

**Manual of Standard Operating
Procedures for Texas LPAI Response**

May 2006

Introduction

Whenever a LPAI is detected in Texas poultry flocks, this SOP Manual will be employed unless modified at the direction of the Incident Commander (IC) placed in charge of the response.

A surveillance plan will be implemented to demonstrate that LPAIV has been eliminated within the surveillance area. This manual provides standard operating procedures (SOP) for task force activities. Additional SOPs will be added as needed.

Definitions

Affected zone

The Affected Zone includes the area within 8 km (5 miles) of any infected flock. All bird premises within the Affected Zone are issued hold orders and will undergo surveillance sampling to determine disease status of the flock.

Surveillance zone

The Surveillance Zone includes the area between 8 km (5 miles) and 16 km (10 miles) from any infected flock. All bird premises within the Surveillance Zone will undergo surveillance sampling to determine disease status of the flock.

Case definition

Infected premises (IP)

A premises will be designated infected when there are birds **with** clinical signs and/or gross lesions consistent with avian influenza **plus one** of the following conditions.

- Isolation and identification of an H5 or H7 AIV
- Positive RRT-PCR with H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies

Or the birds have **no** clinical signs and/or gross lesions consistent with avian influenza infection, **but two** of the following conditions.

- Isolation and identification of H5 or H7 AIV
- Positive AIV RRT-PCR with H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies
- Directigen Flu A or other approved antigen capture positive test (cannot be only criterion even with an epidemiologic link to designate a premises as positive)
- Epidemiologic link

Dangerous contact premises (DC)

A premises will be designated a dangerous contact when a significant risk of exposure to H5 or H7 AIV virus exists due to either proximity or an established epidemiological link to an infected premises. A dangerous contact premises will be issued a hold order.

Required depopulation

The destruction of birds on an infected or dangerous contact premises. Birds on an infected premises will be destroyed. A recommendation regarding the depopulation of birds on a dangerous contact will be made by an epidemiologist. The final decision to depopulate a dangerous contact will be

made by the incident commander. Indemnity will be available for required depopulation.

Voluntary depopulation

An owner-requested depopulation of birds on a premises that is neither an infected premises nor a dangerous contact premises that has been designated for required depopulation. Indemnity will not be available for voluntary depopulation.

Quarantine

A state-enforced action on a premises that restricts the movement of animals and materials onto and off of that premises. Additional special provisions may apply. Movements may be allowed under federally issued permits.

Hold order

A state-enforced *de facto* quarantine.

Commercial premises

Producer operations that participate in the Texas Poultry Federation AI surveillance program.

Non-commercial premises

Operations that raise or hold poultry and/or other avian species, and do not participate in the Texas Poultry Federation voluntary surveillance plan.

Bird owner

A person with immediate control of the birds, or immediate control over the premises where the birds are located. Accordingly, depending on circumstances, the owner could be a resident, an agent, a property owner, a property manager, or any combination thereof. The designation of someone as an "owner" does not preclude others from being considered an "owner" as well.

General Quarantine

Issuing quarantines

All infected premises will be issued quarantines.

Quarantines should be issued to bird owners in person by task force personnel.

At least three attempts to issue the quarantine should be made at different times of the day. Phone calls may be warranted. Unsuccessful attempts to issue the quarantine will be documented.

If the disease situation requires immediate action, the State Animal Health Official may authorize the quarantine on epidemiological grounds.

Releasing quarantines

In order for an infected premises to be eligible for release, the following conditions must be met:

1. The TX AI sampling plan must be completed and all samples tested negative.
2. The premises has been free of birds for 21 days following effective cleaning and disinfection; **or** an assessment of an epidemiologist recommends quarantine release. The absence of birds should be confirmed through visual inspection of the premises.

Hold orders

Issuing hold orders

All premises within the Affected Zone and dangerous contact premises will be issued hold orders.

Hold orders will be issued to premises within the Surveillance and Buffer Zones when sick birds are present and AI is suspected.

Hold orders should be issued to bird owners in person by task force personnel.

At least three attempts to issue the hold order should be made at different times of the day. Phone calls may be warranted. Unsuccessful attempts to issue the hold order will be documented.

