Influenza Surveillance in Swine - Procedures

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Procedures Manual

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USDA

2010 National SIV Surveillance Program – Points of Contact

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Foreword

Developing a better understanding of the epidemiology and ecology of swine influenza virus (SIV) in the U.S. swine population is of significant value to swine industry and to USDA.

Influenza surveillance will facilitate the detection of new swine influenza virus strains and subtypes isolated from the swine population. In addition, this program will assist officials in analyzing the distribution of multiple SIV strains in the United States. In light of the potential public health significance of influenza isolates from swine in humans the U.S. Centers for Disease Control and Prevention (CDC) also has a significant interest in SIV surveillance.

The SIV surveillance plan is designed to detect and identify circulating influenza viruses in swine. Additionally, this surveillance plan will provide epidemiological data related to genomic sequences. Summary epidemiological data generated by this surveillance plan will be made available to the public in a format that protects individual farm identity.

This procedure manual is developed for laboratory and field staffs, as well as Federal and State animal health officials who need to be informed about these efforts. Detailed objectives of each component are described in subsequent sections of this manual. The objectives of the surveillance program are to:

- Monitor genetic evolution of SIV to better understand endemic and emerging influenza virus ecology.
- Make available SIV isolates for research and to establish an objective database for genetic analysis of these isolates and related information.
- Select proper isolates for the development of relevant diagnostic reagents, updating diagnostic assays, and vaccine seed stock products.

This manual also can be accessed on the internet at:

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

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I. Purpose

Full descriptions of influenza in swine and of this surveillance program's goals, objectives, and surveillance streams are fully covered in Appendix A (National Surveillance Plan for Swine Influenza Virus in Pigs).

The body of this document outlines implementation procedures and guidelines for the swine influenza virus (SIV) national surveillance program. The purpose of this document is to clarify:

1. Sample sources, data collection guidelines, and confidentiality safeguards.
2. Sample collection guidelines to maximize virus identification.
3. Sample submission guidelines and shipping instructions including when to refer possible SIV cases to the National Veterinary Services Laboratories (NVSL) for testing.
4. Laboratory guidance on sample testing procedures.
5. Notification guidelines in the event of an isolate of interest.

II. Sample Sources and Data Collection Guidelines

VS and industry agree that case compatible sample submissions into the SIV surveillance program can be enhanced if surveillance data are recorded by default into the SIV surveillance database as anonymous. Anonymous data entry removes all owner and submitting personnel information and limits geographical detail to the state of sample collection. If the owner of a submitted sample specifically wants premises of origin data entered into the SIV database, the sample data is entered as traceable, meaning the detailed premises and sample collector information is recorded in the SIV surveillance database. Submitters must provide written permission from the pig owner for the selected diagnostic lab to enter traceable data into the USDA SIV surveillance database. Further details regarding data elements collected for each option are covered in the description of each sample stream.

The following are stream-specific data sources and sampling methods:

A. Case-compatible swine accessions submitted to veterinary diagnostic laboratories

Producers, veterinarians, or other personnel may collect and submit samples from swine exhibiting ILI for SIV diagnostic testing to participating veterinary diagnostic labs. This may be part of a diagnostic rule out for respiratory disease. Animals to be sampled should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to ten (10) samples from case-compatible animals may be sampled per epidemiologic unit. The samples should be submitted to a participating veterinary diagnostic laboratory according to protocols detailed in section III of this document (see page 12). APHIS will pay the reimburse NAHLN laboratories for testing done in accordance with the established protocols in the SIV Surveillance Manual (see test guidelines in Appendix C). Details related to reimbursement are covered in NAHLN purchase agreements.

Two data entry alternatives exist for swine accessions tested for SIV, as described above. Anonymous data reporting is the default for all qualifying tested submissions unless the submitter provides written herd owner permission for the swine accession to be traceable.

