Quality Assurance of Antibiotic Susceptibility Testing

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What is quality?
- Degree of congruence between expectation (result expected) and realization (result obtained)

Importance of quality assurance in a laboratory
- Results have little meaning unless tests are validated and quality controlled
- System of quality management in place
The diagnostic value of the clinical specimen and microbial isolates are dependent on a Quality Assurance (QA) program.

QA is responsible for providing accurate and relevant information that is of use for clinical diagnosis of a patient or in support of a public health activity (epidemiological surveillance or research).
Quality Assurance

- **QA program**
  - Documents the validity of the test method
  - Assesses the quality of the specimen
  - Monitors the performance of test procedures, reagents, media, instruments & personnel
  - Reviews test results for errors and clinical significance
IQC – a set of procedures undertaken by the laboratory staff for continuously and concurrently assessing their work and emergent results, to decide whether they are reliable enough to be released.

EQA – a system of objectively checking laboratory results by means of an external agency to establish the trueness of results.
A culture of QA is essential in a microbiology lab...
Commitment – recognize value of QA for patient & lab

- Ensures that the information generated by the laboratory is accurate, reliable and reproducible
- Essential component of patient management
- Essential component of a laboratory QA program:
  - Accreditation + licensing
  - Enhance client confidence
  - Motivate for lab funding
  - Identify weaknesses requiring corrective actions
  - Guide training activities
QC of Antimicrobial Susceptibility Testing (AST)

- Kirby-Bauer Disc Diffusion Testing Method
Primary variables to be controlled when performing routine AST

- Media
- Antimicrobial agents (antibiotic discs)
- Bugs: reference strains
- Inoculum
- Incubation conditions
- Endpoint measurements
- Skilled personnel (hands & heads)
- Standard operating procedures (SOP)
Media – essentials

- Formulation: Mueller-Hinton agar or Mueller-Hinton agar + blood
- Agar depth (4mm); pH (7.2 – 7.4)
- Magnesium (12.5mg/l)
- Calcium (25mg/l)
- Storage (2-8ºC)
  - Dry before use
  - Room temp before use
- Note Expiry date
- Check for batch contamination
Antibiotic discs – essentials

- Antimicrobial potency: use discs containing appropriate CLSI-defined drug concentrations
- Storage: long-term (freezer at < -20ºC); short-term (2-8ºC)
  - Take out 1 hour before use
  - Discs must be at room temperature before use
  - Avoid condensation
- Note expiry dates
- Placement:
  - Within 15 minutes of swabbing
  - < 5 discs/ 100mm plate
The Bugs: Reference strains

- Basic QC procedure involves testing reference strains that have defined characteristics of susceptibility (known zone diameters) to the antimicrobial agents

- Sources of ATCC (American Type Culture Collection) strains
  - From ATCC ([www.atcc.org](http://www.atcc.org))
  - Commercial company selling lyophilized vials e.g. BD
The Bugs: Reference strains

- Storage: lyophilized culture vials (containing 12-14 beads each) to be stored at 4°C & re-sealed once opened
- Procedure for preparation of working (bench) stock
  - 4 subcultures per bead
  - Use each subculture for maximum of 1 week
  - Storage of subculture: bench, away from sunlight
  - After 4 weeks use new bead from vial
Inoculum

- McFarland turbidity standards (containing barium sulphate precipitate)
  - Used to standardize the approximate number of bacteria in liquid suspension by visually comparing the turbidities of test suspension & the McFarland standard
- Standard most commonly used in Clinical Microbiology for routine AST

\[0.5 \text{ Mc Farland} = 1.5 \times 10^8 \text{ bacteria/ml}\]
Visual comparison of turbidity must be in presence of good lighting

Hold both tubes in front of Wickerham card

- If suspension too heavy, dilute with saline
- If suspension too light, inoculate with more colonies
Incubation conditions