If the disease situation requires immediate action, the State Animal Health Official may authorize the hold order on epidemiological grounds.

Releasing hold orders

Hold orders will be released when the TX AI sampling plan has been completed and all samples tested negative.

Premises requesting release from a hold order due to extenuating circumstances will be evaluated by an epidemiologist who will submit a recommendation for approval by the incident commander.

Incident and disease reporting

Incident reports are prepared by the Plans section and submitted to the incident commander for review and approval. Reports include:

1. Incident Action Plan,
2. Situation Report,
3. Epidemiology Report and
4. Surveillance Plan

Reports are distributed to the Texas Animal Health Commission and VS regional and national offices.

Situation reports will provide information updated daily and include:

- Critical new information
- New and cumulative IPs and DCs.
- New and cumulative E&D and C&D
- New and cumulative quarantines and hold orders
- Surveillance activities directed by the plan including
 - Negative and positive samples processed
 - Samples submitted and lab results
- New and cumulative movement permits issued, including number of animals moved.
- Summary of response actions and achievements
- Summary of outreach and public information activities

Premises are classified as negative, infected, or dangerous contact by the disease reporting officer (DRO). These classifications are made in accordance with the incident case definition and in consultation with incident

epidemiologists. The DRO is responsible for quality of data (timeliness, accuracy, comprehensiveness) in the EMRS investigation module.

General epidemiology

Epidemiologists will maintain clear notes and organized case files. Epidemiologists will also leave a description of each problem area, history, pertinent epidemiological links, status of unfinished projects, potential leads and traces, current status of all tasks and their progress, and any other vital information to their replacements. These materials and issues will be discussed during the rotation transition.

Case investigation

The lead epidemiologist is responsible for documenting a detailed account of the epidemiological investigation of the incident. The report will include hypotheses for the introduction of an H5 or H7 AIV, investigation of infected and dangerous contact premises, surveillance activities directed toward identifying infected premises and surveillance activities directed toward demonstrating freedom of H5 or H7 AIV virus in the affected, surveillance and buffer zones.

Monitoring the surveillance plan

The surveillance plan will be monitored by the incident's lead epidemiologist. Field activities will be monitored and evaluated to assure the goals of the surveillance plan are being achieved. Concerns and issues regarding the progress of the plan will be brought to the immediate attention of the IC. All modifications to the plan will be submitted to the IC for review and approval.

Procedures for Responding to Sick Calls

1. Epidemiologists will receive information on illness (from tips, leads, field results, epidemiological field visits, etc.).
2. A premises identification number will be assigned to each physical premises. Epidemiologists must have a street address to get a premises identification number assigned.
3. If epidemiologists need a new premises identification number, they will complete an Avian Summary Form and submit it to the data entry group.
4. Epidemiologists, through the EMRS, will "task" the diagnostics group to conduct a Foreign Animal Disease (FAD) investigation involving samples and an epidemiological questionnaire.
5. All premises that undergo diagnostics sampling will receive a hold order, if one does not already exist. Hold orders are in effect until they are released according to specific release requirements.
6. Samples will be couriered or hand-delivered to the appropriate lab in the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) system. Positive identification of AI virus and sequencing of the isolate must be performed at NVSL.
7. Results from these samples will be faxed to the Task Force's Disease Reporting Officer (DRO).
8. The DRO, often with consultation of the epidemiological group, will evaluate the significance of laboratory findings and will declare IPs based on the case

definition of H5 or H7 AIV.

9. DCs will be determined by epidemiologists (based on information from the surveillance or diagnostics groups or a field visit).
10. If deemed necessary to depopulate a DC, epidemiologists will write a justification based on epidemiological evidence. Epidemiologists must establish a justifiable epidemiological link of risk meriting depopulation. The incident commander will make the final decision to depopulate a DC.
11. Epidemiologists will write a request for DC classification and will present it to the DRO. The DRO will verify the accuracy of the information and make the EMRS classification change. The request will identify the premises, describe the epidemiological link, and document the justification for the classification.
12. The DRO will task operations with the depopulation of all IPs and specified DCs. Appraisal, euthanasia, disposal, and cleaning and disinfection all are undertaken by the operations group. Only the DRO can classify a premises and make the necessary entries in the EMRS.
13. The DRO will monitor work done on DCs and IPs and adjust the status of these premises in the EMRS

General biosecurity

Task force personnel visit multiple premises routinely as a part of their job. Inadvertent contact with viruses and bacteria can occur on these premises and without the proper precautions the organisms can be spread to the next premises visited. Therefore, field personnel should make extraordinary efforts to ensure that they do not spread an agent to other facilities or animals.

