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CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 07-02

Veterinary Services

Center for Veterinary
Biologics

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Subject: Qualification of *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* Reference Bacterins for Products Intended for Use in Dogs

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

I. PURPOSE

The purpose of this notice is to inform interested parties of the procedures that may be used to qualify Reference Bacterins for *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* that may be used to test products intended for use in dogs. It also announces the availability of Standard Reference Bacterins distributed by the CVB. These Standard Reference Bacterins are available to all licensees, permittees, and applicants to use under specified conditions in the *in vitro* potency assays described in Supplemental Assay Methods (SAM) 626 and 627. The availability of these standard references does not preclude the use of a suitably qualified alternative reference developed by the licensee.

II. BACKGROUND

The codified potency tests for *Leptospira Grippotyphosa* Bacterin and *Leptospira Icterohaemorrhagiae* Bacterin are hamster vaccination-challenge assays described in Title 9, Code of Federal Regulations (9 CFR), Parts 113.104 and 113.102, respectively. These tests have been targeted by the CVB for replacement as part of the CVB's ongoing commitment to refine, reduce, and replace animal testing.

Enzyme-linked immunosorbent assays (ELISAs) that quantify protective antigens of *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* have been developed by the CVB and are described in SAM 626 and 627, respectively. The ELISAs require the use of a suitably qualified reference bacterin.

Qualifying a new reference bacterin typically involves a 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, provided that the surrogate animal model is deemed to be an acceptable indicator of efficacy in the host animal.



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The CVB conducted studies to determine whether the codified hamster potency test is a suitable surrogate to evaluate the efficacy of *L. grippityphosa* and *L. icterohaemorrhagiae* in dogs. Serials formulated to meet only the minimum potency requirements in hamsters were found to be acceptably efficacious in dogs (data available upon request). These data give the CVB confidence that the hamster test can be used as a surrogate for host animal vaccination-challenge to qualify references for *L. grippityphosa* and *L. icterohaemorrhagiae* bacterins for canine products (i.e., serials passing the hamster potency test will also be efficacious in dogs). Thus, the CVB will consider, under the conditions described in Section III below, references qualified by the hamster potency test.

To further facilitate conversion to *in vitro* testing, the CVB has qualified the following Standard Reference Bacterins for use with SAM 626 or 627:

- IRP 523 (05): *Leptospira Grippityphosa* Standard Reference Bacterin (Lot NRS042805LG)
- IRP 542 (06): *Leptospira Icterohaemorrhagiae* Standard Reference Bacterin (Lot A80752)

These Standard Reference Bacterins are non-adjuvanted preparations, which may be suitable to serve as working references for certain *Leptospira* Bacterins labeled for use in dogs. These Standard Reference Bacterins may be used in serial release testing under the conditions described in Section III below.

III. POLICY

Under 9CFR 113.4, licensees and permittees must request an exemption to the codified hamster potency tests in order to use the ELISAs. Requests for exemption from 9CFR 113.102 and/or 9CFR 113.104 should contain the following:

A. 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 (see Section III.B):

1. 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 *L. grippityphosa* or *L. icterohaemorrhagiae* in dogs.

The stability of the Standard Reference Bacterin will be monitored by the CVB via performance in the codified hamster potency test. New Standard Reference Bacterins will be qualified using the codified hamster test.

2. Produce a product-matched reference bacterin and qualify it via the codified hamster potency test. Submit data from at least 3 independent codified tests. If the reference will be used to test products that are recommended for subcutaneous injection, then the hamsters in the potency test also should be vaccinated 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 113.102 and/or 9CFR 113.104 before final APHIS 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 *L. grippotyphosa* or *L. icterohaemorrhagiae* in dogs.

3. Produce a product-matched reference bacterin and qualify it via a dog vaccination-challenge study acceptable to the CVB. This approach is required to qualify a reference used to release product bearing label claims more stringent than “aids in the prevention of disease” or other non-standard label claims.

Dog vaccination-challenge studies are required to qualify references that do not test satisfactorily by the codified hamster potency test. In such cases, the dog vaccination-challenge study should be designed to demonstrate that the reference generates immunity of acceptable duration (i.e., supports the recommended revaccination interval). Dog vaccination-challenge studies also may be required by the CVB to demonstrate the efficacy of product-matched references for new/novel *Leptospira* Bacterin formulations.

B. Assay Validation Data

Each ELISA must be validated for use with the proposed reference bacterin and the products it will be used to test. Firms are encouraged to submit a validation protocol to the CVB for review and comment prior to initiation of the validation effort.

1. *Specificity data*: Provide data demonstrating that the antibodies used in the ELISA specifically react with the Master Seed strains of the same serovar (*L. grippotyphosa* or *L. icterohaemorrhagiae*) used in the test product(s). These antibodies should not react with any other product component, including bacterial antigens, growth media, or adjuvant. Specificity data should be generated by testing experimental products, made in accordance with the Outline of Production except that the relevant *Leptospira* serovar is not added.

2. *Reproducibility*: Demonstrate that the assay, when conducted by the licensee or permittee, produces reproducible results. Test at least three serials of each product that will be assessed for potency by the ELISA.

Test each serial in at least six independent replicate assays. Assays should be performed by a minimum of two different individuals. Data from a minimum of 18 tests (3 replicates of 3 serials by each of 2 technicians) per product should be submitted.

3. *Dose-response data*: Demonstrate that the ELISA is capable of discriminating between products formulated with the relevant *Leptospira* serovar when the antigen content is $\pm 10\%$ and $\pm 20\%$ of the proposed minimum release value.

4. *Parallelism*: Submit data to demonstrate the degree of parallelism between the reference and each product that will be tested. Data from dilutions delineating the entire dose-response curve (antigen saturation, linear portion, antigen extinction) should be submitted.

C. Applicability of Exemption

For a transition period of 5 years, serials tested against *hamster-qualified* references will continue to be subject to CVB confirmatory testing by 9 CFR 113.102 and/or 9CFR 113.104. The disposition of all such serials tested by the CVB will be based solely on the 9 CFR 113.102 and/or 9CFR 113.104 potency test results. This transition period will be used for ongoing quality assurance.

In the case of currently licensed products tested by *hamster-qualified* references, the minimum antigen content specified in Section IV of the Outline of Production must not be reduced from that established at licensing. Prelicense products must meet all current requirements for establishment of minimum antigen content.

IV. ACTION

Licensees, permittees, and applicants wishing to obtain *Leptospira Grippotyphosa* Standard Reference Bacterin IRP 523 (05) and/or *Leptospira Icterohaemorrhagiae* Standard Reference Bacterin IRP 542 (06) should make a request to the CVB according to the procedures established in Veterinary Services Memorandum 800.97. The Memorandum, which includes a copy of the request form, may be obtained from the CVB website (www.aphis.usda.gov/vs/cvb) or by contacting the CVB.

SAMs 626 and 627 are also available on the CVB website or by contacting the CVB.

/s/Richard E. Hill, Jr.

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