VS Memorandum (VSM) 800.73

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Basic Licensing Requirements and Guidelines for Diagnostic Products

1. Purpose and Background

Diagnostic products are intended for use in detecting an organism or pathogen, or conditions, to determine an animal's state of health to cure, mitigate, treat, or prevent disease. Diagnostic products must be validated to demonstrate that the principle under which they work is scientifically sound, and that they are reliable, reproducible, fit for their intended use, and able to be consistently manufactured.

This memorandum provides guidance to licensees, permittees, and applicants to support an application for a U.S. Veterinary Biological Product License or U.S. Veterinary Biological Product Permit for diagnostic products intended to detect animal disease or immunological status, as authorized by title 9, *Code of Federal Regulations* (9 CFR), part 101.2, and further described in the Appendix of this document. This memorandum outlines requirements for product optimization and validation, establishing serial release specifications, and confirming product dating, as well as other product life cycle maintenance procedures.

The guidance has been updated to emphasize consistency in manufacturing, clarify procedures for validation, and provide flexibility for licensing requirements based on the intended use, target analyte, assay type, and any other pertinent considerations.

2. Document Status

A. Issue Date: 01/12/2024.

B. This document replaces Veterinary Services Memorandum (VSM) 800.73, dated September 24, 2019, which is cancelled.

3. Reason for Reissuance

This Veterinary Services Memorandum has been updated for clarity and consistency, as well as realignment with the Center for Veterinary Biologics' (CVB's) goals.

4. Definitions

A. Diagnostic product: Product proposed for licensure. It has been referred to throughout this document as a diagnostic product to avoid confusion with a reference test. This memorandum includes an Appendix defining CVB's regulatory oversight of diagnostic products.

B. Diagnostic cutoff: Refers to a numeric value, specific to a given diagnostic product, used to classify an unknown sample as positive or negative by comparing the quantitative test result for the unknown to the cutoff. For these products, the same diagnostic cutoff is used to classify samples in the laboratory during serial release and

in the field for samples tested by the end user. Unless otherwise specified, the word "cutoff" in this memorandum refers to the diagnostic cutoff as defined here.

C. Diagnostic accuracy: An assessment of the ability of a diagnostic product to correctly classify diagnostic samples as positive or negative for the target antibody or antigen. This is measured through evaluating the diagnostic sensitivity and diagnostic specificity.

D. Diagnostic sensitivity (or sensitivity): The probability of obtaining a positive test result from the diagnostic product for a truly positive sample. For the purposes of this memorandum, a true positive sample is determined from the reference test. Diagnostic sensitivity can be expressed as a percentage using the following calculation:

$$[\text{true positive}/ (\text{true positive} + \text{false negative})] \times 100\%$$

where true positive samples are those indicated as positive by both the reference test and the diagnostic product and false negative samples are positive samples, as indicated by the reference test, that were classified as negative by the diagnostic product.

E. Diagnostic specificity (or specificity): The probability of obtaining a negative test result from the diagnostic product for a truly negative sample. For the purposes of this memorandum, a true negative sample is determined from the reference test. Diagnostic specificity can be expressed as a percentage using the following calculation:

$$[\text{true negative}/ (\text{true negative} + \text{false positive})] \times 100\%$$

where true negative samples are those indicated as negative by both the reference test and the diagnostic product and false positive samples are negative samples, as indicated by the reference method, that were classified as positive by the diagnostic product.

F. Reference test: The accuracy of a diagnostic product is measured against a reference test. Ideally, a reference test is an error-free method which gives an individual's true disease status; these are considered "gold standard" methods, but it is known that they are rare. In most cases, a reference test may be fallible but deemed acceptable for assessing the accuracy of a new diagnostic product. In some cases, the ideal reference test may be a composite of multiple evaluations, e.g., Johne's disease classification may be based on cumulative results from histopathology, clinical signs, and culture, taking into consideration the herd status and age of the animal. In such situations, an objective and reproducible method of combining the results across

multiple reference tests to get a single classification must be provided *a priori*, or account for the multiple reference methods in the analysis (such as using simulation methods described in STATWI0002, Technical Notes for Estimating Diagnostic Sensitivity and Specificity).

G. Ruggedness: The capacity of the assay to remain unaffected by deliberate small variations in method parameters, such as temperature or time conditions. It provides an indication of assay reliability under normal use and verification of acceptable operating conditions.

H. Sample: In this memorandum, "sample" refers to a diagnostic specimen rather than a statistical sample of units from a population. The few exceptions are clearly noted as sample size and the meaning is also clear from the context.

I. Taxonomic designation: Reflects the target of interest for the diagnostic product. Taxonomic modifiers are used to reflect different levels of the product's specificity. For example, *Salmonella enterica* could include modifiers such as *Salmonella enterica* serogroup D, or *Salmonella enterica* serovar enteritidis (*Salmonella* enteritidis).

5. Authority and References

A. Authorities

- Virus-Serum-Toxin Act (37 Stat. 832-833, 21 U.S.C. 151-159)
- 9 CFR 101.2
- 9 CFR 103.3

B. References

- CVB Data Guide
- CVB Work Instructions STATWI0002.04, Technical Notes for Estimating Diagnostic Sensitivity and Specificity
- VSM 800.112, Guidelines for Validation of In Vitro Potency Assays
- VSM 800.206, General Licensing Considerations: Preparing Outlines of Production for Vaccines, Bacterins, Antigens, Toxoids, and Diagnostic Test Kits

6. Audience

VS employees and members of the veterinary biologics industry.

