Aims of antimicrobial susceptibility testing:

1. Confirm the susceptibility to chosen empirical therapy
2. Detect resistance
Various methods of antibiotic susceptibility testing are:

1. **Quantitative Methods**
2. Qualitative Methods
3. **Automated Susceptibility Tests**
4. Newer Non-Automated Susceptibility Tests
5. Molecular Techniques

**Broth testing methods**

- Broth preparation
- Antimicrobial agent
  - Stock solution
  - Working solutions
- Preparation of plates
- Inoculum preparation
- Inoculation of tubes/plates
- Incubation of tubes/plates
- Reading results
- Quality control

**Reference methods for in vitro susceptibility testing**

1. Macrobroth dilution (Tube dilution)
2. Microbroth dilution

CLSI, EUCAST, BSAC guidelines
Broth preparation

- Mueller–Hinton broth (MHB)
  - General purpose medium (non fastidious)
  - Cation- adjusted
  - Optimum pH

Antimicrobial agent

- Stock solution
  - Potency (from manufacturer)

\[
\text{Wt of powder (mg)} = \frac{\text{Vol of solvent (mL)} \times \text{Concentration (mg/L)}}{\text{Potency of powder (mg/g)}}
\]

- Appropriate solvent for dilution (CLSI Table 5A)
- Stock solutions may be stored at -60°C for more than 6 months for most antimicrobial agents
- Avoid repeated freeze thaw

Inoculum preparation

- Direct colony suspension method
  - Most convenient
  - Fresh colonies; 0.5 McFarland standard
  - Fastidious organisms (e.g., Neisseria gonorrhoeae; Haemophilus spp)

- Growth method
  - When smooth suspension cannot be made
  - Non-fastidious organisms

Working solution

- Serial doubling dilutions
- Dilution scheme available in CLSI M100

Plates preparation

- Store at appropriate temperature
Inoculation of tubes/plates

Incubation of tubes/plates

- Incubate inoculated macrodilution tubes/microdilution trays at 35 +/- 2°C for 16-20 hours in ambient air incubator
- Within 15 minutes of adding inoculum
- Do not stack trays >4 high
- Seal each tray

- CLSI M7A9 Sections 11, 12, Appendix C

Reading results

- QC passed; GC wells +

- The MIC is the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye

Quality control

- To include at least 1 control organism (ATCC) with each batch of testing
- Test values for the control strains should be within the published range

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Limitations

• Labour intensive
• Strict adherence to protocol is required
• The MIC value is a poor predictor of the drug efficacy in vivo
• Resistance by a small subpopulation may not always be detected

Agar dilution method

• Antimicrobial agent
  - Stock solution
  - Working solutions
    • Preparation of agar and plates
    • Inoculum preparation
    • Inoculation of plates
    • Incubation of plates
    • Reading results
  • Quality control

Agar and Plates preparation

• Mullen Hinton agar is considered the reference medium

• Allow the sterilized agar to cool to 50°C in a water-bath, before adding antibiotics
• One concentration of antibiotic/ plate
• Include a drug free control
• Set at room temperature. Do not overdry.
• Store plates at 4-8°C
Inoculation of plates

- Mark the plates for orientation
- Use an inoculum-replicating apparatus to transfer the inocula to the series of agar plates
- Allow the inoculum spots to dry at room temperature before inverting the plates for incubation.

Reading results

Limitations

- Labour intensive
- Strict adherence to protocol is required
- The MIC value is a poor predictor of the drug efficacy in vivo

Automated Susceptibility Testing
Several automated systems for antimicrobial susceptibility testing are commercially available

Examples:
- **Vitek 2 System** (BioMérieux)
- **Phoenix Automated Microbiology System** (SD Diagnostic System)
- **MicroScan Walkaway System** (Siemens Healthcare Diagnostics)
- **Sensititre Aris 2X** (Trek Diagnostic System)

**General advantages**
- Quantitative results (MIC values)
- Reproducibility
- Cost-effective for laboratories with high throughput
- Reduction in labour
- Ease of performance
- Faster reporting of susceptibility results
- Convenient interface with the laboratory information system (LIS)
- “Expert systems” software to interpret susceptibility results involving atypical patterns and unusual resistance phenotypes

**General limitations**
- Space
- Cost
- Regular maintenance required with upgrading of computer software
- Manual preparation of inoculum
- Limited range of organisms
- Limited accuracy in certain organism-antimicrobial combinations
- Limited flexibility in antibiotic panels
- Testing space on the antibiotic susceptibility cards is not infinite, and therefore not all MICs can be tested
- No mixed culture

**Vitek 2**

**Principle:** Utilized growth-based technology

Uses compact colorimetric reagent cards that are incubated and interpreted automatically
Pros

• Closed system that minimized environmental/cross-contamination

• Reliable recheck system that can detect and immediately cease operation of the VITEK 2 system if a specimen card is misplaced on the specimen cartridge

Cons

• Exact MIC may not be available (Depending on the test range. E.g. MIC ≤ 2 μg/ml)

• Limited accuracy in certain organism-antibiotic combination (E.g. Piperacillin-tazobactam)

• Limitation of Advanced Expert System (AES)
  - Reading of raw data rather than modified results

• Lag time in upgrading of new breakpoint in software
Phoenix

Principle:
This system uses an optimized colorimetric oxidation-reduction indicator for susceptibility testing

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td>• Panels can be stored at room temperature</td>
<td>• AST Indicator solution must be added to the AST broth (No vortexing)</td>
</tr>
<tr>
<td></td>
<td>• Exact MIC may not be available (Depending on the test range. E.g. MIC ≤ 2μg/ml)</td>
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MicroScan WalkAway

Principle:
Large self-contained incubator/reader device that can incubate and analyze 40-96 microdilution trays with a photometer/fluorometer to determine growth development.

Sensititre Aris 2X

Principle:
Automated, overnight, incubator/reader device that can incubate and analyze up to 64 microdilution trays. Growth is determined by fluorescence measurement after 18–24 h of incubation.
### Pros

- Plate-specific barcodes provide automatic inventory to allow user to load or unload tests at any time, in any location
- Custom-formatted plates allow for greater antibiotic flexibility and testing actual agents on hospital formulary

### Considerations in evaluating AST systems

- Performance
- Cost
- Practical considerations (Eg: Throughput; Space)
- Software
- Manufacturer’s support
- Technical considerations
- Workflow considerations

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**Thank you**