Case Studies, or "Verification Vignettes"

Vignette #1—Change from One Automated AST to Another

- Your lab is changing from one FDA-cleared automated AST to another
- Is a verification study required?

Vignette #1—Change from One Automated AST to Another

- Yes
- Test ≥30 isolates per panel
  - Lab clinical isolates
  - ATCC or proficiency survey isolates
  - Reflect isolate distribution and resistance phenotypes typically seen

Source: *Cumitech 31A*

Vignette #1—Change from One Automated AST to Another

- Comparator method isn't a reference method
  - Essential agreement (results w/i 2-fold dilution): ≥90%
  - Categorical agreement (S, I, R): ≥90%
  - Calculate overall (for all organisms and drugs) and for each drug

Source: *Cumitech 31A*

Vignette #1—Change from One Automated AST to Another

- Very major error (false susceptible): not possible because neither method is considered true “reference” method
- Major error/discrepancy: one system reports R, other reports S (<5%)
- Minor error/discrepancy: one system reports I, other R or S (combined with ME, <10%)

Source: *Cumitech 31A*

Vignette #1—Change from One Automated AST to Another

- Example
  - 30 GN isolates; 10 antibiotics on new panel
  - 4 isolates produced zosyn results 4-8 fold different than old method. Of these, 2 isolates resistant by new method and intermediate by old method
  - For other drugs, isolates produced identical MIC or within 2-fold difference. No categorical differences

Vignette #1—Change from One Automated AST to Another

	Overall	Target	Zosyn
EA	296/300 (99%)	≥90%	26/30 (87%)
CA	298/300 (99%)	≥90%	28/30 (93%)
ME	None	<5%	None
mE	2/300 (0.7%)	< 10% (M+m)	2/30 (7%)

Action: test isolates with discordant Zosyn results using reference method (vendor usually provides this)

Vignette #1—Change from One Automated AST to Another

- Reproducibility
  - Test several ATCC QC strains (inc. resistant) in triplicate over several (e.g. 2-3) days
  - Repeat results (same day and between days) should be ≥95% essential and categorical agreement overall

Source: *Cumitech 31A*

Vignette #2—Additional or Replacement AST Instrument

- Due to increasing volume, your lab decides to add an additional unit/instrument to your automated blood culture or AST system (or, one of your two AST instruments is replaced by the vendor)
- Are you required to perform a verification study on the new instrument?

Vignette #2—Additional or Replacement AST Instrument

- Answer- no
- Your test system (consists of operators, reagents, QC, instrumentation) is already verified. In this case, a complete instrument qualification (performed by field tech rep) and QC is necessary. Six month parallel instrument check may also be required

Vignette #3—New Cephalosporin Breakpoints

- Your lab has decided to implement the new cephalosporin breakpoints.
- Are you required to perform a new verification study for these antibiotics?

Vignette #3—New Cephalosporin Breakpoints

- Answer- Yes (CLSI recommends)
- Lower breakpoints may change some isolates previously S or I, to R
- You are verifying the ability of your AST system to produce accurate and reliable results (MIC or zone diameter). You are not verifying breakpoints

Vignette #3—New Cephalosporin Breakpoints

- Perform DD (approved CLSI standardized method) and compare the new DD "interpretative" results to the new MIC "interpretative" results to be sure that they agree with your automated system

Vignette #4—Throat and Rectal Swabs for CT/NG NAAT

- How would you design a study to verify (establish) performance characteristics of your CT/NG NAAT for throat and rectal swabs?

Vignette #4—Throat and Rectal Swabs for CT/NG NAAT

- Answer- several options
  - Prospective study using paired specimens
  - Split specimens from another lab
  - Spiked samples
- Additionally, should establish analytical sensitivity, analytical specificity (cross-reacting organisms, interfering substances)

Vignette #4—Throat and Rectal Swabs for CT/NG NAAT

- Additionally, should establish analytical sensitivity, analytical specificity (cross-reacting organisms, interfering substances)
  - Different specimen matrix may affect sensitivity
    - Spike titered Ng into throat and stool matrix and determine LoD
  - *Neisseria gonorrhoeae* assays may cross-react with normal flora
    - Spike titered *Neisseria* spp. into specimens

Vignette #5—Upgrading Automated Blood Culture Instrument

- Replacing Bactec 9240 with FX; bottles not changed
- Is a full verification study required?

Vignette #5—Upgrading Automated Blood Culture Instrument

- Answer- no
- If changes are limited to instrument (hardware and software changes), and bottles not changed, then instrument function check may be sufficient*
- Vendor equipment rep verifies that incubator and optical systems are within specifications

*Source: *Cumitech 31A*

Vignette #6—Changing from EIA to Molecular Assay for *C. difficile* toxin

- Assumptions
  - FDA-cleared nucleic acid amplification test
  - Not modified by the lab (will follow package insert instructions for specimen requirements, performing the assay, interpreting results)
- How would you design a study to verify (validate according to CAP) molecular test prior to reporting patient results?

Vignette #6—Changing from EIA to Molecular Assay for *C. difficile* toxin

- Resources
  - DHHS/CDC brochure "Verification of Test Performance Specifications"
  - CAP Microbiology and All Common checklists
- Assemble a panel of at least 20 specimens, ca. 10 positive and 10 negative, previously tested by EIA. Include both weak and strong positive specimens. Make sure specimen storage is compatible with molecular test.

Vignette #6—Changing from EIA to Molecular Assay for *C. difficile* toxin

- Accuracy (vs. EIA)
  - Perform assay on 20 specimens
  - Do you expect 100% accuracy?
  - Resolve discordant results by repeat testing, perhaps independent method or by clinical diagnosis
- Reproducibility (intra- and inter-run; inter-operator)
  - Select several specimens, perhaps 1 strong positive, 1 wk positive and a negative to test in triplicate on 2 different days (2nd day by different operator)
  - Qualitative (P/N) reproducibility should be 100%
- Reference (normal) range
  - Of the 10 EIA negative specimens (assuming no false negatives), do they all within the "normal" range of the molecular test?
- Reportable range
  - Are specimens with both low and high levels of analyte detected?

Additional cases