Update: USP Chapters on Biological Assays

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Outline

- Chapters history
- Chapters update and next steps
- USP bioassay chapters themes
- New and refreshed concepts
Chapters history

- The USP statistics expert committee was assigned to “modernize” *USP <111> Design and Analysis of Biological Assays*
  - Written over 50 years ago prior to modern computational methods
  - Difficult to implement in practice
  - Work undertaken in 2001 by the USP ad hoc Panel on Biological Assays
    - Panel of biologists and statisticians working in the field of biological assays
Chapters history (cont.)

- A suite of chapters evolved over time
  - <111> was split into two chapters, USP <1032> Biological Assay Development and USP <1034> Biological Assay Analysis
  - <1033> Bioassay Validation added to the suite

“Roadmap” chapter (to include glossary)
Chapters update and next steps

- All but chapter <111> are above 1000 and therefore “informational”
  - Not intended as enforceable (as chapters below 1000)
  - However, the chapters provide a set of best practices which might be considered by regulators in their reviews
- Chapter <111> left to support monographs which reference it
  - USP is working towards addressing product-specific references to prepare it for further revision
Chapters update and next steps

- The bioassay chapters appeared in USP Pharmacopeial Forum (PF) 34(4), July 2010
  - Comments received through October 2010
  - Comments incorporated into final drafts by May 2011
  - Commentary will appear on the USP website
  - Published in USP/NF May 2012
  - Official August 2012
USP bioassay chapter themes

- Relative potency bioassay
  - Although concepts translate well to other dilution based systems
- Lifecycle approach to validation
- Building quality into the bioassay through strategic development
- Proper analysis including acknowledgement of bioassay structure
- Fitness for use validation
New and refreshed concepts

- Fitness-for-use
- Bioassay optimization
- Transformation and weighting
- Geometric coefficient of variation
- Blocking and randomization
- Similarity and equivalence testing
- Characterization
Fitness for use

- The bioassay should be fit for all of its intended uses throughout product development, manufacture, and QC
  - Engineering the process and formulation
  - Linking materials throughout development
  - Developing potency specifications
  - Defining product stability
  - Commercial product release
  - Bridging performance and stability after a process change
Bioassay optimization

- Optimization elements
  - Bioassay system
    - Number of dilutions and dilution steps of standard and test materials
      - Number to support processing
      - Steps to support potency range
    - Intra-run replicates
      - Strategic design to yield optimal performance
      - Aliquots or independent series
    - Inter-run replicates
      - Drives precision of “reportable result”
Bioassay optimization

 Optimization elements

 Bioassay system (cont.)

 - Should be engineered to planned processing
   - Parallel line/curve analysis for log-normally distributed responses using log dilutions
   - Slope ratio analysis for normally distributed responses using arithmetic dilutions

 Bioassay conditions

 - Ranges of assay parameters which yield acceptable bioassay performance (bioassay design space)
 - Using multifactor design of experiments (DOE)
Bioassay optimization (cont.)

- Optimization using multifactor design of experiments (DOE)
  - Step 1 – bioassay process map
  - Step 2 – screening potentially significant factors
  - Step 3 – Perform response surface experiment to determine region of optimal performance (design space)

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Transformation and weighting

- Assumptions of statistical modeling
  - Correct model
  - Parallelism
  - Curvature
  - Normality (of residuals)
  - Equal variance

- A log transformation of the bioassay measurements will typically generate measurements which are approximately normal, with equal variance.

- Alternatively, weighting might be used to favor dilutions with higher precision.
  - Note: never weight by the observed variance – power of the mean (POM)

\[
   w_i = \frac{1}{\text{var}(y_i)}
\]

\[
   \text{POM} = \frac{1}{y^{(x)}}
\]

log – transformation is a member of the POM family.
Geometric coefficient of variation

- The bioassay system yields log-normally distributed measurements
  - Horizontal shift in log dilution/concentration (M)
  - Relative potency is $e^M$ (a geometric mean or GM)

- Measures of variability for “multiplicative” measurements were defined by Kirkwood in 1972
  - Geometric standard deviation (GSD) is defined as $e^s$
  - GSD is a multiplicative factor such that the range $M \pm s$ is directly related to $e^{M \pm s} = (e^M \div e^s, e^M \cdot e^s)$; i.e., GM divided and multiplied by GSD
  - Geometric coefficient of variation is defined as
    \[ GCV = 100 \cdot (\text{GSD} - 1) = 100 \cdot (e^s - 1) \]
Bias is introduced into bioassay measurement through operational factors such as location effects.