The following sections differentiate data elements to be entered into the USDA SIV surveillance database for anonymous and traceable data reporting options:

Anonymous Surveillance Provisions:

1. Diagnostic laboratories including NVSL will process practitioner/producer submitted diagnostic specimens, as requested and necessary to obtain diagnostic results. Results (including SIV results) will be reported back to the submitter per normal business practices.
2. If samples meet SIV case requirements, the diagnostic laboratories will test submissions for SIV per the specified testing guidelines, identifying samples tested for SIV with a non-traceable USDA bar code label. The laboratory will then record only the information listed below (items a. through i.), into the SIV Surveillance database reporting sheet:
    a. NAHLN Laboratory Name* {Note: The NAHLN lab name will not be entered into the compiled SIV anonymous database by APHIS. It is only utilized by NAHLN administrators for accounting purposes, and will be stripped prior to sending data on to NSU for compilation and analysis.}
    b. Each laboratory will sequentially create a new reference codes, starting with 01 and ending at 99 (then returning to 01 again). This reference code substitutes for the laboratory's accession number and will be used along with the unique bar code number to separate accessions in the anonymous data fields without recording individual NAHLN lab accession numbers.
    c. NAHLN bar code (creates anonymity)
    d. Date collected
    e. Collection site State
    f. Age Class of swine associated with case/sample(sow/boar, grower/finisher, nursery or suckling)
    g. Specimen type
    h. Reason for submission / clinical signs associated with the case
    i. All SIV testing, isolation, or sequence results associated with the case/sample[1]
3. For positive samples undergoing individual H, N, and M-gene sequencing by cooperating NAHLN labs, results are to be submitted to GenBank. All sequence submissions to GenBank under the SIV Anonymous Surveillance Plan will include the GenBank minimum required fields of collection date, state of origin, host (swine), serotype, strain and source. Within the strain field the reference code (described above) and bar code number will be used (NOT the diagnostic lab accession number). GenBank accession numbers will be added to the SIV Surveillance database reporting sheet upon receipt from GenBank.
4. If additional testing is required by NVSL, an aliquot is labeled with the NAHLN supplied bar code and forwarded to NVSL.
5. After NVSL analysis and communication of results to the submitting lab, NVSL will provide additional assay results to NSU for inclusion in the SIV Surveillance database. These results will only be identified by the unique sample reference code number and bar code combination. NVSL will also submit results to GenBank entering their minimum required fields of collection date, state of origin, host (swine), serotype, strain and source. Within the strain field the reference code and bar code will be used and not the NAHLN labs' accession number.

[1] For further detail see the testing guidelines in Appendix C

Traceable Surveillance Provisions:

1. Any submission into the traceable stream of the SIV surveillance database is only with written producer permission. Diagnostic laboratories will process practitioner/producer submitted diagnostic specimens, as requested and necessary to obtain diagnostic results. Results (including SIV results) will be reported back to the submitter per normal business practices.
2. If samples meet SIV case requirements, the diagnostic laboratories will test submissions for SIV per the specified testing guidelines, identifying samples tested for SIV with a non-traceable USDA bar code label.
3. Once results are known and reported back to the submitter the diagnostic laboratory will then supply the information listed below (items a. through i.) and the lab results and are entered into the SIV Surveillance database, and sequences are submitted to GenBank. All sequence submissions to GenBank under the SIV Traceable Surveillance Plan will include the information listed below (items a. through i.)
4. If additional testing is required by NVSL and the submission is indicated for the traceable surveillance stream, the laboratory should include at least the information listed below (items a. through i.) when forwarding to NVSL.
5. For ***voluntarily submitted and documented*** traceable submissions only**,** the following information is to be reported in the SIV Surveillance Database reporting sheet:
    a. NAHLN lab name
    b. NAHLN lab submission/accession number
    c. Attending practitioner name and contact information
    d. Date collected
    e. Collection site information (address including zip code and location ID, if applicable)
    f. Age Class of swine associated with case/sample(sow/boar, grower/finisher, nursery or suckling)
    g. Specimen type
    h. Reason for submission /clinical signs associated with the case
    i. All SIV testing, isolation, or sequence results associated with the case/sample[1]
6. Voluntarily submitted individual case data will remain confidential to the maximum extent possible in accordance with APHIS authority and U.S Government privacy safeguards.