7. Guidance

Due to the diversity of diagnostic products in the market and expanding technology, CVB intends the guidance in this memorandum to represent the baseline expected for demonstrating acceptable performance of a diagnostic product for licensure. Requirements will vary based on the target organism/pathogen, target analyte,

technology used in the assay, intended species and sample type(s) to be used with the product, and other influential factors. Validation of diagnostic products occurs in steps which include 1) conceptualization, 2) development, and 3) verification that the product is fit for purpose and can be consistently manufactured to perform in a reproducible and repeatable manner. After successful validation, additional study requirements include confirmation of product dating and other life cycle studies such as replacement of serial release panel members. Each of these studies are further described in this memorandum. CVB has added a separate section to describe special requirements for assays that are only read visually (Section 6. C. 5).

Not all types of diagnostic products will have the same requirements for licensure. To facilitate licensure, submit documents in the order described in this memorandum to allow CVB review of prerequisite information and appropriately outline the path to licensure for a given product. For example, submissions related to development should precede verification as they may identify information that may be required before conducting the verification studies, such as a diagnostic cutoff or ruggedness data, and may also note studies that are not necessary for a particular method or agent. **CVB strongly encourages submitting a protocol prior to initiating any study.** This allows CVB to guide firms in designing appropriate studies that will generate scientifically meaningful data of acceptable quality. Failure to submit a protocol may result in conducting a study that is not fit for purpose and unsuitable to support licensure.

A. Conceptualization

The conceptualization step is intended to be an initial submission to CVB describing the intended product. Based on this information, CVB will provide feedback clarifying which studies may or may not be required for licensure and indicate requirements for the development phase. Submitters can also ask clarifying questions about the product and express potential concerns about licensure. CVB encourages firms to submit a product development plan and Outline of Production prior to other reports or protocols. The following items should be included in this initial submission:

1) Intended label claim.

2) The working principle of the product, including what it is intended to detect and how. Diagnostic analytes are typically antigens, antibodies, or genetic sequences, but may include metabolites specific to an organism.

3) The sample type(s) intended to be tested by the product and sample processing required.

4) Instructions for product use and interpretation by the end user and, if different, how to conduct serial release assays (such as visual read versus densitometry). Describe the intended output (measurement) and if a specific reader/device is required.

B. Development

After CVB reviews the conceptualization submission(s), the firm can conduct development studies to establish the assay conditions required before the verification studies can begin. Unless CVB grants an exception, the firm should perform each of the following studies during the development phase. The results will then be applied to all subsequent verification studies.

1) Ruggedness

Determine the product conditions and reagent concentrations. Evaluate ruggedness by observing the effect of changes in incubation time, incubation temperature, or other test conditions, as applicable, on the results. This should be determined over a minimum of three (3) days using at least two (2) serials of product and by varying the conditions of interest at maximum and minimum limits to support the assay conditions stated in the Outline of Production and product inserts.

2) Validity criteria

Describe the use of controls and methods used to assess the product's performance. Clearly state validity criteria for the controls in the Outline of Production and product inserts so that users know what conditions must be met for the results to be acceptable for use. Use controls and corresponding validity criteria during all serial release testing and other verification studies to ensure all tests are valid prior to using the associated data from tested unknown samples. Validate control ranges prior to conducting the verification studies. Submit a report with a complete description of how you established the control ranges along with the appropriate data.

Controls that are only used to verify assay functionality (such as control lines in a lateral flow device that are independent from the antibody/antigen being targeted) may be treated differently than those required for use in interpreting the assay (such as a positive or negative control in an enzyme linked immunoassay [ELISA]). Both must be described in Section V. C. of the Outline of Production as validity criteria, but the supporting data required will likely be different. See additional information on visual read assays in Section C.5. below to understand leniencies allowed for those types of controls.

Since controls are a component of the diagnostic product, Section V. C. of the Outline of Production must also have specific retest criteria that are followed during serial release testing should the validity criteria not be met. If the retest

fails, the serial will be deemed Unsatisfactory, regardless of the outcome of the serial release panel members.

3) Analytical specificity

Assess the analytical specificity by evaluating potential cross-reacting materials in the test preparation. Test for analytes that are similar to the intended analyte, i.e., those that are genetically related. Samples for this assessment should be diagnostic specimens from the sample type intended for licensure that represent diversity, including any geographic tendencies. This study should take taxonomic modifiers stated on the intended label into account. For example, for a subtype specific label claim, it is expected that domestic subtypes not included on the label are included in this study. Specificity may be evaluated in a separate study or may be included in the diagnostic accuracy study. If a separate study is used, it should be completed during the development phase.

Additional studies that may be required, depending on the type of assay or intended use, are:

4) Analytical sensitivity/limit of detection

Conduct a study to establish the relationship between analyte concentration and the percentage of samples classified as positive for a given concentration. Determining the limit of detection is required for polymerase chain reaction (PCR) assays.

5) Determining the diagnostic cutoff value

For products that require a diagnostic cutoff, establish the cutoff prior to conducting the diagnostic accuracy study using a separate set of diagnostic samples. Describe how the cutoff value was selected and sufficiently describe the data used to set the cutoff in a report submitted to CVB.

