June 28, 2011

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 800.211

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

FROM: John R. Clifford */s/ John R. Clifford*
Deputy Administrator

SUBJECT: Guidelines for Master Reference Qualification and Requalification

I. PURPOSE

The purpose of this Memorandum is to encourage the development of well-designed and rigorously validated assays for new products and revise the policy for determining the dating period for Master References of previously licensed products.

II. BACKGROUND

Many relative potency assays used for serial release testing of veterinary biologics are response-based assays. As a result, the reference preparations for those assays require periodic qualification by vaccination-challenge studies in the target species. Vaccination-challenge studies are time consuming and resource intensive. The time and resources devoted by licensees to maintaining assays for previously licensed products can limit activities aimed at developing improved assays for new products. Title 9, *Code of Federal Regulations* (9 CFR), section 113.8(d)(2) indicates, "The lot of reference used to determine relative antigenic content shall have an initial dating period equal to the dating of the product or as supported by data acceptable to APHIS. … The dating period of the Master Reference and Working Reference may be extended by data acceptable to APHIS if the minimum potency of the Master Reference is determined to be adequately above the minimum level needed to provide protection in the host animal."

To facilitate the development of well-designed and rigorously validated assays for new products, this document presents guidelines intended to minimize the effort needed to maintain and qualify references used in the potency assays of previously licensed products by describing the type of supportive data that is acceptable for extending the dating period for Master References of products licensed prior to January 1, 2011. In addition, this document defines the criteria necessary for qualification of new Master References or requalification of current Master References when the extended dating period has expired.

III. SCOPE

This document outlines requirements for Master References used in relative potency assays. It applies to serial release testing of inactivated vaccines and bacterins by *in vitro*, animal serology, and laboratory animal challenge methods. This document categorizes products into those licensed before and after January 1, 2011, with specific guidance for each category. It applies to all products except those containing significant antigens such as those that have Foreign Animal and Program disease status or zoonotic potential.

IV. GUIDELINES

The Guidelines for Master Reference Qualification and Requalification are appended to this memo.

Appendix

APPENDIX

1. Guidelines for Master Reference Qualification and Requalification

Master References for serial release of products licensed before January 1, 2011, may be used continuously for serial release or to establish new Working References for up to 15 years from the date of initial qualification or 10 years from January 1, 2011, whichever is later, if there are no obviously significant changes in the behavior of the Master Reference in the potency assay from the time of its qualification. This document will describe which products are affected, how changes in the Master Reference are determined, the frequency of this assessment and the type of information that must be submitted to the Center for Veterinary Biologics (CVB).

2. Product Categories

2.1. Previously Licensed Products

This category includes all products licensed or permitted before January 1, 2011. It also includes breakout or combination products derived from previously licensed products, provided no major changes in the manufacturing process have been implemented in the derived products.

Major changes in the manufacturing procedure may result in any product licensed prior to January 1, 2011, being classified as a new product for the purposes of this guidance. An example would be changes that require a new product code and efficacy or safety studies before approval of the manufacturing change.

Combination products containing a previously licensed product and a newly licensed product will be treated as a newly licensed product for the new antigens, and a previously licensed product for those antigens licensed prior to January 1, 2011, provided the combination of fractions does not impact the potency tests for the previously licensed fractions. Multivalent products combining antigens of previously licensed products will be evaluated on a case-by-case basis.

Improvements to the potency assays of previously licensed products will not cause a previously licensed product to be reclassified if such changes do not alter the analytical principle of the test method. Modifications that improve the performance of a previously licensed product's test method will not require complete revalidation of the assay. The firm will need to submit data supporting the proposed change so that the CVB can assess the modification and its impact on the test method. Changes to the analytical principle, *e.g.*, from a serological to an antigen capture ELISA assay, will be considered on a case-by-case basis for validation requirements. Firms should consult with CVB prior to initiating studies in order to confirm that the proposed change is acceptable and the work is sufficient.

2.2. Newly Licensed Products

Newly licensed products are products licensed or permitted after January 1, 2011. *In vitro* potency test methods for new products containing inactivated antigens licensed after January 1, 2011, including those pending licensure on that date, must be validated in accordance with VS Memorandum 800.112. *In vitro* tests include relative potency tests and direct antigen quantification assays.

Serological test methods have both *in vivo* and *in vitro* elements. To be eligible for consideration as a potency assay, a serological test must measure a reproducible serological response that is directly related in a dose-responsive manner to efficacy in the target species and the product's antigen concentration. Such a serological test may be done in the target species or a laboratory animal species provided it has been shown to be meaningful, relevant, reproducible, and robust by validation according to VS Memorandum 800.112.

CVB encourages the development of potency assays that are completely *in vitro* as part of the effort to reduce, refine, and replace the use of animals in testing (the "three Rs"). *In vivo* test methods that cause unrelieved pain and suffering should be considered only when an adequate *in vitro* test cannot be developed, or as an interim method during the development of an *in vitro* test.

