Verification of Gradient Diffusion Strips
Objectives

1. Describe process of FDA clearance of susceptibility tests
2. Describe gradient diffusion tests
3. Discuss CLIA requirements for laboratory verification of susceptibility tests
4. Evaluate strategies for performing susceptibility test verifications

CLIA=Clinical Laboratory Improvement Amendments; FDA=US Food and Drug Administration.
FDA CLEARANCE OF AST DEVICES
Why do AST devices require FDA clearance?

To prove they are safe and effective and pose minimum risks to patients

What does it mean for a commercial AST device to be FDA cleared?

• Submitted by 510(k) process to FDA
• Demonstrated results with AST device are comparable to CLSI broth microdilution (BMD) method
• FDA:
  – Specifies the data that must be submitted
  – Defines criteria for acceptability

Data required by FDA for 510(k)

<table>
<thead>
<tr>
<th># Testing sites (including 1 in-house)</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms</td>
<td>100/site</td>
</tr>
<tr>
<td>Fresh/stock (clinical)</td>
<td></td>
</tr>
<tr>
<td>CDC challenge*</td>
<td>75/one site</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>25/site or</td>
</tr>
<tr>
<td></td>
<td>10x3x3/site</td>
</tr>
<tr>
<td>Stability (3 lots)</td>
<td>Real time</td>
</tr>
<tr>
<td>QC</td>
<td>20 results/site</td>
</tr>
<tr>
<td>CLSI reference method</td>
<td>MIC</td>
</tr>
</tbody>
</table>

CDC=Centers for Disease Control and Prevention; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration; QC=quality control.

*MICs near “R” breakpoint.

Criteria used to determine if AST device results are acceptable

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Definition</th>
<th>Acceptable limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential Agreement (EA)</td>
<td>MIC within +/- 1 2-fold dilution of the REF MIC</td>
<td>&gt;89.9 %</td>
</tr>
<tr>
<td>Category Agreement (CA)</td>
<td>S, I, and R results agree</td>
<td>&gt;89.9 %</td>
</tr>
</tbody>
</table>

PLUS: minimal “false susceptible” (very major) & “false resistant” (major) errors

I=intermediate; R=resistant; S=susceptible.

Calculating Agreement

Essential agreement (EA) =
\[
\frac{\text{# isolates within 1 two-fold dilution of reference MIC}}{\text{Total # isolates tested}} \times 100
\]

Category agreement (CA) =
\[
\frac{\text{# isolates with same S, I, or R result as reference MIC}}{\text{Total # isolates tested}} \times 100
\]
Calculating Discrepancies

Very major error (VME) =
\[
\frac{\text{No. with VME (false S)}}{\text{Total # isolates that tested “R” by reference method}} \times 100
\]

Major error (ME) =
\[
\frac{\text{No. with ME (false R)}}{\text{Total # isolates that tested “S” by reference method}} \times 100
\]

Minor error (mE) =
\[
\frac{\text{No. with mE}}{\text{Total # isolates tested}} \times 100
\]
Example: Ceftolozane/tazobactam and *P. aeruginosa*

### Breakpoints (µg/mL)

<table>
<thead>
<tr>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Ref</th>
<th>Test</th>
<th>EA</th>
<th>CA</th>
<th>Any error?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 S</td>
<td>4 S</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>4 S</td>
<td>1 S</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>8 I</td>
<td>4 S</td>
<td>Yes</td>
<td>No</td>
<td>Minor</td>
</tr>
<tr>
<td>4</td>
<td>4 S</td>
<td>32 R</td>
<td>No</td>
<td>No</td>
<td>Major</td>
</tr>
<tr>
<td>5</td>
<td>32 R</td>
<td>4 S</td>
<td>No</td>
<td>No</td>
<td>Very major</td>
</tr>
</tbody>
</table>

References:
- EA: Susceptible
- CA: Intermediate
- Any error: Sensitive, Intermediate, Resistant
Example: Calculating Agreement