A diagnostic cutoff is not required for visual read assays or other qualitative assays that use a visual interpretation or other qualitative response that does not rely on comparison to a cutoff for sample classification. If a visual read assay has instructions for the end user to be able to classify samples using a machine reader, then a diagnostic cutoff for use with the machine reader will have to be validated with data (see Section C.5. for more on visual read assays). A cutoff is not required for use with a machine reader that is only used for the serial release test.

6) Uniformity

Where applicable, conduct a uniformity study analogous to that described in VSM 800.112. Often for plate-based diagnostic assays, samples may only be plated in a single well by the end user so understanding edge effects or other potentially influential plate location effects may help define an appropriate plate layout for end use.

C. Verification

The studies described in this section are intended to determine the diagnostic performance characteristics, which include establishing diagnostic sensitivity and specificity, demonstrating the repeatability and reproducibility of the diagnostic product through an interlaboratory comparison, validating the serial release process, and confirming product dating. These assessments define the parameters under which a given product could be considered "consistently manufactured". They illustrate how much serial-to-serial variability may be allowed in the manufacturing process while still yielding product that has consistent diagnostic accuracy and is appropriate for release to the market. bed in the Outline of Production and product inserts and use the conditions established from the development studies for controls and validity criteria.

For each of these studies, submit a report and raw data to CVB for review. CVB strongly recommends submitting a detailed protocol describing these proposed studies prior to study initiation. Recommended formats for raw data may be found in the CVB Data Guide.

1) Diagnostic sensitivity and specificity

These performance characteristics are estimates that depend on the quality of the sample set and the reference methods used in their estimation. In the case of fallible reference methods, the estimates of diagnostic sensitivity and specificity will be biased towards the reference method. Further bias results from the general impracticality and inability to randomly select diagnostic samples from the population of interest. Account for these potential biases when designing the diagnostic accuracy study to reduce their impact as much as possible.

This testing establishes the amount of variability to be expected from the consistent manufacturing process by using multiple serials and showing they can achieve similar diagnostic accuracy. That information will be used later to help set the serial release specifications.

CVB intends the study design recommendations below as a baseline for consideration.

a. Samples

Identify the species, sample type(s) (specimens), proposed number of samples, and the acquisition of samples, which could include any pertinent information such as geographic source, stage of infection, sample treatment, storage conditions, shipping conditions, etc. Provide a justification for the sample set and explain proposed sample sizes. Describe how the sample set represents the target population.

1. Sample type: Use the same sample type intended for use on the product label. If the label claim for the product includes more than one sample type (e.g., blood and tissue homogenates) or more than one species, estimate diagnostic sensitivity and specificity for each sample type and each species. There may be instances where a reduced number of samples can be used when products are intended for use with whole blood, serum, and plasma; see Section C.1) f Abbreviated Study for Blood Claims for further information.

2. Sample set composition: Test an adequate number of positive, especially weak positive, and negative samples of each required sample type (species/sample type combinations). Typically, this would include one hundred to three hundred (100-300) positive samples, at least ten (10) percent of those being considered weak positive, and another one hundred to three hundred (100-300) negative samples. The positive samples must cover the range of reactivity from weak positive to strong positive to demonstrate the product's ability to function adequately with samples that differ in reactivity. Avoid including multiple samples from the same animal, such as blood draws taken at different times. You may include such data as supplemental information but do not use it in assessing sensitivity and specificity.

   Ideally, the sample set is composed of samples from animals selected randomly from an intended target population, but in practice that is often not feasible. Instead, the sample set may be constructed from a group of available specimens. Random sampling from a group of available samples is strongly encouraged but not required. Describe methods used to select or randomize samples in detail in the report.

3. Sample source: Generally, diagnostic samples should originate from the United States to account for the specific disease agent and animal genetics common to the United States. Collect samples, both positive and negative, from a minimum of three sources, ideally from geographically diverse locations, for each sample type intended for licensure. Varying

sample sources avoids confounding similarities in animal breeds, husbandry, production methods, etc., within the samples collected. For transboundary diseases, it may be necessary to source samples from other countries, but this should be described in a protocol prior to conducting the study with adequate information for CVB to evaluate.

4. Diversity of agents/organisms: Within each sample type, obtain samples that reflect the diversity of the target analyte of interest and support the intended label claim. Any taxonomic modifiers in the label claim need to be appropriately accounted for in the sample set by using sequencing data or subtype specific assays to demonstrate that the diversity present in the sample set matches the intended label claim. For example, a study to support a non-subtype-specific label claim should include all subtypes found in the United States, whereas one that is subtype specific need only include samples from subtypes stated on the label. Limiting diversity in the sample set may result in adjustments to the label claim to appropriately fit the sample set for which diagnostic sensitivity and specificity was demonstrated.

