Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Bacterial Plate Count of *Erysipelothrix rhusiopathiae* Vaccines

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Erysipelothrix rhusiopathiae Vaccines

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1. Introduction

1.1 Background

This is a Supplemental Assay Method (SAM) for the analysis of *Erysipelothrix rhusiopathiae* vaccine, live culture, to determine the colony-forming units (CFU) in final container samples, as prescribed in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.67. This method uses 5% bovine blood agar and 1% peptone saline as a diluent.

1.2 Keywords

Erysipelothrix rhusiopathiae, potency test

2. Materials

2.1 Equipment/instrumentation

2.1.1 Vortex mixer

2.1.2 Colony counter

2.1.3 Inoculum spreader

2.1.4 Bunsen burner

2.1.5 Disposable syringes and needles--appropriate sizes

2.1.6 Sterile disposable pipettes--appropriate sizes

2.1.7 Sterile screw-capped (sc) culture tubes, 20 x 150 mm

2.1.8 Pipetting aid

2.1.9 35° ± 2°C incubator

2.1.10 Biosafety cabinet

2.1.11 Gloves and colored lab coat or frock

2.1.12 Sterile gauze pads, 4 x 4 in

2.1.13 Test tube rack

2.2 Reagents/supplies

2.2.1 1% Peptone-saline solution **(Section 9.1)** National Veterinary Services Laboratories (NVSL) Media No. 10138

2.2.2 Blood agar with 5% bovine blood **(Section 9.2)** NVSL Media No. 10006 or as stated in the Outline of Production (OP) from the biologics manufacturer

2.2.3 *E. rhusiopathiae* reference culture (American Type Culture Collection #19414**)**

2.2.4 70% ethyl alcohol

2.2.5 Sterile water in serum vials--volumes determined by referring to the biologics manufacturer's OP or as stated on the vaccine vial

3. Preparation for the test

3.1 Personnel qualifications/training

The personnel performing this test must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures, policies, and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn on the biosafety cabinet 1 hr before use and turn off after use.

3.2.2 Monitor the incubator daily for temperature according to the current version of GDOCSOP0001.

3.2.3 Monitor freezers and coolers used for storing samples daily for temperature according to the current version of GDOCSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Warm samples and reference culture to room temperature before rehydrating to the appropriate volume.

3.3.2 Prepare *E. rhusiopathiae* reference control samples according to the current version of STRPP0001.

3.3.3 Negative and Positive Controls: Incubate 2 uninoculated plates of 5% bovine blood agar with test sample plates as negative control plates. *E. rhusiopathiae* reference culture (positive control) is diluted the same as the test samples, but plated depending on the titer found in **Section 3.3.2.**

3.3.4 Store plates used for making counts at refrigerator temperature. Place plates to be used for counts in a 35° ± 2°C incubator overnight prior to use or dry in a biosafety cabinet before use. At the time of use, plates are no more than 14 days old.

3.4 Preparation of the sample

Samples are *E. rhusiopathiae* and/or combination products containing this fraction. They are received according to the current version of STSOP0001.

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4. Performance of the test

4.1 Remove 2 vials of product to be tested and 1 vial of *E. rhusiopathiae* reference control sample from the freezer or cooler storage and allow to warm to room temperature.

4.2 Disinfect the cap with 70% ethyl alcohol. If needed, rehydrate the vials with the accompanying diluent or sterile water. Allow the contents of the vials to reconstitute for at least 5 min. Shake the vials by inversion until thoroughly mixed.

4.3 Prepare a tenfold dilution series of the product by setting up a rack of 20 x 150-mm sc tubes and pipetting 9 ml of 1% peptone saline solution into each tube using a 10-ml pipette. Label the tubes 10^{-1} to 10^{-x} as needed.

4.4 Transfer 1 ml of the first sample from **Section 4.2** into the first tube of 1% peptone saline solution by using a pipette. Cap the tube and vortex. Continue the dilution series by using a pipette to transfer a 1-ml sample of this tube to the tube labeled 10^{-2}. Repeat this method using a sterile pipette for each transfer until the required number of serial tenfold dilutions, as determined from the release titer listed in the firm's OP, is attained.

4.5 Repeat this dilution series with the second sample.

4.6 Deposit 0.1 ml of the sample from the last 3 dilution points of the dilution series for the product onto the surface of media in **Section 2.2.2** using a pipette. Label 3 plates containing the appropriate media for each dilution point with the sample number or name, vial number, and dilution. Use a sterile pipette when inoculating plates with the diluted sample.

4.7 Use a sterile inoculum spreader to evenly distribute the inoculum over the surface of the agar medium.

4.8 Prepare tenfold dilutions of the reference culture as determined from **Section 3.3.2**. Prepare 3 plates of media as in **Sections 4.6** and **4.7** from each of 3 reference control dilutions as determined from **Section 3.3.2**. Use 2 uninoculated plates of media as negative controls.

4.9 Invert all plates and incubate at 35° ± 2°C for up to 72 hr. After incubation, count plates from each series that contains 30 to 300 CFU. Multiply the CFU by the dilution factor and determine the CFU per dose for the dilution series. Determine the mean CFU per dose for the number of vials tested.

5. Interpretation of the test results

5.1 If on the initial test the CFU per dose is equal to or exceeds the required minimum as written in the firm's OP, the serial or subserial is satisfactory (SAT) for bacterial count without additional testing.

5.2 If on the initial test the CFU per dose is less than the required minimum as written in the firm's OP, the serial or subserial may be retested using 4 new vaccine samples, provided that if the retest (RT) is not done, the serial or subserial is unsatisfactory (UNSAT). Compare the firm's OP method to this SAM method when retesting the 4 vials. If on the RT, the average count of the 4 vaccine samples with the firm's OP method is less than the required minimum, the serial or subserial is UNSAT.

5.3 If on the RT with 4 vials the average count using the firm's OP method is equal to or exceeds the required minimum, the serial is SAT.

5.4 If on the initial test the reference culture or positive control culture is not within the titer range determined in **Section 3.3.2**, but the serial being tested has a SAT result, the serial or subserial is a no test (NT) for bacterial count and the serial is released on the results of the firm's test. If the reference culture is not within its titer range and the serial being tested is below its minimum release titer, the serial is retested using 2 new vaccine samples. If on the initial test there is growth on the negative control plates, the serial or subserial is a NT for bacterial count without additional testing.

6. Report of test results

6.1 Record the CFU per dose along with the final conclusion for the product tested on the log book record sheet and the computer worksheet after calculating the CFU per dose and interpreting the results. Enter the results and conclusions into the computer under the ST test code 067-PT1 as stated in the current version of STSOP0021.

6.2 Initial and date the log book record sheet and the computer worksheet. Forward all paperwork to the CY/ST supervisor or microbiologist to review and sign.

6.3 Validate the test results as stated in the current version of STSOP0021 and file all paperwork appropriately.

7. References

Code of Federal Regulations, Title 9, Part 113.67, U.S. Government Printing Office, Washington, DC, 1999.

8. Summary of revisions

This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.

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9. Appendices

9.1 NVSL Media Formulation No. 10138

Peptone solution 1% + 0.5% NaCl

Bacto peptone	10.0 g
NaCl	5.0 g
QH_2O	1000 ml

Autoclave for 20 min at 121°C.

9.2 NVSL Media Formulation No. 10006

Blood agar base with 5% bovine blood

Blood agar base (Difco)	40.0 g
H_2O	950 ml

Autoclave for 20 min at 121°C.

Cool to 47°C and add:

Defibrinated bovine blood	50 ml