



## VETERINARY SERVICES MEMORANDUM 800.202

Animal and Plant  
Health Inspection  
Service

Veterinary Services

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**TO:** Veterinary Services Leadership Team  
Directors, Center for Veterinary Biologics  
Biologics Licensees, Permittees, and Applicants

**FROM:** Jack Shere  
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**SUBJECT:** General Licensing Considerations: Efficacy Studies for Prophylactic and Therapeutic Biologics

### I. PURPOSE

General licensing considerations provide guidance to licensees, permittees, and applicants concerning the submission of documents to support an application for a U.S. Veterinary Biological Product License or U.S. Veterinary Biological Product Permit for Distribution and Sale, according to title 9, *Code of Federal Regulations* (9 CFR), parts 102.5 and 104.5. This memorandum addresses basic principles for conducting efficacy studies for prophylactic and therapeutic biological products.

### II. REPLACEMENT

This memorandum replaces Veterinary Services (VS) Memorandum No. 800.202 dated October 27, 2014.

### III. BACKGROUND

Veterinary biological products work via immunological mechanisms to prevent, treat, or diagnose animal disease. This memorandum addresses prophylactic and therapeutic products. Prophylactic products, intended to prevent or control the occurrence of disease, include vaccines, bacterins, and toxoids. Therapeutic products, such as antitoxins, hyperimmune serum, or immunostimulants, are intended to treat existing conditions.

Efficacy is the capacity to produce a desired or intended result. The ability of a biological product to perform as indicated on product labeling must be adequately demonstrated prior to issuance of a USDA license or permit.

### IV. GUIDELINES

“General Licensing Considerations: Efficacy Studies for Prophylactic and Therapeutic Biologics” is appended to this memorandum.

### V. IMPLEMENTATION/APPLICABILITY

This guidance is applicable upon publication of this memo.

**General Licensing Considerations  
Efficacy Studies for Prophylactic and Therapeutic Biologicals**

**1. Introduction**

- 1.1 Products.* This document applies to prophylactic products, herein called vaccines, and therapeutic products. Diagnostic products are discussed in Veterinary Services (VS) Memorandum No. 800.73.
- 1.2 Efficacy.* Efficacy is the direct effect of a medical intervention on an individual subject. The effect of an intervention program in the population is often termed effectiveness. The concept of effectiveness includes both direct effects and the indirect effects of the intervention at the herd or population level. Herd immunity, which protects nonvaccinated individuals in a group by reducing disease transmission through the vaccinated individuals, is an example of an indirect effect. Vaccine efficacy may be isolated from effectiveness by design or analysis.
- 1.3 Design.* The preferred design for animal vaccine efficacy studies is the prospective, placebo-controlled, randomized, and double-blinded vaccination-challenge trial. In such studies, each subject receives the same exposure to the virulent pathogen by active challenge. By design, challenge studies aim to isolate the direct effect of the vaccine on individual subjects. Other types of studies, such as those relying on natural exposure, may be considered where warranted. Immunotherapeutic trials should aim to compare the responses of product- and placebo-treated subjects that have the existing condition. Deviations from these design features should be noted and justified in the study protocol.
- 1.4 Protocols.* Applicants are strongly encouraged to submit detailed efficacy study protocols to the Center for Veterinary Biologics (CVB) for review and comment at least 60 days prior to the initiation of a study. The CVB may arrange with the applicant to observe the study at selected times. If protocols are highly similar to previously reviewed studies, it is helpful to cite the prior protocol and list the differences.

Succeeding sections of this document list important considerations for the materials, methods, and criteria of efficacy studies. Protocols of proposed efficacy studies and final reports should address these considerations in addition to those noted in the General Licensing Considerations for Study Practices and Documentation (see VS Memorandum No. 800.200).

**2. Materials**

- 2.1 Experimental product.* The applicant is responsible for establishing the validity of the experimental product used to demonstrate efficacy. The experimental product must accurately represent the product that will be produced once a product license is granted. Describe in detail its composition, including antigenic mass. Give its potency and state

the potency test method. Provide the passage level from Master Seed and Master Cell Stock from which each antigen lot in the product was produced.