5. Experimental infection: Experimentally infected samples will be considered as supplemental information only. An exception might be granted in rare circumstances, such as transboundary diseases.

b. Reference tests

Determining the true status of the sample, for the purposes of this study, requires testing by one or more reference tests. If multiple reference tests are used, the objective procedure for diagnosing a sample needs to be clearly defined prior to starting the study, and preferably in a protocol to allow CVB to comment on the appropriateness. Test all samples using the reference test(s) and experimental product, concurrently. Use the results of the reference test(s) to determine each sample's diagnostic status. Do not rely on historic reference test results for a given sample when comparing to the diagnostic product. Ideally, run both the reference tests and diagnostic product on the same day. Do not retest samples with discrepant results between the reference test(s) and the diagnostic product.

c. Serials

Use three consecutive serials produced within the allowable range of manufacturing conditions. If these serials demonstrate similar diagnostic accuracy, despite having potentially different numeric responses when tested on the same sample set, they will be used to establish the acceptable

manufacturing process. That variability will be later used to set the serial release criteria.

1. Sensitivity and specificity will be estimated separately for each serial within sample type and species but make every effort to test the same samples on all serials. Testing different sample sets on each serial loses the ability to claim that the serials have similar diagnostic accuracy.

2. Use the same serials for this study to validate the serial release panel ranges (defined in Section C.3) which can also be used for confirmatory testing in the CVB laboratory if testing is conducted in an appropriate timeframe. Plan appropriately to conduct studies while all these serials are within dating for both the sensitivity and specificity study and the serial release panel validation study.

d. Device output requirements

Provide all data for each sample from testing every serial of the diagnostic product and from the reference test(s) in an appropriate electronic format. Include both the positive/negative classifications and numeric values for the diagnostic product, regardless of the assay type. The classifications will be used to estimate sensitivity and specificity, but the numeric results will be used to characterize the limitations of the diagnostic product and the ability to perform on a wide range of samples. If the assay has an innate quantitative component, such as a sample/positive (S/P) ratio in an ELISA or cycle threshold (Ct) value for PCR, that is sufficient. If the assay is a visual read device, each device must have a machine read (such as a densitometry reading) that will be submitted with the final report. For each reference test, provide the classification of every sample electronically along with any quantitative data used to characterize the sample.

e. Study design and analysis

When designing this study, consider other study design components, such as study day and technicians, to avoid skewed results due to confounding variables. Provide estimates of diagnostic accuracy for each serial, sample type, and species combination. It is not necessary to have multiple technicians test the same sample for a single serial. If multiple technicians test the same serial, separate estimates for sensitivity and specificity will result from each technician.

Randomization is highly encouraged, when possible. This may apply to the order devices are tested, the order samples are tested, or even the location of

samples on a plate. The technicians performing these studies should be blinded to sample status during the testing.

Estimates of diagnostic accuracy should reflect the product's end use. If a product is to be used multiple ways by the end user, provide data for each method in the study report. An example is a visual read lateral flow device that will also seek approval for the end user to use a machine read; sensitivity and specificity estimates would be determined for both the visual reads and the machine reads. CVB will use data from this study to estimate diagnostic sensitivity and specificity and provide a ninety-five (95) percent confidence interval, when appropriate. Propose an analysis in the protocol.

Depending on the sensitivity and specificity of the chosen reference methods, it may be advantageous to use simulation-based methods that do not automatically penalize the diagnostic product for discordant results with the reference tests. One such option is available in STATWI0002.

f. Abbreviated study for blood claims

When pursuing claims for a combination of serum, plasma, and/or whole blood samples, due to the similar nature of these sample types, and the difficulty in preserving whole blood, it may be an option to conduct a full sensitivity/specificity study for one sample type (i.e., serum) and an abbreviated study for the other sample types. Submit a request to CVB with supporting information that the target analyte is detectable in all requested sample types prior to conducting the study.

g. Pooling and group samples

For the purposes of this memorandum, CVB considers pooled samples to be controlled collections of samples from individual animals with intentional contribution to the pool. Group samples, also known as aggregate samples, are collections of samples where contributions from each individual are unknown or difficult to define (such as oral fluid rope tests).

One claim will be allowed on the label for each product based on the samples used in the diagnostic sensitivity and specificity study. If that study is performed using individual samples, the claim will be for individual samples. If that study is performed using pools of size *n,* then a label claim will state use of pools of size *n.* For such claims, test pools of size *n* using both the experimental product and the reference test(s) and explain how pools were created in the final report. CVB expects the sample pools to span the range of reactivity expected for the proposed product.

Group samples require special considerations. CVB will not consider claims for group samples at this time.

2) Inter-laboratory comparison (field study)

Product reproducibility is established by conducting an inter-laboratory comparison to evaluate the suitability of the diagnostic product when used by different laboratories. For this study, participating laboratories must follow all final assay procedures, including the use of product controls and associated validity criteria.

a. Test panel

The manufacturer will create a test panel consisting of at least twenty (20) samples spanning the expected range of reactivity demonstrated as the functioning range of the product in the diagnostic accuracy study. Include no more than five (5) negative samples. The negative samples could be positive for another analyte which might cross-react with the kit to further assess analytical specificity. CVB recommends one (1) to two (2) positive samples be duplicated within the panel to further indicate consistency in the manufacturing and usability of the product. Make every effort to use samples from natural infection/exposure rather than spiked samples. If the test panel cannot be created from diagnostic samples from unique, naturally infected animals, discuss the rationale and provide details regarding the samples to be used in the panel in a protocol prior to conducting the study. The samples should be randomized and blinded on the panel.