Basic Biosecurity Equipment

- Disposable plastic boots or rubber boots that can be cleaned and disinfected
- Bucket and brush for cleaning boots and equipment
- Broad-spectrum surface disinfectant such as Virkon S or Nolvasan.
- Disposable or clean, reusable coveralls or smocks
- Fingernail brush
- Hand sanitizers
- Disposable masks
- Disposable hair covers
- Disposable gloves
- Protective eyewear
- Garbage bags
- Zip-lock bags
- Water supply where water is unavailable or possibly contaminated

Biosecurity measures

All Task Force personnel will take the following minimum biosecurity measures:

1. Wear rubber boots (or other footwear that can be cleaned and disinfected) or disposable plastic boots. When visiting low-risk areas, such as offices or homes away from animal areas, clean street shoes or boots are acceptable. It may be possible to store footwear at facilities that would only be worn there. NOTE:

Some animal owners provide rubber boots or disposable plastic boots for visitors. Ask if they would prefer that you used their footwear, if provided.

2. Remove all dirt and organic matter from your boots and then thoroughly disinfect them using a bucket, brush, and an appropriate broad-spectrum disinfectant prior to entering and when leaving premises.
3. Wear disposable or clean coveralls, laboratory coats, smocks, or other suitable outerwear when you plan to come into contact with animals, manure, or animal secretions. If visiting multiple facilities, have an ample supply of disposable or clean coveralls so a fresh pair can be used at each site. Outerwear need not be sterile, but if it has come into contact with animals or is soiled with manure, blood, or other secretions change into a clean replacement when leaving a premises. Place dirty materials in a double plastic bag and seal it.
4. Thoroughly wash your hands with antimicrobial soap prior to entering and when leaving a premises. Liberal use of an appropriate hand sanitizer may be used if soap and water is unavailable. Disposable latex gloves should also be used, but not as a substitute for proper hand washing. NOTE: Remove watches, jewelry, and other items prior to washing your hands. Lather your hands with soap and then rub hands together vigorously for 15-20 seconds. Finish by rinsing under a stream of water if available.
5. Avoid driving through manure and wastewater. Park your vehicle away from pens, pastures, or areas where animals may be held. Park on concrete or paved areas when available. NOTE: Do not enter animal production areas unless a facility employee is present or you have been authorized to do so by the facility owner.
6. Clean your vehicle between visits, including tires and floor-mats (carpets should be covered with plastic floor mats). A commercial car wash is adequate. Tire sprays may be necessary in some situations.
7. Place disposable items in a double plastic garbage bag and seal them for later disposal in the designated dumpster at the Task Force headquarters.
8. Keep all equipment used in the field clean. Disinfect equipment that comes into contact with animals or their secretions before taking it to another premises, or use disposable equipment. NOTE: When making visits to premises, select equipment that is easily disinfected. Plastic clipboards are easier to disinfect than wooden and organic material is easier to see.
9. Keep clean and dirty clothing, equipment, and supplies separate. Designate “clean” and “dirty” storage areas in your vehicle.
10. If you come into contact with a sick or dying animal, consider yourself a carrier of disease and clean your shoes, shower, put on clean clothing, and wash your car before coming into contact with other animals.

Procedures for Biosecurity before Leaving Premises

1. Task Force personnel will thoroughly clean and disinfect all reusable equipment and eyewear.
2. Task Force personnel will place disposable coveralls (turned inside out), boots, and other solid items in a double plastic garbage bag to be placed in the “dirty”

area of their vehicles. These items will be disposed of in the designated dumpster at the task force headquarters.

3. Task Force personnel will wash their hands with soap and water or antibacterial gel.
4. Task Force personnel will disinfect vehicle tires upon exiting the premises.