Prepare the experimental product:

- 2.1.1 In accordance with the filed Outline of Production. Cite the applicable Outline approval date in the study report. If an Outline has not yet been filed at the time the experimental product is made for an efficacy study, or if changes in manufacturing have occurred since the last Outline was filed, ensure that the study report contains sufficient detail on the manufacture of the experimental product to support a subsequently filed Outline.
- 2.1.2 In licensed production facilities in accordance with filed facility documents. If prepared in research facilities, establish that the experimental product fully represents the product that will be prepared in production facilities. Validation of production may be required after scale-up.
- 2.1.3 At or below minimum potency provided in the Outline of Production for the antigen under study and any other product antigens that may provide cross-protection (e.g., bovine viral diarrhea virus (BVDV) type 1 when demonstrating efficacy against BVDV type 2).

The potency of the efficacy serial is used in determining the specifications for the potency of post-license serials at release into the marketplace and throughout dating.

- 2.1.4 At the highest passage from the Master Seed and Master Cell Stock allowed by the Outline of Production. Generally, the 5th passage from the Master Seed and the 20th passage from the Master Cell Stock are the highest allowed unless a higher passage is justified.
- 2.2 *Placebo*. State the composition of the placebo or active control treatment. Placebos may be “product matched” to contain all constituents of a vaccine except the antigen of interest and cross-reacting antigens (preferred), or they may be immunologically inert (e.g., normal saline). Justify the type of placebo or active control selected.
- 2.3 *Challenge*. State the source, composition, and quantity/concentration of the challenge material. In general, the challenge material must be a single pathogenic agent. In those rare cases where co-challenge with multiple agents may be scientifically justified, contact the CVB for further guidance.

### 3. Methods

#### 3.1 Subjects.

- 3.1.1 *Signalment.* Specify the age, breed, sex, source, and other distinguishing features of the animals to be used in the study. Describe how they represent the target population in which the product is likely to be used after licensure.
- 3.1.2 *Enrollment criteria.* List the inclusion/exclusion criteria for enrollment into the study. Generally, subjects should be immunologically naïve to the agent under study; exceptions must be justified. For therapeutic trials, it may be prudent to consider the stage/severity of the existing disease and any prior treatments.
- 3.1.3 *Removal criteria.* Once enrolled, animals should be removed from the study only with ample justification. If certain scenarios are a material possibility (e.g., removing an injured animal for humane euthanasia), describe removal procedures.
- 3.1.4 *Identification.* Unless otherwise justified, identify each animal uniquely. Individual animal identification typically is not required for fish or large-scale poultry studies where observations are summarized at the level of the experimental unit, which is typically the housing unit or tank.
- 3.1.5 *Environment.* State how the subjects will be grouped, housed, and managed in each phase of the study. A floor or site plan often facilitates regulatory review of protocols and reports. Specify whether different treatment groups are in contact or separated.
- 3.1.6 *Auxiliary procedures.* Indicate any concomitant treatments or procedures to be done during the study. Where it is plausible that these procedures might bias study conclusions, justify their use.

#### 3.2 Group assignment and treatment allocation.

- 3.2.1 *Experimental unit.* Identify the experimental unit. The experimental unit is the individual animal or the smallest group of animals that may be randomly assigned to a distinct treatment, where treatment means a unique set of applied conditions (e.g., vaccination) and environmental conditions. Indicate any clustering or grouping of units.

Housing factors may impact the designation of the experimental unit for statistical analysis. For example, if it is necessary to house vaccine and placebo groups separately rather than commingle all treatment groups, the housing unit becomes the experimental unit. It then becomes necessary to include replicate housing units for each treatment group.

Include enough units for the study to produce sufficiently precise estimates of efficacy. Although not required, sample size calculations based on information from pilot studies and other sources may help in planning the study.

