Quality Control and Quality Assurance for Antibiotic Testing

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Microbiology Technical Workshop

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Quality assurance

“practice of assessing performance in all steps of the process to promote excellent outcome in medical care”

Quality control

“aggregate of processes and techniques to detect, reduce, and correct deficiencies in an analytical process.”

Processes

Quality Assurance

With thanks to Derek Brown @ EUCAST Educational Workshop: 31 March 2012
External quality assurance

The challenge of laboratory procedures with specimens of known but undisclosed content

**EQA**

Examples of EQA organizations

- College of American Pathologists
- Royal College of Pathologists (Australasia)
- UKNEQAS
- Local reference laboratories

**EQA: benefits**

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Highlights problem areas
- Performance related to guidelines and methods
EQA: benefits

• International differences highlighted

• Gives practical experience of difficult tests (especially if resistance is uncommon)

• Provides background information and guidance on appropriate methods

• Performance indicator for accreditation

EQA: limitations

• 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

EQA: @ CGH Laboratory

• Survey subscription based on services offered

• Survey sample management

• Result submission management

• Performance assessment

Internal Quality Assurance

The challenge of laboratory procedures by repeat testing of specimens of unknown content

Sample

↓
routine testing

↓
results

↓
reported

↔
compare & evaluate

↓
results

↓
routine testing

↓
blind
**Internal Quality Assurance**

The challenge of laboratory procedures by repeat testing of specimens of unknown content

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**IQA: Benefits**

- Check of laboratory processes
- Identifies:
  - typographical errors
  - Inconsistencies between different technologists
  - process errors

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**IQA: Practical issues**

- Different organisms picked from mixture on primary plates
- Borderline susceptibility leads to variation
- Discrepancies with “difficult” tests
- Labor intensive
- Sample variability (stored samples)
IQA: @ CGH Laboratory

Target: 50 specimens issued monthly

Frequent issues:
- Variation in zone diameters
- Enumeration of cells
- Urine viable count

Audit: @ CGH Laboratory

Processes
Documentation
Skills
Knowledge

Audit: @ CGH Laboratory

Accreditation audit

Internal surveillance
- Target 10% of total test specimens
  - July 2013: 1,198 of 13,209 specimens

Frequent issues:
- Variation in zone diameters
- Enumeration of cells
- Urine viable count

Audit: @ CGH Laboratory

Errors

Internal surveillance
- 10% of total test specimens
  - July statistics: 1,198 of 13,209 specimens
**Documentation**

Standard operating procedures
- user-friendly, not reference text!
- updated regularly
- document control

Readily accessible

**Examples from:**

- WHO SOP (SE Asia)
  http://apps.searo.who.int/PDS_DOCS/B0217.pdf
- HPA (UK) SOP repository
  http://www.hpa.org.uk/SMI
- Mount Sinai SOP
  http://microbiology.mtsinai.on.ca/manual/anti/index.html

**Evaluation & Validation**

For new testing methods / antibiotics introduced into laboratory

<table>
<thead>
<tr>
<th>Analytic accuracy</th>
<th>Compare new against “gold standard”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>Repeatability</td>
</tr>
</tbody>
</table>

Evaluation & Validation @ CGH Laboratory

Introducing use of yeast AST testing by Vitek

<table>
<thead>
<tr>
<th>Analytic accuracy</th>
<th>Compare results obtained by Vitek against reference microbroth dilution method using a range of various species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>20 consecutive days of parallel testing</td>
</tr>
</tbody>
</table>

Education & Training

Competency
- Define skills to be assessed
- Define how to assess
- Define who will assess
- Documentation
- Remediation

Continuing professional education

Skills upgrading

Reference
Microbiology Training Record

<table>
<thead>
<tr>
<th>Date</th>
<th>Date</th>
<th>Topic</th>
<th>Trainer</th>
<th>LNG</th>
<th>ZE</th>
<th>ZO</th>
<th>ZS</th>
<th>ZI</th>
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<td>Talk</td>
<td>Vancomycin binding proteins in relation to staphylococcal growth and drug resistance</td>
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</table>

Quality Control

Antibiotic testing

- Temperature
- Atmosphere
- Disc potency
- Media
- Organism

QC: Antibiotic testing

Specified routine quality control strains are used to monitor test performance.

