



January 16, 2007

United States
Department of
Agriculture

VETERINARY SERVICES MEMORANDUM NO. 800.203

Animal and Plant
Health Inspection
Service

Subject: General Licensing Considerations: Compatibility of Components

Washington, DC
20250

To: Veterinary Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

This memorandum provides guidance for evaluating the compatibility of components in combination biological products formed by assembling previously licensed products.

II. CANCELLATION

This memorandum cancels Veterinary Services Memorandum No. 800.203, dated May 28, 2002.

III. BACKGROUND

The products covered by this memorandum are vaccines and similar prophylactic immunobiologicals, such as bacterins or toxoids, which contain antigens intended to actively stimulate an immune response in the recipient. Antigenic fractions with previously established efficacy in licensed products may be combined to form a new polyvalent product. It must be verified that the efficacy of each fraction has not been compromised in the new product when compared to a product with known efficacy.

IV. DEFINITIONS

- A. Component Interference - Component interference is an adverse alteration of the expected immune response to one antigen by the presence of another antigen or component in the same product.
- B. Excessive Interference - Interference is excessive when there is reason to believe the product's efficacy against disease has been decreased by the alteration of the immune response due to interference.
- C. Fraction - A fraction of a prophylactic immunobiological product refers to an antigen (organism) and the form in which it appears (e.g., modified live,



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inactivated, subunit, toxoid, vectored, etc.).

- D. Reference Product - A reference product is a product for which efficacy in the target species has been directly demonstrated.
- E. Test Product - A test product is a new polyvalent product formed by combining fractions from previously licensed product(s) and/or adding new antigens to licensed combinations.

V. GUIDELINES

Material submitted to support the efficacy of a test product must include information verifying the absence of excessive component interference. Support for the absence of excessive interference may be provided by one of the following.

- A. Efficacy Study - A satisfactory efficacy study conducted with the test product may be used to verify the absence of excessive interference on the efficacy of the vaccine antigen that was challenged. Efficacy studies may be conducted when licensing any test product and must be conducted for the following:
 - 1. *Avian and Fish Products* – Conduct an efficacy study for each fraction of new polyvalent products intended for use in poultry or fish.
 - 2. *Mammalian Products* – Conduct an efficacy study for each fraction of new polyvalent products intended for use in mammals if the new product differs significantly from the licensed products in composition, production methods, or recommended vaccination regimen.
- B. Existing Information - Submit convincing objective data documenting the absence of excessive interference. Such information may include previous studies or documented experience with the fractions comprising the test product.
- C. Potency Test - Validated potency tests which accurately reflect a fraction's efficacy may be sufficient support for the absence of excessive interference. Acceptable potency tests have been limited to the *in vivo* tests found in the Standard Requirements for *Leptospira* species, *Clostridium* species, and the equine viral encephalitides (Code of Federal Regulations, Title 9, Part 113).
- D. Comparative Serology - In comparative serology studies, serum titer is used as an indicator of the overall immune response. If the humoral responses to vaccination with the test and reference products are equivalent, then the critical assumption is made that cell-mediated processes and their relative contributions to the overall immune response are unlikely to be materially affected as well. A change in that balance may be signaled by an antibody response that is either increased or reduced. To support the lack of excessive interference by comparative serology, conduct a study in the target species comparing the serological responses between a group vaccinated with the test product

and a group vaccinated with a reference product. In many instances, the serologic response of interest will be a serum titer, and the group geometric mean serum titers (GMT) would be compared. The absence of excessive interference would then be supported if the GMTs were equivalent. For the typical comparative serology studies described in this section, lack of excessive interference would be supported by the noninferiority of the test product GMT relative to the reference product GMT, and full equivalence need not be demonstrated. If, however, the GMT of the new product is dramatically higher than the reference product GMT, further study of the potential impact on the overall protective immune response may be warranted.

1. *Methods*

- a. Equivalence - Two values are equivalent if they differ by less than an amount which is considered meaningful in a clinical or practical sense. The range within which two values are considered equivalent is termed the equivalence margin.
 - b. Formulation - Formulate the test and reference products from the same bulk lot of each antigen common to both products. The potency of each common antigen should be the same in both products.
 - c. Serum titer - Use a validated assay that measures an antibody response that has been shown to be related to efficacy.
2. *Design* - Design the study for statistical analysis and inference by accepted equivalence methodology.

- a. Route of administration - Equivalence should be evaluated for each route of administration separately.
- b. Serum collection time - Collect serum near the time of peak response to the reference product. If serum is collected on more than one occasion, adjust for multiplicity by estimating simultaneous confidence intervals for all occasions. Important differences at any time point may be an indication of excessive interference. If there is reason to believe the product's antibody response profiles over time are different, design the study so that the profiles may be directly evaluated.
- c. Extraneous variance - The objective of a serological interference study is to draw conclusions about the immunogenicity of the test product based on the average serological response of vaccinated animals. Such studies may benefit from reducing the impact of assay variance on interval estimates. Studies intended to do so should be designed to include multiple titrations on each serum specimen.

3. *Criteria*

- a. Noninferiority - The serological noninferiority of the test product must be demonstrated. Serological noninferiority means that the expected GMT of the group vaccinated with the test product is not likely to be lower than the expected GMT of the group vaccinated with the reference product by more than the equivalence margin.
- b. Lower margin - Protocols proposing serological equivalence studies must

explicitly state the noninferiority margin. For studies of component interference, use a 63% margin, which aims to show that the test product GMT is at least 63% of the reference product GMT, unless another criterion is justified. A 63% titer ratio corresponds to a difference of about two thirds of a twofold dilution in a serial dilution assay. (The 63% margin does not necessarily apply to applications other than component interference studies.)

- c. Confidence - For studies of component interference, use a 0.05 level of significance and a confidence interval based on one minus twice the significance level, i.e. a 90% confidence interval. For noninferiority, compare only the lower end of the confidence interval to the lower end of the equivalence margin. For full equivalence, no part of the confidence interval should lie outside the equivalence margin.
 - d. Lack of seroconversion - If more than a few animals in a group do not seroconvert, it is not appropriate to estimate the group GMT. In that case, the presence of many nonresponders indicates the need to evaluate whether or not serology is suitable to evaluate component interference. If a serological comparison is considered valid because the distribution of serum titers is typical of the product, use statistical methods that are appropriate for the nature and distribution of the data. (For example, a mixture model or a nested model which simultaneously evaluates the fraction of animals that seroconvert and the GMT of those animals that have seroconverted.)
4. *Field Studies* - The variability of the humoral response in some cases may require that a serological equivalence study include more subjects than available for an experimental study. Serum derived from appropriately designed field studies may be used to study serological equivalence. For example, subjects in a field safety trial may be randomized to new and existing products. While such subjects would not necessarily be seronegative, they would emulate the target population as well as the rest of the field safety study sample.

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