

## EFFICACY STUDIES (including Interference Studies)

### Overview

Each antigenic fraction of a licensed/permitted product must be supported by an efficacy study acceptable to APHIS. If label claims for cross protection are desired, separate efficacy studies must be performed to support each claim. Likewise, separate efficacy studies must be performed to support multi-syndrome claims for diseases with more than one distinct disease syndrome.

Once efficacy has been proven for an antigen in a given product formulation, that antigen often may be combined with other antigens in related products with reduced requirements for efficacy. If each antigen in a proposed new product previously has been proven efficacious individually (or in other combinations), it may be necessary only to demonstrate that the antigens do not excessively interfere with each other in the new combination.

After licensure, efficacy-type studies may be performed to qualify reference serials for potency tests, or to confirm appropriate revaccination intervals. Although reference qualification studies may sometimes utilize slightly smaller treatment groups than pivotal efficacy studies, all other guidelines for review and interpretation of results apply.

### Flow of Information

1. Efficacy reports are routed directly to the reviewer upon receipt.
2. Electronic data submitted with efficacy reports are posted by the program assistant to the electronic mail log. The existence of electronic data is noted in the mail log record by an associated Statistical Data File document record.
3. Most efficacy studies warrant evaluation by the statistics unit. A preliminary review of the report should be done as soon as possible to confirm if statistical input is necessary. If statistical input is desired, preliminary information should be filled out in the efficacy licensing study summary (see chapters 4.4.2 Efficacy study licensing summary and 4.4.2.1 Efficacy study licensing template for additional information). Adding the preliminary information to the efficacy licensing template facilitates statistical review. The report may then be forwarded to Statistics. . Statistics will return the submission to the reviewer with written comments.
4. When the reviewer has reviewed the submission and prepared a response, it is handled like all other correspondence (see Office Procedures chapter).
5. The reviewer then completes the final portion of the Efficacy Study Licensing Summary to document the basis for the regulatory decision.
6. If the study is accepted to support licensure, the reviewer evaluates the co-submitted Draft Individual Summary (submitted as part of the single-tier initiative) for major deficiencies. [REDACTED]



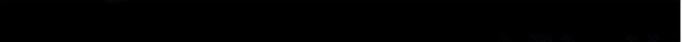
## Definitions

**Efficacy:** The direct effect of medical intervention on an individual subject. In our context, it refers to the ability of a product, when used as directed, to protect an animal against disease.

**Immunogenicity:** Ability of a substance to elicit an immune response in animals. Sometimes used synonymously with **antigenicity**. Strictly speaking, antigenicity does not necessarily mean that the immune response is protective; any substance that is seen as foreign will elicit some type of immune response. In practice, however, immunogenicity and efficacy are often used interchangeably in the biologics industry.

## Reviewing Efficacy Studies

Guidelines regarding efficacy studies are found in [VS Memorandum 800.202](#). Reports should be prepared in accordance with the documentation guidelines found [in VS Memorandum 800.200](#). Consider the following:

1. Nearly all pivotal efficacy studies need to be performed in host animals and nearly all require vaccination-challenge. Serological studies are acceptable only when serological titer is highly predictive of efficacy. 
2. The serial used to demonstrate efficacy should be representative of how production lots will be made (according to the Outline of Production and in production-scale equipment).   
 Although it is common practice to prepare a small efficacy serial, it should not be less than 1/3 of the anticipated average volume of a production serial (defined in Section IV.E.2 of Outline of Production). The characteristics of the efficacy serial will define the minimum permissible antigen content (Section IV.I. of Outline) and the highest permissible passage levels from the Master Seed(s) and Cell(s) (Section II.B. of Outline). For modified live products, the potency of the efficacy serial is the basis for calculating the minimum release titer. If multiple vials are used in a pivotal efficacy study, the vials should be pooled to ensure consistency and homogeneity of the test article (). For lyophilized products, the test serial for the efficacy serial should be rehydrated with the diluent that will be marketed with the product .

