Overview of Analytical Validation of Donor Screening Tests

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Presentation Outline

• Overview of analytical validation
  – Why, When, and What

• General requirements for IVD analytical validation
  – Analytical sensitivity, specificity, precision, reproducibility and repeatability, interference, etc.

• Considerations and review issues for IVDs used for infectious disease screening
  – Study design, controls, standards
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**Why:**
To demonstrate that the manufactured product meets its prescribed requirements for safety and effectiveness
– Preclinical and analytical both are being discussed here
– Analytical performance is as critical as clinical performance

**When:**
Preferably before the IND
Definitely before the final submission!
“What” to Perform (1)

• Setting the blank
• Setting the cut-off
• Demonstration of the dynamic range
• Setting the calibration curve
• Setting the Positive and Negative Controls
“What” to Perform (2)

• Rationale and demonstration of a re-test algorithm, where applicable
• Linearity (quantitative and semi-quantitative assays)
• Establishment of gray zone where applicable
  – To demonstrate true positives/negatives
• Sample and matrix suitability, including analyte stability
Regulations, Guidance, and Standards

- **21 CFR 58** – Good Laboratory Practice for Nonclinical Laboratory Studies.
- **ICH Guideline**: Validation of Analytical Procedures: Text and Methodology Q2(R1), November 2005.
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Analytical Validation Establishes Device Performance

- Precision
- Reproducibility
- Analytical sensitivity & specificity
- Cross reactivity and interference
- Matrix comparison
- Measuring range, reference range
- Stability studies
Precision and Reproducibility

- Evaluates how well the assay yields the same result on repeated determinations.
- Statistically valid approach to evaluate multiple aliquots, multiple lots at multiple sites, via multiple runs on multiple days, etc.
  - Intra- and intra-assay variability
  - Intra- and inter-lot variability
  - Inter-operator variability
  - Inter-instrument variability, if needed
- Other assay-critical, or system-critical variables (e.g., plate A/plate B when there is a specific order recommended)
- Total variability
Analytical Sensitivity

Definition: “slope of the calibration curve”; capacity of a test method to differentiate between two very close concentrations of an analyte (CLSI EP17-A2)

Study design

- End-point dilutions
- Contrived specimens as needed
- > 3 concentrations of the analyte/panels
- Multiple replicates

Analytical Sensitivity ≠ Limit of Detection (LoD)
Limit of Detection (LoD)

$L(LoD)$: the lowest concentration of an analyte that can be consistently detected (typically in ≥ 95% of samples tested) (CLSI EP17-A2)

Study design

- Known-positive or standards/panels
- 5 dilutions/panel
- At least 20 replicates/panel (include non-reactives)
- Statistical analysis: > 95% reactivity
Analytical Specificity/Interference Testing/Cross-Reactivity

*Cause of significant difference in the test result due to the effect of another component or property of the sample (CLSI EP07-A2)*

- Samples to test for cross-reactivity
  - Other species/serotypes/genetic variants
  - Other disease conditions (autoimmune, infections, etc.)
- Sample size – variable for different conditions
- Interference testing
  - Endogenous (albumin, bilirubin, hemoglobin, lipid, IgG)
  - Exogenous interferents (various drugs/supplements)
Matrix Comparison

- Matrices claimed: whole blood, plasma, and serum
- Lysed/prepared specimen vs neat/diluted
- Different anticoagulants
- Cadaveric claims
  - Analytical sensitivity
  - Analytical specificity
  - Reproducibility
Stability

• **Samples**
  - Room temperature, refrigerated, or frozen
  - Neat, pooled, prepared (lysed), on-board

• **Kit**
  - Calibrators, and controls
  - Real-time: basis for shelf-life claims
  - Open kit on-board

• **Labeling claim**
  - Test one time point beyond the proposed claim for shelf-life
  - Based on data, not extrapolated or interpolated points
  - Based on real-time stability, not accelerated studies
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Complex Donor Screening IVDs

• Diverse IVDs with different final analytes
  – Antigen, antibody, nucleic acid

• Technology
  – EIA, chemiluminescence, PCR, transcription-mediated amplification (TMA)

• Different limit of detection (LoD) parameters
  – g/mL, IU/mL, copies/mL, iRBC/mL

• Different clinical samples/sample size
  – Genetic variants, prevalence/risk of transfusion-transmission (TT)
Precision and Reproducibility

- Panel of 6-10 well-characterized specimens, representing a clinically relevant range:
  - Minimum of one positive and negative sample near assay cut-off
  - Assay controls and calibrators

- Different group/panel of specimens
  - Each type of specimen matrix
  - Each analyte
  - Genotype (or variant)

- Tested using at least three kit lots
Analytical Sensitivity (1)

- Each specimen group, genotype or strain
- Each sample matrix (e.g., serum, EDTA-plasma)
- Approaches
  - End-point dilution
  - Earliest time of reactivity in serially-collected specimens
  - Comparison to reference standards
  - Comparison to an independent method
  - Quantitative biochemical characterization
- Direct comparison to a FDA-licensed, approved, or cleared test
- Controls targeted to clinical decision points
  - Low positive between 1-3 S/CO or 1-3 x LoD
- Validation of assay’s gray zone(s)
Analytical Sensitivity (2)

- Appropriate standards or CBER reference panels
e.g., HIV, HCV, HBsAg, *Babesia*
- Seroconversion panels, when available
e.g., multiple specimens from at least 10 subjects undergoing seroconversion
- Low-titer panels for each strain/analyte/matrix, if applicable
e.g., 6-10 specimens per panel
- Dilution series
e.g., at least 10 specimens from 10 subjects for each strain/matrix
- Known-positives from relevant populations
e.g., samples from an HIV-1 high risk group
- NAT: confirm sequence identity for strains/genotypes claimed
Analytical Sensitivity (3)

• FDA prepares and provides panels of samples
  – Different panels for all analytes
  – Analytes at various levels

• Not to be confused with lot release panels

• Number of samples correctly detected is evaluated
Seroconversion Panels

• Panels collected from plasmapheresis donors who are in the process of seroconverting

• Real clinical samples from blood donors with values near the cut-off are rare

• Seroconversion panels are real samples with analytes at relevant clinical concentrations
Analytical Specificity

• Samples to include
  – Other strains/variants – confirm identity
  – Other disease/medical conditions (autoimmune, infections)
  – Potentially interfering substances
  – Endogenous (albumin, bilirubin, hemoglobin, IgG, etc.)
  – Exogenous interferents (various drugs/supplements)
  – Different anticoagulants/collection tubes

• Sample size – variable for different conditions

• Labeling claim: include interference/cross-reactivity
Device Performance - What to Submit

• Summaries of study designs
  – Materials, procedures, analysis, and oversight
  – Sample collection, selection criteria, handling, and storage
  – Statistical and clinical considerations
  – Documentation that all testing performed at an approved facility using Good Laboratory Practice (GLP)

• Summaries of results and line data for all studies
  – Data for each specimen
  – Each assay run performed, including failed runs
  – Documentation and justification of excluded data
  – Documentation and justification of deviations, outliers, etc.
Common Review Issues

- Results don’t meet pre-specified acceptance criteria
- Inconsistent definition of LoD
- Validation not performed on final device (including algorithm and cut-off)
- Insufficient samples around the cut-off
- Intended Use too broad (such as “for infectious diseases testing”)
- No definition of guard bands
- Gray zone included in final device
- Not all claims are validated
Summary

• Analytical studies = Foundational studies

• Device performance - final results that are precise with high sensitivity and specificity, reproducible across variables, demonstrating no effect of interferents
References
Thanks!

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