

October, 2010

**PSEUDORABIES SURVEILLANCE PROCEDURE
MANUAL- VERSION 1.3**



USDA APHIS Veterinary Services



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Table of Contents

FY2010 National PRV Surveillance Program Contacts	3
I. Introduction	5
A. Purpose	5
B. PRV- The Description	5
C. Surveillance Plan Overview.....	6
1. Investigations and Diagnosis of Suspicious PRV Cases.....	6
2. Testing of Tissues from Sick Pigs Submitted to Diagnostic Laboratories	7
3. Serological Testing of Swine Cases Submitted to Diagnostic Laboratories.....	7
4. Serological testing of Herds Classified as High-Risk	8
5. Serological Testing of Herds Reporting Exposure to Feral Swine	10
6. Serological Testing of Cull Sows-Boars at Slaughter	11
7. Collection and Testing of Meat Juice from Market Hogs at Slaughter	11
8. Sampling of Feral Swine.....	12
II. Detailed Sample Collection Procedures.....	13
A. Tools Needed	13
B. Necessary Tissues for PRV Testing.....	13
C. Proper Labeling of Tissue Samples Including Serology Submitted to Labs	17
III. Shipping of Specimens	18
A. Packing	18
B. Shipping to the Designated NAHLN laboratory.....	19
IV. Submitting specimens to NAHLN Laboratories.....	20
V. Discretion of Diagnostician	21
Appendix A: Designated NAHLN PRV Testing Laboratories, Addresses and Contact Information	22
Appendix B: PRV Surveillance Activities by State, Agency and/or Slaughter Plant.....	24
Appendix C: AVIC and State Veterinarian Office Directory	27
Appendix D: USDA PRV Surveillance Submission Datasheet.....	28
Appendix E: PRV Clotted Blood/Serum Testing Algorithm for NAHLN Laboratories	30
Appendix F: The 95/10 Serological Sampling Protocol for Blood Testing	33
Appendix G: PRV Notification and Response Actions.....	34
Appendix H: The National Pseudorabies Surveillance Plan	38

I. Introduction

A. Purpose

This document outlines implementation procedures and guidelines for the pseudorabies virus (PRV) national surveillance program. This program's goals include enhanced surveillance for rapid detection, demonstration of disease freedom, and monitoring international or domestic sources of PRV.

The purpose of this document is to clarify:

- The procedures necessary to meet the objectives of PRV surveillance
- When to refer suspicious or possible PRV cases to the AVIC for investigation
- What is considered a high risk swine herd and when should samples be collected
- Communication protocols
- Where to ship specimens
- Laboratory guidance on sample testing procedures
- What information is necessary for reporting and recording surveillance samples
- Sample submission guides

B. PRV- The Description

Pseudorabies virus (PRV), also known as Aujeszky's disease, emerged as an economically important disease of swine in the late 1960s. Pigs are the primary host and reservoir of PRV making them the principal source of infection. Non-swine species infected with PRV often experience an intense pruritis and death usually occurs within 48 hours. Sometimes, the only sign noticed in non-swine species is sudden death. Horses, birds, and humans are considered resistant to PRV infection.

The United States Department of Agriculture's (USDA's) Animal and Plant Health Inspection Service (APHIS), began plans to eradicate PRV in the late 1970's and early 1980's. The resulting eradication program led to elimination of PRV in 2004 from commercial swine; however, the infection still exists in non-commercial and feral swine.

More information pertaining to etiology, clinical signs, epidemiology, diagnosis, and rationale for PRV surveillance can be found in the PRV surveillance plan (Appendix H).

C. Surveillance Plan Overview

The goal of this plan is to target swine populations and diagnostic samples considered high risk for PRV infection. The goal of surveillance is to rapidly identify infection if it enters the commercial swine industry. This targeted population includes: 1) investigations and diagnosis of suspicious PRV cases; 2) antigen testing of tissues submitted from sick pigs to diagnostic laboratories; 3) random serological testing of swine cases submitted to diagnostic laboratories; 4) serological testing of herds classified as high risk; 5) serological testing of herds with known feral swine exposure; 6) serological testing of cull sow-boar slaughter animals; 7) testing of meat juice from market animals; and 8) select feral swine for the presence of PRV.

The surveillance streams listed above support the objectives of the surveillance plan. Each stream is outlined in the PRV surveillance plan (appendix H). The general method of action pertaining to each sampling stream is listed below. For questions regarding the PRV surveillance plan, contact John Korslund 970-494-7217 or John.a.korslund@aphis.usda.gov

1. Investigations and Diagnosis of Suspicious PRV Cases

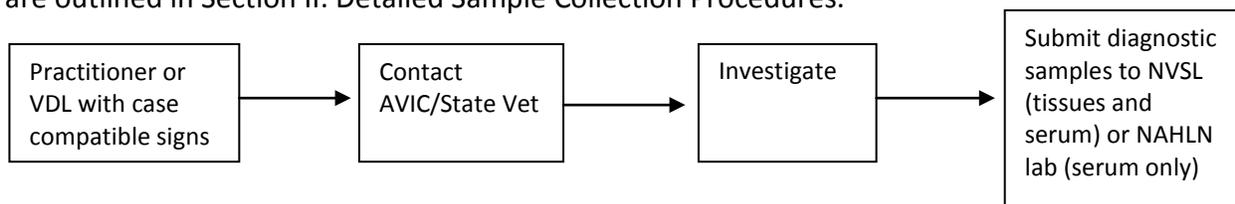
Diagnosis of PRV infection is achieved through a combination of herd history, clinical signs, and diagnostic sampling information. Clinically ill swine showing symptoms consistent with PRV should be reported to the AVIC, and the State animal health official (SAHO) or designee.

Suspect swine cases fitting this category include:

- Suckling pigs exhibiting high mortality (approaching 100 percent) accompanied by histological CNS lesions or rarely observed gross lesions consistent with PRV;
- Sows with respiratory signs and, or reproductive failure; and
- Respiratory signs in weaned market pigs accompanied by evidence of CNS symptoms or increased mortality consistent with PRV.

All swine herds fitting these categories should be referred to the AVIC/SAHO in the State (appendix C). The AVIC or designee will determine if a full investigation is warranted. If an investigation is warranted and only serum is collected from the farm, submit the serum to a PRV NAHLN laboratory using the on-farm datasheet (appendix D). If both serum and tissues are collected submit samples to National Veterinary Services Laboratory (NVSL) for analysis. NVSL bar-codes (see page 17 for information on obtaining bar codes) should be placed on all samples. Additional information on performing investigations can be found in VS Memo 580.4

Note: the procedures for obtaining barcodes and collecting specimens for investigating disease are outlined in Section II: Detailed Sample Collection Procedures.



Questions regarding investigation of suspicious PRV cases should be directed to your APHIS-VS Regional Swine Epidemiologist: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter-Spalding in the Eastern Region, 919-855-7250.

2. Testing of Tissues from Sick Pigs Submitted to Diagnostic Laboratories

*****Testing of tissues other than sera for this stream is to be implemented after successful test validation*****

This sampling stream involves any swine accessions submitted by practitioners to PRV NAHLN laboratories. **Tonsil, brain and lung samples will be eligible for PRV antigen PCR testing (once validated) and serum for serological testing** if the case meets any of the following criteria:

- submissions for dramatic respiratory or neurological disease in market or breeding aged swine;
- submissions related to sow abortions.

