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.
- **Guidance for Industry and FDA Staff** – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests. March 2007.
- **CLSI EP05-A3** – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline. Third Edition. October 2014.
- **CLSI EP06-A** – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. April 2003.
- **CLSI EP07-A2** – Interference Testing in Clinical Chemistry; Approved Guideline, Second Edition. November 2005.
- **CLSI EP09-A3** – Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Third Edition. August 2013.
- **CLSI EP12-A2** – User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline, Second Edition. January 2008.
- **CLSI EP17-A2** – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition. June 2012. (Replaced CLSI EP17-A2 Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. October 2004.)
- **CLSI EP25-A** – Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline. September 2009.
- **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, hemoglobulin, 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, hemoglobulin, 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.

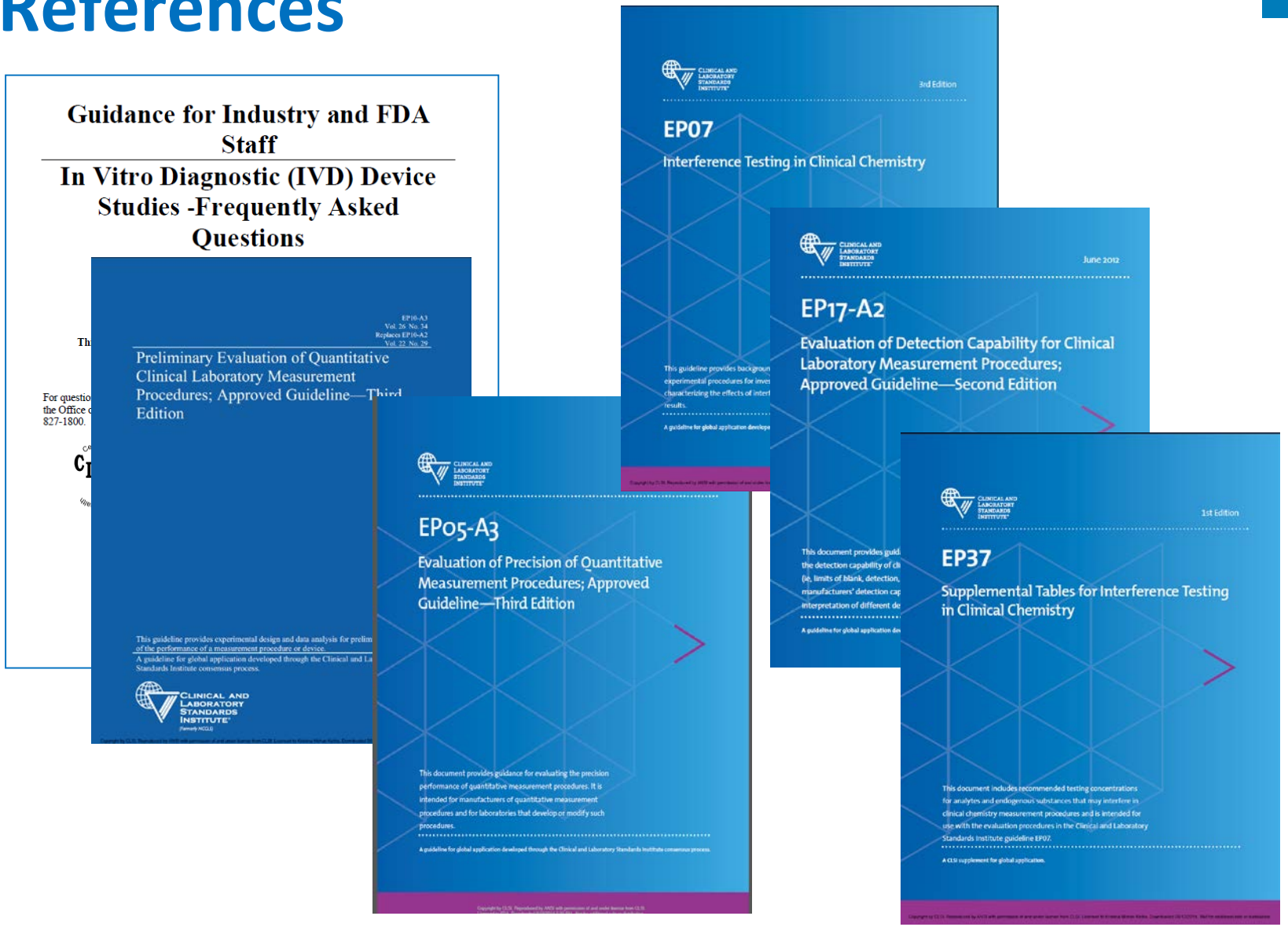
Common Review Issues

- 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


Guidance for Industry and FDA Staff
In Vitro Diagnostic (IVD) Device Studies -Frequently Asked Questions
EP10-A3
Vol. 26 No. 34
Replaces EP10-A2
Vol. 22 No. 29
Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition
CLINICAL AND LABORATORY STANDARDS INSTITUTE
EP05-A3
Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.
A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.
3rd Edition
EP07
Interference Testing in Clinical Chemistry
June 2012
EP17-A2
Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition
1st Edition
EP37
Supplemental Tables for Interference Testing in Clinical Chemistry
This document includes recommended testing concentrations for analytes and endogenous substances that may interfere in clinical chemistry measurement procedures and is intended for use with the evaluation procedures in the Clinical and Laboratory Standards Institute guideline EP07.
A CLSI supplement for global application.

Thanks!

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