Submit a report to CVB describing the panel members in detail, including how reactivity of each was determined and any known cross-reactive agents that are in the samples. Describe all randomization and blinding procedures in the final report.

b. Study design

Ship the panel to each of three (3) different laboratories, along with two (2) serials, in accordance with 9 CFR 103.3. Each laboratory will test the entire panel on both serials. Avoid retesting samples with discrepant results across serials. The final report must include a table displaying all test results for each sample. Provide all raw data to CVB in electronic format.

c. Suitability using interval samples

In addition to the panel, participating laboratories should evaluate the diagnostic product by testing field specimens submitted to their laboratory when possible. This is especially valuable for fresh specimens, such as whole

blood and fecal samples, and particularly when there are processing steps needed for fresh specimens that are not necessary for the prepared test panel members. Include all available information about the additional samples tested, and the data obtained from those samples, in the final report.

3) Serial release testing

Every serial of product must be tested according to the serial release test described in Section V.C. of the Outline of Production prior to release. CVB expects that serials released to market have been consistently manufactured to yield similar diagnostic accuracy to the serials used in the validation studies. The serial release test uses classifications and numeric measurements from a panel of test preparations referred to as the serial release panel (SRP) to determine the consistency of manufactured product. Panel members in the SRP are expected to have a relationship to the diagnostic accuracy study regarding the range of reactivity represented. To make that connection, test the same serials used in the diagnostic sensitivity/specificity study to establish the serial release specifications for each panel member. The study to set initial serial release specifications should be conducted within a short timeframe, when possible, so the data collected do not confound consistency expected from serial release specifications with potential stability issues.

a. Serial release panel

1. The SRP must consist of a minimum of three (3) panel members – a negative, a weak positive, and a strong positive. CVB strongly encourages firms to include a fourth panel member that could be considered a “moderate positive” to help track manufacturing consistency but will not require this. For diagnostic products intended to detect more than one (1) analyte, additional panel members might be necessary.

2. Target formulation of positive panel members to reflect the range of observed reactivity observed in the diagnostic sensitivity and specificity study. The low positive panel member should represent the low end of observed responses from the diagnostic accuracy study, and the high positive should represent the high end. If a moderate positive is used, it should fall somewhere in the middle of the observed range.

3. When creating panel members, use the same matrix as diagnostic samples. For instance, if using serum for diagnostic samples, use serum for all panel members, including the negative. Negative panel members should not be sterile diluent, buffer, or any other medium. If the diagnostic product is approved for multiple sample types, select one that is most feasible and representative. If the approved sample type is

impractical for use in a serial release panel (i.e., fecal swabs), propose an alternative composition for the panel members in the protocol.

4. Each panel member must be lot controlled in the Outline of Production with the required serial release specifications and recommended storage conditions described.

5. Submit panel members to the CVB laboratory for confirmatory testing.

6. Controls are part of the serial release test and are expected to have criteria stated in the Outline of Production, but these are considered validity criteria and are not serial release panel members. See Section 6.B.2. on Validity Criteria.

b. Release specifications

All panel members must have both a diagnostic classification (positive/negative) and data-driven numeric serial release specification stated in the Outline of Production. For tests that produce an increasing response with an increasing analyte concentration, set the specification as follows and reverse as appropriate if the product produces a decreasing response with increasing concentration.

1. Clearly state the serial release procedure in the Outline of Production. Include the number of wells/replicates/etc. required for a single test and any calculations performed to yield a single test response. If a diagnostic product is a single use device, the response from one device likely defines a single serial release test for a given panel member. If diagnostic product uses multiple wells for each panel member within a single test, the procedure for combining information across wells to produce a single response from each test run needs to be clearly defined in the Outline of Production.

   As an example, if the Outline of Production indicates each panel member should be plated in five (5) wells of a ninety-six (96)-well plate and the arithmetic mean is determined from those five (5) wells, then that is the test method that is expected to be used in each serial release test.

   Note that it is expected that the steps to test the diagnostic product for serial release are the same as stated in the insert for the end user. Firms may choose to test serial release panel members more than what is required for end use samples in the field. For example, a serial release test may require a panel member be tested in multiple wells on the plate, whereas the end user may plate each sample in a single well. The other

steps for processing the plate should be the same during serial release as for end use.

2. The diagnostic classification of a panel member may come from a comparison to a pre-established diagnostic cutoff (validated during the development phase) or be based on a visual read depending on the assay type.

3. Establish numeric serial release specifications from repeated testing of each panel member following the serial release procedure and using the serials tested in the diagnostic accuracy study. Set the serial release specifications as no more than three (3) standard deviations from the mean calculated for each panel member using summary statistics from the repeated testing.

    i. Follow the procedure described in the Outline of Production for each run of the test including replicates/wells involved in a single test and the calculations performed to yield a single response.

    ii. Conduct a minimum of five (5) tests from each serial for each panel member to set serial release specifications. If other study design factors, such as the day of testing or multiple technicians, will be incorporated into this study, additional tests may be required to adequately account for the other factors.

    iii. Every serial should be used to perform the same number of tests for each panel member. Determine a mean response from each serial, separately, for each panel member as well as a within serial only variance estimate (such as a pooled standard deviation, grouping by serial).

    iv. For each serial, set the upper bound using the serial with the lowest mean response. This is the link between the diagnostic accuracy study and the serial release test. If the serials have demonstrated similar diagnostic accuracy, the variability that was demonstrated to not affect the sample classification can be used when setting serial release panel specifications if the same serials are used in both studies. Set the upper bound as the highest mean from Section 6. C. 3) b. 3. iii plus three (3) pooled standard deviations and the lower bound as the lowest mean from Section 6. C. 3) b. 3. iii minus three (3) pooled standard deviations.

    v. For products compared to a diagnostic cutoff, CVB does not require testers to incorporate the cutoff into the serial release ranges.

