Analytical Methods for Vaccines: FDA Perspective

Sara Gagneten, PhD
Associate Division Director for Policy
Division of Viral Products
CBER/FDA

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My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate FDA.

Overview

- Analytical assays: control of product quality
  - Setting specifications
  - Assay performance (qualification, validation)
  - Maintenance of assay suitability
- Assay changes
  - Assay replacement
  - Modification of assay methods (change of reagents or equipment)

Use of analytical tests

- **Product development**
  - Assays used to define product characteristics
- **Manufacturing consistency**
  - Critical in-process parameters
  - Process validation
  - Lot-to-lot consistency
- **Product release**
  - Quality of final product
  - Stability of final product
- **Comparability:** Lots made presently are the same as lots shown to be safe and efficacious clinically

Setting specifications (1)

- Driven by
  - Clinical relevance
  - Process/product variability
  - Assay variability
- Balancing
  - Risk to the product/consumer
  - Cost to the manufacturer
    - Retesting
    - Loss of commercial lots

Setting specifications (2)

- No one statistical approach to setting product specifications is appropriate for all situations
- Most approaches rely on the distribution of the assay results incorporating both product and assay variability
- Specifications should indicate a potential loss of product quality
- All specifications should be considered in the context of manufacturing and other testing

Assay selection considerations

- Capability:
  - measures the relevant analyte
- Reagents/Materials
- Optimization
  - Capable of precision, accuracy, robustness
- Qualification
- Validation

Assay qualification

- Prevalidation studies to evaluate assay performance
  - Precision
  - Accuracy
- Establishes assay control
  - System suitability criteria and limits
- Sets stage
  - "Lock" the SOP
  - Develop validation protocol based on intended use

Assay validation

Formal study to demonstrate that the assay performs adequately for its intended use when run according to the SOP

- Protocol driven
  - Samples – mock and incurred
  - Sources of variability - design
  - Statistical analysis
- Predefined validation criteria determined by intended use
- The SOP precedes the validation
  - The SOP describes the final assay to be validated
    - Validation mimics routine sample analysis
  - The SOP includes validity criteria and system suitability criteria
    - Accept or reject assay runs as during routine use

System suitability criteria (SSC) for routine assay use and control

SSC are selected during development, limits set during qualification, verified in validation studies, and tracked and trended in routine control

System suitability criteria used for:

- Long term verification of assay performance based on analysis of accumulating results (e.g., analysis of variability compared to the variability during validation)
- Routine assay monitoring
  - Tracking and trending
- Reagent qualification
- Revalidation
- Proficiency testing (e.g., assess calibration curve variability/goodness-of-fit, reference standard variability)

Perspective

- Maintaining product quality combines process control and appropriate testing
- Specifications should attempt to identify questionable products and/or process failures
- Reliable analytical method results rely on adequately developed, optimized, characterized, validated and controlled assays
- All testing results should be considered in context to ensure product quality

Analytical Assay Changes

Analytical assay changes

Assay changes are anticipated as part of product life-cycle management due to scientific advances, gained experience based on assay use, ethical considerations (animal use), assay superiority (improved sensitivity, accuracy, precision)

- Replacement of assay methods
- Changes to existing methods

Analytical assay changes: Replacement assay

- New or modified assays must have equal or better sensitivity and accuracy compared to the approved assays: Assure that current lots are comparable to those shown to be safe and effective in clinical trials
- Animal-based (*in vivo*) potency tests replaced with *in vitro* tests
  - Significant interest to find alternatives to animal-based tests due to animal welfare and assay variability consideration
- Improved superiority (LOD, specificity, repeatability, accuracy) and efficiency (time, cost)

Examples of assay method replacement (1)

Alternatives to animal testing

- Inactivated poliovirus vaccine
  - Rat immunogenicity vs. antigen ELISA
- Inactivated rabies virus vaccine
  - Lethal challenge in mice vs. immunogenicity vs. antigen ELISA
- HepB vaccine
  - Immunogenicity in mice vs. ELISA
- DTaP vaccine
  - Lethal histamine sensitization assay in mice vs. in-vitro CPE

Examples of assay method replacement (2)

- **Mycoplasma and Mycobacteria testing**
  - Conventional testing: Culture method
  - Replacement: qPCR
- **Viral adventitious agent testing**
  - Conventional testing: Culture (3 cell types), animals (four species), PCR
  - Replacement testing: High Throughput Sequencing

Challenges for implementation of method replacement (1)

- **Replacement of animal-based immunogenicity assays with ELISA-based assays require**
  - Characterization of antibodies: binding to conformational epitopes or well defined neutralizing epitopes
  - Assay should be useful for predicting product performance in humans

Challenges for implementation of method replacement (2)

- **Replacement of in vitro and in vivo adventitious agent testing with High Throughput Sequencing**
  - Assay standardization and validation: methods are complex and evolving
  - Bioinformatic analysis requires: data format and analysis guidelines and development of reference virus data bases
  - Guidelines needed for analysis and investigation of positive signals

Examples of modification to existing assay methods

- Implementation of automated readers for ELISA, plaque and SRID assays
- Change of cell substrates used for potency or inactivation assays
- Reagent changes: replacement or preparation method
- Changes of mathematical methods for calculations results

Challenges for implementation of assay modifications

- Lack of data demonstrating stability of parent reference standard used to qualify the new reference standard
- Introduction of assay bias
  - Change from manual to automated readers (plaque reader, SRID reader)
  - Assay transfer to new laboratory
- Use of partial validation protocols with inadequate demonstration of assay validity (e.g., accuracy and linearity in entire range)

Perspective

- Often bioassays need to be replaced or modified to address scientific advances, assay suitability over time, and reagent replacement
- New or modified assays must be validated and must assure that current lots are comparable to those shown to be safe and effective in clinical trials
- Supplements to the original license application must be submitted to support assay modifications or replacements