

**United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol**

SAM 123

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

Date: **October 21, 2014**

Number: SAM 123.05

Supersedes: SAM 123.04, September 9, 2011

Standard Requirement: 9 CFR, Parts 113.213(c)(2)(vi) and 113.318(b)(3)

Contact: Alethea M. Fry, (515) 337-7200
Peg A. Patterson

Approvals: /s/Geetha B. Srinivas Date: 22Dec14
Geetha B. Srinivas, Section Leader
Virology

/s/Byron E. Rippke Date: 29Dec14
Byron E. Rippke, Director
Policy, Evaluation, and Licensing
Center for Veterinary Biologics

/s/Rebecca L.W. Hyde Date: 29 Dec14
Rebecca L.W. Hyde, Section Leader
Quality Management
Center for Veterinary Biologics

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may be suitable.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

Table of Contents

- 1. Introduction**
- 2. Materials**
 - 2.1 Equipment/instrumentation**
 - 2.2 Reagents/supplies**
- 3. Preparation for the Test**
 - 3.1 Personnel qualifications/training**
 - 3.2 Preparation of equipment/instrumentation**
 - 3.3 Preparation of reagents/control procedures**
 - 3.4 Preparation of the sample**
- 4. Performance of the Test**
- 5. Interpretation of the Test Results**
- 6. Report of Test Results**
- 7. References**
- 8. Summary of Revisions**

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

1. Introduction

This Supplemental Assay Method (SAM) describes an *in vivo* method which employs challenge of swine to determine the potency of killed Pseudorabies virus (PRV) vaccines or the immunogenicity of PRV Master Seed viruses as specified in title 9, *Code of Federal Regulations* (9 CFR).

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

2.1.1 Digital thermometer

2.1.2 Centrifuge with rotor

2.1.3 Water bath

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below. All reagents and supplies must be sterile.

2.2.1 PRV-susceptible swine at the minimum age recommended for vaccination and shown to be negative for PRV serum neutralizing (SN) antibodies by the current version of **SAM 117**, *Supplemental Assay Method for Titration of Pseudorabies Virus Neutralizing Antibody (Constant Virus – Varying Serum Method)*. Swine shall be considered susceptible if there is no neutralization at a final serum dilution of 1:2. Other tests of equal sensitivity acceptable to the Animal and Plant Health Inspection Service (APHIS) may be used.

Numbers of animals required for testing:

- 1.** 9 CFR 113.213: 5 vaccinated swine (VS) + 5 controls (CONT)
- 2.** 9 CFR 113.318(b)(4): 5 VS + 5 CONT
- 3.** 9 CFR 113.(b)(1)-(3): 20 VS + 5 CONT

2.2.2 PRV Challenge, Becker strain only with permission from State Veterinarian approving the procedure.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

2.2.3 Minimum Essential Medium (MEM) (National Centers for Animal Health (NCAH) Media #20030)

1. 9.61 g MEM with Earles salts without bicarbonate
2. 1.1 g sodium bicarbonate (NaCHO_3)
3. Q.S. to 1000 mL with deionized water (DI), adjust pH to 6.8-6.9 with 2N hydrochloric acid (HCl)
4. Sterilize through a 0.22- μm filter.
5. Store at 2°- 7°C.

2.2.4 Serum separation tubes with 20-gauge x 1 1/2-inch Vacutainer[®] needles

2.2.5 Polystyrene tubes, 12 x 75-mm

2.2.6 Syringes, 3- and 10-mL and needles, 20 gauge x 1 1/2-inch

3. Preparation for the Test

3.1 Personnel qualifications/training

Personnel must have training and experience in evaluating swine for clinical signs of disease due to PRV infection. A current Animal Use Application must be available prior to ordering the swine for the test. All constraints in the application must be followed.

3.2 Preparation of equipment/instrumentation

Set the water bath at $36^\circ \pm 2^\circ\text{C}$.

3.3 Preparation of reagents/control procedures

Rapidly thaw the PRV Challenge in a $36^\circ \pm 2^\circ\text{C}$ water bath; dilute in MEM as recommended on the Center for Veterinary Biologics (CVB) Reagent Data Sheet.

3.4 Preparation of the sample

3.4.1 No preparation of Test Vaccine/Master Seed is required.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

3.4.2 Preparation of blood samples

1. Allow blood samples to clot in the serum separation tubes at room temperature for 20 ± 5 minutes.
2. Separate serum from the clot by centrifuging the tubes at $1000 \times g$ for 20 ± 5 minutes (2,000 rpm, Model J6B centrifuge with a JS-4.0 rotor).
3. Pour off the serum into labeled 12 x 75-mm polystyrene tubes. Maintain each animal's serum separately.
4. Store serum samples at $-20^{\circ} \pm 5^{\circ}C$ until tested for SN antibodies according to the current version of **SAM 117**.

4. Performance of the Test

4.1 On the day of the first vaccination, bleed, using the Vacutainer[®] system, from the anterior vena cava of all VS and CONT swine for SN antibody susceptibility determination. Using a 10-mL syringe and needle, administer 1 dose of Test Vaccine/Master Seed as recommended on the label to all VS. Replace the needle between swine. Follow label recommendations for interval between vaccinations if 2 doses are to be administered to the swine; repeat the vaccination if required.