<table>
<thead>
<tr>
<th>Organism</th>
<th>N</th>
<th>EA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>100</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>94</td>
<td>89</td>
<td>94.6</td>
</tr>
</tbody>
</table>

*Unacceptable according to FDA criteria*
## Example: Calculating Percent Errors

<table>
<thead>
<tr>
<th>Organism</th>
<th>N</th>
<th>Reference result</th>
<th>Very major</th>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td># S</td>
<td># R</td>
<td>#</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>100</td>
<td>60</td>
<td>30</td>
<td>1</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Package Insert or “Labeling” Sections for AST Device

<table>
<thead>
<tr>
<th>Intended use</th>
<th>If device falls short in a category, will be listed as a “limitation”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents</td>
<td></td>
</tr>
<tr>
<td>Reporting of results</td>
<td></td>
</tr>
<tr>
<td>Performance characteristics</td>
<td></td>
</tr>
<tr>
<td>QC</td>
<td></td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>
A note on AST device labeling: RUO, IUO, IVD

• **Research use only (RUO):**
  – “For Research Use Only. Not for use in diagnostic procedures” must be in labeling
  – Devices are in research phase of development

• **Investigational use only (IUO):**
  – “For Investigational Use Only. The performance characteristics of this product have not been established” must be in labeling
  – Products being tested or evaluated prior to regular marketing

• **In vitro diagnostic device (IVD):**
  – FDA-cleared

GRADIENT DIFFUSION TESTS
Gradient Diffusion Tests

Determine MIC of antimicrobial agent

Strip of paper impregnated with concentration gradient of antibiotic

When applied to inoculated agar gradient of antimicrobial transferred to agar

After incubation, ellipse of inhibition is formed

MIC read where edge of ellipse intersects with strip

Photo credit: Liofilchem

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Notes on Reading Gradient Strips

• Carefully follow manufacturer instructions on how to interpret imperfect ellipses

• In particular, colonies within ellipse can be difficult (arrow), and instructions may vary

ETEST is a registered trademark of bioMérieux SA or one of its subsidiaries.
Notes on Reading Gradient Strips (continued)

• Gradient diffusion strips provide MICs between traditional 2-fold dilutions. Round up to nearest 2-fold dilution when interpreting results.

**MIC read as 0.75 µg/mL**
Round up to 1 µg/mL $\rightarrow$ “S”

**MIC breakpoints for ceftolozane/tazobactam**

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>≤4/4 µg/mL</td>
<td>8/4 µg/mL</td>
<td>≥16/4 µg/mL</td>
</tr>
</tbody>
</table>

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VERIFICATION STUDIES
Verification Studies: CLIA 493.1253

• Each lab that introduces an unmodified, FDA-cleared or approved test system must do the following before reporting patient test results:

  – Demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics:
    • Accuracy
    • Precision (reproducibility)
    • Reportable range of test results for the test system

  – Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population

QC is not sufficient!

Verification Guidance

M52
Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems

Verification Process

**PLAN**
- Write a **verification plan**
- What isolates will you use?
- What will the comparator method be?
- What will the acceptance criteria be?
- How will you resolve discrepancies?
- Write the **SOP**

**VERIFY**
- Verification study
- On the bench
- Perform statistics
- Write **verification report**

**EVALUATE**
- Were acceptance criteria met?
- Troubleshooting
- Review and approval by laboratory director

**VALIDATE**
- Daily QC, proficiency testing, supervisor review

SOP=standard operating procedure.