[1] For further detail see the Testing Guidelines in Appendix C

B. Targeted surveillance of sick pigs at first points of concentration or commingling events

Veterinarians who observe resident pigs displaying ILI at public swine events (e.g., fairs, expos, etc), markets, auctions or zoos should report the findings to the appropriate state animal health officials. The state animal health official will determine the appropriate follow up actions. If sampling occurs, sampled animals should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to ten (10) samples from case-compatible animals may be sampled per epidemiologic unit. The attending veterinarian and/or regulatory officials should submit samples from these animals for SIV diagnostic testing to participating NAHLN SIV veterinary diagnostic labs for analysis per established protocol (see section III on page 12 of this document). Unless written permission is granted by owners of pigs sampled for traceable surveillance, all collection data and testing results will be entered on the SIV surveillance database data sheets via the anonymous protocols listed above.

C. Surveillance of swine populations epidemiologically linked to a human case of SIV

These cases are investigated only with the consent of the pig owner/producer. In cases where swine are linked to human cases of SIV, Animal health officials, in cooperation with public health investigators will determine whether swine are epidemiologically linked to human infections on a case by case basis. These investigations will occur in cooperation with the swine operation's licensed veterinarian having a valid client patient relationship (if applicable). Under the direction of the State Animal Health Official (SAHO), personnel may collect nasal swabs and/or lung from swine meeting the test eligible case definition of ILI in swine. Sampled animals should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to ten (10) samples from case-compatible animals may be sampled per epidemiologic unit. The samples should be submitted to NVSL according to protocols detailed in section III of this document (see page 12). These samples will be entered into the SIV surveillance database data sheets via the traceable protocol listed above.

D. Data Management

Data for SIV testing are currently being collected on NAHLN lab-specific spreadsheets and forwarded to the NAHLN coordinator. The spreadsheet is viewed as a temporary data management solution until data can be collected via a permanent APHIS data management system that meets the above data requirements.

The NAHLN lab or NVSL lab will enter only those sample collection data elements listed above for anonymous surveillance or traceable surveillance provisions into the SIV surveillance database data sheets. The following list more clearly defines data elements common to both anonymous and traceable data reporting, as well as additional elements captured with traceable data collection:

Data elements to be included in both anonymous and traceable SIV data records:

- Reference Code (2 digits, unique to each separate accession)
- Specimen Barcode Number (creates anonymity)
- Specimen Collection Date
- Collection Site State
- Reason for submission
- Age Class
- Specimen Type

- Test DATE and Test RESULT for each SIV test conducted (e.g., test types are Matrix PCR, N1 PCR, virus isolation, hemagglutinin typing, neuraminidase typing, matrix typing)
- GenBank accession numbers
- Remarks (optional)

ADDITIONAL data elements to be included in all traceable data entries:

- NAHLN Laboratory Name
- NAHLN Laboratory Accession Number
- Animal identification number
- Sample Collector: Attending practitioner name and contact information
- Collection Site Location information: address including zip code and location ID, if applicable
- Remarks (optional)

E. Response to Swine Influenza Virus Surveillance Findings in Swine

Swine influenza commonly occurs in modern production facilities. Many swine influenza viruses cause only mild illness, however due to the reassortment capabilities of influenza, the potential exists for more pathogenic viruses to occur.

The objectives for SIV surveillance are to:

- Monitor genetic evolution of SIV to better understand endemic and emerging influenza virus ecology.
- Make available SIV isolates for research and to establish an objective database for genetic analysis of these isolates and related information.
- Select proper isolates for the development of relevant diagnostic reagents, updating diagnostic assays, and vaccine seed stock products.

This surveillance program standardizes a system by which isolates are collected, analyzed and made available for further study. The strategies used to respond to SIV surveillance data and analysis will be case-specific but, in general, are:

- Enhanced biosecurity measures; and
- Retention of swine until clinical signs have resolved sufficient for movement as determined by or under the supervision of a licensed veterinarian.