As per VS Memorandum 800.202, the efficacy serial should be formulated at or below the minimum potency provided in the Outline of Production for the product. Historically, for most live virus vaccines, the titer required throughout dating has been expected to be at least 0.7 log<sub>10</sub> greater than the titer of the product used in the efficacy study. An

additional  $0.5\log_{10}$  overage has historically been added, so that in general, modified live products have been released at a level of 1.2 logs greater than the minimum immunizing dose observed in the efficacy study. For most live bacterial vaccines, the bacterial count required throughout dating has been expected to be twice that of the product used in the efficacy study. To explore the possibility that release titers may be lowered based on scientifically sound data, Draft Document 440 was posted for public comment. If a firm chooses to determine serial release (for products that are tested using an in vitro potency test) using the guidance in Draft Document 440, the following points should be considered:

- Overage for assay variability should be based on data evaluated during assay validation. When serial release is based on a well validated assay, antigen overage to account for assay variability should not be as high, as for an assay in which a great deal of variability is observed. Therefore, historical overages for assay variability may be decreased based on the appropriate data.
- Codified antigen overages, (for example as described in 9 CFR 113.330, 113.331, 113.332 etc) must still be met, regardless of guidance in Draft Document 440.
- Final release specifications and throughout dating specifications are based on confirmation of dating study data. Initial specifications may be adjusted based on confirmation of dating study data.

3. The efficacy study should be performed according to the study protocol. Firms are strongly encouraged to submit study protocols for review prior to initiating pivotal studies (see chapters on Protocols and Statistics). Ideally, the protocol also is appended to the final report for reference.

4. The characteristics of animals used in the efficacy (and safety) study will be used to define permissible labeling statements. These include the minimum age at vaccination and any special recommendations (e.g., use in pregnant, lactating animals).

5. Each animal in the study should be uniquely identified. An exception is often made in large poultry studies or fish studies, where animals are identified by house/tank instead of individually.

6. Animals enrolled in efficacy studies should be immunologically naïve for the antigen under study. The report should include pre-vaccination data that demonstrate the animal's eligibility for inclusion in the study. For some ubiquitous antigens and depending on the sensitivity of the screening assay, it may be necessary to use animals with low pre-existing titers. In such cases, it may be prudent to block the animals by titer when allocating them to treatment groups.

7. The study should include the proper types of treatment groups. A separate treatment group should be included for each proposed route of administration. (Note: Needle-free administration is treated as a distinct route of administration [REDACTED])

The nature of the vaccine and the disease under study should be considered to determine whether “control” animals should receive a placebo containing everything in the test product except the antigen under study [REDACTED]

[REDACTED]

If a nonspecific disease parameter (e.g., lung consolidation) is used as the primary measure of efficacy, then a small group of non-vaccinated, non-challenged controls should be included to evaluate the baseline incidence/severity in the test population. An experimental challenge should be strong enough to generate significant differences between the challenged and non-challenged controls.

8. The persons evaluating /scoring test subjects should be blinded to treatment allocation *and* group membership. For extremely subjective measures (e.g., lung scores), multiple independent scorers are highly recommended.
  9. Ensure that scoring systems are scientifically sound. Each progressive category in a scoring system should reflect a *clinically relevant* progression of disease.
  10. The study should generally be evaluated by the primary outcome defined in the protocol, *provided that* the proposed primary outcome is appropriate. The criteria for an acceptable study should not be changed after the study is completed merely to accommodate the actual data generated. Scientific judgment is critical, however; regulatory decisions should be based on a thorough review of all of the data associated with a study.
  11. Differences between treatment groups must be **clinically relevant** as well as statistically significant. The statisticians provide a statistical analysis, but it is up to the reviewer to evaluate the clinical relevance. [REDACTED]
- [REDACTED]

Statistical measures such as prevented fraction and mitigated fraction are often used to analyze vaccination effects. Mitigated fraction measures the likelihood that vaccination reduces a particular disease effect, but in itself does not provide information regarding the magnitude of the reduction. Example: An efficacy study may demonstrate a highly repeatable mitigation of viral shedding, but if the magnitude of the shift is only one day (e.g., vaccinates shed 13 days and controls shed 14 days), the clinical relevance of the effect is questionable.