Procedures for Biosecurity at the End of the Day

1. Task Force personnel will clean and/or launder all reusable clothing and equipment.
2. Task Force personnel will take a shower and shampoo their hair, clean under their fingernails, and clear their respiratory passages by blowing their nose, clearing their throats, and spitting into a sink with running water.
3. Task Force personnel will ensure that their vehicle is washed thoroughly at the end of each work day.

Protocol for Foot Baths

Equipment and Supplies Needed

- Plastic grass rugs
- Holding tray
- Rubber-backed carpets
- Prepare a 1 percent solution (1.3 ounces of Virkon S concentrate to 1 gallon of water)
- Water

Procedures for Maintaining Foot Baths

1. Logistics personnel are responsible for maintaining foot baths to prevent the spread of virus.
2. Foot baths will be located in the front of entrances to building doors.
3. Plastic rugs will be placed inside fiberglass trays and covered with a 1 Stroke solution (½ ounce to a gallon of water) or Virkon S solution (1.3 ounces to a gallon of water).
4. Rubber-backed carpets (4' x 6') will be placed in front and behind the trays. Trays have nonskid carpet under them to keep them from moving when used.
5. Foot baths will be cleaned at least twice a day.

Surveillance sampling

The tables below provide the timeline and necessary samples to be collected in the zones.

Non-commercial Farms:

Zone	Round 1		Round 2*	
	Serum	Swab**	Serum	Swab**
Affected Zone	YES	YES	YES	YES
Surveillance Zone	YES	YES	YES	YES

* Sampling for Round 2 will begin 14 to 21 days after Round 1 sampling.

**Tracheal swab samples will be collected from all gallinaceous birds and cloacal swab samples will be collected from all waterfowl.

Commercial Farms*:

Zone	Round 1		Round 2**	
	Serum	Swab***	Serum	Swab***
Affected Zone	YES	YES	YES	YES
Surveillance Zone	YES	YES	YES	YES

*Samples from commercial farms may be collected by trained commercial farm employees rather than task force personnel.

**Sampling for Round 2 will begin 14 to 21 days after Round 1 sampling.

***Only tracheal swab samples will be collected from commercial farms.

Dangerous Contacts and Sick Calls

Flocks determined epidemiologically to be dangerous contacts will have both serum and swab samples collected initially. If results of the initial sampling are negative, a second set of serum and swab samples will be taken 14 to 21 days after the initial sampling. Any flocks in which sick birds are reported will have both serum and swab samples collected. Tracheal swabs will be collected from gallinaceous birds and cloacal swabs will be collected from waterfowl. All dangerous contact and sick call samples will be submitted either to Texas Veterinary Medical Diagnostic Laboratory or the National Veterinary Services Laboratories in Ames, Iowa. The number of birds to be sampled from each house is presented in Table 1 below.

Number of birds in each house	Minimum number of birds to be sampled
10 or less	Sample all
20	15
30	15
40	15
50 or greater	20

Surveillance Team supplies:

- Biosecurity supplies - Tyvek coveralls, boot covers, gloves, hair net, dust mask, protective eyewear,

disinfectant spray bottle, disinfectant, waterless hand cleaner.

- Brain Heart Infusion broth (BHI) - must be stored in cooler located in "clean" area with ice packs (should be clear amber color, if discolored or cloudy discard).
- Sterile Dacron swabs
- Cooler, ice packs or wet ice
- Zip lock bags, large trash bags
- GPS unit
- Forms: Avian Influenza Surveillance

Procedures before arriving at a premises:

1. Surveillance Teams will receive their assignments each morning from the operations chief or his or her designate.
2. The Surveillance Teams should prepare the vehicle for the day's assignments by re-stocking all needed supplies and forms.
3. Maintain a clean and dirty area of the vehicle at all times.
4. The Surveillance Teams will attempt to notify premises owner that their arrival is imminent. BHI tubes, blood tubes, and zip lock bags should be labeled with waterproof marker with premises identification number.
5. The vehicle should be parked in an area near the premises but in a "clean" area. Windows should be kept closed and the vehicle should be kept locked when personnel are away from it.
6. One team member is identified as the sampler and the other is the catcher.