- **Atmosphere**
  - Humidified ambient air – **aerobic**
  - Unless CO$_2$ specified – e.g. Haemophilus spp

- **Temperature**
  - 35°C
  - Within 30 mins of preparation

- **Reading after 16-18 hours**
  - 24 hours for Staph (Oxacillin)
  - 16-18 hours incubation adequate for Cefoxitin disc
Incubation conditions

- 24 hours for Enterococci
  (Vancomycin & HL gentamicin)

- Stacking of plates
  - No more than 5 plates high
Endpoint measurement

- Use reflected light
  - Hold plate against or few inches above black background
  - Lawn of growth must be near-confluent
  - Measure zones from back of plate for transparent media; or remove lid and measure from top of plate
  - Measure zone of inhibition of growth and not inhibition of haemolysis for haemolytic organisms

- Transmitted light for Staph (Ox) & Enterococci (Va)
  - Reflected light may be used for Cefoxitin disc to predict Oxacillin resistance in Staph

- Use callipers to measure zone sizes
Correct technique
- Storage of discs
- Choice & storage of media
- Standard inoculum preparation
- Swabbing of plates
- Incubation conditions
- Measurement of zone sizes
- Recording of results

Personnel: Technologist competency
QC SOP

- Who will perform the AST QC?

- How often – weekly/daily?
  - Test ATCC control organisms with each new batch of antimicrobial discs/media
  - Do **weekly** AST QC routinely with ATCC control organisms (CLSI guidelines)

- Assistance with troubleshooting – assign an individual

- How & when will problems be communicated to the clinicians?
When things go wrong... Troubleshoot!

- Ask
  - Is procedure correct?
  - Is it the correct test strain?
  - Check other test materials
  - Check equipment – refrigerators/incubators/freezer
- Review technique of personnel
Troubleshooting
Possible errors

- Inoculum too heavy?
- Antibiotic deteriorated?
- Correct content of antibiotic disc?
- Mueller Hinton agar too deep?
- Appropriate control strain?…
## Troubleshooting guide for AST QC

<table>
<thead>
<tr>
<th>Factor</th>
<th>Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media (depth of agar)</td>
<td>Thin media yield excessively large inhibition zones &amp; vice versa</td>
</tr>
<tr>
<td>Composition of medium</td>
<td>Affects rate of growth of organisms</td>
</tr>
<tr>
<td></td>
<td>Affects activity &amp; diffusion of antibiotics</td>
</tr>
<tr>
<td>Antibiotic discs (potency)</td>
<td>Deterioration in content leads to smaller zone sizes</td>
</tr>
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<tr>
<td>Antibiotic discs – spacing</td>
<td>Disc too close together will cause overlapping zones. Smaller plate accommodates fewer discs</td>
</tr>
<tr>
<td>Timing of application</td>
<td>If placed long after swabbing plates, small zones may form</td>
</tr>
<tr>
<td>Reference strains for QC</td>
<td>Incorrect reference strain used for specific AST will lead to incorrect zone diameters – false alarm</td>
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<tr>
<td>Inoculum density</td>
<td>Larger zones with a light inoculum and vice versa</td>
</tr>
<tr>
<td>Incubation time</td>
<td>Ideal 16-18 hours; less time gives unreliable results</td>
</tr>
<tr>
<td>Temperature</td>
<td>If &lt; 35°C larger zones are seen and MRSA may go undetected</td>
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<tr>
<td>Endpoint measurements reading</td>
<td>Subjective errors in determining the clear edge</td>
</tr>
<tr>
<td>Colonies within zone of inhibition</td>
<td>Mixed culture/ contaminant</td>
</tr>
<tr>
<td></td>
<td>Resistant subpopulations</td>
</tr>
<tr>
<td>Indistinct zones</td>
<td>Poorly streaked plates</td>
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CLSI standards

- **M100** – Performance Standards for Antimicrobial Susceptibility Testing, 29th Edition (Dec 2018)
Thank you

Acknowledgement
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