There are three situations by which an isolate can enter the surveillance program:

A) Case-compatible swine accessions submitted to veterinary diagnostic laboratories.

Normal diagnostic work-up protocols and communications are followed prior to entry of the testing results data into the anonymous or traceable surveillance database. Pandemic H1N1(2009) is a special case and when isolated from swine will be reported to the SAHO prior to entry of information into the anonymous or traceable surveillance database and through links that include VS NCAHEM, CDC Infectious Diseases, AVIC of state of origin, VS CDC Liaison, and the swine industry.

Data from the anonymous SIV surveillance stream will be monitored and analyzed by USDA, in collaboration with industry stakeholders, to identify sequences and data that will assist in meeting the USDA surveillance animal health objectives. SIV sequences or data that are identified to a state may initiate further research or targeted surveillance in that state in cooperation with the SAHO and industry of that state.

Data from the traceable SIV surveillance stream will be monitored and analyzed by USDA, in collaboration with industry stakeholders, to identify sequences and data that will assist in meeting the USDA surveillance animal health objectives. Response to SIV sequences or data that are identified to an owner, site or operation will be handled on a case by case basis under the authority of the SAHO. Normal operations continue when clinical signs have resolved sufficient for movement as determined by or under the supervision of a licensed veterinarian.

B) Targeted surveillance of sick swine at first points of concentration or commingling events.

Swine arriving at an auction, market, fair, or other swine exhibition event, exhibiting clinical signs of any disease, including ILI, must be managed in accordance with State and Federal regulations and should not be off loaded. Groups of swine that are exhibiting ILI after off-loading should be isolated and:

- Held until the group has recovered as determined by a licensed veterinarian or,
- Relocated to an offsite location (may be the premises of origin) where the group can be isolated, cared for and allowed to recover.

If not already implemented, biosecurity measures to prevent interspecies transmission should be adopted until the animals have recovered from ILI as determined by or under the supervision of a licensed veterinarian.

Normal diagnostic work-up protocols and communications are followed prior to entry of the testing results data into the anonymous surveillance database or prior to entry into the traceable surveillance database upon permission of the producer/owner. The SAHO is responsible for appropriate response based on the communicated information prior to entry of the testing results data into the anonymous or traceable surveillance database.

Normal operations are allowed to continue for groups of swine not showing ILI.

C) Surveillance of swine populations epidemiologically linked to a human case of SIV.

In the event of a human case of SIV infection in which exposure to swine may have been involved, USDA will work closely with Federal and State public health officials, the SAHO, the affected producer and their herd veterinarian, and the swine industry to determine the appropriate course of action. If it is determined that there is an epidemiological link between swine and an infected person, the health status of the swine should be assessed under direction of the SAHO and in cooperation with the owner/producer and the licensed veterinarian.

I. No swine with ILI are observed.
If swine linked to a human infection with SIV are observed to have no signs of ILI, there will be no action.

II. Swine with ILI are observed.
If swine linked to a human infection with Swine Influenza Virus are observed to have signs of ILI that meet the case definition established in the "*National Surveillance Plan for Swine Influenza Virus in Pigs*" normal operations continue when clinical signs have resolved sufficient for movement as determined by or under the supervision of a licensed veterinarian.

Pandemic H1N1(2009) is a special case and when isolated from swine will be reported to the SAHO and through links that include VS NCAHEM, CDC Infectious Diseases, AVIC of state of origin, VS CDC Liaison, and the swine industry.

F. Evaluation and Next Steps

This flexible approach of anonymous surveillance will be evaluated 12 months after implementation of an outreach effort to evaluate attainment of SIV surveillance program objectives. Further modifications may be considered at that time.

G. Non-swine Sampling and Testing

Non-swine species animal testing is not covered in this manual, however if you have samples from outside the scope of this swine surveillance program please contact NVSL Diagnostic Virology Laboratory for submission approval and information on appropriate non-swine samples and handling.