Procedures upon arrival at a premises:

1. Prior to exiting the vehicle, each team member should put on one pair of boot covers.
2. Team member will ask the owner to come to the "clean" area or near the vehicle to complete the necessary paperwork. The "clean/dirty" line should be strictly adhered to.
3. Team members will put on biosecurity equipment (refer to Biosecurity SOP) and gather necessary supplies to collect samples. The sampler will duct tape a large plastic bag and two zip lock bags to the Tyvek suit. The necessary number of BHI tubes, blood tubes, and/or swabs will be put into one of the two zip lock bags. The other bag is for the samples once they are ready for laboratory submission. Team members will wear two pairs of gloves.

Procedures while on the premises:

1. The Surveillance Team will observe all of the birds to get an accurate inventory of the flock to record on the Avian Influenza Survey Form. They will also determine the health status of the birds.
2. If dead or sick birds are observed, exit the bird housing area immediately and contact a supervisor.

3. Refer to the table to determine which samples need to be collected for each time period and zone.

Guidelines for serum sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- Blood is collected from the wing vein into a 3 ml red top tube. Collect at least 1 ml of blood from each bird using a sterile blood collection tool (scalpel or needle (22g) and syringe (3cc)).
- Place tubes in zip lock bag taped to Tyvek suit. Place blood collection instruments in trash bag.

Guidelines for swab sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- The sampler will use dry swabs to collect an oropharyngeal/tracheal swab from each gallinaceous bird. Collect only cloacal swabs on waterfowl species.
- Put up to 5 birds' samples in each BHI tube, keeping species separate and cloacal and oropharyngeal/tracheal swabs separate. Write the premises identification number, the species and the location on each tube.
- Swirl each swab in the media and wring out against the side of the tube then remove the swab. DO NOT LEAVE SWABS IN THE TUBE.
- Dispose of used swabs in trash bag taped to the Tyvek suit of the sampler.

Procedures for exiting the premises:

1. Pick up all samples, bag(s) of trash and take to clean/dirty line established earlier.
2. At this area the sampler will remove all biosecurity clothing using appropriate procedures (see Biosecurity SOP). The sampler will return to the vehicle to obtain trash bags and zip lock bags in order to double bag samples. At the clean/dirty line the catcher will hold the bags with serum and swab samples so the sampler can spray with virucidal disinfectant. The samples will then be placed in the second bag, handed over the line and the second bag will be disinfected with a virucidal disinfectant. All nets or other equipment should be disinfected at this time.
3. The catcher will remove all Biosecurity clothing using appropriate procedures and cross the clean/dirty line. Double bagged samples will now be placed in a third bag and labeled with the premises identification number, owners name, and species of birds sampled. The third

bag is then sprayed with virucidal disinfectant and placed in the dirty cooler. The trash bag of used biosecurity clothing and trash is sprayed with virucidal disinfectant and placed in a second bag which is then sprayed and placed in the dirty area of the vehicle.

4. Team members will spray their hands with virucidal disinfectant and then wash their hands with waterless hand cleaner.

Procedures for disinfecting the vehicle and surrounding area:

1. One team member will use the sprayer to disinfect the vehicles tires, the ground where the trash had been placed and any other areas of the vehicle that may have been contaminated.
2. Team members will spray their shoes with virucidal disinfectant and will disinfect their hands again with waterless cleaner just prior to entering the vehicle.

Procedures upon returning to the ICP:

1. Place bags containing trash in the designated dumpster.
2. Place ice-packs from the dirty cooler, soiled nets, and any other items to be cleaned and disinfected in the designated C&D area.
3. Deliver samples to be transported to the laboratory for testing to the designated sampled receiving and processing area.
4. Vehicles should be washed at a commercial car wash that night or in the morning before going out on any other assignments.
5. Team members will shower and change clothes before the next days assignments.

Procedures for transporting samples to the laboratory:

1. A designated courier will transport samples to the laboratory once per day.
2. Make copies of laboratory submission forms and include with other paperwork for each premises. The original forms are to accompany the samples to the laboratory.
3. Seal plastic bags to be transported to the laboratory with security tape to ensure integrity of sample tracking and chain of custody.
4. Deliver sealed bags containing samples to laboratory personnel at curbside along with laboratory submission forms.