Plan: Study details

• CLSI recommends laboratories evaluate:
  – Accuracy
    • Test a minimum of 30 isolates one time
    • Compare results to a reference method
    • Desire mix of “S” and “R” isolates
  – Precision
    • Test minimum of 5 isolates (QC and clinical strains) three times each
    • Include at least some clinical isolates (not just QC) when doing this

Plan: What isolates to use

- 30 isolates needed
- ZERBAXA® (ceftolozane and tazobactam) has antimicrobial activity* vs:
  - Enterobacter cloacae
  - Escherichia coli
  - Klebsiella oxytoca
  - Klebsiella pneumoniae
  - Proteus mirabilis
  - Pseudomonas aeruginosa

*Only select pathogens indicated for ZERBAXA are listed.
Indications and Usage

Indications

ZERBAXA is indicated in adult patients for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis, caused by the following Gram-negative microorganisms: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa.*

ZERBAXA used in combination with metronidazole is indicated in adult patients for the treatment of complicated intra-abdominal infections (cIAI) caused by the following Gram-negative and Gram-positive microorganisms: *Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Bacteroides fragilis, Streptococcus anginosus, Streptococcus constellatus,* and *Streptococcus salivarius.*

Usage

To reduce the development of drug-resistant bacteria and maintain the effectiveness of ZERBAXA and other antibacterial drugs, ZERBAXA should be used only to treat infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.
Important Safety Information

Patients with renal impairment: Decreased efficacy of ZERBAXA has been observed in patients with baseline CrCl of 30 to ≤ 50 mL/min. In a clinical trial, patients with cIAIs with CrCl > 50 mL/min had a clinical cure rate of 85.2% when treated with ZERBAXA plus metronidazole vs 87.9% when treated with meropenem. In the same trial, patients with CrCl 30 to ≤ 50 mL/min had a clinical cure rate of 47.8% when treated with ZERBAXA plus metronidazole vs 69.2% when treated with meropenem. A similar trend was also seen in the cUTI trial. Monitor CrCl at least daily in patients with changing renal function and adjust the dose of ZERBAXA accordingly.
Plan: Where to find isolates?

CDC/FDA AR Bank


AR=antibiotic resistance; CDC=Centers for Disease Control and Prevention.
Plan: Isolates

CDC/FDA AR Bank panel contains:
- 30 isolates
  - 5 *K. pneumoniae*
  - 3 *E. cloacae*
  - 8 *E. coli*
  - 2 *K. oxytoca*
  - 2 *P. mirabilis*
  - 10 *P. aeruginosa*
- 11 S, 2 I, and 17 R

Testing this collection fulfills the ‘accuracy’ part of verification. Compare ceftolozane/tazobactam results to those obtained by CDC.

Plan: Reference method

• If laboratory has never tested ceftolozane/tazobactam, CLSI recommends using results from a reference laboratory as the comparator

• CDC/FDA AR Bank isolates come provided with reference MICs

Plan: Acceptance criteria

CLSI recommends:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acceptance Criteria</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>≥90% EA</td>
<td>If testing 30 isolates:</td>
</tr>
<tr>
<td></td>
<td>≥90% CA</td>
<td>- Max 3 isolates can be out of EA or CA</td>
</tr>
<tr>
<td></td>
<td>VME &lt;3%</td>
<td>- Max 1 isolate with VME or ME</td>
</tr>
<tr>
<td></td>
<td>ME &lt;3%</td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td>95% of results in EA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>95% of QC strains</td>
<td></td>
</tr>
<tr>
<td></td>
<td>within QC specifications</td>
<td></td>
</tr>
</tbody>
</table>

CA=category agreement; EA=essential agreement; ME=major error; VME=very major error.

Verify: Testing

• On the bench testing
• Accuracy performed over ≥1 day
• Precision performed over ≥1 day
• QC should be performed each day of verification testing
  – Note this may be included as part of the precision
## Example: Test Schedule