NAHLN Laboratories should use NVSL bar codes on all specimens tested. Since these specimens are submitted from practitioners, the laboratory will need to bar code the samples. NAHLN labs receiving inconclusive or positive PCR results for PRV are to forward samples to NVSL for confirmatory testing. NAHLN labs testing serum should follow the testing algorithm (appendix E). Additionally, labs are to follow appendix G on notification of sample results if a non-negative is received.

VS or State personnel will not be required to collect samples for this stream. These samples are submitted to NAHLN labs by practitioners.

Questions regarding samples eligible for testing of can be directed to the NAHLN laboratory or to the appropriate APHIS-VS Regional Swine Epidemiologist: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter Spalding in the Eastern Region, 919-855-7250.

3. Serological Testing of Swine Cases Submitted to Diagnostic Laboratories

This stream involves testing serological submissions from veterinary practitioners to PRV NAHLN laboratories for diagnostic or routine monitoring of diseases (other than PRV). It does not include samples submitted for PRV serology to qualify for movement or herd validation. If the practitioner specifies PRV testing, then the sample does not qualify for surveillance unless the serum sample qualifies for testing in the “testing of tissues from sick pigs submitted to diagnostic laboratories” surveillance stream.

This stream can include submissions for herd profiling, as well as serum from animals experiencing clinical disease. A maximum of five serological samples are allowed to be tested from each submission or premises.

Qualifying submissions are to be pulled for testing at program-specified rates. Since laboratories receive numerous samples, participating PRV NAHLN laboratories will have testing quotas and specifications (appendix B). Laboratories are to follow the established testing algorithm for screening samples (appendix E) and the notification protocols (appendix G). Laboratories are also to apply a NAHLN barcode to each serum sample tested.

Sample submission information and testing results will be captured on a PRV lab entry sheet (spreadsheet-based) and submitted monthly by the 5th of each month, or as directed by your NAHLN coordinator for collation. Laboratories with no data to report need to report that they have no data to report to the NAHLN coordinator, Ms. Barb Martin. Eventually a web-based information capturing system will be created to improve and automate data entry.

Samples tested should be representative of all submissions over space and time. No more than five samples per submission will be tested for this sampling stream.

Questions regarding this sampling stream should be directed to your APHIS-VS NAHLN coordinator, Barb Martin at 515-337-7731.

4. Serological testing of Herds Classified as High-Risk

AVICs, State Veterinarians, or designees can review bio-security safe-guards and authorize testing of swine herds considered to be at high-risk for transfer of PRV from its feral reservoir into the commercial swine sector. The overall goal of this surveillance plan is to rapidly identify disease in the commercial industry. Therefore, high risk herds that should be considered for testing are herds that may have a direct impact on the commercial swine industry.

To determine which herds will be considered high-risk and qualify for testing under this portion of the surveillance plan, officials could consider the following factors:

- concentration of feral swine and their proximity to the operation
- direct or indirect interstate sales of swine from the operation
- risk of infection from purchased swine
- housing/facilities/management that may allow exposure to feral swine (i.e. effectiveness of biosecurity measures), etc.

Appendix B (PRV High-Risk Herd Testing Surveillance Activities) provides a list of US counties that could deserve extra emphasis due to the presence of significant commercial swine populations coupled with the presence of feral swine. This list is only a guideline of possible high risk areas. States should test herds they feel necessary to protect or rapidly find disease in their States' commercial herds.

Examples of high-risk herds could include:

- commercial swine with outdoor access and located within 10 miles of known populations of feral swine
- swine herds located within 10 miles of feral swine hunt clubs
- herds with exposure to feral swine that supply show pigs especially those that may show interstate.

Testing of pigs for exhibitions is not considered high-risk testing and not part of this surveillance plan. Confined herds of swine that were at one time feral, or herds that incorporate feral swine into production will **NOT** be considered high-risk herds for testing purposes. These herds are considered an extension of the feral swine population.

High-risk testing specimens can be collected through on farm testing, market testing, high-risk garbage feeder testing, small slaughter plant testing or other methods as necessary. If on-farm sampling is determined the most appropriate collection method, the herd should be tested using the 95/10 methodology (appendix F). A schedule of testing of high-risk herds may be devised dependent on housing type.

- Those with outdoor access would be sampled once every two years
- Those in total confinement without a perimeter fence or those outdoor facilities with an effective perimeter fence would be tested once every five years
- Those in total confinement with adequate biosecurity, including effective perimeter fencing, would not be tested

Please consult with your Regional Epidemiologist to determine number of herds to sample and sampling methodology. Specimens should be submitted to an activated PRV NAHLN laboratory (see appendix A). States should contact an activated laboratory prior to submitting specimens to confirm testing ability.

The USDA On-Farm Sample Collection datasheet must be included with the sample shipment. An example can be located in appendix D. Specimens sent to a laboratory without this datasheet will **not** be analyzed. All specimens submitted must have a separate bar code place on every sample. Bar codes can be obtained through NVSL by contacting Jason Bunn, NVSL at phone 515-337-7350. The AVIC or designee is responsible for recording required data. A current version of the datasheet can be found at

http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/pseu_surv_proced_manual.shtml

Questions regarding serological testing of high risk herds can be directed to the appropriate APHIS VS Regional Swine Epidemiologists: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter-Spalding in the Eastern Region, 919-855-7250.

5. Serological Testing of Herds Reporting Exposure to Feral Swine

Swine eligible for testing include swine from high risk outdoor commercial herds with the possibility of interstate movement that are exposed or potentially exposed to feral swine. This stream relies on voluntary reporting by the producer or owner. When the producer suspects feral swine incursions or observes feral swine in his/her herd, the producer may contact authorities to report the feral swine intrusion. Feral swine intrusions include either direct exposure to feral swine or visual evidence of fence line contact with feral swine.

When reported, the AVIC, State Veterinarian or designee will determine eligibility and appropriate timing for enhanced herd monitoring and herd surveillance, as needed, by discussing the situation with their Regional Epidemiologists. Sampling may include on-farm blood collection at the 95/10 sampling level for serology testing at an appropriate time interval following suspected exposure to allow for sero-conversion (appendix F).

Data capturing methods are described above in the high risk herd testing stream (surveillance stream 4).

For this stream, direct exposure to feral swine should be observed or strongly suspected. If exposure is reported, consult your Regional Epidemiologist for sampling methodology and sample submission laboratories. Samples are to be submitted to an activated PRV NAHLN lab for analysis.

The PRV On-Farm swine surveillance datasheet must be completed and submitted with individual bar-coded samples to an activated NAHLN laboratory. Specimens sent to a laboratory without this datasheet will **not** be analyzed. Bar codes can be obtained from NVSL by contacting Jason Bunn, NVSL at phone 515-337-7350.

Questions regarding on-farm sampling of herds exposed to feral swine can be directed to the appropriate APHIS-VS Regional Swine Epidemiologist: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter Spalding in the Eastern Region, 919-855-7250.