Quality control strains must be from a reliable source.

Proper storage to maintain characteristics.
QC Strains: specific tests

| Organism/Characteristics | Disk Diffusion Tests | MIC Tests | Screening Tests | Other
|--------------------------|----------------------|-----------|----------------|-------
| ATCC 10011               |                      |           |                |       |
| K. pneumoniae ATCC DAA-105 | C/S producing, C/S sensitive |           |                |       |
| K. pneumoniae ATCC DAA-106 | C/S sensitive to cefepime by chloramphenicol, C/S sensitive |           |                |       |
| K. pneumoniae ATCC DAA-107 | C/S sensitive to cefepime by chloramphenicol, C/S sensitive |           |                |       |
| E. aerogenes ATCC DAA-108 | Weak-aminoglycoside producing strains |           |                |       |

QC Disc: acceptable ranges

Acceptable range for ATCC strains

Table 3A. Disk Diffusion: Quality Control Strains for Non-Pathogenic Organisms (Unsuppressed Mueller-Hinton Broth)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Disk Content</th>
<th>E. coli ATCC 25922</th>
<th>P. aeruginosa ATCC 27853</th>
<th>E. cloacae ATCC 13047</th>
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<tbody>
<tr>
<td>Amoxicillin</td>
<td>25</td>
<td>15-30</td>
<td>10-30</td>
<td>20-40</td>
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<tr>
<td>Ceftazidime</td>
<td>30</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
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<tr>
<td>Cefotaxime</td>
<td>25</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
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<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
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<tr>
<td>Cefuroxime</td>
<td>25</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
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<tr>
<td>Gentamicin</td>
<td>15</td>
<td>10-20</td>
<td>15-30</td>
<td>20-40</td>
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<tr>
<td>Tobramycin</td>
<td>15</td>
<td>10-20</td>
<td>15-30</td>
<td>20-40</td>
</tr>
<tr>
<td>Amikacin</td>
<td>20</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20</td>
<td>15-30</td>
<td>20-40</td>
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<tr>
<td>Tobramycin</td>
<td>20</td>
<td>15-30</td>
<td>20-40</td>
<td>25-50</td>
</tr>
</tbody>
</table>

QC Disc: testing

15-Replicate (3 x 5 day) Plan Flow Chart:

- Test 3 replicates of each QC strain for 5 days using individually prepared inoculum.
- 0-1 of 15 out of range?
  - 2-3 of 20 out of range?
  - 24 of 30 out of range?
  - Fail. Continue to incorporate QC each test day, take corrective action.
- Pass. Convert to weekly QC.
- Test another 3 replicates for 5 days.
- 0-1 of 15 out of range?
- Pass. Convert to weekly QC.
Westgard rules

multiple rules used to monitor a charted process determines if the process is within acceptable limits.

Westgard rules

A. \( T \) = \( T \) rule detects serious error. \( T \) rule is violated when one control value is outside the mean ±3SD control values in the previous run should be considered to be out of control.

B. \( R \) = \( R \) rule detects systematic errors. \( R \) rule is violated when two consecutive control values are equal (same) (mean ±3SD or mean ±2SD) level.

C. \( A \) = \( A \) rule detects systematic error. \( A \) rule is violated when four consecutive control values exceed the same (mean ±2SD or mean ±2SD) level.

D. \( D \) = \( D \) rule detects rapid change. \( D \) rule is violated when 10 consecutive control values are on the same side of the mean. Its violation often indicates the deterioration of assay reagents.

Consecutive values must be on the same side of the mean.

Westgard relevant

reject when 10 consecutive control measurements fall on one side of the mean.