<table>
<thead>
<tr>
<th>Day</th>
<th>Accuracy</th>
<th>Precision</th>
<th>QC*</th>
<th>Total # tests</th>
</tr>
</thead>
</table>
| 1   | Isolates 1-5 from AR Bank | 1 clinical isolate in triplicate | *P. aeruginosa* ATCC 27853 x 3  
*E. coli* ATCC 25922 x 3 | 5+3+6 = 14 |
| 2   | Isolates 6-10 from AR Bank | 1 clinical isolate in triplicate | *P. aeruginosa* ATCC 27853 x 3  
*E. coli* ATCC 25922 x 3 | 5+3+6 = 14 |
| 3   | Isolates 11-15 from AR Bank | 1 clinical isolate in triplicate | *P. aeruginosa* ATCC 27853 x 3  
*E. coli* ATCC 25922 x 3 | 5+3+6 = 14 |
| 4   | Isolates 16-23 from AR Bank | - | *P. aeruginosa* ATCC 27853 x 3  
*E. coli* ATCC 25922 x 3 | 8+6 = 14 |
| 5   | Isolates 24-30 from AR Bank | - | *P. aeruginosa* ATCC 27853 x 3  
*E. coli* ATCC 25922 x 3 | 7+6 = 13 |

* Allows fulfillment of 3x5 QC plan & conversion to weekly QC; alternatively can perform only on day 1 and perform daily QC after test is verified

# Example: Data Accuracy

<table>
<thead>
<tr>
<th>Isolate #</th>
<th>CDC MIC</th>
<th>CDC Interpretation</th>
<th>Lab MIC</th>
<th>Lab Interpretation</th>
<th>EA?</th>
<th>CA?</th>
<th>Error Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0351</td>
<td>4</td>
<td>S</td>
<td>4</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0352</td>
<td>32</td>
<td>R</td>
<td>32</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0353</td>
<td>128</td>
<td>R</td>
<td>128</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0354</td>
<td>1</td>
<td>S</td>
<td>1</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0355</td>
<td>2</td>
<td>S</td>
<td>2</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0356</td>
<td>32</td>
<td>R</td>
<td>32</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0357</td>
<td>128</td>
<td>R</td>
<td>128</td>
<td>R</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0358</td>
<td>8</td>
<td>I</td>
<td>4</td>
<td>S</td>
<td>Y</td>
<td>N</td>
<td>Minor</td>
</tr>
<tr>
<td>0359</td>
<td>2</td>
<td>S</td>
<td>2</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>0360</td>
<td>1</td>
<td>S</td>
<td>1</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
</tbody>
</table>
One minor error observed, for *P. aeruginosa* with MIC of 4 µg/mL by gradient strip in lab but 8 µg/mL by CDC

Verification “passed” → can start testing upon laboratory director review & approval
### Example: Data Precision

#### Clinical Isolates

<table>
<thead>
<tr>
<th>Isolate #</th>
<th>CDC MIC</th>
<th>CDC Interpretation</th>
<th>Lab MIC #1</th>
<th>Lab MIC #2</th>
<th>Lab MIC #3</th>
<th>% EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0351</td>
<td>4</td>
<td>S</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3/3=100%</td>
</tr>
<tr>
<td>0352</td>
<td>32</td>
<td>R</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>3/3=100%</td>
</tr>
<tr>
<td>0353</td>
<td>128</td>
<td>R</td>
<td>128</td>
<td>64</td>
<td>128</td>
<td>3/3=100%</td>
</tr>
</tbody>
</table>

#### QC Isolates

<table>
<thead>
<tr>
<th>QC Strain</th>
<th>CLSI QC Range</th>
<th>Day 1 Results</th>
<th>Day 2 Results</th>
<th>Day 3 Results</th>
<th>Day 4 Results</th>
<th>Day 5 Results</th>
<th>% in QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25922</td>
<td>0.12-0.5</td>
<td>0.12</td>
<td>0.25</td>
<td>0.12</td>
<td>0.25</td>
<td>0.06</td>
<td>14/15=passed</td>
</tr>
<tr>
<td>ATCC 27583</td>
<td>0.25-1.0</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>15/15=passed</td>
</tr>
</tbody>
</table>
Example 2: Data Analysis

What if there are more errors?