6. Serological Testing of Cull Sows-Boars at Slaughter

Swine eligible for testing include cull sows-boars located at selected federal/state slaughter establishments.

Samples from all cull sows-boars with appropriate identification for surveillance will be collected and sent to the USDA laboratories located in Kansas and Kentucky. The samples will be sorted and tested to ensure that States meet the existing surveillance requirements found in Program Standards. Sample collection reduction protocols may be applicable in certain slaughter plants. These protocols will be slaughter plant specific and implemented with AVIC assistance.

Questions regarding serological testing of cull sows-boars at slaughter establishments can be directed to the appropriate APHIS-VS Regional Swine Epidemiologist: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter-Spalding in the Eastern Region, 919-855-7250.

7. Collection and Testing of Meat Juice from Market Hogs at Slaughter

Market swine slaughtered at select federally-inspected slaughter plants are eligible for meat juice testing. Meat collected from representative lot-identified market swine carcasses will be processed and analyzed using approved program meat juice assays. Samples collected for this stream will be collected by VS-employees and/or contracted personnel. Collectors will send samples to specified laboratories for sampling.

Data will be collected in the short-term in spreadsheets and forward quarterly to program staff for review and analysis.

Questions regarding collection and testing of meat juice from market swine at slaughter can be directed to the appropriate APHIS-VS Regional Swine Epidemiologist: Mark Schoenbaum in the Western Region at 970-494-7314 or Barb Porter-Spalding in the Eastern Region, 919-855-7250.

8. Sampling of Feral Swine

Sampling of feral swine for PRV may be closely linked to the classical swine fever (CSF) surveillance program to improve sampling efficiencies. Eligible feral swine include free-roaming feral swine in states where Wildlife Services (WS) biologists have access to public and private land to capture and sample feral swine. Disease surveillance often overlaps with operational activities to conduct feral swine damage management; however, state level surveillance and monitoring approaches vary based on input from local experts.

WS collecting samples for the CSF program may also collect serology samples from the same animals for PRV testing to determine the presence of PRV. Samples collected by WS wildlife disease biologists for PRV testing will be submitted to laboratories directed by WS and in accordance with their WS Comprehensive Feral Swine Disease Surveillance Procedure Manual. WS personnel submitting serum for PRV testing should complete the datasheet (appendix D) and submit it with samples.

Samples collected in areas where previous assays of feral swine are known to be positive will not be re-tested unless determined necessary by WS. Testing should be restricted to samples from areas with previous negative or unknown PRV status, particularly areas with recent feral swine incursions and/or significant commercial swine production. Feral swine samples collected by WS will not be forwarded to NVSL for confirmatory testing.

AVICs requesting feral swine sampling in their state should consult with WS. Samples submitted for PRV analysis from anyone other than WS or through cooperation with WS will not be analyzed under the USDA program.

Questions regarding sampling of feral swine for PRV can be directed to Brandon Schmit (970-266-6079) or Troy Bigelow at 515-284-4121.

II. Detailed Sample Collection Procedures

This section describes how to collect tissues and blood samples in the field. Most of the tissue samples collected for the PRV surveillance program will be submitted by practitioners. However, in the event that blood collection or tissue samples are needed for investigations and diagnosis of suspicious PRV cases, the following explains the proper procedures for collection of tissues and blood for PRV testing. Details of submitting serum samples can be found in Section IV. Submitting Samples to NALHN Laboratories.

A. Tools Needed

- For removing tonsils and tissues from euthanized or expired animals (sick pigs only)
 - Knife (and/or scalpel) scissors
 - Forceps
 - Plastic whirl bags or screw top-plastic tubes
- For serology collection
 - Centrifuge (if possible)
 - 10 - 12ml Red or red/grey top blood collection tube or vacutainer
 - Needles (size of choice) for collection of blood from swine
 - Syringes with luer lock tips
 - Falcon “snap cap type” tubes
 - Hog snare or other swine restraining device
- Tools and items necessary for both tissue and serology collection
 - Fine-point permanent marker (smear proof/water proof)
 - Ball-point pen
 - Pan or bucket for disinfecting instruments and rinsing hands
 - Bleach (disinfectant)
 - Paper towels
 - Freezer bags
 - Trash Bags for soiled personal protective clothing
 - Mailers to submit samples

B. Necessary Tissues for PRV Testing

When collecting tissues for PRV testing is necessary, at minimum, collect the fresh tissues listed below for PRV testing. Blood will be collected from all animals, other tissues are only needed if pigs are exhibiting clinical signs (sick pigs.) Each tissue should be kept separate in bags or tubes, labeled with program-approved bar codes (if applicable) and refrigerated or chilled immediately.

- Tonsil
- Lung
- Brain (if possible to remove)
- Blood (fill red-top tube)

The following details explain how to collect tonsil specimens. Tonsils only need collected by a VMO when he/she is investigating a herd with possible PRV, and the VMO will be performing a post mortem examination as part of the investigation. Tonsil collection and testing for other PRV surveillance streams has not been implemented.

Removing Tonsils (dead pigs)

1. Lay the pig in dorsal recumbency.



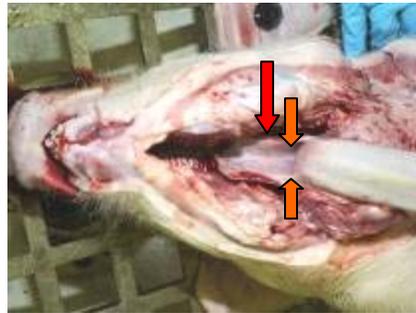
2. Beginning near the chin, use a knife to reflect the skin caudally to expose underlying tissues in the intermandibular and proximal cervical regions.



3. Incise soft tissue along the medial aspect of each mandible. Extend proximally to the mandibular symphysis on each side in order to free the attachments of the tongue.



4. After freeing up the proximal attachments of the tongue, reflect the tip of the tongue caudally to expose the hard and soft palate. The palatine tonsil, a flat bi-lobed structure with a prominent medial septum, is located caudal to the soft palate (red arrow). Cut the lateral attachments that restrict further retraction of the tongue (orange arrows). The entire tonsil (both lobes) should be collected.



5. Reflect the tongue further to expose the tonsil and epiglottis. Note the dimpled appearance of the flattened tonsil, due to invaginations of the epithelium to form crypts.



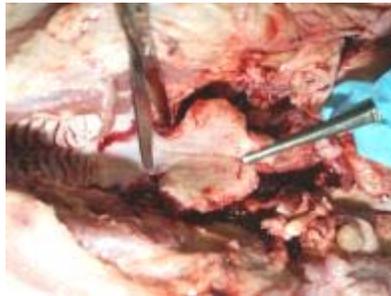
6. Use scissors to separate the tonsil from laryngeal structures caudal to it.



7. Grasp the caudal aspect of the tonsil with forceps and use scissors to cut the deep attachments of the tonsil.



8. Cut the proximal attachments of the tonsil to the soft palate. The tonsil is now freely excised and can be removed.



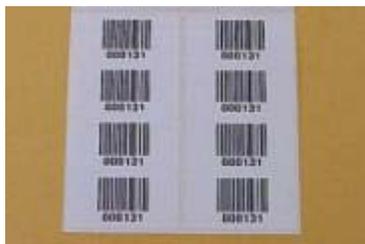
9. Place tonsils in collection container.
10. Wipe container surfaces free of gross contamination (blood, fecal material, etc.) before packaging for shipment.

