk contents used e.g.
  - Ampicillin 10 µg instead of 2 µg for *H. influenzae*
- Borderline susceptibility leads to variable results e.g.
  - *S. aureus* erythromycin R changed to S
  - *S. aureus* mupirocin S changed to I
  - *S. aureus* fusidic acid S changed to R
- Discrepancies with “difficult” tests
  - Cefoxitin with hetero-resistant MRSA
  - Vancomycin with VanB enterococcus
- Typographical errors
Benefits of IQA for antimicrobial susceptibility testing

- Tests reproducibility of all aspects of processing a specimen
- Covers areas not tested by EQA
- More samples than EQA
- Locally responsive
- Rapid turnaround so problems investigated early
- Recognised by accreditation authorities
Limitations of IQA for antimicrobial susceptibility testing

- Discrepancies may not be related to susceptibility testing
- No reference results so the correct answer is unknown - both results could be wrong
- Cost
Quality assurance of antimicrobial susceptibility testing

- Multiple components contribute to maintaining the quality of antimicrobial susceptibility testing

- Quality assurance is essential to ensure reliable results