Historically, different claim “levels” would be given, depending on the strength of the vaccination effect; see chapter on single-tier policies for details on the historical claim levels. As of 2016, we implemented regulations for single-tier effectiveness statements. All label claim statements are to be in the codified format of: “This product has been shown to be effective for the vaccination of healthy (insert name of species) \_\_ weeks of age or older against \_\_.” (9CFR 112.5) Basic data regarding the design and outcome of pivotal efficacy studies is published on the CVB website for users that wish to evaluate a particular product further.

12. Post-challenge observations should continue until all relevant clinical signs have resolved in the surviving vaccinates. This is especially critical for products being considered on the basis of reduced disease severity or duration. Label claims should not be granted for products that merely delay the onset of disease or increase the time to peak disease severity.

13. The study report should include all data generated, even those that do not support licensure. If you feel that data are missing, do not hesitate to ask for them!

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15. We have, on occasion, accepted efficacy studies that were conducted in other countries. Consideration must be given to the facilities available, the use of relevant

challenge strains, potential differences in animal breeds, susceptibility to disease, and differences in husbandry practices, To maintain consistency, proposals to conduct efficacy studies in foreign countries should be discussed with the applicable Section Leader and at the Reviewer Staff meeting.. [REDACTED]

17. [VS Memorandum 800.211](#) establishes a policy for legacy vs. new products. Efficacy expectations for legacy products may differ from new products. See the memorandum for details.

### Efficacy for Related Products

Once a firm demonstrates efficacy for products made with certain Master Seeds, it is not uncommon to mix and match those antigens in different product combinations. If a proposed combination product is identical to an already licensed product except for the addition of a new antigen (i.e., same adjuvant in same concentration, same schedule and route of administration, same minimum antigen concentrations), then it is not generally necessary to repeat host animal efficacy studies for each fraction in the new combination, *provided that* data are submitted to demonstrate that the new antigen(s) does not excessively interfere with the immunogenicity of the other(s).

Sometimes a firm will plan for an entire product line before the first product in the line is licensed. If a company wishes to license two or more related products, efficacy is often demonstrated with the product containing the largest combination of antigens. Smaller combinations are then licensed as “break-out” or “fall-out” products with minimal, or no, additional efficacy data. If there is concern that a certain antigen (e.g., a gram-negative bacterium) not present in all break-out products may potentiate the immune response, then data to demonstrate that the removal of that antigen did not adversely impact the immunogenicity of the remaining antigens should be required.

In each of these scenarios, a study to demonstrate lack of excessive interference (“interference study”) is usually performed. See [VS Memorandum 800.203](#) for additional guidance. Frequently, comparative serology is performed using serials of product that are matched except for the presence or absence of the antigen being added. The comparison product should be one for which efficacy was demonstrated by vaccination-challenge [REDACTED]. Avoid cascading “generations” of products each licensed

on the basis of serology compared to the generation before it. Over time, such practices can result in substantial drops in required efficacy.

If serological equivalence can be demonstrated, no further data are usually required. If, however, serological equivalence cannot be demonstrated, then a host animal efficacy study is usually necessary to demonstrate that an antigen in a new combination remains adequately efficacious. If adding a new fraction to a previously licensed multivalent product, the firm should formulate the new fraction at the MID and the other fractions at/above release values to demonstrate lack of interference. If a firm is proposing initial licensure of a multivalent product, it may be acceptable to formulate the efficacy serial with all fractions at the MID, which would require only a single serial to be formulated as the efficacy serial, if there are no known issues regarding interference with the fractions involved. An exception to this would be a multivalent product that contains Newcastle Disease Virus and Avian Bronchitis Virus. Since there are known issues regarding interference with these viruses, the antigen being tested should be formulated at MID and other fractions at/above release values. [REDACTED]

If a firm proposes doing a serological interference study to demonstrate lack of interference on one fraction, and a vaccination/challenge study to demonstrate lack of interference on another fraction, it may be acceptable to use the same vaccine formulation and thus the same animals in both studies [REDACTED]

Serology is often acceptable to demonstrate lack of excessive interference when it would not be acceptable to demonstrate efficacy directly. The rationale for this is that with an interference study, we are attempting to gain confidence that the immune response elicited by the new product combination is qualitatively and quantitatively similar to another matched product for which efficacy has been demonstrated. We are seeking to demonstrate consistency of response, and serology is usually considered to be an acceptable indicator for this purpose. If the serological response is not considered to be equivalent, then the conclusion simply should be that the immune response elicited by the product may have changed. No attempt should be made to use serology to determine the impact that the altered immune response has on the overall efficacy of the product.