To be eligible for consideration as a potency assay under such conditions, an *in vivo* potency test must measure a response in the laboratory animal model (LAM) that is related in a dose-responsive manner to efficacy in the target species and the product's antigen concentration. The dose relationship for the parameters measured in the LAM must be correlated to protection of the target species and be able to discriminate between a satisfactory serial and a marginally unsatisfactory serial. Such an *in vivo* test must be shown to be meaningful, relevant, reproducible, and robust, by validation in a manner analogous to the guidelines for *in vitro* tests in VS Memorandum 800.112.

3. Monitoring

3.1. Previously Licensed Products

In order for the Master Reference of a previously licensed product to be eligible for extended dating, it must be monitored continuously from the time of initial qualification using data from serial release testing.

The report must include the following:

1. Raw data for the Master Reference, test serials, and controls from each test in an electronic format suitable for data analysis. Include all data from the initial qualification through the most recent test.
2. Graphical and/or tabular summaries of the data as appropriate.
3. Summary statement of the storage conditions and temperature monitoring procedure for the Master Reference.
4. The potency reference submission worksheet for each Master Reference (Appendix I of VS Memorandum 800.92).

The raw data sets should be complete. For example, ELISA raw data would include the optical density for every well on each plate, along with related information such as plate layout and dilution sequences. For serological assays, include serum titers of each animal, and for laboratory animal challenge tests, include the response of every animal, including those used for back titration of the challenge material. On a case-by-case basis, CVB's Policy, Evaluation, and Licensing division (CVB-PEL) may consider historical data sets that are not entirely complete as adequate for a retrospective assessment. For prospective monitoring, the complete data should be recorded. (Example formats for submission of these data sets are available on the CVB website.)

Where appropriate, data summaries should include plots of each individual test. This is important, for example, with test methods utilizing dilutions sequences, such as ELISAs or animal vaccination-challenge tests. The plots should include reference and serials. It may also be useful to plot the parameter estimates from regression models fit to the individual tests.

Before submitting the initial round of monitoring data and the summary report, CVB-PEL advises firms to prepare a draft proposal for each category of test method (ELISA relative potency, serological potency, etc.) for submission to their reviewer for comment. If the monitoring proposal is acceptable, the reference will be assigned an expiration date of 15 years.

The first stability monitoring report for each Master Reference must be submitted no later than June 30, 2013. If the firm has a substantial number of Master References, they should stagger submission of the first reports so that approximately 20% of the Master References are submitted every 6 months.

Subsequent reports summarizing all the data from time zero must be submitted to CVB-PEL at 2½ year intervals after submission of the first report. The firm's Staff Reviewer will respond to these by letter. If there are no obviously significant changes in the behavior of the assay and reference, the reference will be designated as satisfactory by letter and may continue to be used until its expiration.

3.2. Newly Licensed Products

Monitoring of assays for newly licensed products should follow VS Memorandum 800.112. Specific guidance for ELISA relative potency assays may be found in Appendix III of that document. Those guidelines may be used as a template for monitoring other types of assays, although there may be some differences.

4. Reference Dating

4.1. Previously licensed products

With concurrence by CVB that there are no obviously significant changes in the behavior of the assay and reference, the Master Reference may be used up to the maximum designated dating period (15 or 10 years). Firms should monitor references continuously between reporting intervals, and Master References exhibiting an obvious decline in stability at any time must be replaced. Either 15 years from the time of initial approval of the Master Reference or 10 years from January 1, 2011, whichever is later, the firm must requalify the same Master Reference or qualify a new Master Reference by conducting host animal efficacy trials using the same animal model and study design used in the original efficacy trial that supported the current label claim. An outcome that is similar to that in the pivotal efficacy study will be sufficient for qualification or requalification. The guidance in this document will then apply to the newly qualified or requalified reference. The firm may also elect to propose changes to the original study design. In that case, the study protocol should explain and justify the proposed changes.

4.2. Newly licensed products

The Master Reference of a product licensed after January 1, 2011, may be used continuously as long as it meets the criteria establishing it and has remained stable. The stability monitoring plan and periodic submissions should follow the guidance in VS Memorandum 800.112.

5. Storage Conditions of Master References

The Master Reference must be stored under constant conditions for a stability monitoring program to be effective. Consequently, there must be a system described in a standard operating procedure (SOP) for recording the conditions of storage and verifying that they do not deviate from limits specified in the SOP. The temperature should be recorded at regular intervals by automated or manual means using calibrated equipment. There may also be verification that a standardized storage condition is maintained by other means (*e.g.*, liquid nitrogen vapor phase). Data should be stored electronically and available for inspection. Reports should include a summary statement describing the storage conditions and monitoring tools.