June 25, 2008

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 800.112

TO: VS Management Team (VSMT)
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: John R. Clifford /s/ Jerry W. Diemer for
Deputy Administrator
Veterinary Services

SUBJECT: Guidelines for Validation of *In Vitro* Potency Assays

I. PURPOSE

The purpose of this document is to provide guidance concerning the information a firm should provide when submitting a new potency assay for consideration by the Center for Veterinary Biologics (CVB). This document further clarifies information found in Title 9 Code of Federal Regulations (9CFR) Parts 102.3(b)(2)(ii), 113.8(a)(3)(ii) and Veterinary Services Memorandum 800.50.

II. BACKGROUND

Assay validation is a process that provides evidence that an assay does what it is intended to do. All assays, regardless of format or function, must be relevant, reliable, reproducible, and scientifically sound. The formal process for evaluating these characteristics is commonly known as validation. This document provides guidance for validating veterinary biologics potency assays.

III. SCOPE

These guidelines apply to *in vitro* assays used to determine the potency of veterinary biological products. They provide a framework for evaluating the suitability of reports that detail the specifics of a particular assay. Additional guidance relative to specific assay formats will be provided as it is developed.

IV. GUIDELINES

The Guidelines for Validation of *In Vitro* Potency Assays and Suggested Validation Report Topics are appended to this memorandum.

Appendix I - Guidelines for Validation of *In Vitro* Potency Assays

1. *Introduction.*

1.1. *Aim*. This guidance includes general principles intended to apply to *in vitro* assays. While specific methods or criteria may vary due to the nature of particular assay types, a common set of concepts underlies the idea of assay validation. The guidance in this document outlines a general approach to validation, but not every recommendation in it may be applicable to every assay in every circumstance.

1.2. *Validation phases*. The validation of a potency assay is a process that begins when the assay is first proposed and its relationship to efficacy in the target species is first investigated. Validation continues through the development of the assay as it is first optimized and then various aspects of its precision and accuracy are characterized. Subsequent validation activities involve the transition to implementation and monitoring the behavior of the assay over time when it is in routine use.

1.3. *Validation vs. Use*. Before implementation, the assay is optimized and its essential characteristics are demonstrated. When that has been done, the assay is considered validated for its intended use; and, so long as the test is conducted according to the optimized procedure, the results of the assay are considered to be valid. Consequently, procedures that were required to be performed during optimization of the assay may not be necessary after adoption for routine use. For example, the range of dilutions that are used during validation of the assay may be greater than the range necessary for routine use. Consequently, the validation process must include an evaluation of the performance of the assay under conditions of routine use.

2. *Outline of the Validation Process*. Validating a test procedure occurs in steps that include conceptualization, development, optimization and verification that the test does what it is supposed to do. Reports may be submitted to CVB after completion of any step in the process. The final report typically includes data from the verification steps, and may also refer to earlier work on the conceptualization, development, and optimization of the assay.

2.1. *Conceptualization:* Issues that should be addressed early in assay development

* The relationship between the response measured in the potency test and efficacy in the target species.
* The composition of reference or standard
* The availability of reagents
* The ability of the assay to measure the analyte or parameter of interest
* The ability of the assay to measure the analyte in the range of concentrations expected in test and control preparations.
* The type of sample processing required
* The potential effect of interfering or cross-reacting materials in the test preparation

2.2. *Development and optimization:* During development and optimization, the firm should:

* Evaluate assay reactivity against placebo material.
* Determine the optimum extraction and/or antigen elution conditions.
* Assess the effect of long term contact of the entity of interest with adjuvant, if appropriate
* Assess the effect of adjuvant saturation, (a critical concern when comparing a monovalent reference to multivalent products)
* Determine the final assay conditions and reagent concentrations
* Determine the criteria for acceptance for reagents, references, and controls.
* Determine assay performance with actual unknown
* Incorporate the use of controls and methods of monitoring assay and reagent performance. (The use of an Internal Control (IC) is described in the definitions.)

2.3. *Verification.* The firm should determine accuracy, precision, selectivity, sensitivity, and ruggedness with the test method and provide that data to CVB. The guidance in this section should be taken as suggestions that are not necessarily applicable to every assay in every circumstance.

