



Animal and Plant
Health Inspection
Service

Veterinary Services

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VETERINARY SERVICES MEMORANDUM NO. 800.102

December 12, 2013

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

FROM: John R. Clifford /s/ Jack A. Shere, for
Deputy Administrator

SUBJECT: Exemption from *Leptospira* Bacterin Testing Under 9 CFR
113.101-104 and the Associated References and Studies

I. PURPOSE

This memorandum provides guidance for obtaining an exemption to the requirement for testing *Leptospira* bacterins for potency in hamsters. It also provides information on alternative *in vitro* potency assays and reagents available from the Center for Veterinary Biologics (CVB).

II. REPLACEMENT

This memorandum replaces Veterinary Services (VS) Memorandum No. 800.102, dated May 23, 2002. In addition, it compiles information on policies and reagents supplied in the following CVB notices:

1. CVB Notice No. 07-02, "Qualification of *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* Reference Bacterins for Products Intended for Use in Dogs," dated March 1, 2007.
2. CVB Notice No. 07-12, "Qualification of *Leptospira pomona* and *Leptospira canicola* Reference Bacterins for Products Intended for Use in Dogs," dated July 13, 2007.
3. CVB Notice No. 09-16, "Qualification of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira icterohaemorrhagiae*, and *Leptospira pomona* Reference Bacterins for Products Intended for Use in Swine and/or Cattle," dated August 3, 2009.

III. BACKGROUND

Of the several animal models established for leptospirosis, hamsters are commonly used due to their high susceptibility to infection. The hamster model is used in *Leptospira* bacterin potency tests codified as Standard Requirements in title 9, *Code of Federal Regulations* (9 CFR), parts 113.101-104. The codified

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tests utilize a vaccination challenge format in a hamster model. Product is diluted based on the host animal dose volume. The route used is described as “in accordance with label recommendations for use.”

The CVB considers exemptions to codified tests, per 9 CFR 113.4 (a), if the replacement test is at least equivalent to the Standard Requirement and the test methods are described in the filed Outline of Production for the product.

In keeping with its commitment to replace animal tests with *in vitro* tests, enzyme-linked immunoassays (ELISA) and associated reagents for potency testing *Leptospira* products have been developed by the CVB. Test details are provided in Supplemental Assay Methods (SAM) 624-627, and reagents are available per VS Memorandum No. 800.97. We encourage firms to obtain exemptions to use the ELISA tests.

The ELISAs require a suitably qualified reference bacterin. The CVB supplies non-adjuvanted *Leptospira* reference bacterins that may be used to test products intended for use in dogs, cattle, or swine. The stability of the Standard Reference Bacterins will be monitored by the CVB via performance in the relevant codified hamster test. New Standard Reference Bacterins will be qualified using the relevant codified hamster test. The availability of Standard Reference Bacterins does not preclude the use of a suitable qualified alternative reference developed by the licensee, and an alternative reference may be necessary for certain products. Qualifying a new reference bacterin typically involves the demonstration that the reference is acceptably efficacious in a host animal vaccination-challenge model. A surrogate animal model may be used in lieu of host animal studies to qualify a reference (not for pivotal efficacy), provided that the surrogate animal model is deemed to be an acceptable indicator of efficacy in the host animal. The CVB conducted studies to confirm that the codified hamster test is a suitable surrogate to evaluate the potency of the following *Leptospira* fractions in dogs, cattle, and swine: *Leptospira* serogroups *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, and *pomona*.

IV. POLICY

- A. Host Animal Efficacy Studies. Although the codified hamster test is considered to be correlated to host animal efficacy, in most cases it does not reflect the natural transmission of the pathogen in the host animal. Therefore, the CVB requires host animal efficacy studies to license products containing new Master Seed Bacterins (MSB) or to evaluate substantial production method changes. The antigen input of the efficacy serial should be characterized in objective terms (e.g., organism counts prior to inactivation), not as potency relative to a reference, and the efficacy serial should also pass the hamster test. If these conditions are met, subsequent reference bacterins prepared to contain at least as much antigen as the efficacy serial may be qualified using the relevant codified hamster test. Summarize host animal efficacy studies in section V.C of the Outline of Production, and include the challenge strain identified to the serogroup.