Note: CONT swine are not vaccinated.

4.2 At 14 to 28 days postvaccination after the last vaccination, draw blood samples, using the Vacutainer[®] system, from the anterior vena cava of all VS and CONT swine.

4.3 For a Test Vaccine, if at least 4 of the 5 VS have not developed titers of at least 1:8 and the remaining VS have not developed a titer of 1:4, the VS and CONT swine may be tested by challenging as stated below. For an MSV, all swine are challenged.

4.4 Using a 3-mL syringe without a needle, administer intranasally, 2 mL of the diluted PRV Challenge per swine (1 mL/nostril), during inhalation to both VS and CONT swine. Swine should be held vertically with head up and not sedated or anesthetized for the challenge.

4.5 For -1 and 0 days prechallenge exposure, determine and record rectal temperatures.

4.6 Observe swine daily for 14 days postchallenge in the morning before and during feeding. Note and record all clinical observations. After clinical observations are recorded, determine rectal temperatures and record daily for 7 days postchallenge.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

4.7 Clinical observations

4.7.1 For 9 CFR 113.318(b)(3)(i) CONT swine, severe central nervous system (CNS) signs include, but are not limited to: Falling, difficulty rising, inability to rise, head tilt, head pressing, paralysis, tremors, spasms, convulsions, paddling, opisthotonos, circling, and coma.

4.7.2 For 9 CFR 113.213(c)(2)(vi) CONT swine, CNS signs include all of the signs listed previously: Plus pruritus, teeth grinding, empty chewing, persistent or unusual vocalization, disorientation, ataxia, stumbling, loss of postural control, and proprioceptive placing deficits.

4.7.3 Clinical signs of PRV infection in VS include, but are not limited to: All of the CNS signs listed previously (Sections 4.7.1 and 4.7.2), plus anorexia on 2 or more consecutive DPC, fever of μ 106°F (μ 41.1°C) on any 2 or more DPC, stunting, weakness, vomiting, diarrhea, constipation on any 2 or more DPC, blindness or other ocular disease, persistent sneezing, persistent or deep coughing, labored breathing, and pneumonia.

4.7.4 For the purposes of this testing, the following clinical signs of PRV infection will not be included in the evaluations, due to their ambiguity: Transient inappetence, depression, shivering, occasional sneezing, occasional upper respiratory cough, reduction in rate of weight gain, and fever which is transient or $< 106^{\circ}\text{F}$ ($< 41.1^{\circ}\text{C}$).

4.7.5 Moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power may be humanely euthanized and considered as deaths as outlined in 9 CFR 117.4.

5. Interpretation of the Test Results

5.1 For swine challenge-exposure no point system or weighted scoring system is allowed.

5.2 Validity requirements

5.2.1 9 CFR 113.318 (b)(3)(i): If at least 4 of the 5 CONT swine do not develop severe CNS signs or die, the test is inconclusive and may be repeated. For each swine, this development will be considered the observations on 1 or more days as described in Section 4.7.1.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

5.2.2 9 CFR 113.213 (c)(2)(vi): If at least 4 of the 5 CONT swine do not develop CNS signs or die, the test is inconclusive and may be repeated. For each swine, this development will be considered the observations on 1 or more days as described in Section 4.7.2.

5.3 Test Vaccine evaluation criteria

5.3.1 9 CFR 113.213: If 2 or more of the VS develop clinical signs of PRV infection or die, the Test Vaccine is unsatisfactory.

5.3.2 9 CFR 113.318(b): If 2 or more of the VS develop clinical signs of PRV infection or die, the Master Seed is unsatisfactory.

6. Report of Test Results

Record all test results on a test record.

7. References

7.1 Title 9, *Code of Federal Regulations*, parts 113.213 and 113.318, U.S. Government Printing Office, Washington, DC.

7.2 Vannier P. Experimental infection of fattening pigs with Pseudorabies (Aujeszky's disease) virus: efficacy of attenuated live and inactivated virus vaccines in pigs with or without passive immunity. *Am J Vet Res* 1986; 46:1478-1502.

8. Summary of Revisions

Version .05

- The Contact information has been updated; however, the Virology Section has elected to keep the same next review date for the document.

Version .04

- The phrase "available from the Center for Veterinary Biologics/CVB" has been removed from the document as these reagents are no longer supplied by the CVB.

Version .03

- The Contact information has been updated.

UNCONTROLLED COPY

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

Version .02

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the following changes were made to the document:

- The document number has been changed from MVSAM123 to SAM 123.
- The Contact has been changed from Larry Ludemann and Steve Hanson to Joseph Hermann and Peg Patterson.
- **2.2.2:** A statement regarding virus permissions has been added.
- **2.2.3:** The amount of sodium bicarbonate (NaHCO_3) has been changed from 2.2 g to 1.1 g.
- **4.7.5:** The 9 CFR 117.4 statement has been added.
- The phrase “Reference and Reagent Data Sheet” has been changed to “Reagent Data Sheet” throughout the document.
- The refrigeration temperatures have been changed from $4^{\circ} \pm 2^{\circ}\text{C}$ to $2^{\circ} - 7^{\circ}\text{C}$.

UNCONTROLLED COPY