C. Proper Labeling of Tissue Samples Including Serology Submitted to Labs

Specimens collected by animal health officials, should be labeled consistent with other disease programs like CSF. All samples must have a NVSL supplied unique barcode.

Barcodes can be obtained by contacting the shipping and sample department at NVSL . Contact NVSL 515-337-7530 at to obtain bar codes.

- Label each tube or sample collection container with a smear/waterproof pen and barcode. Include on each label:
 - Sample number
 - Type of specimen in tube or container (tissue type i.e. tonsil, lung, liver etc.)
 - Barcode identification label
 - Bar codes are printed in sets of 4 individual labels. Each sample should receive a **different** bar code, even if several samples are collected from the same animal.
 - Bar codes should be used as follows:
 - One label on each sample tube – be sure to place bar-code **lengthwise** along the tube.
 - One label on the submission form
 - **Any labels that are not used should be destroyed.** Re-usage of previously used bar codes will “confuse” automated sample handling protocols in laboratories.
 - **All serology specimens must have a unique barcode**



- Place the samples in a cooler and/or on cold packs. **Do not freeze specimens.**
- Properly dispose of tissues and/or carcasses.
- Send the tissues/serology to the appropriate NAHLN laboratory

Questions regarding sampling techniques can be directed to the program managers at APHIS-VS-National Center for Animal Health Programs, Troy Bigelow (515-284-4121).

III. Shipping of Specimens

For samples collected by animal health officials, the following protocol should be used in submitting samples to NAHLN or USDA laboratories

A. Packing

- Place labeled sample tube/containers into the clear bio-hazard bag (STP-741) with absorbent and seal.



- Place this bag into white bio-hazard bag (STP-740) and seal.



- Place the white bag into the shipping box.
- Place frozen ice packs on top of the bag.



- Place completed U.S. Department of Agriculture (USDA) On- farm **Swine** Surveillance Submission form (if available) on top of inner styrofoam lid.



- Seal box
- Place address shipping label on the box addressed to designated laboratory conducting PRV testing for this collection site.
- Place the other required shipping labels on the box to meet current IATA shipping requirements (e.g. UN 3373 label, etc.)
- Ship by overnight delivery with a contract carrier service.

****If shipping on a Friday, be sure to mark/label box for Saturday delivery. Make sure the lab can receive and refrigerate a shipment on a Saturday.****

NOTE: Individuals who ship surveillance samples to laboratories are responsible for meeting requirements for the shipment of biological substances. At a minimum, these individuals should be familiar with the IATA Packing Instructions

Contact NVSL or the submitting NAHLN laboratory for information regarding shipping materials and shipping regulations. If you need further assistance with shipping, you may contact the shipping department at:

National Veterinary Services Laboratories

Ph: (515)337-7530

Fax: (515) 337-7378

B. Shipping to the Designated NAHLN laboratory

Ship specimens via the overnight contract delivery service only to the designated NAHLN laboratory. Appendix A contains a list of designated PRV NAHLN laboratories.

IV. Submitting specimens to NAHLN Laboratories

VS personnel can obtain disposable blood collection supplies by contacting your VS area office. If possible, blood samples should be centrifuged and serum separated. Serum is then packed in Falcon tubes and sent with submission form to the appropriate NAHLN laboratory or USDA laboratory. All blood/serology tubes submitted to NAHLN labs must be identified with a unique bar code. Bar codes can be obtained by contacting with NVSL shipping at 515-337-7350.

Recommended Supplies/Equipment for Blood Collection and Serum Submission

- Cooler
- Ice packs
- Needles of choice for blood collection
- 10-15 ml syringes with luer lock tips
- 10 ml red or red/grey topped vacutainers
- Swine surveillance specimen barcode labels
 - WS-contact Brandon Schmit for barcodes
 - VS-contact NVSL for barcodes
- Falcon tubes or cryovials for serum
- Falcon tube shipping containers
- Ziploc freezer bags to use as primary and secondary shipping containers
- Paper towels to be used as absorbent material in-between primary and secondary shipping containers
- Centrifuge to spin off serum
- GPS units to record location of sample collection
- Snares to catch and immobilize swine for sample collection
- Submission forms (see appendix D)

If possible, please provide a minimum of 2.0 ml of clear, non-hemolyzed, separated serum per animal for PRV testing.

V. Discretion of Diagnostician

Samples submitted to NAHLN laboratories by practitioners that qualify for PRV serology and or antigen testing (surveillance streams 2 and 3 above) must meet minimum information requirements. **Samples that arrive without the minimum information necessary for surveillance purposes do not qualify for PRV testing under the USDA National PRV Surveillance program and are not to be analyzed for PRV using USDA supported funds.** In addition, diagnosticians are to follow the sampling criteria listed throughout this document.

Minimum information requirements pertain to the traceability of the sample. Required information on diagnostic form must be able to provide:

- Submitting practitioner information
 - Name
 - City
 - State
 - Zip code
- Animal ID number
- Date of sample submission
- Production site information shall identify the actual location of the pigs sampled to qualify for sampling.
 - Premises ID or Premises name (if provided) If provided must be included
 - Street
 - City
 - State
 - Zip code

Samples tested should be representative of all submissions over space and time. The maximum number of samples per submission to test is five.

On each qualifying sample that is submitted from practitioners the laboratories are to place a unique barcode supplied through NVSL.

Diagnosticians with questions pertaining to sample testing and necessary information should contact USDA, VS, NAHLN coordinator at 515-337-7731.

Appendix A: Designated NAHLN PRV Testing Laboratories, Addresses and Contact Information

The following NAHLN laboratories are considered designated PRV testing laboratories. Shipping addresses and contact information for the veterinary diagnostic laboratories is outlined below in the chart.

AL	Thompson Bishop Sparks State Diagnostic Laboratory 890 Simms Road PO Box 2209 Auburn, AL 36832	Phone: 334-844-7207 Fax: 334-844-7206
CA	California Animal Health & Food Safety Lab University of California, Davis School of Veterinary Medicine W. Health Science Drive Davis, CA 95616	Phone: 530-752-8709 Fax: 530-752-5680
GA	University of Georgia Tifton Veterinary Diagnostic Laboratory 43 Brighton Road Tifton, GA 31793-3000	Phone: 229-386-3340 Fax: 229-386-3399
IA	Veterinary Diagnostic Laboratory College of Veterinary Medicine Iowa State University 1600 S. 16th St. Ames, IA 50011-1250	Phone: 515-294-1950 Fax: 515-294-3564
KS	Kansas State Veterinary Diagnostic Laboratory Kansas State University, CVM L232 Mosier Hall, 1800 Dennison Ave Manhattan, KS 66506-5606	Phone: 785-532-5650 Toll Free: 866-512-5650 Fax: 785-532-4039
MN	University of Minnesota Veterinary Diagnostic Laboratory 1333 Gortner Ave, 244 Veterinary D L St. Paul, MN 55108	Phone: 612-625-8787 Fax: 612-624-8707
MO	Veterinary Medical Diagnostic Lab University of Missouri 1600 East Rollins PO Box 6023 Columbia, MO 65211	Phone: 573-882-6811 Fax:

