United States
Department of
Agriculture

Animal and Plant
Health
Inspection
Service

Veterinary
Services

Washington, DC
20250

VETERINARY SERVICES MEMORANDUM NO. 800.104

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

FROM: for Jack A. Shere
Deputy Administrator

BURKE HEALEY Digitally signed by BURKE HEALEY
Date: 2018.12.13 15:20:20 -05'00'

SUBJECT: *In Vitro* Serial Release Potency Test for Completed Product Containing *Clostridium chauvoei*

I. PURPOSE

This memorandum provides guidance to licensees, permittees, and applicants concerning the Center for Veterinary Biologics (CVB) policy for obtaining an exemption to use an *in vitro* potency test in place of the current Standard Requirement (SR) test for releasing serials of product containing *Clostridium chauvoei* antigen.

II. REPLACEMENT

This memorandum cancels Veterinary Services (VS) Memorandum No. 800.104, dated May 29, 2003.

III. BACKGROUND

Currently, CVB requires testing of completed product containing *Clostridium chauvoei* antigen prior to serial release using a guinea pig vaccination-challenge potency test as specified in title 9, *Code of Federal Regulations* (9 CFR), part 113.106. The CVB encourages implementation of *in vitro* testing to replace the guinea pig vaccination-challenge potency test as part of our ongoing commitment to refine, reduce, and replace animal testing.

The CVB-Policy, Evaluation, and Licensing (CVB-PEL) has developed an *in vitro* test for quantifying *C. chauvoei* antigen in completed product, which may be used as a serial release potency test for products containing inactivated *C. chauvoei*. This test is an antibody sandwich enzyme-linked immunosorbent assay (ELISA) that uses *C. chauvoei* flagella-specific monoclonal antibody and *C. chauvoei* flagella-specific polyclonal rabbit antibodies to capture and detect protective flagellar antigen.

The amount of flagellar antigen in the test serial is compared to that in an approved reference preparation using the *in vitro* test developed by CVB. The protocol, designated BBPRO0220 and entitled "Potency Testing of *Clostridium chauvoei* Bacterins using an ELISA Procedure," is available onCVB web site and may also be requested directly from CVB-PEL.

IV. POLICY

A. Exemption Request

According to 9 CFR 113.4, the licensee or permittee must comply with the test methods and procedures contained in all applicable SRs, unless the CVB-PEL has provided an exemption, and must cite any exemption in the filed Outline of Production for the product. The licensee or permittee submits a letter to CVB-PEL requesting an exemption from 9 CFR 113.106 SRs for *Clostridium chauvoei* Bacterin. CVB-PEL will consider granting an exemption request if the firm has performed all of the following:

1. Test validation data

   a. *Products*. The ELISA is validated for testing the specific products for which the test is intended and in accordance with VS Memorandum No. 800.112.

   b. *Correlation data*. The ELISA correctly identifies potent and subpotent serials when compared to the SR guinea pig vaccination-challenge test. The firm may submit data generated by testing diluted products used for reference qualification as part of the test correlation data.

2. Changes to the Outline of Production

   a. *Test method*. The BBPRO0220 procedure or an acceptable alternative ELISA procedure for quantifying the flagellar antigen of *C. chauvoei* is described in section V.C. of the Outline of Production or a Special Outline.

   b. *Information regarding reference preparations.* The identity, stability monitoring protocol, and expiration date of the approved reference preparation is included in section V.C. of the Outline of Production.

   c. *Test criteria*. The requirements for a satisfactory test, along with validity requirements, are included in section V.C. of the Outline of Production. If specified in section V.C. of the Outline of Production, the CVB-PEL allows the firm to use the guinea pig vaccination-challenge test described in the SR as a second-stage test on serials failing the ELISA potency test. The firm must designate the ELISA test results as “Inconclusive” if the guinea pig vaccination-challenge test is used for second-stage testing.

   d. *Date of exemption*. The exemption to the SR test is documented in section V.C. of the Outline of Production in accordance with 9 CFR 113.4 and VS Memorandum No. 800.206.

3. Qualified reference data

   The firm has described the methodology for qualifying a reference. Section IV.B of this document describes the methodology required.

B. Qualification of *C. chauvoei* Reference for *in vitro* Potency Assay

1. *Initial reference qualification*. The initial reference qualification is applicable to products for which the *in vitro* potency assay has not previously been approved for serial release. The firm must make qualifying serials for all proposed reference preparations according to a filed Outline of Production. The firm must prove the efficacy of references by a vaccination-challenge study that includes at least 10 vaccinated and 5 control cattle. All cattle are challenged with a virulent *C. chauvoei* spore suspension. If at least 8 of 10 of the vaccinated cattle survive the challenge, and at least 4 of 5 of the control cattle die as a result of the challenge, the reference is satisfactory. CVB-PEL highly recommends evaluation in guinea pigs according to 9 CFR 113.106, as described below, concurrently with the initial reference qualification study in cattle to establish a guinea pig 50% protective dose (PD_{50}).

   The firm establishes the PD_{50} of the reference preparation in guinea pigs using the vaccination-challenge methods outlined in Supplemental Assay Method 200 for Potency Testing Products Containing *Clostridium chauvoei* Antigen. The firm vaccinates groups of at least 5 guinea pigs with fractional doses of the reference preparation, using serial dilutions no greater than two-fold. CVB-PEL prefers the firm to establish the PD_{50} determination using the geometric mean of at least 5 independent assay replicates. CVB-PEL allows the firm to use the established guinea pig PD_{50} as a standard to requalify and/or monitor existing approved reference preparations and to qualify new reference preparations.

2. *Reference qualification or requalification of previously approved reference*. If a product has an established guinea pig PD_{50}, CVB-PEL allows the firm to use the guinea pig vaccination-challenge for future reference qualification and requalification of an approved reference. If the firm has not established a guinea pig PD_{50}, CVB-PEL requires the firm to perform future reference qualification and requalification of an approved reference in cattle.

C. Confirmatory Testing

CVB-PEL may conduct confirmatory testing on serials according to 9 CFR 113.106 after approval of the *in vitro* potency assay.

V. IMPLEMENTATION/ APPLICABILITY

Firms wishing to use the ELISA test for serial release should request an exemption through their assigned reviewer. The request should be accompanied by the data and documents described above.