QC Disc: monitoring

QC Disc: failures
Antibiotic testing: Common sources of error

Media
- pH
- Thickness
- Storage
- Contamination
- Wrong media

QC strains
- Wrong strains
- Inocula
- Storage
- Contamination
- Age

Antibiotics
- Storage
- Wrong disc

Procedures
- Incubation conditions
- Disc placement
- transcription

Antibiotic testing: Common sources of error

Media
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Antibiotic testing: Common sources of error

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- Wrong strains
- Inocula
- Storage
- Contamination
- Age

Antibiotics
- Storage
- Wrong disc

Procedures
- Disc placement
- Incubation condition
- transcription
QC Disc: Troubleshooting

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>QC Strain</th>
<th>Observation</th>
<th>Probable Cause</th>
<th>Comments/Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>E. coli ATCC 25921</td>
<td>Zone too small</td>
<td>pH of media too low</td>
<td>Acceptable pH range 7.2-7.4, avoid CO2, incubation, which lowers pH</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>P. aeruginosa ATCC 27853</td>
<td>Zone too small</td>
<td>pH of media too high</td>
<td>Acceptable pH range 7.2-7.4</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>P. aeruginosa ATCC 27853</td>
<td>Zone too small</td>
<td>Ca++ and/or Mg++ content too high</td>
<td>Use alternative lot of media</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>P. aeruginosa ATCC 27853</td>
<td>Zone too small</td>
<td>Ca++ and/or Mg++ content too low</td>
<td>Use alternative lot of media</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>E. coli ATCC 35218</td>
<td>Zone too small</td>
<td>Ceftriaxone acid is stable, disk has lost potency</td>
<td>Use alternative lots of disks, check storage conditions and package integrity</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>E. coli ATCC 35218</td>
<td>Zone too large</td>
<td>Spontaneous loss of the plasmid encoding the β-lactamase</td>
<td>See comment (1) on QC organism maintenance</td>
</tr>
<tr>
<td>β-Lactam group</td>
<td>Any</td>
<td>Zone initially acceptable, but decreases and possibly out of range over time</td>
<td>Disk has lost potency</td>
<td>Use alternative lots of disks, check storage conditions and package integrity, Imipenem, clavulanic acid, and ceftriaxone are especially stable</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>K. pneumonia ATCC 700603</td>
<td>Zone too large</td>
<td>Spontaneous loss of the plasmid encoding the β-lactamase</td>
<td>See comment (1) on QC organism maintenance</td>
</tr>
</tbody>
</table>

Some concluding thoughts

QC of Automated Systems

- Use the recommended quality control strains
- Follow manufacturer’s instructions
- Purity check of used inocula

Restricted range of test concentrations means that the available range may not include the MIC of the control strain.
### Quality measures and effort (%)

<table>
<thead>
<tr>
<th>Quality measure</th>
<th>Effort (%)</th>
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<tbody>
<tr>
<td>Clinically relevant testing strategies</td>
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<tr>
<td>Testing of reference QC strains</td>
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<tr>
<td>Technical competency</td>
<td>15</td>
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<tr>
<td>Organism antibiogram verification</td>
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<tr>
<td>Supervisor review of results</td>
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<tr>
<td>Procedure manual</td>
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<tr>
<td>Cumulative antibiogram</td>
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<tr>
<td>Proficiency surveys</td>
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<tr>
<td>Other</td>
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</table>


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**Conclusion**

Can we afford quality assurance in microbiology laboratories?

“15% of Microbiology reports are wrong!”

**Can we afford NOT TO HAVE quality assurance in microbiology laboratories?**
Resources

WHO Laboratory Quality Management System Handbook 2011

CLSI M40 susceptibility testing
www.clsi.org

EUCAST quality tables
www.eucast.org/antimicrobial_susceptibility_testing/qc_tables/

General Review
http://www.intechopen.com/books/latest-research-into-quality-control/quality-assurance-in-antimicrobial-susceptibility-testing#823