NC	Rollins Diagnostic Laboratory North Carolina Department of Agriculture & Consumer Services 2101 Blue Ridge Rd. Raleigh, NC 27607 Mailing Address: 1031 Mail Service Center Raleigh, NC 27699-1031	Phone: 919-733-3986 Fax: 919-733-0454
NE	Veterinary Diagnostic Center University of Nebraska 1900 N. 42nd Street Lincoln, NE 68583 Mailing Address : Fair Street & East Campus Loop Lincoln, NE 68583-0907	Phone: 402-472-1434 Fax: 402-472-3094
OH	Ohio Department of Agriculture Animal Disease Diagnostic Laboratory 8995 E. Main Street, Building 6 Reynoldsburg, OH 43068-3399	Phone: 614-728-6220 Fax: 614-728-6310
OK	Oklahoma Animal Disease Diagnostic Laboratory Oklahoma State University College of Veterinary Medicine Farm & Ridge Road, Room 127 Stillwater, OK 74078	Phone: 405-744-6623 Fax: 405-744-8612
SD	Animal Disease Research & Diagnostic Laboratory South Dakota State University Box 2175, N. Campus Dr. Brookings, SD 57007-1396	Phone: 605-688-5171 Fax: 605-688-6003
TX	Texas Veterinary Medical Diagnostic Laboratory 1 Sippel Road College Station, TX 77843 Mailing Address: PO Box Drawer 3040 College Station, TX 77841-3040	Phone: 979-845-3414 Toll Free: 888-646-5623 Fax: 979-845-1794
WI	Wisconsin Veterinary Diagnostic Laboratory 1512 E. Guy Avenue Barron, WI 54812-0097	Phone: 715-637-3151
WA	Washington Animal Disease Diagnostic Laboratory Washington State University P.O. Box 647034 Bustad Hall Room 155-N Pullman, WA 99164-7010	Phone: 509-335-9696 Fax: 509-335-7424

Appendix B: PRV Surveillance Activities by State, Agency and/or Slaughter Plant

Sow-boar market slaughter samples (surveillance stream 6) will continue to be collected in slaughter plants as previously conducted. Slaughter sample testing will occur at the two Federal PRV laboratories in KS and KY. **States should submit all slaughter surveillance or testing necessary to meet the minimal regulatory surveillance requirements (5%) to the USDA Kansas and Kentucky Regional testing laboratories.**

Regulatory officials collecting serology samples (other than slaughter) collected for surveillance purposes or **traceback investigations** should send properly labeled and bar-coded samples to an activated PRV NAHLN Laboratory. States should contact an activated NAHLN laboratory prior to submission to determine if the activated NAHLN PRV testing laboratory can process the samples without going over specified sample quotas or States can contact their Regional Epidemiologist to determine where to send samples (i.e. which NAHLN lab.)

Sample selections for surveillance stream 3, “Serological Testing of Swine Cases Submitted to Diagnostic Laboratories”, should be representative of all submissions to that NAHLN laboratory over space and time. A maximum of five samples per submission are to be tested. Laboratories will use a unique USDA provided bar code on each sample.

The following summary applies to the implemented streams listed in the introduction. Expected sample targets are listed for each state:

NAHLN labs testing for PRV will be phased in over time.

State	NAHLN Laboratory	FY 2011 convenience serology swine submissions
AL	Thompson Bishop Sparks State Diagnostic Laboratory	500
CA	California Animal Health & Food Safety Lab	500
GA	University of Georgia Veterinary Diagnostic Laboratory	1000
IA	Iowa State University Veterinary Diagnostic Lab	9750
KS	Kansas State Veterinary Diagnostic Laboratory	1500
MN	Uni. of MN Veterinary Diagnostic Laboratory	10500
MO	Veterinary Medical Diagnostic Laboratory	150
NE	Veterinary Diagnostic Center, Uni. of Nebraska	275
NC	Rollins Diagnostic Laboratory	2500
OH	Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory	200
OK	Oklahoma Animal Disease Diagnostic Laboratory	150
SD	Animal Disease Research & Diagnostic Laboratory, SDSU	5000
TX	Texas Veterinary Medical Diagnostic Laboratory	2000
WA	Washington Animal Disease Diagnostic Laboratory	60 plus feral swine submissions
WI	Wisconsin Veterinary Diagnostic Laboratory	90 plus feral swine submissions

Wildlife Services Feral Swine PRV Surveillance

WS National Wildlife Disease Program will determine areas for PRV sampling with assistance from VS officials. WS is responsible for determining sample size and allocating sample distribution among the states with known feral swine populations. Through a joint decision by WS and VS, samples collected by WS biologists for PRV testing will be submitted to NAHLN laboratories as directed by WS. Only samples submitted from WS qualify for the program using USDA funds.

PRV High Risk Herd Testing Surveillance Activities

AVIC's and State Veterinarian should identify high risk herds based on the criteria outlined in the high risk herd testing section (surveillance stream 4). AVICs / State veterinarians shall work with their Regional epidemiologist to determine the most practical means to obtain samples from the high risk herds and number of samples to take.

To assist states in determining where their high risk farms may reside, the National Surveillance Unit has provided the following guideline to those high-risk states. This chart lists counties with documented feral swine populations that also report over 1000 breeding animals and their breeding swine density. This chart could be used as a guideline for sampling in states in consultation with their Regional Epidemiologist. This chart is not all inclusive and States may or may not consider the list as areas where high risk swine reside. Additionally, States may consider other areas and herds as "high risk". Please remember that the goal of surveillance is to rapidly identify PRV infection in commercial herds. A priority is testing commercial herds with high risk of contact with feral swine. These herds may or may not exist in the areas NSU provided. High risk herds are to be determined by local VS and Regional officials. Only those herds defined as "high risk" are to be tested through federal funding mechanisms for the high risk herd testing surveillance stream number 4.

State	County
Arkansas	Conway, Hempstead, Howard, Logan, Montgomery, Newton, Pike, Polk, Pope, Sevier, Yell
California	Fresno, San Bernardino, Stanislaus, Tulare
Colorado	Yuma
Georgia	Tattnall, Bulloch , Oglethorpe
Illinois	Effingham, Monroe, Fulton
Indiana	Orange, Jackson, Martin, Wayne, Washington, Jay, Spenser, Dubois, Adam
Iowa	Van Buren
Kansas	Smith, Montgomery
Michigan	Hillsdale, Montcalm, Huron, Ionia, Branch, Gratiot, Calhoun, Allegan
Missouri	Cedar, St Clair, Wright, Maries, Bates, Cole, Webster, Franklin, Henry, Cass, Osage, Callaway, Barton
Nebraska	Butler, Custer, Platte, Seward
North Carolina	Anson, Beaufort, Bertie, Bladen, Brunswick, Cabarrus, Columbus, Craven Duplin, Edgecombe, Harnett, Hertford, Johnston, Nash, North Hampton, Onslow, Pender, Pitt, Richmond, Robeson, Sampson, Union, Wayne
Ohio	Auglaize, Champaign, Darke, Mercer, Morgan, Morrow, Pickaway, Preble, Shelby, Williams
Oklahoma	Canadian, Grady, Haskell, Hughes, Latimer, Le Flore, McCurtain, Okfuskee, Pottawatomie
South Carolina	Orangburg
Tennessee	Lawrence
Wisconsin	Crawford

