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United States  
Department of  
Agriculture

## VETERINARY SERVICES MEMORANDUM 800.201

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

**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*



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*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.

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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.

# **TARGET ANIMAL SAFETY: EXAMINATION OF LIVE VETERINARY VACCINES IN TARGET ANIMALS FOR ABSENCE OF REVERSION TO VIRULENCE**

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## **1. INTRODUCTION**

The absence of reversion to or increase in virulence test is generally an essential requirement for the registration or licensure of live vaccines in the EU, Japan and the USA. International harmonization of this test will minimize the need to perform separate studies for regulatory authorities of different countries. Appropriate international standard methods will reduce research and development costs by avoiding, whenever possible, duplication of tests. Animal welfare will benefit because fewer animals will be needed by eliminating repetition of similar tests in each region.

This guideline has been developed under the principle of VICH and will provide a unified standard for government regulatory bodies to facilitate the mutual acceptance of reversion to virulence data by the relevant authorities. The use of this VICH guideline to support registration of a product for local distribution only is strongly encouraged but is up to the discretion of the local regulatory authority. Furthermore, it is not always necessary to follow this guideline when there are scientifically justifiable reasons for using alternative approaches.

### **1.1. Objective**

This guideline establishes agreed criteria and requirements for the conduct of studies that examine the potential for reversion to or increase in virulence of live veterinary vaccines in target animals.

### **1.2. Scope and General Principle**

This guideline is intended to cover live vaccines. Live vaccines <sup>(1)</sup> 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. The guideline covers the following species: bovine, ovine, caprine, feline, canine, porcine, equine, poultry (chickens and turkeys). This guideline will not provide information for the design of tests in other species including aquatic animals. For other species, tests should be designed following local guidance. Guidance on laboratory tests to determine adequate attenuation of the vaccine strain is not within the scope of this guideline.

( 1 ) In case of vector vaccines this only covers vector vaccine seeds that replicate in the target species.

## 2. STUDY DESIGN

This study is carried out using the master seed. If the quantity of the master seed sufficient for testing is not available, the lowest passage seed used for production that is available in sufficient quantity should be examined. Use of another passage option must be justified. Generally, serial passages should be made in target animals through five groups of animals, unless there is justification to make more passages or the organism disappears from the test animals sooner. The time interval between inoculation of the animal and harvest for each passage must be justified based upon the characteristics of the test organism. If recovery is successful, passages should continue through five groups of animals. Appropriate methods, preferably *in vitro* propagation, should be used to confirm the presence and to determine the number of the test organisms at each passage. *In vitro* propagation may not be used to expand the passage inoculum.

Where a reasonable explanation for the sudden loss of the organism exists, e.g. experimental error, the previous passage may be repeated. When the organism is not recovered from any intermediate *in vivo* passage, a reasonable attempt should be made to repeat the test in 10 animals (90% probability of isolating the organism at 20% probability of recovery – see Appendix) using *in vivo* passaged material from the last passage in which the organism was recovered. If the target organism is recovered from one or more animals in the repeat test, the passages should continue using the material recovered in the repeat test as the inoculum for the next passage. The repeat test will be counted as a passage. If the target organism is not recovered, the experiment is considered to be completed with the conclusion that the target organism does not show an increase in or reversion to virulence.

Generally, for each target species, the most sensitive class, age, sex and serological status of animals should be used. In cases where alternative approaches are used, alternatives should be justified. Generally, a minimum of two animals is used for the first four groups and a minimum of eight animals is used for the fifth group.

Housing and husbandry should be adequate for the purpose of the study and conform to local animal welfare regulations. Animals should be appropriately acclimatized to the study conditions. Appropriate prophylactic treatment should be completed before the initiation of the study. Reduction or elimination of suffering during the study is essential. Euthanasia and necropsy of moribund animals is recommended.

The initial administration and subsequent passages shall be carried out using a recommended route of administration or natural route of infection that is the most likely to lead to reversion to or increase in virulence and result in recovery of the organism following replication in the animal. The route used must be justified.

The initial inoculum should contain the maximum release titer expected in the recommended dose or, in the cases where the maximum release titer to be licensed is not specified, then a justifiable multiple of the minimum release titer can be used. Passage inocula should be collected and prepared from the most likely source of spread of the organism, unless there is scientific justification to use another material.

General clinical observations should be made during the study. Animals in the fifth group should be observed for 21 days unless otherwise justified. These observations should include all relevant

parameters typical for the disease which could indicate reversion to or increase in virulence. If signs consistent with the target disease are observed, then causality needs to be investigated. No evidence of an increase in virulence, indicative of reversion, should be seen with passage.