Serological equivalence should not be confused with serological noninferiority. In the past, it was often considered acceptable to generate serological titers with the proposed product that are substantially higher than that obtained with the product of proven efficacy, just as long as the response was not inferior. The scientific wisdom of this is questionable, given our current knowledge of type I vs type II immune responses. If the immune response in the proposed product is shifted to a more predominantly type II response, it may be characterized by a higher antibody titer but a weaker cellular response and may be less protective overall. More is not necessarily better.

For certain antigens with a codified *in-vivo* potency test that is adequately linked to efficacy (e.g., certain clostridial products), demonstrating that the new combination passes the potency test may be acceptable. For poultry products, it is common to perform a host animal efficacy study to demonstrate lack of excessive interference.

## Reviewing Interference Studies

1. Determine whether demonstrating lack of excessive interference is an acceptable alternative to demonstrating efficacy directly:
  - 1.1. The new product combination must contain the same adjuvant system (in the same concentration) and have the same schedule and route of administration as does the licensed product that will be used as the basis for comparison. It must use the same Master Seeds at the same minimum concentrations.
  - 1.2. Efficacy must have been demonstrated directly for the product used as the basis for comparison. In other words, a product that was licensed on the basis of interference studies cannot, in turn, serve as the basis for licensure of another product.
2. Ensure that the serials being compared are matched except for the antigen of interest. They should be made from the same bulk antigen lots, with equal amounts of antigen.
3. Ensure that data are generated for each antigen in the licensed product. For example, if a fourth virus is being added to a licensed 3-way viral product, serological titers against each of the 3 original viruses must be compared to ensure that the addition of the fourth antigen did not interfere with any of the other viral antigens.
4. For serological studies:
  - 4.1. Compare the geometric mean titers from each treatment group. Ensure that serological equivalence is demonstrated to an acceptable degree of confidence. Currently, a 63% equivalence margin with a 0.05 level of significance is acceptable for serological noninferiority. The estimated confidence interval must not have a lower limit less than 63%.

Although [VS Memorandum 800.203](#) states that we are looking for serological noninferiority, any product testing outside of these parameters (higher OR lower) should be suspect. Use your professional discretion when evaluating interference data in which the new product generates substantially higher titers.
  - 4.2. Ensure that the serological assay used to demonstrate equivalence is adequately validated (especially with respect to repeatability) and properly controlled.
5. If serological equivalence cannot be demonstrated initially, the firm may elect to:
  - 5.1. Increase the group sizes for serological comparison. Data may be generated from appropriately designed field studies randomized to the proposed and existing product.
  - 5.2. Challenge vaccinated animals to demonstrate protection with the proposed product. If this is done, the review considerations for pivotal efficacy studies apply.

6. If potency tests are used:

6.1. Some potency tests are sufficiently correlated to host animal efficacy so that demonstrating that the proposed product passes the potency test is sufficient.

Example: clostridial antitoxin titers in host animal or acceptable laboratory animal

6.2. Other codified potency tests are sometimes used as the basis for comparison.

[Redacted]

### Efficacy Licensing Summaries for internal use (aka efoia summaries)

Reviewers are expected to prepare internal summaries of efficacy studies, including the basis for their regulatory decisions [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

### Individual Study Summaries for Single-Tier

1. When a firm submits a pivotal efficacy study, they should also include a draft Individual Study Summary, which will eventually be posted on the public website as part of the single-tier effectiveness. The Individual Study Summary contains far less detail and technical lingo than the internal “efoia” summary. The reviewer is responsible for approving (“clearing”) the Individual Summary for publication at the time of efficacy study review. [REDACTED]

### References

[VS Memorandum 800.200](#) Study Practices and Documentation

[VS Memorandum 800.202](#) Efficacy Studies

[VS Memorandum 800.203](#) Antigen Interference

[VS Memorandum 800.206](#) Preparing Outlines of Production for Vaccines, Bacterins, Antigens, and Toxoids

[VS Memorandum 800.211](#)

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