- 3.2.2 *Group assignment.* Describe the randomization structure and method of randomly assigning subjects to treatment groups. Design the study so that randomization takes into account features affecting the independence of observations or the confounding of effects. For example, blocking by subject characteristics such as antibody titer, age, weight, litter, or parity may be important. If so, include the blocking plan. Or, if subjects are naturally clustered, indicate whether treatment allocation or sample selection is within or between clusters.
- 3.2.3 *Group treatment allocation.* Efficacy studies usually include at least one group treated with the experimental product and one treated with a placebo. If, instead of a placebo, the control group is to be given an alternative active treatment or left entirely untreated, explain and justify this in the protocol. Additional groups may be needed for other treatment regimens/doses or for non-treated sentinels. Clearly outline any nonparallel group configurations, such as factorial or cross-over type designs. If cohorts of animals are enrolled in stages, ensure that each treatment group is equitably represented in each stage.

For products intended to protect neonates by maternal antibodies from vaccinated dams, studies should be designed according to the proposed vaccination regimen.

- 3.2.3.1 If the recommended regimen is to vaccinate the dam for the passive immunization of her offspring, treatment groups should be comprised of adults whose responses are measured by the immunity of their offspring.
- 3.2.3.2 If the recommended regimen is to vaccinate both the dam and her offspring for the protection of the offspring, treatment groups should be comprised of adult-neonate units.
- 3.2.3.3 If there is more than one recommended vaccination regimen, the efficacy of each recommended regimen must be supported by appropriate treatment groups. Studies of products recommended for both passive and active immunization should include separate groups of passively and actively immunized subjects.

3.2.4 *Group proportions.* Prior information may suggest the proportions for dividing subjects between treated and control groups to optimize the study's efficiency and minimize the total number of subjects. In the absence of such information or codified requirements, equally sized groups are recommended.

*3.3 Treatment and challenge.*

3.3.1 *Vaccine trials.* Describe the vaccination regimen and challenge method. If the proposed design does not include experimental challenge, but relies on natural exposure or other factors, explain the reason for the design.

3.3.2 *Immunotherapeutic trials.* Describe the existing disease condition and treatment regimen.

*3.4 Observations.*

3.4.1 *Observation times.* State the timing and frequency of observations. The overall duration of the observation period should be sufficient to monitor all relevant events occurring after challenge. This period is typically based on the expected incubation period of the disease agent and the expected duration of clinical disease. The presence of clinical signs of disease at the end of the planned observation period may indicate the planned observation period is not long enough to assess the study objectives properly. All reports describing studies in which post-challenge observations are terminated before relevant disease signs are resolved should include a justification why continued observation would not have materially affected the conclusions drawn from the study.

3.4.2 *Blinding.* Individuals performing clinical or postmortem observations or laboratory analyses should be blinded (masked), so that the subject's status in the study is unknown. Blinding should include at least the following two levels, or the protocol should justify their absence.

3.4.2.1 *Masked treatment allocation.* The observer does not know which treatment a group has received.

3.4.2.2 *Masked group membership.* The observer does not know the group to which a subject is assigned.

3.5 *Verification of challenge.* Provide adequate evidence that the observed disease was due to the challenge agent. This is of particular importance when the study measures clinical signs or lesions that are not pathognomonic for a particular agent.

3.6 *Adverse events.* Record and report all adverse events occurring during the study whether or not they are considered related to vaccination, treatment, or challenge. See

VS Memorandum No. 800.204 for additional guidance on classifying and reporting adverse events.

*3.7 Laboratory procedures.* Describe procedures for all laboratory analyses. Where laboratory testing outcomes are fundamentally involved in the definition of the primary outcome, validation reports for individual laboratory methods should be provided before conducting the study. Methods conducted in institutional laboratories accredited by the American Association of Veterinary Diagnostic Laboratories or other recognized quality assurance standards may be acceptable without submitting direct proof of validation.

*3.8 Clinical outcomes.* An outcome is an observation on an individual subject that includes the clinical event and the unit of measurement.

*3.8.1 Outcome specification.* Define outcomes in accordance with VS Memorandum No. 800.200, appendix section 2.3.6.7. Outcomes may be specified in terms of a case definition, severity categorization, or a natural scale of measurement.

*3.8.1.1 Case definition.* A case definition is the outcome used to study disease prevention. Subjects meeting the case definition are considered positive, whereas others are negative. Disease case definitions should be explicit and have a natural clinical interpretation as representing a case of the disease. An arbitrary dichotomization of disease severity is not a case definition.