Appendix C: AVIC and State Veterinarian Office Directory

A current list of AVIC offices and detailed AVIC information can be found by viewing the following website http://www.aphis.usda.gov/animal_health/area_offices/

State Animal Health Offices

A current list of State Animal Health offices and officials can be found by viewing the following website <http://www.nasda.org/cms/7195/8617.aspx>

Appendix D: USDA PRV Surveillance Submission Datasheet

The following datasheet is an **example** used by USDA APHIS WS personnel. Only approved personnel should complete the form as directed and in accordance with WS National Wildlife Disease Program. This is not a complete form as parts have been deleted for illustration purposes

USDA- APHIS- Wildlife Services Feral Swine Disease Surveillance Datasheet - FY2010			Page _____	of _____
Wildlife Services Information:		Referral Number:		Date Collected:
Collector Name: _____		_____		____/____/____
Phone: _____		state initials month day year		mm dd yyyy
GPS Location: N _____ (DD, WGS-84) W- _____		Collection Site: _____ County: _____ State: _____		
1	Subject ID: Barcode _____ state initials number	CSF Barcode	FMD Oral Swab Barcode	FMD Nasal Swab Barcode
Age Class: Juvenile / Sub-adult / Adult	CSF Diagnostic Lab: FADDL or _____	FMD Diagnostic Lab: (circle one) CA - CAHFSL / KS - KSVDL / TX - TVMDL / NC - RADDL		
Gender: Male / Female	Date Shipped: ____/____/____ Sample Type: Serum / Tonsil	Date Shipped: ____/____/____		
PRV Barcode Serum only	SB Barcode Serum only	Archive Barcode Serum only	# of Archive Vials:	
			Date Shipped: ____/____/____	

Check here if submitter info is same as collector

Submitter Name: _____

Number of Feral Swine in Referral: _____

Phone: _____

CSF Storage Condition Prior to Shipping: Refrigerated / Frozen

(Please fax all feral swine datasheets to NWDP: 970-266-6215 or scan and email to nwdpdata@aphis.usda.gov)

The following datasheet is an **example** used by USDA VS or State personnel performing surveillance or epidemiological tracing activities. This datasheet must be included with the samples and sent to the appropriate PRV NAHLN laboratory to qualify for testing. The datasheet can be found on the web at:
http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/on-farm_collect_submiss_form.pdf

Remember all samples submitted must have a unique bar-code attached. The following form is not complete as parts were deleted for illustration purposes.

USDA Swine Health Surveillance : Data Sheet for On-Farm/High Risk Collections		Page of
Veterinarian (Collector) Name: _____ Address: _____ City: _____ State ___ Zip _____ Accred #: _____ Phone: _____		Testing Laboratory Name: Collection Date:

Collection (Production) Site Information					
Premises Name:	Premises ID:	City:	State:	Zip:	GPS Location N: _____ GPS Location W: (-) _____
Reason for Submission: <input type="checkbox"/> waste feeding operation <input type="checkbox"/> premises w/ known or suspect feral swine exposure <input type="checkbox"/> high risk premises <input type="checkbox"/> sero-testing / traceback		Collection Site Type: <input type="checkbox"/> farm <input type="checkbox"/> waste feeding operation <input type="checkbox"/> market / auction <input type="checkbox"/> small slaughter establishment		For Waste Feeding Operations Waste Feeder License #: Type of waste food: <input type="checkbox"/> waste with meat <input type="checkbox"/> waste without meat	

Animal and Specimen Information						
*Other specimen type may be nasal swab, tonsil, tonsil scraping, meat juice, lung, brain, spleen, lymph, whole blood (EDTA) or whole blood heparin						
Animal ID	Bar Code	Age Class: <input type="checkbox"/> fetus <input type="checkbox"/> juv / nursery <input type="checkbox"/> subadult/grower-finisher <input type="checkbox"/> adult/ sow, boar	Clinical Signs: <input type="checkbox"/> abortion <input type="checkbox"/> CNS signs <input type="checkbox"/> fever <input type="checkbox"/> respiratory <input type="checkbox"/> septicemic lesions	<input type="checkbox"/> wasting <input type="checkbox"/> diarrhea <input type="checkbox"/> none	Specimen Type: <input type="checkbox"/> serum other:	Condition to Test: <input type="checkbox"/> CSF <input type="checkbox"/> PRV
Animal ID	Bar Code	Age Class: <input type="checkbox"/> fetus <input type="checkbox"/> juv / nursery <input type="checkbox"/> subadult/grower-finisher <input type="checkbox"/> adult/ sow, boar	Clinical Signs: <input type="checkbox"/> abortion <input type="checkbox"/> CNS signs <input type="checkbox"/> fever <input type="checkbox"/> respiratory <input type="checkbox"/> septicemic lesions	<input type="checkbox"/> wasting <input type="checkbox"/> diarrhea <input type="checkbox"/> none	Specimen Type: <input type="checkbox"/> serum other:	Condition to Test: <input type="checkbox"/> CSF <input type="checkbox"/> PRV

Remarks:

Date samples shipped to testing lab: _____
 Name of submitter: _____
 Fax or email results to: _____

Number of samples shipped: _____
 Submitter phone number: _____

Revised 7/13/10

Appendix E: PRV Clotted Blood/Serum Testing Algorithm for NAHLN Laboratories

****Reminder, slaughter surveillance and qualification for movement including exhibition (export/intrastate movement) samples do not qualify for this testing algorithm****

Prior to testing samples for PRV, NAHLN Laboratories selecting swine clotted blood/serology admissions for PRV testing need to assure that: (1) the required epidemiological traceback information has been provided; (2) there is sufficient serum quantity; and, (3) the serum must be of good quality, free of bacterial contamination and excessive hemolysis.

If the sample has the necessary traceback information, analyze the sample to assure it meets the quantitative and qualitative requirements. Since samples are submitted to laboratories by practitioners for other testing purposes, surveillance testing is only to be performed if enough serum is present to perform all required tests. If the sample has enough serum to perform multiple tests, including PRV, please consider that if the sample is inconclusive/presumptive positive for PRV antibodies, the sample must be forwarded to the NVSL for confirmatory testing. Therefore, samples should have a minimum of two ml of quality serum.

Laboratories testing samples for PRV for other than USDA surveillance program purposes are to follow the manufacturer's recommendations on testing/retesting as required by the CFR and forward all positive samples to NVSL for confirmation.

Laboratories performing serological screening for USDA surveillance purposes are to use the following algorithm on all PRV surveillance clotted blood/serology samples.

- Review paperwork and information for proper traceback information. The information at minimum should provide a zip code or a premises ID of where the pigs are actually located (not where the corporate office is located)
- Observe the sample for sufficient serum quantity and quality.
- If the sample qualifies for testing, apply an USDA supplied bar-code to the sample
- Testing the sample:
 - Screening tests
 - PRV gB ELISA, **or**
 - Automated latex agglutination (ALA)

*Laboratories can choose which screening test to use for initial sample screening but should only perform one test.