2.3.1. *Specificity/selectivity.* Evaluate the ability of the assay to selectively detect the analyte without being significantly affected by cross-reactive substances. This may be done by assessing the response curve for placebo vaccines, vaccines spiked with potentially interfering substances, or vaccines containing similar but non-identical analytes. Ideally, such preparations would show no evident dose-response in the assay, and any detectable signal would be trivial compared with the signal of the analyte. Test methods that are used for potency testing multivalent vaccine formulations must show that the dose-response curve of each formulation is similar to the reference or standard dose-response curve. More than one serial or serial prototype of each formulation should be evaluated. CVB may request that additional data be generated if the data for specificity/selectivity suggests multivalent formulations have different dose-response curves.

2.3.2. *Analytical Sensitivity*.

* *Limit of Detection (LOD).* Determine the least amount of analyte that can be distinguished from background, but not necessarily quantified.
* *Limit of Quantitation (LOQ).* Determine the lowest and highest concentrations of an analyte that can be quantified with an acceptable level of accuracy and precision.
* *Signal to Background (S/B).* Evaluate the ratio of the signals of analyte and reagent blank.

2.3.3. *Accuracy*. To evaluate accuracy, compare measurements produced by the assay to values that are assumed to be correct, such as those associated with an accepted standard, or the nominal values of preparations formulated for that purpose. The concentrations of the analyte should span a range appropriate for the assay.

2.3.4. *Precision*. Design a study to evaluate precision appropriate to the application which may include within-assay, between-run and between-day variance

components. Depending on the analyte level, interest may be focused on the precision of a raw measurement (e.g. optical density) or the final value (e.g. titer). A nested design with adequate replication for each variance is often appropriate. (For example, several runs on each of several days by each of several operators.)

2.3.5. *Discrimination*. The ability to discriminate between close values is a function of both accuracy and precision. Formulate preparations with a range of potencies (analyte concentrations) suitable for determining the ability of the assay to discriminate between satisfactory levels of analyte and marginally unsatisfactory levels. The discrimination of a potency assay reflects its diagnostic sensitivity, in the sense of reliably detecting an unsatisfactory serial.

2.3.6. *Ruggedness*. Evaluate ruggedness by observing the effect of changes in incubation time, incubation temperature, operators, reagent lot, or other test conditions on the test result. Also consider the possibility that systematic features of the assay's structure and design may have an effect on the test results. An example is ELISA plate location effects, which may be evaluated with a uniformity plate.

2.3.7. *Other*. Verification of particular types of assays may need to include the evaluation of other critical elements specific to the assay type. Critical elements for specific assay types are outside the scope of this document.

2.4. *Serial release*. Determine the format for routine use of the assay in serial release testing. Describe the method of calculating the potency estimate and proposed validity specifications for an individual test. Requests to consider an assay that does not entirely meet ideal assay assumptions for use in serial release testing should be accompanied by:

* explanation of the mechanism producing the departure from the ideal
* experimental evidence supporting the explanation
* quantitative assessment of the size of the departure and its impact on potency estimation

2.5. *Monitoring.* Include a plan in the final validation report for monitoring the performance of the assay in routine use to show it continues to behave as expected. A monitoring plan typically includes control preparations, statistical and graphical tools for assessing the performance and stability of the assay, standards, and references.

2.6. *Report*. Validation reports may be submitted in stages. Upon completion of validation and compilation of preliminary monitoring data the firm should submit a validation report containing information on the work that has not been previously submitted. A suggested list of topics for the report (can be found in Appendix II). Accompany the report with the raw data in electronic format according to Veterinary Services Memorandum 800.96. Plot the complete dose-response data graphically. Provide relevant estimates of potency, variance components of potency, and variances or coefficients of variation of other quantities (e.g. ODs) where appropriate.

3. *Definitions*.

3.1. *Accuracy.* The closeness of agreement between the value produced by the assay and the correct value.

3.2. *Analyte.* The component of an unknown that is measured by the test system.

3.3. *Internal Control.* The internal control (IC) is a preparation included in an assay to serve as an independent measure of the assay's performance. ICs maybe crude preparations, semi-purified or purified, fractions of the unknown or other materials that respond similarly to the reference and analyte in the assay. An IC is stored under conditions that preserve stability and maintain consistent performance. An independent measure would be another test method or panel of test methods that rely on a different principle(s) than the test system being monitored. These independent test method(s) must address qualitative and quantitative features of the IC.