III. Detailed Sample Collection Procedures for Regulatory Officials

This section explains which samples are to be collected and the procedures to follow for regulatory sample collection. All collection procedures in this section are validated for SIV diagnostic work and validated for SIV surveillance activities. Diagnostic labs may use this to advise veterinarians on collection procedures.

A. Tools Needed

- For removing lung tissues from euthanized or expired animals
    - Knife and scissors
    - Forceps
    - Plastic Whirl-Pak® bags or screw top-plastic tubes
- For collection of nasal swabs
    - Dacron or synthetic nasal swabs (no wooden handle swabs)
    - Collection tube with viral transport media [such as Tris Buffered Tryptose Broth (TBTB) or Brain Heart Infusion (BHI) from a Foreign Animal Disease kit]
    - Hog snare or other swine restraining device
    - Ear plugs
- Tools and items necessary for both lung tissues and nasal swab collection
    - Cooler
    - Ice packs
    - Fine-point permanent marker or other waterproof marker
    - Ball-point pen
    - Pan or bucket for disinfecting instruments after appropriate contact time to ensure disinfection
    - Bleach (disinfectant)
    - Personal protective equipment (PPE) to protect yourself from potential exposures
    - Trash Bags for soiled PPE
    - Submission form(s) (VS Form 10-4 for regulatory officials)
- If bar code labels are needed, contact NVSL Shipping. Contact information for NVSL appears on page 2 of this manual (2010 National SIV Surveillance Program – Points of Contact).

B. Steps in Collecting Specimens for SIV

Nasal swab or lung tissue should be taken for SIV surveillance testing. Disposable tissue and nasal swab sample collection supplies can be obtained by contacting your AVIC, as listed in Appendix C**.**

Nasal swab: DO NOT pool swabs from individual pigs.

- The pig should be properly restrained with the head positioned upward to allow easy access to the nasal cavity. Anesthesia is not needed.

- Insert a sterile Dacron swab into the nasal cavity and gently swab the surface of the nasal mucosa, using a circular motion to cover as much of the nasal mucosal surface as possible. Avoid touching the skin with the swab as you enter the nasal cavity.

- The swab will collect nasal mucosal secretions and surface epithelium. It is important not to scrape too hard, as drawing blood is undesirable.

- Remove the Dacron swab from one nostril and repeat the same procedure in the other nostril, using the same swab.

- Once the nasal swab (both nostrils) has been collected, mix the swab in a transport media designed for maintaining viruses (TBTB or BHI viral transport media).

- The volume of viral transport media should be sufficient to cover the head of the swab.

- To remove the swab handle, back the swab out of the tube slightly and bend the portion of the handle that the collector touched back and forth over the edge of the tube until it breaks. This portion of the handle is considered contaminated and should be discarded. Alternatively, scissors or wire cutters may be used to cut the swab handle.

- Clearly label with appropriate ID (using a waterproof marker), place the sealed tubes in a Whirl- Pak® bag, and immediately refrigerate or chill.

- Store and ship in an upright position to reduce chances of leakage.

Lung Tissue

- When collecting lung tissue, the tissue should be fresh, or as fresh as possible. Collect multiple sections of lung tissue from affected areas.

- Samples should be **at least** half-dollar size.

- Be sure to include the junction of normal and abnormal lung tissue.

- Double bag, using Whirl-Pak® bag for at least the inner bag, clearly label with appropriate ID (using waterproof marker), and immediately refrigerate or chill.

- Lung tissue from each individual animal should be kept in a separate bag.

C. Proper Labeling of Samples

Samples should be labeled with bar code labels, if available. Samples collected by Animal Health Officials should be labeled consistent with other animal disease programs, such as the Classical Swine Fever (CSF) program or the Pseudorabies Virus (PRV) program.