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- B. Codified Hamster Test. The route used in the hamster potency test should be consistent with the label recommendations for the host animal.

Host Animal routes		Codified Hamster Test routes	
Efficacy study	Labeling	Hamster vaccination route for Serial release	Hamster Vaccination route for ELISA reference maintenance
IM	IM	IM	IM
SC	SC	SC	SC
IM & SC	IM & SC	IM or SC but must specify in Outline	IM or SC but must specify in Outline
IM & SC	SC	SC	SC
IM & SC	IM	IM	IM

When the hamster test is used, we encourage firms to use humane end points wherever possible.

- C. Exemptions to use ELISA based tests. The licensee or permittee must request an exemption to tests prescribed in 9 CFR 113.101-104. The ELISA procedure must be described in the relevant Outlines. To maintain the exemption, the minimum antigen content specified in section IV of the Outline must not be reduced from that established at licensing. Prelicense products must meet all current requirements for establishment of minimum antigen content. Cite the exemption as described in VS Memorandum No. 800.206.

Submit the following supporting information to request an exemption to codified potency testing:

1. *MSB*. Identify the MSB to the serogroup level to ensure the reagents utilized in the ELISA protocol are appropriate.
2. Production methods should be detailed to ensure significant changes can be noted.
3. Validate the performance of the ELISA with the specific product for which the exemption is sought. Follow the guidance in VS Memorandum No. 800.112 to generate specificity, reproducibility, and parallelism data. *Leptospira* bacterins do not react identically in the ELISA; firms with some data collected to validate the test may submit preliminary data for CVB review and comment.

- D. Identification of a reference bacterin. The need for a qualified reference may be addressed by one of the following methods, provided the potency assay can be acceptably validated using the proposed reference, as indicated above.
1. *Standard Reference Bacterin*. Use the Standard Reference Bacterin supplied by the CVB at the recommended working dilution. The Standard Reference Bacterin can be used to release product labeled as an aid in the prevention of disease due to the applicable *Leptospira* in dogs, cattle, or swine.
 2. *Product-matched Reference Bacterin (qualified by hamster test)*. Produce a product-matched reference bacterin containing at least as much antigen input as the efficacy serial and qualify it via the codified hamster potency test. Submit data from at least three independent codified tests (beginning with three separate vials of product). If the reference will be used to test products that are recommended for subcutaneous injection, vaccinate the hamsters subcutaneously. Suitable references should repeatedly generate satisfactory results in the codified test. All candidate reference preparations will be subject to confirmatory testing by 9 CFR 113.101-104, as appropriate, before final Animal and Plant Health Inspection Service approval is granted.

This approach may be required for adjuvanted products that do not demonstrate acceptable parallelism with the non-adjuvanted Standard Reference Bacterin in the potency assay. References qualified in this manner can be used to release product labeled as an aid in the prevention of disease due to the relevant *Leptospira* in the relevant host animal.
 3. *Host qualified reference bacterin*. If data submitted to support the hamster test is not appropriate for a product, this may be considered by the CVB on a case-by-case basis. For those references which are not suitable for monitoring in hamster assay, the reference should be requalified in the host animals at regular intervals of five years.
- E. Valid Challenge Ranges. When the hamster vaccination challenge tests are conducted via 9 CFR 113.101-104, a range of 10-10,000 hamster LD50 is codified. Valid test results are not obtained at challenge doses above 10,000 LD50. This may be due to an immunizing effect or the induction of a chronic disease state. Over-challenges are considered a no test and must be repeated. No changes to this policy will be considered unless additional guidance is released.
- F. Stability Monitoring of References
1. Standard Reference Bacterins will be monitored by CVB utilizing the hamster test.

2. Firm's reference bacterins should be firm monitored using trend analysis and periodic reevaluation (approximately every 2.5 years) using the codified hamster test. The CVB may monitor the firm's reference bacterins at their discretion via the codified hamster potency assay. For products unable to be monitored by the hamster laboratory model, please refer to section IV.D.3.

V. IMPLEMENTATION/APPLICABILITY

This memo is applicable immediately and applies to all *Leptospira* bacterins with codified potency tests and intended for use in dogs, cattle, or swine. It applies to serials manufactured after the signature date of this memo. Firms not in compliance with this memorandum should contact their assigned reviewer to propose alternative strategies.