However, panel members must meet both the diagnostic classification and the numeric range to pass serial release so it is implied that the serial release ranges must correspond to that cutoff appropriately for a sample to meet both.

vi. In most circumstances, CVB expects the serial release specifications for the strong positive and weak positive will not overlap, although this may not be possible in rare cases.

vii. The negative panel member is only required to have an upper bound for the serial release specifications.

c. Serial release test

For releasing serials of product to the market, perform the potency test in accordance with the filed Outline of Production. Conduct five (5) replicate tests; four (4) of the five (5) replicate tests must pass for each panel member to release the serial. For a plate-based assay, the plate layout must follow that indicated in the Outline and five (5) separate plates must be tested for serial release.

d. Replacing serial release panel members

If a panel member is depleted or must be replaced, to keep the connection to the diagnostic accuracy study, formulate a replacement panel member to meet the existing approved serial release specifications. Design studies to demonstrate newly formulated panel members and emulate the current panel members they intend to replace.

1. For products licensed after this guidance: There are two options for demonstrating proposed replacement panel members have been appropriately formulated to match the current panel members. In both options, the studies are intended to indicate no change to the serial release specifications is necessary.

i. Plan ahead and test concurrently. Test a single serial that has already been released to the market, using the current and proposed panel members concurrently and show they have similar responses to keep the existing serial release specifications. Using this approach, one or all panel members may be replaced at the same time.

ii. Replace one panel member at time. If side-by-side testing or using an already released serial is not a desirable option, panel members must be replaced one at a time. A single serial that may or may not have been tested using the current serial release panel may be used for this

testing. The proposed panel member should be tested alongside the other approved panel members (without the panel member intended to be replaced) to suggest the serial meets the current specifications and that the new panel member is appropriately formulated.

iii. To change the existing specifications. Conduct a small-scale sensitivity study with multiple serials to determine the link between serial release panel members and the diagnostic accuracy of the product. If requesting changes to the established serial release specifications, submit a protocol prior to study initiation to describe your proposal.

2. For products licensed prior to this guidance. To continue using the current serial release specifications, test the proposed and current panel members according to the Outline simultaneously on one (1) or more available released serials and demonstrate that the responses are similar. There will be no exception to side-by-side testing in this situation. If side-by-side, concurrent testing is not possible, firms will be required to validate new data-driven ranges using a small-scale sensitivity study on diagnostic samples.

4) Confirmation of dating

Conduct a real-time stability study to confirm the initial dating period. CVB assigns a dating period of twelve (12) months to diagnostic products before confirming dating and will consider requests for longer dating periods if justified.

a. Perform testing according to Section V.C. of the Outline of Production.

b. Test three (3) consecutive serials at release and at the end of the proposed dating period using the serial release panel specified in the filed Outline of Production. CVB recommends, but does not require, interim testing. Use the same serial release panel for all tests performed on a given serial.

c. Before completing the stability study, include a statement in Section VI.A of the Outline of Production that dating has not been confirmed. Once the study has been completed and approved, include the Mail Log number and date of approval in the Outline of Production.

5) Special considerations for visual read assays

Products that are only read visually present a unique scenario because the validation requirements are typically not as extensive as needed for other assays. This section addresses some exceptions and special considerations that may simplify the licensure of these products, as well as reiterating some that have already been stated in other sections. However, visual read devices that are also to be licensed with a machine reader for use by the end user will be expected to go through full validation procedures using the machine reader. If a machine read is only used for serial release, the following special considerations apply.

a. Most visual read assays have a control line alongside a test line to indicate the device worked as expected and the response on the test line is interpretable. Control lines are expected to have both a numeric and qualitative (positive/negative) criterion that is used to determine validity of the test during the serial release test. If the control line is not related to the target analyte (such as bovine serum albumin), the numeric validity criterion will not be required to be data driven. These lines/spots on the device are used to show the reagents properly ran through the instrument, so the leniency will be granted for setting those values for these types of controls. These controls will still fall under the validity criteria requirements stated above (Section 6.B.2)).

b. A diagnostic cutoff, the quantitative value used to classify samples from a machine read, is not required unless the reader is validated for use by the end user. The panel members should be formulated in the same way as other diagnostic products and the numeric serial release ranges will be established in the same way, but the diagnostic classification for serial release will only be based on the visual read.

c. If multiple technicians are to be used during any of the studies, CVB prefers that multiple technicians not perform visual reads of a single device, as that becomes more a measurement of user vision than the repeatability or reproducibility of the product.

d. Submit data for all validation studies that includes both the visual read and the serial release machine read. Record the visual read prior to obtaining the machine read to avoid biasing the technician doing the visual read.