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reference Result</th>
<th>EA</th>
<th>CA</th>
<th># VME</th>
<th># ME</th>
<th># mE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#S</td>
<td>#I</td>
<td>#R</td>
<td>#S</td>
<td>#I</td>
<td>#R</td>
<td>#S</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>5 1 4</td>
<td>70</td>
<td>70</td>
<td>3 (75%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>5 2 13</td>
<td>90</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

In this example, three VME were observed for *P. aeruginosa* with MIC of 4 µg/mL by gradient strip in lab but >32 µg/mL by CDC.

Verification “failed” → what to do?
Evaluate: Troubleshooting

1. Repeat test for these isolates
   - Resolve problem? STOP
     - Resolved? STOP
     - Not Resolved
       - Use a 3rd method (e.g., disk diffusion)
         - Resolved? STOP
         - Not Resolved
           - Contact manufacturer
           - Request new isolate(s)*

*Consider obtaining isolates with known MICs to ceftolozane/tazobactam from other notable institutions.

Validate: Ongoing Monitoring

What else?

• Standard operating procedure
  – When to test; how to interpret; any special reporting considerations (body site reporting, intrinsic resistance, etc.)

• Information technology
  – Must build test & interpretations in Lab IT system
  – Interface?

• Quality control
  – Write IQCP or daily QC

• Proficiency testing

• Review of instructions for use

• Training & competency of staff

• Correlate with clinical findings

IQCP=individualized quality control plan.

SELECTED SAFETY INFORMATION FOR ZERBAXA® (ceftolozane and tazobactam) 1.5 g
**Important Safety Information**

**Patients with renal impairment:** Decreased efficacy of ZERBAXA has been observed in patients with baseline CrCl of 30 to ≤ 50 mL/min. In a clinical trial, patients with cIAIs with CrCl > 50 mL/min had a clinical cure rate of 85.2% when treated with ZERBAXA plus metronidazole vs 87.9% when treated with meropenem. In the same trial, patients with CrCl 30 to ≤ 50 mL/min had a clinical cure rate of 47.8% when treated with ZERBAXA plus metronidazole vs 69.2% when treated with meropenem. A similar trend was also seen in the cUTI trial. Monitor CrCl at least daily in patients with changing renal function and adjust the dose of ZERBAXA accordingly.

**Hypersensitivity:** ZERBAXA is contraindicated in patients with known serious hypersensitivity to ceftolozane/tazobactam, piperacillin/tazobactam, or other members of the beta-lactam class. Serious and occasionally fatal hypersensitivity (anaphylactic) reactions have been reported in patients receiving beta-lactam antibacterials. Before initiating therapy with ZERBAXA, make careful inquiry about previous hypersensitivity reactions to cephalosporins, penicillins, or other beta-lactams. If an anaphylactic reaction to ZERBAXA occurs, discontinue use and institute appropriate therapy.
Important Safety Information (continued)

*Clostridium difficile-associated diarrhea (CDAD)*, ranging from mild diarrhea to fatal colitis, has been reported with nearly all systemic antibacterial agents, including ZERBAXA. Careful medical history is necessary because CDAD has been reported to occur more than 2 months after the administration of antibacterial agents. If CDAD is confirmed, antibacterial use not directed against *C. difficile* should be discontinued, if possible.

**Development of drug-resistant bacteria:** Prescribing ZERBAXA in the absence of a proven or strongly suspected bacterial infection is unlikely to provide benefit to the patient and increases the risk of the development of drug-resistant bacteria.

**Adverse Reactions:** The most common adverse reactions occurring in ≥5% of patients were headache (5.8%) in the cUTI trial, and nausea (7.9%), diarrhea (6.2%), and pyrexia (5.6%) in the cIAI trial.
Summary

In summary, we have discussed the following:

• The process of FDA clearance of susceptibility tests
• Gradient diffusion tests
• CLIA requirements for laboratory verification of susceptibility tests
• Strategies for performing susceptibility test verifications

Please see accompanying ZERBAXA Prescribing Information. Select link to access.