Broth and Agar testing methods
Automated susceptibility testing

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Outline

• Introduction

• Broth testing methods
  - Macrodilution
  - Microdilution

• Agar dilution

• Automated Antimicrobial Susceptibility Testing (AST) systems
  - Vitek-2
  - Phoenix
  - Microscan
  - Sensititre

• Considerations in evaluating AST systems
Introduction
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
Reference methods for *in vitro* susceptibility testing

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

CLSI, BSAC, EUCAST guidelines
• Broth preparation
• Antimicrobial agent
  - Stock solution
  - Working solutions
• Preparation of tubes/plates
• Inoculum preparation
• Inoculation of tubes/plates
• Incubation of tubes/plates
• Reading results

• Quality control
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 M100 Table 5A)
- Stock solutions may be stored at -60°C for more than 6 months for most antimicrobial agents
- Avoid repeated freeze thaw
➢ Working solution
- Serial doubling dilutions
- Dilution scheme available in CLSI M100

Tubes/ Plates preparation
• Store at appropriate temperature
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
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 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
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Antimicrobial agent in mg l⁻¹

Growth control
Sterility control

A: MIC = 64 mg l⁻¹
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
Limitations

- Labour intensive
- Strict adherence to protocol is required
- The MIC value is not the sole predictor for clinical outcome
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

- Mueller–Hinton agar is considered the reference medium
- One concentration of antibiotic/plate
- Include a drug free control
• Allow the sterilized agar to cool to 50°C in a water-bath, before adding antibiotics
• 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 not the sole predictor for clinical outcome
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
- Lag time in upgrading of new breakpoints in 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 (e.g., Test range MIC ≤ 2 μg/ml)
- No mixed culture
Vitek 2

Principle:
Utilized growth-based technology

Uses compact colorimetric reagent cards that are incubated and interpreted automatically
Navigate to “virtual cassette” workspace.

Inoculate tubes and place in cassette.

Scan the appropriate card and place it in the cassette.

Scan in the isolate identification number, or use the keyboard to type it in.

Place cassette in filler.

Place cassette in reader.

Results print automatically when card is complete.

Finished cards are automatically sent to waste bin for safe disposal.
Phoenix

Principle:
This system uses an optimized colorimetric oxidation-reduction indicator to detect organism growth in the presence of an antimicrobial agent for susceptibility testing.
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.
Considerations in evaluating AST systems

• Performance
• Cost
• Practical considerations (Eg: Throughput; Space)
• Software
• Manufacturer’s support
• Technical considerations
• Workflow considerations
Thank you
Vitek 2  http://www.youtube.com/watch?v=1bVlcY30YU0

Sensititre Arix X 2  http://www.youtube.com/watch?v=l21VMzHLhqQ

?Direct inoculums