Pharmaceutical Outsource SOLUTIONS

Part 2 of a Series

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Microbiological and Ligand-Binding Assays *Summary*

Background:
In May 2001 the FDA promulgated its first official guidance for bioanalytical methods. This guideline is titled Guidance for Industry Bioanalytical Method Validation and represents the latest word in what is expected in bioanalytical methods supporting clinical and non-clinical studies. The following is a summary of what the FDA document says so that sponsors may better manage future and existing bioanalytical method validations. This summary specifically addresses the guidance for microbiological and ligand-binding assays. A copy of the actual FDA document can be obtained at www.fda.gov/cder/guidance/4252fnl.htm.

Overview:
Microbiological and ligand-binding assays, including immunoassays/antibody assays, are required to meet all of the established requirements for chemical assays. However, due to the nature of the microbiological assays and ligand-binding assays, certain additional elements such as specificity must be more explicitly established and other elements such as accuracy and precision may meet a lesser standard, or be evaluated on a sample-to-sample basis.

The principal elements of a method validation are discussed at length in the first part of this series titled: Bioanalytical Method Validation – Summary. The exceptions or additional elements as they apply to microbiological and ligand-binding assays are discussed below.

Specificity
Microbiological and ligand-binding assays should additionally be validated for cross-reactivity to metabolites and co-medications. This evaluation should be performed at high levels and in combination to thoroughly demonstrate selectivity or to determine specific cross-reactivity for each competing analyte.

When possible, the microbiological or ligand-binding assay should be cross-validated with a chemical assay method such as LC/MS.

Linearity
Microbiological and ligand-binding assays are inherently non-linear. Because 4- and 5-parameter logistic curves are used to create calibration curves, a large number of calibrators should be used to most accurately describe the curve.

- Anchoring points in the asymptotic high (above ULOQ) and low (below LLOQ) concentrations
- Calibrators should be prepared in the same matrix as study samples or alternate matrix of equivalent performance
- Both the ULOQ and LLOQ should meet pre-defined accuracy and precision
- Multiple curves may be generated and combined to create the calibration curve.

Precision
Greater latitude in precision is allowed for these types of assays. The precision should be evaluated during the method validation by analyzing 4 sets of QC samples at LLOQ, low, medium, and high levels in duplicate in 6 different batches.

For assays not capable of meeting the nominal acceptance criteria, greater criteria can be set but the precision should be evaluated for each sample analyzed by preparing and analyzing multiple aliquots of each sample. The precision should be evaluated for each sample and then the standard procedure for reporting results followed as for any sample.

Accuracy
The accuracy of the method should be determined during the method validation by analyzing 4 sets of QC samples at LLOQ, low, medium, and high levels in duplicate in 6 different batches. For each batch 4 out of 6 QC samples must be within ± 15% of nominal concentration but the two failed QC samples may not be at the same level.

Recovery
When separation (or cleanup) is used for samples but not for calibrators the recovery of this separation or cleanup step must be determined and used to correct reported sample concentrations. Possible approaches to assess recovery are the use of a radio labeled tracer analyte or an internal standard not recognized by the antibody and measured using another technique.

Stability
Assessment of analyte stability should be performed in whole matrix, not treated, stripped, or prepared matrix.

Matrix Effects
Matrix effects are particularly troublesome in immunoassay methods. The affect of matrix and non-specific binding must be evaluated and documented in a number of different ways during the method validation:

- serial dilution of reference analyte with matrix and then the evaluation of response to known concentration;
- the calibration curve in matrix vs. buffer;
- parallelism between diluted samples and reference analyte;
- non-specific binding.