*3.8.1.2 Severity categorization.* Severity categories are outcomes used to study reductions in disease severity. Ideally each category should reflect a discrete progressive disease state. Use only those categories that reflect sharp distinctions in severity. Excessive categories often undermine clinical relevance and statistical validity.

Example: The categorization dead/sick/well includes three undeniably discrete states. If the sick category is further subdivided into, for example, mild and severe disease, they may still be discrete if mild disease includes transient low-grade clinical signs and severe disease reflects serious morbidity.

*3.8.1.3 Natural scales of measurement.* Outcomes may be based on a measured quantity. The scale of measurement may be discrete, such as the number of days elapsed between the first and last positive disease observation, or relatively continuous, such as body temperature. Select natural scales of measurement, clearly relevant to clinical disease. Avoid contrived, complex scoring systems that are difficult to analyze and interpret because they incorporate many disparate types of observations.

3.8.2 *Primary outcome.* Specify a single (primary) outcome on which the conclusion criterion for each clinical effect will be based. (For example, a study might be designed to study both disease prevention and pathogen shedding, two different types of clinical effects.) The primary outcome should provide the most relevant evidence directly supporting clinical efficacy. If clinically relevant, the primary outcome may be designed as a composite of more than one type of observation.

Serological responses are not usually sufficient for establishing efficacy. An efficacy claim based on serological data alone will be considered only when there is a substantial scientific basis for considering the serological test to be indicative of disease protection. Protocols proposing serologic outcomes, along with scientific documentation supporting these outcomes, should be submitted for review and comment by the CVB prior to initiating the study.

3.9 *Conclusion criterion.* State the criterion for concluding whether the study findings support efficacy. Conclusion criteria should be based on the size and precision of estimated treatment effects and their clinical relevance. Where appropriate, state the intended comparison of primary outcomes between treatment groups, such as by an estimated difference or ratio.

Study statisticians may use any scientifically sound method of comparison, but the two most commonly used by the CVB when evaluating submitted studies are prevented fraction and mitigated fraction.

3.9.1 *Prevented fraction.* The prevented fraction is the complement of the risk ratio ( $1 - p_2/p_1$ ), where  $p_2$  is the affected fraction in the experimental product group and  $p_1$  is the affected fraction in the placebo group. The precision of the estimate is evaluated by determining the 95% confidence interval.

3.9.2 *Mitigated fraction.* To evaluate products that reduce the severity of disease, vaccine efficacy is frequently assessed by mitigated fraction (Siev, D. 2005. *J Mod App Stat Meth*, 4:500-508). Note that mitigated fraction estimates the *probability* vaccinated animals that will be less severely affected than control animals. Alone, however, mitigated fraction does not provide a measure of clinical relevance, as it does not indicate how great the expected reduction in severity may be. Estimates of mitigated fraction should always be accompanied by an estimate, on the original scale of measurement (e.g., duration of viremia in days), of the magnitude of the vaccine effect (e.g., the difference in days between treatment groups).

3.10 *Data analysis.* Describe the proposed method of data analysis and indicate how it is appropriate to the study design and nature of the data.

*3.11 Submit all data.* Submit and summarize all data from each study. Submit the results of every study involving a product proposed for USDA licensure. This applies whether the results were satisfactory, the study was repeated, or the study was exploratory.

#### **4. Effectiveness Indications Statements**

*4.1 Label claims.* As of 2016, labeling for most prophylactic and some therapeutic products will transition to the same generic Indications statement: “This product has been shown to be effective for the vaccination of healthy (insert name of species) \_\_\_ weeks of age or older against \_\_\_.” (9 CFR 112.2(a)(5)). Users are then directed to [productdata.aphis.usda.gov](http://productdata.aphis.usda.gov) for summaries of specific studies conducted to support product licensure.

4.1.1 The data must demonstrate that the product provides a statistically significant, clinically relevant level of efficacy. The product may prevent clinical disease or reduce its clinical severity.

4.1.2 Studies showing that a vaccine merely delays the onset of disease after exposure to a disease agent are typically not suitable to support product licensure.