3.4. *Precision.* Degree of scatter among a series of measurements obtained from multiple observations of the same homogeneous sample under specified conditions. Precision may be considered at several levels. To illustrate the idea of variance components, the following levels of precision may be considered for plate-based assays. The list is neither prescriptive nor exhaustive.

3.4.1. *Within-plate.* The precision among replicated specimens on the same plate. This is evaluated by residual error.

3.4.2. *Between-plate.* The precision of tests run under the same operating conditions concurrently or within a short time interval. This is also called repeatability.

3.4.3. *Intermediate precision.* The precision among the results of tests run under varying conditions within the same laboratory. Elements of intermediate precision may include:

3.4.3.1. *Between assay.* Precision among assays run independently under similar, but not necessarily identical, operating conditions such as assays run at different times on the same day.

3.4.3.2. *Within-laboratory.* Precision among assays run within the same laboratory under different conditions, such as on different days or by different operators.

3.5. *Reagent Blank.* The reagent blank (RB) consists of all components of the test sample except the analyte being measured. The RB is processed the same as the unknown.

3.6. *Reference.* A reference is a preparation that has had its clinical or immunological activity or analyte concentration established in a valid, well controlled study, series of studies or assays.

3.7. *Reproducibility.* The precision of the assay run on the same specimen by different laboratories. (E.g. R&D vs. QC).

3.8. *Ruggedness.* The capacity of an assay (method) to remain unaffected when small changes in environment or operating conditions are made.

3.9. *Sensitivity*.

3.9.1. *Limit of Detection (LOD)*. The limit of detection is the lowest concentration of analyte in a sample that can be distinguished from background, but not necessarily quantified.

3.9.2. *Limit of Quantitation (LOQ)*. The limits of quantitation are the lowest and highest concentrations of an analyte in a specimen that can be quantified with an acceptable level of accuracy and precision. The lower LOQ is greater than the LOD.

3.10. *Specificity/selectivity*. Specificity is the ability of an assay to measure the analyte of interest to the exclusion of other relevant components, that is, it detects only one analyte. Selectivity is the extent to which an assay can measure a particular analyte in a complex mixture without interference from other components in the mixture. During optimization, the goal is to enhance selectivity by carefully choosing conditions, pretreatments and controls.

3.11. *Standard.* A standard is a preparation with known analyte concentration.

3.12. *Signal to Background Ratio (S/B).* S/B is the ratio of the signal of the analyte to the RB signal. It is important for the RB to be identical to the test sample except for the analyte being measured unless otherwise justified during the validation process. Buffer or air blanks are usually not satisfactory indicators of background because they may not account for all extraneous signals. (In assay validation studies, the term signal-to-noise ratio is often used, and it is understood that noise refers to background rather than the random scatter.)

3.13. *Unknown.* The unknown is the test preparation that is assayed to determine the content of the analyte.

3.14. *Validation.* A process that provides evidence that an assay method does what it is intended to do.

3.15. *Verification.* The term used in this document to describe a subset of the validation process where specificity/selectivity, accuracy, precision, discrimination and ruggedness of the test method are evaluated.

Appendix II - Suggested Validation Report Topics

1. Title of Method
2. Principle of method and its relationship to efficacy
3. Development and optimization
   - 3.1. Developmental work description
   - 3.2. Optimization work
     - 3.2.1. Description of approach
     - 3.2.2. Materials and methods
     - 3.2.3. Results
     - 3.2.4. Analysis
     - 3.2.5. Discussion and conclusion. (Include critical specifications of reagents equipment and the test procedure.)
4. Description of procedures for assessing:
   - 4.1. Accuracy
   - 4.2. Precision
   - 4.3. Ruggedness
   - 4.4. Sensitivity
   - 4.5. Specificity
5. Results (for each of the items in 4)
   - 5.1. Data summary
   - 5.2. Graphs
   - 5.3. Analysis
   - 5.4. Discussion and conclusions
6. Finalized procedure
7. Description of monitoring plan
   - 7.1. References
   - 7.2. Controls
   - 7.3. Standards
   - 7.4. Preliminary data,
   - 7.5. Proposed action plan. May include control charts, statistical methods, specifications.