Uniformity testing should be performed during development to establish if there are location effects in the bioassay.

- Cage effects in an *in vivo* bioassay
- Plate effects in an *in vitro* bioassay
- Effect of time from beginning to end of testing a series of samples

A trend is observed across columns of the plate.
Blocking and randomization (cont.)

- The potential bias due to location effects can be moderated through blocking and randomization

  - A poor plate layout
    - Reference (R) and test samples (A & B) grouped together on the plate
    - A plate effect is likely to impact both series

  - Strip plot design
    - Randomize samples to rows
    - Reverse dilutions in the bottom half of the plate
    - A potential plate effect is averaged away through randomization
Similarity and equivalence tests

- Similarity is the condition in bioassay that a test sample behaves as a simple dilution/concentration of the standard.

\[ F_T(z) = F_S(\rho \cdot z) \]

- "Equivalent" slopes
  In parallel line analysis

- "Equivalent" shape (a,b,d)
  In parallel line analysis

- "Equivalent" intercepts
  In slope ratio analysis
Similiarity and equivalence tests (cont.)

- Test of parallelism
  - Paradox: a formal test of parallelism rewards sloppy work, and penalizes good work
    - The greater the precision in the data, the more likely you will fail the test of parallelism
  - Solution – use an equivalence test
    - Determine an acceptable range in a metric of difference (LAL, UAL)
    - Demonstrate (with confidence) that there’s an acceptable difference

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Note: Rewarded for good design
Implementing Equivalence Testing for Similarity

- Choose a measure of non-similarity
  - In the parallel line case, could be the difference or ratio of slopes
  - For slope-ratio assays the measure of non-similarity is the difference of y-intercepts
  - For the four-parameter logistic model, similarity must be addressed on the basis of three parameters: the slope and the upper and lower asymptotes
    - Can be addressed for each parameter separately
      - Parameters or parameter combinations which are “biologically meaningful”
      - Issue with statistical multiplicity
  - A single composite measure of “similar performance”
Implementing Equivalence Testing for Similarity (cont.)

- Four bases for determining an equivalence margin are discussed in USP Chapter <1032>, Development of Biological Assays
  - **Approach 1**: compile historical data that compare the reference to itself, and derive a tolerance interval* for the measure of non-similarity
    - Standard statistical process control (SPC) approach
    - Controls “manufacturer’s risk” (risk of failing a good assay) but not “consumer’s risk” (risk of passing a bad assay) – see approach 3

* A tolerance interval is a interval containing a fixed percentage of values with specified confidence. Thus a 95%/99% tolerance interval contains 99% of future values with 95% confidence.
Implementing Equivalence Testing for Similarity (cont.)

- **Approach 2**: determine a tolerance interval for the maximum departure from similarity of the confidence interval on the measure
  - Similarity concluded if the confidence interval falls within the interval
  - Protects against passing assays with larger than usual amounts of within-assay variation
Implementing Equivalence Testing for Similarity (cont.)

- Four bases for determining an equivalence margin are discussed in USP Chapter <1032>, Development of Biological Assays
  - **Approach 3**: add data comparing standard to known failures (e.g., degraded samples)
    - Determine a measure of non-parallelism which discriminates between the distributions of ref/ref and ref/failure
    - Note: this method can be used to determine which parameters are sensitive to failures for nonlinear models
  - **Approach 4**: based on what is known about the product and the assay
    - Conventional limits such as (0.80,1.25)
    - Sensitivity might be driven by therapeutic index of the drug
Characterization

- Since bioassay has multiple uses throughout development and post licensure, the bioassay validation might be considered a characterization of the variability of the method.
  - Variance components can be used to design the release assay, stability protocols, and comparability studies.

\[
\text{Format Variability} = 100 \cdot \left(\frac{e^{\frac{\sigma_{\text{Run}}^2}{k} + \frac{\sigma_{\text{Replicate}}^2}{n-k}} - 1}{n-k}\right) \\
\]

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- Significant inter-analyst variability
  - Analyst training
  - Repeat over analysts
Summary

- The USP bioassay chapters offer best practices in bioassay development, validation, and analysis to biologists and statisticians alike

- Adoption of these practices will result in a more reliable tool for product development, and better assurance of quality to patients

- USP Science & Standards Symposium on Biologics & Biotechnology: Advancing Quality Standards through Analytics and Assays, October 3-6, Seattle, WA