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United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM 800.201

TO: VS Management Team (VSMT)
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: John R. Clifford /s/ Jerry W. Diemer for
Deputy Administrator
Veterinary Services

SUBJECT: General Licensing Considerations: Backpassage Studies

I. PURPOSE

This guideline provides information and recommendations about the design and conduct of backpassage studies 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 9 CFR 102.5 and 104.5.

Although this guideline represents current policy regarding reversion to virulence studies, it does not confer rights for, or on, any person and does not operate to bind APHIS or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

II. CANCELLATION

This memorandum cancels Veterinary Services Memorandum 800.201, dated February 22, 2000.

III. BACKGROUND

The Center for Veterinary Biologics-Policy Evaluation and Licensing (CVB-PEL) requests that license and permit (for distribution and sale) applicants conduct backpassage studies to evaluate the stability of Master Seeds for conventional modified live or live recombinant vaccines to provide assurance that such vaccine microorganisms will not revert to virulence when administered to the host animal. Live vaccines are those that may be capable of replication in the target animal, stimulate a useful immune response, and generally cannot be completely characterized by chemical and physical tests alone. This policy is consistent with the International Cooperation on Harmonization of Technical Requirements for the Registration of Veterinary Medicinal Products (VICH) requirements for licensure of live vaccines. VICH Guideline 41, *Examination of live veterinary vaccines in target animals for absence of reversion to*

virulence, outlining the requirements to demonstrate a lack of reversion to virulence is attached to this memorandum as Appendix 1. One objective of VICH is to promote harmonization of regulatory requirements for veterinary medicinal products by reducing the differences in technical requirements for such products among regulatory agencies in different countries. As a VICH member, APHIS is committed to seeking scientifically based harmonized technical requirements for veterinary biological products. VICH has adopted a reversion to virulence test guideline to provide a unified standard for government regulatory bodies to facilitate the mutual acceptance of reversion to virulence data by relevant authorities. This guideline has been developed under the principles of the VICH and will provide a unified standard for the European Union (EU), Japan, and the United States (US) to facilitate the mutual acceptance of clinical data by the relevant regulatory authorities. This guideline was developed with consideration of the current practices in the EU, Japan, and the US together with those of Australia and New Zealand. If a study is conducted as per VICH guidelines, or per the guidelines in Veterinary Services Memorandum 800.201, the results should be acceptable to APHIS to demonstrate lack of reversion to virulence.

Backpassage studies consist of successively propagating vaccine Master Seed through a series of backpassages *in vivo*. Applicants administer the Master Seed microorganism to a group of susceptible host animals, and after an appropriate incubation time, recover the microorganism from these animals and administer it to a second group of susceptible host animals. Applicants should conduct a minimum of five such successive passages.

IV. GUIDELINES

A. General

1. *Study Protocols* - Applicants should submit a detailed protocol, including the criteria for determining reversion, for CVB-PEL review before initiating a backpassage study.

2. *Preliminary Data* - The CVB recommends that applicants submit preliminary data from studies conducted to evaluate the route of administration and procedures for recovery and to assess the expected rate of recovery of the vaccine microorganism from test animals with the proposed protocol. CVB-PEL will consider the backpassage requirement fulfilled when the applicant confirms preliminary data indicating that the applicant cannot recover the vaccine microorganism from vaccinates by using a group of 10 animals in a follow-up study performed as outlined in Section IV.B of this guideline.

3. *Passage Procedures* - In progressing from one backpassage to the next, applicants may concentrate recovered material between passages but are prohibited from *in vitro* propagation between passages.

4. *Study Animals* - Applicants should conduct the backpassage studies using the most susceptible species, age, and sex of animal that is in the product's label recommendations. These test animals should also be susceptible (seronegative) to the vaccine microorganism being tested.

5. *Combining Backpassage Studies with Shed-and-Spread Studies* - If the route of administration for backpassage studies determined from preliminary work is the same as the route of administration recommended on the label, applicants may expand backpassage studies to also collect data on shed and spread of the vaccine microorganism; otherwise, CVB-PEL will require a separate shed-spread study.

B. First Backpassage

1. *Route of Administration* - Administer the vaccine Master Seed to a group of host animals by the route most likely to lead to replication and to reversion of the microorganism to virulence.

2. *Numbers of Animals* - Use two to five animals, as needed, to ensure reisolation and continued backpassage (see table on probability of reisolation). Use 10 animals to confirm failure to recover the vaccine microorganism from a preliminary study (see section IV. A. 2. above).

3. *Dosage* - Administer test animals at least a typical vaccine dose (not an immunogenicity test dose). A typical vaccine dose would be formulated at a titer that would be above the targeted release dose of the product and would include overage for expected loss of titer over dating and testing variation.

4. *Recovery of the Microorganism* - After a time-period consistent with the pathophysiology of the progression of the disease in a naturally infected animal, attempt to recover the vaccine microorganism from the most appropriate tissues or secretions collected from treated animals.

C. Successive Backpassages

1. *Passage Procedures* - Administer recovered material (pooled material is acceptable) from animals in the preceding treatment group to animals in successive groups by the same route as in the first passage.

2. *Number of Animals for Each Successive Passage* - Based on the expected rate of recovery, treat two to five animals as needed to provide a high probability of reisolation.

3. *Observations* - Observe treated animals for clinical signs indicative of reversion of the vaccine strain to virulence. Clinical signs that indicate administration of the material caused adverse effects to the animal should be assessed.

4. *Number of Passages* - Make at least five backpassages (four successive backpassages beyond the first backpassage).

5. *Maintenance Period* - Maintain test animals from the last backpassage group for at least 21 days after administration of the recovered microorganism, unless otherwise justified.

6. *Characterization* - Characterize the microorganism isolated from the last backpassage phenotypically and/or genotypically and compare it with the Master Seed to evaluate genetic stability and reversion to virulence.