D. Shipping Diagnostic Products

It may be desirable to ship diagnostic products outside the recommended storage conditions specified on the label. To do so, request an exemption to 9 CFR 114.11 by submitting data and a report to CVB demonstrating that the product performs as

expected after being subjected to the temperatures outside the recommended storage conditions for a specified period of time. Submit a protocol to CVB before initiating the study. If approved, include the date CVB granted the exemption and the specific shipping conditions in section VI.B of the Outline of Production.

E. Program and Transboundary Diseases

The National Veterinary Services Laboratories (NVSL) and the National Animal Health Laboratory Network evaluate diagnostic test kits intended for U.S. Federal and/or State eradication/control and surveillance programs, or other NVSL priorities, in support of U.S. agricultural activities. CVB provides prelicense serials intended for program diseases to NVSL for evaluation. The license and/or permit for such kits may restrict distribution to APHIS-approved laboratories. A U.S. Veterinary Biologics License or Permit does not guarantee the test kit will be used in an official program. Significant changes in disease prevalence over time may affect the diagnostic implication of a test result and, hence, the role of the kit in disease eradication/control programs.

8. Implementation/Applicability

This update is effective immediately.

VS Memorandum (VSM) 800.73

APPENDIX - Regulatory Oversight of Veterinary Diagnostics

Table of Contents

1. Purpose and Scope

This appendix addresses jurisdiction considerations, determination of materials that require licensure versus those that do not, and key foundations of the licensure process.

2. Related Documents

Regulations:

9 CFR 101.2 – Administrative Terminology
9 CFR part 102 – Licenses for Biological Products
9 CFR part 104 – Permits for Biological Products

Policy:

CVB-SOP-5109, Import Permits for Research and Evaluation or Transit Shipment Only

NCAH Portal User Guides for Import Permits for Research and Evaluation or Transit Shipment Only

Legal:

Creekstone Farms Premium Beef v. Department of Agriculture, 539 F.3d 492 (D.C.Cir., 2008)

3. Regulatory Authority for Diagnostics

3.1. CVB's authority over diagnostics is broad and has been tested in legal challenges. The Circuit Court of Appeals for the D.C. Circuit ruled in Creekstone Farms Premium Beef v. Department of Agriculture: "We must give substantial deference to an agency's interpretation of its own regulations ... unless it is plainly erroneous or inconsistent with the regulation." Creekstone Farms, 539 F.3^{d} at 500, And subsequently found: "Given the 'degree of deference' we owe USDA, we uphold its definition of 'treatment' as including diagnosis and therefore its authority to regulate BSE testing for the purpose of diagnosis." Creekstone Farms, 539 F.3d at 502.

3.2. The following language from 9 CFR 101.2 encompasses jurisdiction over veterinary diagnostics and provides a framework for regulated versus non-regulated materials:

3.2.1. A product's intended use shall be determined through an objective standard and not a subjective one, and would be dependent on factors such as representations, claims (either oral or written), packaging, labeling, or appearance.

3.2.2. The term "analogous products" shall include:

3.2.2.1. Substances, at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals and which are similar in function to biological products in that they act, or are intended to act, through the stimulation, supplementation, enhancement, or modulation of the immune system or immune response; or

3.2.2.2. Substances, at any stage of production, shipment, distribution, or sale, which are intended for use in the treatment of animals through the detection or measurement of antigens, antibodies, nucleic acids, or immunity; or

3.2.2.3. Substances, at any stage of production, shipment, distribution, or sale, which resemble or are represented as biological products intended for use in the treatment of animals through appearance, packaging, labeling, claims (either oral or written), representations, or through any other means.

3.2.2.4. The term treatment shall mean the prevention, diagnosis, management, or cure of diseases of animals.

3.3. Diagnostic products are not specifically defined in Title 9 of the *Code of Federal Regulations* but synthesizing the information present in the above they are a subset of biological products intended to provide information in support of the treatment of disease, meaning the prevention (including through determination of state of health), management (including mitigation or prevention), or cure of diseases of animals.

3.4. CVB has used regulatory discretion to limit the scope of diagnostic regulatory oversight to focus on areas which present the most risk to the animal agriculture industry, as well as companion animals and their owners.

3.5. The identification of a diagnostic product in this document is not a precise description of every diagnostic product that is, or will be, subject to the regulation.

3.6. CVB does not have regulatory jurisdiction over some diagnostics such as serum chemistries, metabolic diseases, or hormone level test kits.

4. Considerations for Licensure

4.1. CVB's approach to the regulation of diagnostics focuses on three (3) tenets:

4.1.1. Assuring the tests are fit for purpose for the end user.

4.1.2. Assuring manufacturing consistency.

4.1.3. Assuring transparency in the licensing and labeling process.

4.2. Critical versus interchangeable reagents. Always use the reagent used for licensing for testing. Other users can evaluate interchanging reagents. Items such as taq polymerase, nucleic acid extraction kits, and buffers used for licensure should be stated. Post transparency of materials and methods in labeling on the web in study summaries. Implement methods to highlight critical reagent or "version" changes.

4.3. Pen-side considerations: Consider the most extreme environmental conditions; these conditions may be required on the label. Non-pen-side diagnostics may require a label statement to run only at specific conditions in an environmentally controlled laboratory setting.

4.3.1. CVB may allow foreign animal disease kits with a pen-side claim on the label. However, CVB will determine the user and site considering the reportable disease list and veterinary use only (not producer) restrictions.