- Label each tube or sample collection container with a waterproof pen. Include on each label:
  - Sample number
  - Type of specimen in tube or container (i.e., lung tissue, nasal swab)
  - Bar code identification label should be used if available,
    - 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

- Place the sealed samples in a Whirl-Pak® bag, and then place in a cooler and/or on cold packs. **Do not freeze specimens**.
- Properly dispose of non-submitted tissues and/or carcass.
- Send the samples overnight to a participating NAHLN SIV testing lab or NVSL.
- Questions regarding sampling techniques can be directed to the program managers at APHIS-VS-National Center for Animal Health Programs (NCAHP). Contact information for NCAHP appears on page 2 of this manual (2009 National SIV Surveillance Program – Points of Contact).

IV. Influenza Virus Biosafety Guidelines

A. Laboratory Workers

See: CDC web site, **http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm**

Interim Biosafety Guidance for All Individuals Handling Clinical Specimens or Isolates containing 2009-H1N1 Influenza A Virus (Novel H1N1), including Vaccination Strains

NOTE: This guidance was developed by CDC specifically for individuals working with clinical specimens or isolates from **human** patients with suspected pandemic influenza A (H1N1) virus infection. Although not written specifically for individuals who work with clinical specimens or isolates from swine exhibiting signs of influenza-like illness, this document is provided as a source of information about biosafety measures that could be considered.

For additional information: **Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition**

B. Collectors and Submitters of Samples

Personnel should take all necessary precautions to prevent themselves from exposure to pathogens during collection of samples. Safe hygienic practices are necessary to prevent exposure and possible infection when collecting samples from ILI swine with an unknown etiology. Personal protective equipment should include but not be limited to coveralls, rubber / polyurethane boots, disposable gloves, safety goggles, disposable head covering and respirator. Proper biosecurity measures must be followed to prevent contaminants from spreading on a premises or off a premises (Biosecurity recommendations of the National Pork Board can be found at www.pork.org).

V. Shipping of Specimens to NAHLN labs or to NVSL (including Specimens being shipped from NAHLN Laboratories to NVSL for Confirmatory Testing)

The following protocol should be used to submit samples to NAHLN or NVSL. NAHLN laboratories sending samples to NVSL for confirmatory testing should follow standard laboratory sample submitting protocols and regulations for submitting samples to NVSL.

A. Packing and Shipping Specimens

Packaging material

- Paper towels to be used as absorbent material in-between primary and secondary shipping containers
- To prevent leaks, Whirl-Pak® bags are to be used as primary shipping containers for tissue samples and tubes are primary shipping containers for swabs. Whirl-Pak® bags or Ziploc freezer bags are used as secondary shipping containers
- Mailers to submit samples

- Place labeled sample tubes/containers into the clear bio-hazard bag (STP-741) or Whirl-Pak® bag along with absorbent and seal.

- Place this bag into white bio-hazard bag (STP-740), or another approved mailer, and seal.

- Place the white bag into the shipping box.
- Place frozen ice packs around the bag (both sides).

- Add enough additional packing material to the box to prevent the sample and the ice from shifting around.
- Place completed VS Form 10-4 on top of inner Styrofoam lid.

- Seal box
- Place address shipping label on the box addressed to designated laboratory conducting SIV testing for this collection site.
- Place the other required shipping labels on the box.

- Ship by overnight delivery with a contract carrier service.
- Airline shipments should be in compliance with current International Air Transport Association (IATA) regulations (**www.iata.org**) for dangerous goods.

  ****If shipping on a Friday, first confirm that the receiving lab can receive on a Saturday and be sure to mark/label box for Saturday delivery. Priority of shipments will be determined on a case-by-case basis.****

  [NOTE: Participating NAHLN laboratories will be reimbursed for the expense of shipping positive samples to NVSL for confirmatory testing. This includes the Saturday delivery surcharge.]

Contact NVSL for information regarding shipping materials. If you need further assistance with shipping, you may contact the shipping department at:

National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, IA 50010
Ph: (515) 337-7530
Fax: (515) 337-7378

B. Designated NAHLN Laboratory

Ship all SIV specimens via your local overnight contract delivery service to the designated NAHLN laboratory or NVSL. See Appendix D to identify a designated NAHLN laboratory, or contact the AVIC or State Animal Health Official (SAHO) for specific instructions. Appendix B contains contact information for AVICs and SAHOs.