If the fifth group of animals shows no evidence of an increase in virulence indicative of reversion during the observation period, further testing is not required. Otherwise, materials used for the first passage and the final passage should be used in a separate experiment using at least 8 animals per group to directly compare the clinical signs and other relevant parameters. This study should be done by the route of administration that was used for previous passages. An alternative route of administration may be used if scientifically justified.

When attenuation of a test organism is known to be the result of a well characterized specific marker or genetic change, additional tests using suitable molecular biological methods for comparison of the initial seed organism and the organism recovered from the final passage should be performed, thus confirming the genetic stability of the attenuation marker in the vaccine strain.

If available data or assessment indicate a substantial risk that the test organism may revert to or increase in virulence, additional studies may be required to provide further information on the organism.

Except in exceptional and justified cases, if the completed studies show that the test organism does revert to or increase in virulence after passage in the target animal, the test organism will be deemed to be unsuitable for use as a live vaccine.



### 3. GLOSSARY

**Class.** Subset of target animal species which is characterized by factors such as reproductive status and/or use (dairy vs. beef, broiler vs. layer)

**Master seed.** A collection of aliquots of a micro-organism suspension for use in the preparation of the product, obtained from single culture, distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability.

**Maximum release titer.** The expected highest number of viable organisms allowed per dose in vaccines at the time of release, verified by safety studies. In regions where a maximum release potency is not established, a justifiable multiple of the release antigen content is applied.

**Minimum release titer.** The expected lowest number of viable organisms required per dose in vaccines at the time of release, verified by efficacy and stability data.

**Passage.** Transfer of organisms through a group of inoculated animals, either from the beginning seed material or from a previous passage in animals.

The table gives the probability that the organism will be recovered from at least one animal of a group of inoculated animals. The probability of recovery from a single animal is in the left margin. The number of inoculated animals is in the top margin. The corresponding entry shows the probability that the organism will be recovered from at least one animal in the group.

Probability of Recovery from a Single Animal	Probability of Recovery from At Least One Member of a Group				
	Number of Animals in Group				
	2	3	5	7	10
0.975	0.999	>0.999	>0.999	>0.999	>0.999
0.950	0.997	>0.999	>0.999	>0.999	>0.999
0.925	0.994	>0.999	>0.999	>0.999	>0.999
0.900	0.990	0.999	>0.999	>0.999	>0.999
0.875	0.984	0.998	>0.999	>0.999	>0.999
0.850	0.978	0.997	>0.999	>0.999	>0.999
0.825	0.969	0.995	>0.999	>0.999	>0.999
0.800	0.960	0.992	>0.999	>0.999	>0.999
0.775	0.949	0.989	0.999	>0.999	>0.999
0.750	0.938	0.984	0.999	>0.999	>0.999
0.725	0.924	0.979	0.998	>0.999	>0.999
0.700	0.910	0.973	0.998	>0.999	>0.999
0.675	0.894	0.966	0.996	>0.999	>0.999
0.650	0.878	0.957	0.995	0.999	>0.999
0.625	0.859	0.947	0.993	0.999	>0.999
0.600	0.840	0.936	0.990	0.998	>0.999
0.575	0.819	0.923	0.986	0.997	>0.999
0.550	0.798	0.909	0.982	0.996	>0.999
0.525	0.774	0.893	0.976	0.995	0.999
0.500	0.750	0.875	0.969	0.992	0.999
0.475	0.724	0.855	0.960	0.989	0.998
0.450	0.698	0.834	0.950	0.985	0.997
0.425	0.669	0.810	0.937	0.979	0.996
0.400	0.640	0.784	0.922	0.972	0.994
0.375	0.609	0.756	0.905	0.963	0.991
0.350	0.577	0.725	0.884	0.951	0.987
0.325	0.544	0.692	0.860	0.936	0.980
0.300	0.510	0.657	0.832	0.918	0.972
0.275	0.474	0.619	0.800	0.895	0.960
0.250	0.437	0.578	0.763	0.867	0.944
0.225	0.399	0.535	0.720	0.832	0.922
0.200	0.360	0.488	0.672	0.790	0.893
0.175	0.319	0.438	0.618	0.740	0.854
0.150	0.277	0.386	0.556	0.679	0.803
0.125	0.234	0.330	0.487	0.607	0.737
0.100	0.190	0.271	0.410	0.522	0.651
0.075	0.144	0.209	0.323	0.421	0.541

0.050	0.097	0.143	0.226	0.302	0.401
0.025	0.049	0.073	0.119	0.162	0.224

Probability of Recovery from At Least One Animal of a Group of Inoculated Animals

