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Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

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April 21, 2006

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 06-11

Subject: Replacement of Potency Assay Reference Samples for Immunodiagnostic Test Kits

To: Biologics Licensees, Permittees, and Applicants
Veterinary Services Management Team
Directors, Center for Veterinary Biologics
Area Veterinarians in Charge, VS
State Veterinarians

Veterinary Services Memorandum (VS Memo) 800.73, General Requirements for Immunodiagnostic Test Kits for the Detection of Antibody or Antigen, provides guidance to licensees, permittees, and applicants concerning the requirements to support an application for a U.S. Veterinary Biological Product License or a U.S. Veterinary Biological Product Permit for antigen/antibody-based immunodiagnostic test kits for the diagnosis of animal disease and/or immunological status, as authorized by Title 9 Code of Federal Regulations (9 CFR), Part 114.9(f). In VS Memo 800.73, Part VII. Serial Release Testing C. Potency, guidance regarding the serial release potency requirements is provided. The purpose of this Center for Veterinary Biologics (CVB) Notice is to provide additional guidance for replacement of potency panel reference samples.

VS Memo 800.73 states: "The manufacturer must perform a potency test on each serial of assembled test kits...as specified in the filed Outline of Production, using reference samples that are acceptable to APHIS. The purpose of the potency test is to ensure that each serial of the assay is producing accurate test results when properly performed. Serial release testing provides confidence that each serial will perform to the specificity and sensitivity standards determined at the time of licensure." The 9 CFR Part 113.5, General Testing, states, "(a) No biological product shall be released prior to the completion of tests prescribed in a filed Outline of Production or Standard Requirement for the product to establish the product to be pure, safe, potent, and efficacious." Performance characteristics for diagnostic test kits are determined during prelicense evaluation. The potency assay does not determine performance characteristics; it is intended to demonstrate that minimum potency standards are met throughout the dating of the product.

VS Memo 800.73 further defines the panel of reference samples for potency testing of diagnostic test kits to include examples of negative/uninfected animals; strongly positive animals; weakly positive animals; samples generating assay values just above and just below the cutoff value between positive and negative classification; samples from animals with reactivity to closely related (potentially cross-reactive) antigens and/or vaccinated animals; and samples from animals reactive for only one, or a subset, of



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antigens for kits that detect reactivity to more than one antigen. Although there is not a specific requirement that all samples are collected from individual animals, the CVB encourages firms to attempt to locate and characterize samples from individual animals that meet the target objectives. When it is not possible to locate appropriate samples, dilutions of positive samples may be approved in order to ensure availability of samples with desired reactivities.

VS Memo 800.73 states, “The reference samples must be identified in the Outline of Production by lot number, date of preparation, purpose, and acceptable assay range. When the inventory of a reference sample is low, a replacement sample should be identified and validated. The replacement sample should serve the same purpose as the original sample (e.g., a strongly positive sample is replaced by another strongly positive sample). Validation data must be accepted by APHIS prior to use of the replacement sample in serial release testing; confirmatory testing at the CVB-L may be required.”

To support replacement reference samples for potency panels, manufacturers should conduct an abbreviated validation study that aims to compare the reactivity of replacement and current reference samples. The CVB will evaluate the data based on the following criteria:

1. The replacement reference sample should serve the same purpose as the sample it is replacing. Its performance should demonstrate its ability to fulfill the role of the sample it is intended to replace, but it need not have the precisely same reactivity (e.g., O.D. or S/P value) as that of the previously approved reference sample.
2. The assessment should seek to illustrate the important characteristics of the reference samples’ reactivity, for example by estimating appropriate parameter estimates (e.g. mean and variance if the data are normally distributed) and/or graphical displays of the data distribution (e.g. scatter plots or box plots) for both new and current reference samples. The appropriate assessment of the validation data will vary by reference panel member.
 - a. For reference samples with reactivity that is not near the extremes of the dynamic range (i.e. not near the diagnostic cutoff value, extinction, or saturation region), the following considerations will generally apply:
 - i. For most reference panel members (e.g., positive samples not near the saturation or critical decision points of the assay), the replacement reference sample will be acceptable if the distributions of the current and replacement reference samples are similar. Minimum and maximum values for acceptable performance (reactivity range) should be established. If the current reference data are not available, reasonable judgment should be used to approve the acceptable replacement reference sample range of reactivity.

- ii. Replacement low positive reference sample reactivity should be close to the critical decision point (cutoff value between positive and negative classification). For visual-read kits, the reactivity of the replacement reference sample throughout the dating period of the product should remain within the targeted response range (i.e., should remain positive). For kits in which instrumentation is used to determine reactivity (ELISAs or other kits read using instrumentation), reactivity ranges should be established with an appropriate response range near the cutoff value. Stability data may be helpful in predicting the appropriate target reactivity.
 - b. For reference samples at the extremes of the dynamic range, such as negative reference samples, the reactivity of the replacement reference sample should be within the response range, (e.g., all negative reference samples should test within the negative range).
 - i. For negative samples, the range should be stated as “equal to or less than (or more than, depending on the architecture of the assay) (the negative cut-off value).”
 - ii. In general, samples that are so strongly positive that they test in, or near, the saturation region should not be used. If circumstances dictate that such samples are necessary, the low reactivity should be established and the reactivity range should be stated as “equal to or greater than (or less than, depending on the architecture of the assay) (the low reactivity).”
 3. Sufficient validation data should be developed to demonstrate the reference sample’s performance. The reference sample should be tested multiple times by multiple users, typically using multiple in-date serials of the licensed diagnostic test kit. One example of a generally acceptable protocol for replacing reference samples would include independent testing of the proposed reference sample by two or more competent technicians experienced in running the assay; using a minimum of two serials, each tested a minimum of five times; using the number of replicates of the replacement reference sample and current reference sample in each independent test as specified in the current filed Outline of Production. All serials used to develop the data must have satisfactory potency using the previously approved reference sample panel. We recommend that one of the serials used to develop the data should be near the release date and one of the serials should be near the expiration date. This will provide data to support the acceptable range of reactivity for the reference panel sample throughout product dating. The statistical, mathematical, or other method used to establish the proposed reactivity range should be stated. Reduced testing may be approved when sample quantities or serials are limited.

Prior to filing the Outlines of Production for approval of replacement reference samples, the CVB may require confirmatory testing at the CVB Laboratory to ensure that the replacement reference sample is appropriate and comparable to the previously approved reference sample.

/s/ Richard E. Hill

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