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CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 09-16

**United States
Department of
Agriculture**

Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

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

FROM: Richard E. Hill, Jr. /s/ Byron E. Rippke, for
Director
Center for Veterinary Biologics

SUBJECT: Qualification of *Leptospira Canicola*, *Leptospira Grippytyphosa*,
Leptospira Icterohaemorrhagiae, and *Leptospira Pomona* Reference
Bacterins for Products Intended for Use in Swine and/or Cattle

I. PURPOSE

The purpose of this notice is to inform interested parties of the procedures used to qualify Reference Bacterins for *Leptospira canicola*, *L. grippytyphosa*, *L. icterohaemorrhagiae*, and *L. pomona* which may be used to test products intended for use in swine and/or cattle. 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) 624, 625, 626, and 627 for testing swine and/or cattle products. The availability of these standard references does not preclude the use of a suitably qualified alternative reference developed by the licensee or an alternative validated potency test acceptable to the Center for Veterinary Biologics (CVB).

II. BACKGROUND

The codified potency tests for *Leptospira Canicola* Bacterins, *Leptospira Grippytyphosa* Bacterins, *Leptospira Icterohaemorrhagiae* Bacterins, and *Leptospira Pomona* Bacterins are hamster vaccination-challenge assays described in Title 9, Code of Federal Regulations (9 CFR), Parts 113.103, 113.104, 113.102, and 113.101, 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. Two previous notices, CVB Notice Nos. 07-02 and 07-12, announced the availability of these Standard Reference Bacterins distributed by the CVB in conjunction with their use in testing canine leptospira bacterins.

Enzyme-linked immunosorbent assays (ELISAs) that quantify protective antigens of *Leptospira canicola*, *L. grippytyphosa*, *L. icterohaemorrhagiae*, and *L. pomona* have been developed by the CVB for use as serial release tests and are described in SAM 625,



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CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 09-16

Page 2

626, 627, and 624, 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.

The CVB conducted studies to determine whether the codified hamster potency test (utilizing either the IM or SC route) is a suitable surrogate to evaluate the efficacy of canine leptospira bacterins and found this to be the case. This was then tested for swine products and again found to be an acceptable surrogate. Serials formulated to meet only the minimum potency requirements in hamsters were found to be acceptably efficacious in swine. These data give the CVB confidence that the hamster test can be used as a surrogate for host animal vaccination-challenge studies to qualify references for swine and cattle, as well as 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 625, 626, 627, or 624, respectively:

- IRP 524(05): Leptospira Pomona Standard Reference Bacterin (Lot # 061505LP)
- IRP 555(07): Leptospira Canicola Standard Reference Bacterin (Lot # 82518)
- IRP 523(05): Leptospira Grippityphosa Standard Reference Bacterin (Lot # 061505LG)
- IRP 542(06): Leptospira Icterohaemorrhagiae Standard Reference Bacterin (Lot # A80752)

The CVB will monitor these references over time to ensure their stability. As indicated in the previous notices, these Standard Reference Bacterins are nonadjuvanted preparations, which may be suitable to serve as working references. These Standard Reference Bacterins may be used in serial release testing under the conditions described in Section III below.

III. POLICY

Under 9 CFR 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 9 CFR 113.101, 113.102, 113.103, and/or 113.104 should be addressed to the firm's reviewer and 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 or as an aid in the reduction of disease due to leptospirosis in swine or cattle. The Standard Reference Bacterin will be requalified with the codified hamster potency test at regular intervals by the CVB. New Standard Reference Bacterins will be qualified using the codified hamster test by the CVB.
2. Produce a product-matched reference bacterin and qualify it via the codified hamster potency test. Submit data from at least three 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 113.101, 113.102, 113.103, and/or 113.104 before final APHIS approval is granted. This approach may be required for adjuvanted products that do not demonstrate acceptable parallelism with the nonadjuvanted 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 or as an aid in the reduction of disease due to the four leptospira serotypes in swine and cattle.
3. Produce a product-matched reference bacterin and qualify it via a swine and/or cattle 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. Swine and/or cattle vaccination-challenge studies are required to qualify references that do not test satisfactorily by the codified hamster potency test. In such cases, the swine and/or cattle vaccination-challenge study should be designed to demonstrate that the reference generates immunity of acceptable duration (i.e., supports the recommended revaccination interval on the labeling). Swine and/or cattle 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. canicola*, *L. grippotyphosa*, *L. icterohaemorrhagiae*, or *L. pomona*) 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 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 period of 5 years, serials tested against hamster-qualified references will continue to be subject to CVB confirmatory testing by 9 CFR 113.101, 113.102, 113.103, and/or 113.104 with the reference bacterin at the current use dilution in addition to the test serial. The CVB will conduct the ELISA for correlation purposes, only. The disposition of all such serials tested by the CVB will be based solely on the 9 CFR potency test results. This transition period will be used for ongoing quality assurance. Further guidance on this issue will be provided at a later date. In the case of currently licensed products tested by hamster-qualified

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 09-16

Page 5

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 Standard Reference Bacterins for testing canine, swine, or bovine products should make a request to the CVB according to the procedures established in Veterinary Services Memorandum No. 800.97. The memorandum, which includes a copy of the request form, may be obtained from the CVB website (www.aphis.usda.gov/animal_health/vet_biologics) or by contacting the CVB.

SAMs 624, 625, 626, and 627 are also available on the CVB website or by contacting the CVB.