4.1.3 Products with additional beneficial effects other than direct disease control, such as the control of infectiousness through the prevention of pathogen shedding or a reduction in its duration, may be eligible for licensure if the size of the effect is clinically relevant and statistically significant. Reductions in the magnitude of pathogen shedding are typically not sufficient to support product efficacy when vaccinates still shed in appreciable amounts.

4.1.4 Prior to 2016, there were 4 tiered effectiveness claim levels. See the CVB website for [historical details](#). Products licensed prior to 2016 may still have tiered claims on product labeling during a transition period of approximately 4 years.

*4.2 Disease form.* When a microorganism is associated with more than one clinical form of disease, limit claims to the disease form(s) for which efficacy has been demonstrated, such as “respiratory form” or “reproductive form.” Use specific disease or syndrome names whenever applicable.

*4.3 Administration regimen.* Establish efficacy separately for each route (e.g., intramuscular, subcutaneous, intranasal, in ovo) and regimen (e.g., age and frequency) of administration recommended on the label.

*4.4 Animal Species.* Establish efficacy in each non-fish species for which the product is recommended. When products are recommended for multiple species of fish raised at

the same water temperature, demonstrate efficacy in two of the recommended species.

*4.5 Age and susceptibility.* Conduct efficacy studies in fully susceptible animals of the youngest age for which the product is to be recommended. (Weight may be used in lieu of age for aquatic species.) Minimize age variations in juvenile study subjects, as the designated age of the study population as a whole is based on its oldest members. The age of the study population in efficacy studies, along with the youngest animals used in field safety studies (see VS Memorandum No. 800.204), determines the minimum age in which the product may be labeled for use. If the animal age differs between efficacy and safety studies, the older age applies.

If interfering levels of maternal antibody may still be present at the youngest recommended age, do one of the following:

4.6.1 Provide data to demonstrate efficacy of the product in the face of expected levels of maternal antibody, or

4.6.2 Indicate on the labeling that the product is for the vaccination of susceptible animals of the minimum age used in the efficacy study and indicate that the effect of maternal antibody on product efficacy has not been specifically studied.

*4.6 Onset of immunity.* Support with acceptable data any specific claims concerning onset of immunity. The onset of immunity is defined as the interval between final vaccination and challenge in the supporting efficacy study.

*4.7 Duration of immunity.* Support with acceptable studies any explicit claims for the duration of immunity (DOI) for any product fraction. Studies to support minimum DOI also may be required to support the recommended revaccination interval after the primary vaccination series, even when specific DOI claims are not made. This is determined on an agent-by-agent basis, but in general includes all “newer” agents (i.e., those for which biologics were first developed after ca. 2000), agents known to induce short-lasting immunity (e.g., influenza), and rabies virus. Data are also required when a revaccination interval greater than a year is proposed.

Conclusions drawn from DOI studies should be as robust as short-term efficacy studies, although it is understood that disease due to certain pathogens may be milder in animals exposed at an older age. The following considerations apply:

4.7.1 To prevent the confounding effect of immunity boosted by natural exposure to an agent after vaccination, implement the appropriate biosecurity to protect all subjects from inadvertent natural exposure in the extended interval prior to experimental challenge. Monitor study animals for exposure at regular intervals between vaccination and challenge.

- 4.7.2 Ideally, formulate the experimental product for DOI studies to the same potency used in previously approved short-term efficacy studies. If DOI is demonstrated using a product with greater potency, the DOI potency will be used as the minimum immunogenic dose for the potency specification for serial release.
- 4.8 *Product lines.* When there is a group of products, manufactured identically in composition and potency except for the total number of microbial fractions, efficacy is typically demonstrated with the product containing the greatest number of fractions. The results then may be extrapolated to the remaining products (i.e., breakouts/fallouts) having a subset of fractions found in the highest combination product. Extrapolation of conclusions to breakouts may not apply if the test product has a potentially immunopotentiating (e.g., gram-negative bacteria) or cross-reactive fraction not found in the breakout product. Extrapolations may not be made to products containing more fractions than the tested product because of the potential for immunological interference by the non-tested fractions; see VS Memorandum No. 800.203 for further guidance.