



Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

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CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 17-06

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

FROM: Byron E. Rippke
Director

SUBJECT: Option to Remove Back-titration Hamsters from In Vivo Potency Tests for
Leptospira Serogroups Pomona and Grippotyphosa

*Re-signed on
February 5, 2026
with the inclusion
of the footer below*

I. PURPOSE

The purpose of this Notice is to inform interested parties that the Center for Veterinary Biologics (CVB) will allow an exemption from the titration requirement in vaccination-challenge potency assays for *Leptospira* Serogroups Pomona and Grippotyphosa. Removal of the back-titration hamsters could reduce animal use by 50 percent when potency testing these two fractions.

II. BACKGROUND

The codified potency tests for *Leptospira* Pomona Bacterins and *Leptospira* Grippotyphosa Bacterins are hamster vaccination-challenge assays described in title 9, *Code of Federal Regulations* (9 CFR), parts 113.101 and 113.104 respectively. The leptospiral codified potency tests have been targeted by the CVB as part of our ongoing commitment to refine, reduce, and replace animal testing. CVB Notice No. 15-13 already allows the option to remove back-titration hamsters from *in vivo* potency tests for *Leptospira* Serogroups Canicola and Icterohaemorrhagiae. This Notice expands the option to include the remaining codified leptospiral potency assays.

Currently, a valid test requires $\geq 80\%$ (8/10) of challenge control hamsters to succumb to disease and an LD₅₀ between 10 and 10,000. The minimum LD₅₀ requirement is met if at least 80% of challenge controls succumb to disease; the back-titration hamsters are not required to confirm a minimum challenge dose is administered. The maximum LD₅₀ constraint has been based on 1) reports that subpotent bacterins may occasionally test potent when $\geq 10,000$ LD₅₀ is administered and 2) historical concerns that a potent serial may be deemed subpotent when challenged with a higher concentration of *Leptospira* than normally seen in the field.

Tests were conducted at the CVB to evaluate the risk associated with the removal of the LD₅₀ limit. An increased risk of either a subpotent serial release or a potent bacterin being deemed unsatisfactory was not specifically identified with removal of the LD₅₀ requirement. Notably, the calculated LD₅₀ often did not correlate to

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Leptospira concentration, suggesting the LD₅₀ requirement may not be a functional validity requirement.

III. ACTION

Firms may request an exemption from the titration requirement for the vaccination-challenge potency assays for *Leptospira* Serogroups Pomona and Grippityphosa when using the CVB supplied strains. The CVB will accept the following alternative in the Outline of Production:

A 9 CFR vaccination-challenge two-stage assay *without* back-titration hamsters.

1. Vaccinated hamsters and challenge control hamsters will be tested according to the 9 CFR specifications except the LD₅₀ of the challenge will not be monitored.
2. Any serial that is deemed UNSAT cannot be retested due to concerns of an overchallenge.

The potency assays outlined in 9 CFR 113.101 and 113.104 remain acceptable if the firm prefers not to request an exemption from the titration requirement. If a firm chooses to test by the codified methods, all tests in which the challenge exceeds 10,000 LD₅₀ will be considered invalid.

IV. IMPLEMENTATION

According to 9 CFR 113.4, licensees and permittees must request an exemption to the codified hamster potency tests if they are interested. The Outline of Production must specify the backtitration exemption for each fraction. This policy is effective at the receipt of this notice.