- If the screening test indicates an inconclusive¹ or presumptive positive result, retest the sample using the PRV g1 ELISA test. Perform the g1 within one business day of the presumptive positive or inconclusive screening result.
- If the g1 indicates an inconclusive or presumptive positive result, submit the sample with all pertinent information including tests conducted and numeric results to the NVSL for confirmatory testing. Include information on which test method was used if the gB screening test was used as cutoff values vary with the method. Sample(s) should be shipped overnight to the NVSL
- Prior to shipping the sample (s), notify NVSL representatives via telephone. The section head of NVSL's Diagnostic Virology Laboratory can be reached by calling (515) 337-7551

After processing the sample(s), laboratories are to record all information on the PRV laboratory datasheet. The sheet is located on the USDA website at:
http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/pseu_surv_proced_manual.shtml

Sample Retention Criteria

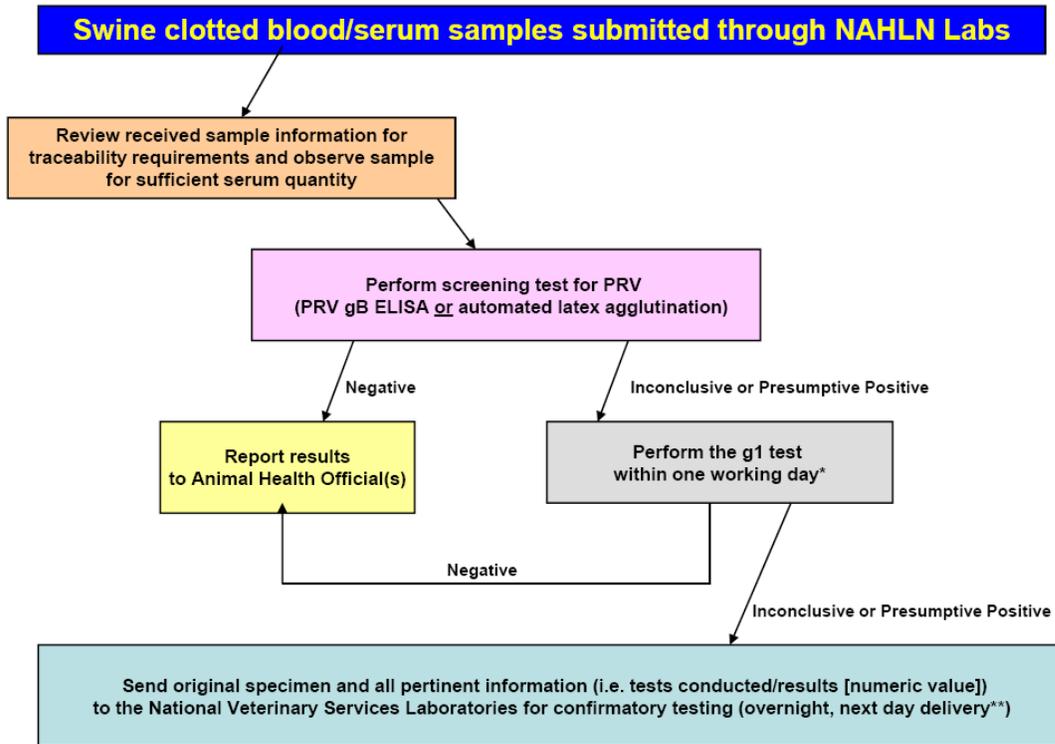
If a sample is negative on the primary screen, the laboratory can discard the sample according to your laboratory policy.

If a sample is positive on the primary screen and negative on the secondary screen, you may discard the sample according to your laboratory policy.

If a sample is positive on both the primary and secondary screens, you should forward the sample to NVSL for additional testing and should retain it and all other samples from that plate for at least 15 days

The following flow chart visually displays the USDA surveillance sample program PRV clotted blood/serum testing protocol.

¹ For PRV surveillance purposes, inconclusive is defined as a surveillance sample with interpretation values indicating the sample is neither positive nor negative (i.e., retest) on the initial test and after following the manufacturer's retesting recommendations



*Does not include weekends

**Samples shipped on Friday for overnight delivery need to have a Saturday delivery label adhered to the box

NOTE:

USDA PRV feral swine surveillance samples submitted to laboratories from USDA Wildlife Services are only to be tested via the screening tests (PRV gB ELISA or Automated latex agglutination). All screening test results, including inconclusive, positive and negative results, shall be reported back to USDA Wildlife Services, National Wildlife Disease Program (wslabresults@aphis.usda.gov or faxed to 970-266-6215). The g1 ELISA is not needed for testing feral swine sera. Additionally, positive samples from feral swine do not require submission to the NVSL for confirmatory testing unless they are needed for other regulatory purposes.

Appendix F: The 95/10 Serological Sampling Protocol for Blood Testing

This sampling procedure is to be used for all herds tested for PRV including those herds tested for traceback purposes. The 95/10 sampling procedure is a procedure that provides a 95-percent probability of detecting one positive animal in a herd where at least 10 percent of the swine are seropositive. Each segregated group of swine (different location, same owner) should be tested as a separate herd. The following guideline outlines the number of animals needing to be tested to meet the 95/10 protocol:

- For herds less than 100 head—test 25
- For herds with 100–200 head—test 27
- For herds with 201–999 head—test 28
- For herds with 1,000 and over—test 29

Appendix G: PRV Notification and Response Actions

Introduction

This appendix specifies the notifications and response actions resulting from a positive or inconclusive PRV laboratory finding. This document does not supersede any Veterinary Services Memos or Notices. Notifications and response actions will begin upon the report of an inconclusive or positive result from either a NAHLN laboratory or the NVSL. Full response will be indicated after the reporting of a confirmed PRV positive sample from the NVSL.

1.0 Response Actions

Samples tested as part of the PRV surveillance program will be initially screened at designated NAHLN laboratories by trained personnel. These personnel must follow specified protocol(s) for screening of serum, meat juice, and tissues for PRV Surveillance purposes. If results of the screening tests indicate an inconclusive or positive result, confirmatory testing will be performed at the NVSL in Ames, Iowa.

If serum from feral swine tests positive or inconclusive in a NAHLN laboratory, results should be reported to Wildlife Services. Wildlife Services will disseminate laboratory results through approved communication protocols involving their wildlife disease biologists to VS. These samples do not need forwarding to NVSL unless there is a need for other regulatory or research purposes. PRV positive feral swine samples do not initiate the described action below. Based on local regulatory authority and feral swine population status in a particular state, state agencies and Wildlife Services will likely implement varying responses to PRV findings in feral swine.

1.1 Inconclusive or positive result on sera (including meat juice samples) or tissues submitted to approved NAHLN testing laboratories, excluding routine feral swine samples

The following actions will be taken when an inconclusive or positive result is identified at a NAHLN laboratory.

1.1.1 Actions by NAHLN laboratories

- Immediately report² via telephone and fax the result to NVSL in Ames, IA and submit all of the sample to NVSL in Ames, IA for confirmatory testing as indicated in appendix E. **Do not split the sample.** Include all necessary paperwork and previous test results with the sample.
- Enter results and required data in the database or spreadsheet.