5. Products Currently Under Regulatory Oversight

5.1. CVB will use regulatory discretion to determine which products it will actively regulate versus those which will only warrant regulatory oversight if problems in the marketplace occur.

5.2. CVB will exert active regulatory oversight at this time over diagnostic products which include the following characteristics:

5.2.1. The products make claims or could reasonably be construed to be used to diagnose the presence or absence of an infectious agent, a biological intoxication, or the immune status of an animal.

5.2.2. The products detect, quantify, or determine antigens (including biological toxins and prions), antibodies, or nucleic acid sequences which are, or are components of, or are directed against, infectious disease-causing agents. The diagnostic products would identify specific disease-causing agents; for bacteria, to a minimum of the genus level and for viruses, to the minimum of the family level. CVB will not at this time regulate more general diagnostic products that do not differentiate to the levels specified.

5.2.3. The product test is run using samples directly or indirectly originating from animals including, but not limited to: Blood, serum, feces, urine, saliva, mucous, lavage fluid, milk, vesicular fluid, tissues, or lacrimal secretions.

5.2.4. The product test is run using food or environmental samples if the presence of an agent would reasonably imply disease in animals.

5.2.5. The product contains all the critical specific components and information required to set up, conduct, and interpret the results. The components do not have to be packaged together, and CVB will likely exert jurisdiction over manufacturers who try to compartmentalize components of the testing for the sole purpose of avoiding licensure.

5.2.6. Labeling a product "for research purposes only" will not automatically exclude a diagnostic from CVB regulatory oversight.

6. Products Currently Excluded from Regulatory Oversight

6.1. CVB currently will not exert active regulatory oversight over the following related diagnostic materials:

6.1.1. Analyte specific reagents (ASRs) are antibodies, both polyclonal and monoclonal, specific proteins, nucleic acid sequences, and similar reagents which may be used in a diagnostic application for detecting and identifying an agent in a biological specimen. ASRs that otherwise fall within this definition are not within the scope of this document when they are sold to organizations that use the reagents for purposes other than providing diagnostic information to practitioners or owners, e.g., forensic, academic, research, and other nonclinical laboratories.

6.1.2. Laboratory services offered by diagnostic laboratories. While some of the diagnostic products marketed to the laboratories may require CVB licensing, the laboratories themselves, and the tests the laboratories develop in-house, perform, and report are not licensed by CVB if those tests are not transferred, bartered, or sold to other diagnostic entities.

6.1.3. Products clearly marketed for detecting infectious disease agents in food or environmental samples (e.g., air, water, litter, and soil) unless the presence of such an agent would reasonably imply disease in animals in the same environment (e.g., a foreign animal disease agent).

6.1.3.1. CVB may require licensing along with distribution restrictions of products labeled for environmental sampling, but which also could be used on clinical samples if the infectious disease diagnosed is part of a regulatory control or eradication program, and inappropriate use of the kits may bypass regulatory reporting requirements.

6.1.3.2. If a manufacturer wishes to make both disease and environmental claims, CVB will regulate the product. The product would require a disclaimer stating

that CVB did not review data supporting the environmental claim and CVB did not consider it in the licensing process.

6.1.4. Broad technologies such as nucleic acid sequencing and mass spectrometry in general would not be under CVB jurisdiction unless products fit the categories outlined above, and very specific disease diagnostic claims were being targeted for products being marketed directly to anyone other than a recognized diagnostic or research laboratory.

6.1.5. The method is completely electronic or chemical, and the device itself does not have any biological material incorporated (e.g., antibodies, nucleic acids, proteins).

6.1.6. Genomic sequencing of the host for mutations which cause diseases or where disease associations are known are not being considered for licensing by CVB at this time.

6.1.7. Diagnostics for cancer in animals are not being considered for licensing by CVB at this time.

6.1.8. Diagnostics which result in a general disease result but do not specify/characterize an etiologic agent to the required level are not being considered for licensing by CVB at this time. Examples would include a test for mastitis where the cause is reported as “gram positive,” or “coliform” bacteria).

7. Importation of Unlicensed Diagnostics

7.1. CVB has the authority to issue Research and Evaluation (R&E) permits for unlicensed animal diagnostic kits manufactured in countries other than the United States as described in 9 CFR part 104 and CVB-SOP-5109. State veterinary diagnostic laboratories routinely request these permits, particularly when no similar licensed product is available in the United States.

7.2. The CVB policy regarding these permits is to accommodate U.S. veterinary diagnostic laboratories if the importation does not pose a significant risk to U.S. agriculture or animal or public health. Any requested products will be subject to a risk analysis which may result in use restrictions, safety test requirements prior to entry, or denial of the importation.

7.3. R&E permits for diagnostic test kits being requested for routine use for entities other than governmental institutions will receive more scrutiny because of the potential to evade the use of accredited veterinarians and associated animal disease reporting obligations.

7.4. Unlicensed diagnostic test kits made with ingredients of animal origin and manufactured in countries with foot and mouth disease will not be eligible for R&E permits from CVB. Please refer to VSM 800.51 for additional requirements.

7.5. R&E permits for test kits which have licensed analogs available will also receive increased CVB scrutiny and require a justification. Requestors will be encouraged to transition to licensed test kits.