² Diagnostic Virology Laboratory, NVSL Telephone 515-337-7551; Fax number 515-337-7348

1.1.2 Actions by NVSL

- Perform confirmatory testing on samples received from the NAHLN laboratory.
- If the results are negative, notify the submitting laboratory of the test result
- If the results are positive, notify the submitting laboratory, the AVIC of the State where the lab is located, the AVIC of the State of animal origin, the Regional Epidemiologist and Swine Health Program Staff³.

1.1.3 Actions by VS and/or SAHO in the State where the NAHLN laboratory is located, the State where the sample was collected, and/or the State of origin of the source pig. **These actions are only performed if the sample is confirmed positive by NVSL.**

- Secure all paperwork and any identification materials associated with the submission.
- Determine the source of the submission.
- Determine the last known premises of residence of the animal of interest.
- If the sample was collected at slaughter, collect initial traceback information.
- Communicate findings to personnel in the Regional Office.
- Follow protocol as indicated in 'Pseudorabies Eradication State-Federal-Industry Program Standards' located at http://www.aphis.usda.gov/animal_health/animal_diseases/pseudorabies/downloads/program_std.pdf

1.1.4 Actions by Swine Health Program staff when a sample is confirmed positive

- Notify NCAHEM staff
- If necessary, assist Regional Officials in obtaining needed information.
- Review information received from the Region(s).

1.1.5 Notification

- External to VS
 - AVIC ensures that the State Veterinarian has been notified.
 - If appropriate, Staff or Regional officials will notify Legislative and Public Affairs (LPA) to inform industry and the public

Note: If parts of this section do not apply or need to be revised, applicable revisions will be made. Communications should be made to Troy Bigelow, Swine Health Programs by emailing troy.t.bigelow@aphis.usda.gov

1.2 Negative PRV result at NVSL for an Inconclusive or Positive NAHLN Result

If tests at NVSL indicate a negative result after a NAHLN laboratory reported and forwarded an inconclusive or positive result, all parties previously notified of the inconclusive or positive NAHLN result will need to be re-notified of the negative results. No further investigative actions need to be initiated.

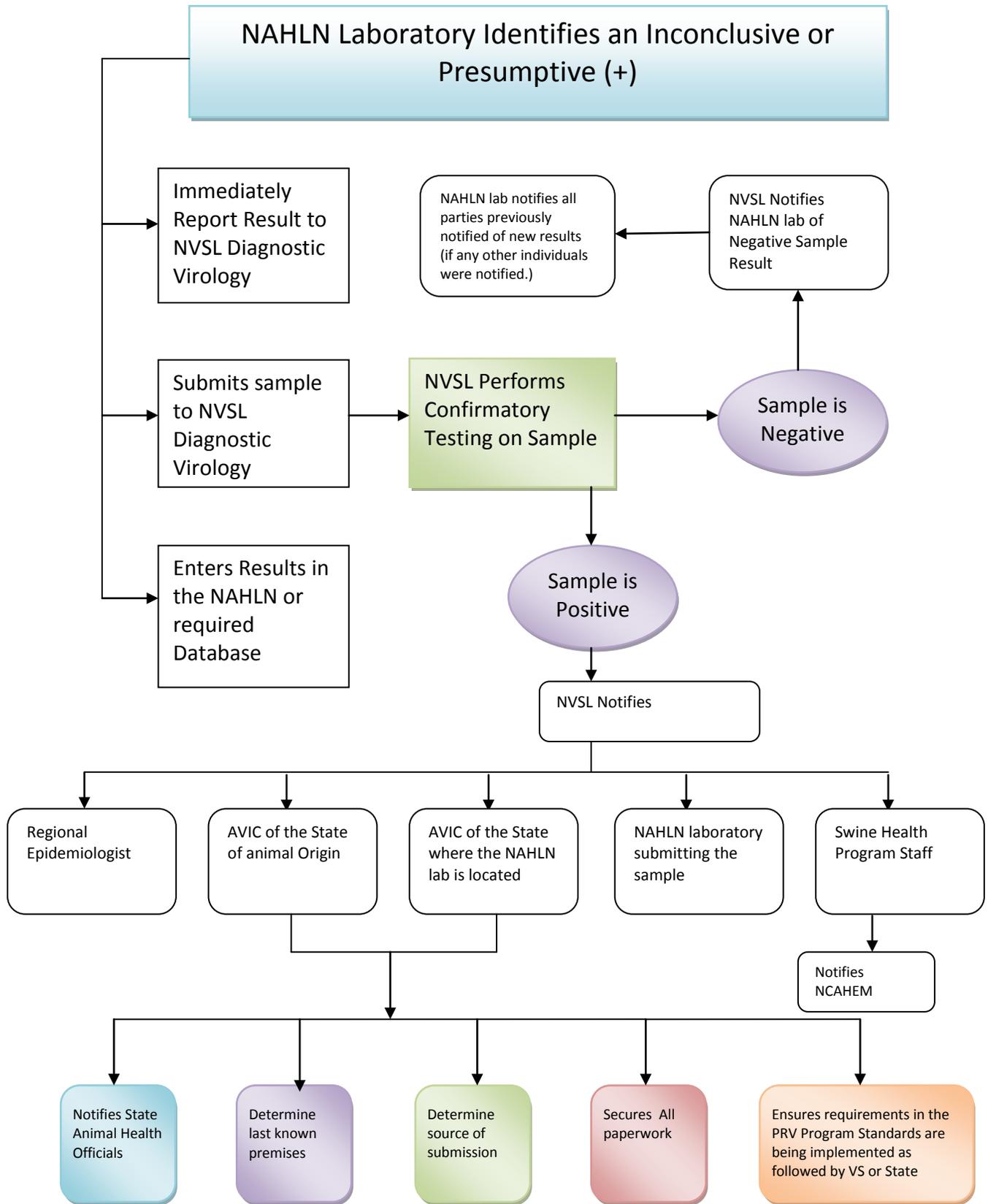
³ Swine Health Program Staff include: 1) troy Bigelow; 2) Dave Pyburn;

1.3 Confirmation of Positive Result at NVSL

If a positive result is confirmed at NVSL, the procedures outlined in section 1.1.3 thru 1.1.5 will be performed. The response to control and remove PRV from the infected herds will commence as quickly as possible. State Animal Health Officials in the State where the NAHLN lab is located and the State where the Animal originated need to be immediately notified. In addition, Animal Health Officials should begin performing all necessary activities to identify origin of the disease, collect all traceback information, and initiate quarantines to prevent the spread of disease. Animal Health Officials are to follow disease control and removal methods outlined in the Pseudorabies Eradication State-Federal-Industry Program Standards⁴.

The following page contains a flow chart of response and communication actions.

⁴ The Pseudorabies Eradication State-Federal-Industry Program Standards can be found by viewing http://www.aphis.usda.gov/animal_health/animal_diseases/pseudorabies/downloads/program_stds.pdf



Appendix H: The National Pseudorabies Surveillance Plan

The national pseudorabies surveillance plan can be viewed on the internet. The website is:

http://www.aphis.usda.gov/vs/nahss/swine/prv/prv_surveillance_plan_final_draft_04_16_08.pdf