PRODECURES FOR Sampling Commercial premises

This commercial surveillance sampling may be performed by the personnel employed by the poultry company and will be required in the affected and surveillance zones in addition to sampling already occurring in accordance with the Texas Poultry Federation Surveillance Plan.

Commercial poultry operations (broilers, breeders, and/or layers) located in the affected or surveillance zone should initially collect serum and tracheal swab samples from 30 birds in each biosecurity unit (house) located on the

operation for testing. A second round of sampling should occur 14 to 21 days after the initial sampling and should again include both serum and tracheal swab collection. Samples should be collected from birds 3 weeks of age and older.

Collect samples from test eligible birds once during the initial sampling period. If birds will be sent to slaughter before the second sampling period, then slaughter surveillance protocols will suffice.

Guidelines for Swab Sample Collections:

- The swabber will use a dry swab to collect an oropharyngeal/tracheal swab from each bird.
- Pooled swabs from oropharyngeal/tracheal samples of 5 birds will be placed in BHI (Brain-Heart Infusion broth) tubes.
- Swirl each swab in the media, wring the swab out against the side of the tube, and then discard the swab. DO NOT LEAVE SWABS IN THE TUBE.
- Number all tubes in succession and label as tracheal (T). Indicate which samples are from which house on submission form.

Laboratory Information:

- Samples should be transported to the biosecure commercial sample processing center at the Incident Command Post.
- TAHC Taskforce personnel will process the samples and deliver them by courier to the appropriate laboratories in the Texas Veterinary Medical Diagnostic Laboratory system.

Euthanasia and disposal

Once a premises has been declared infected or a dangerous contact and depopulation has been approved by the IC, an appraisal will be made on the birds to be destroyed. An indemnity form (VS 1-23) must be completed by a VS or TAHC representative and signed by the owner or the owner's authorized representative before depopulation operations can be undertaken.

An owner requesting voluntary depopulation of birds on a premises that is neither infected or a dangerous contact will not be paid indemnity. The voluntary depopulation should be documented by the TAHC or VS personnel on-site, including an inventory of birds destroyed and the signature of the owner or the owners authorized representative.

Depopulation can be done by regulatory personnel or by the

premises owner under the supervision of regulatory personnel. The procedures for euthanasia will depend upon the type of birds to be destroyed and the facilities available. The use of CO₂ as a euthanasia agent is an approved method of humane euthanasia and should be used for the depopulation of birds on premises where indemnity will be paid. In depopulations where CO₂ will not be used as the euthanasia agent, the procedure must be approved in advance by the IC.

If possible, the euthanized birds should be disposed of on-site; if it is not possible to accomplish on-site disposal, the movement and disposal off-site must be approved by the TAHC.

Cleaning and disinfection

Commercial premises

Cleaning and disinfection of an infected or contact premises will be the primary responsibility of the owner, who may be reimbursed for certain expenses to be established prior to C&D activities. Texas Animal Health Commission (TAHC) personnel will monitor the progress of C&D activities and conduct inspections of each phase to insure compliance with this protocol.

All trash and debris will be removed from around the exterior of the poultry houses. If possible, trash and debris should be disposed of on the affected premises; if it is not possible to accomplish disposal on-site, the movement and disposal off-site must be approved by the TAHC.

All poultry litter will be removed from the poultry houses. Poultry litter will be handled in a manner pre-approved by the TAHC.

All equipment, including feeder lines, cables, waterers, fans, heaters and side curtains, and interior walls and ceilings will be power washed to remove all organic material. After cleaning, each poultry house will be disinfected with a virucidal agent approved by the TAHC. Flaming of metal surfaces with a propane torch may also be used in the disinfection process.

Following final disinfection, all poultry houses will remain empty for a minimum of 21 days prior to restocking.

Non-commercial premises

Cleaning and disinfection (C&D) activities on non-commercial premises should be limited to areas inhabited by or exposed to poultry. C&D of an infected or dangerous contact premises will be the primary responsibility of the owner, who may be reimbursed for certain expenses to be established prior to C&D activities. Texas Animal Health Commission (TAHC) personnel will monitor the progress of C&D activities and conduct inspections of each phase to insure compliance with this protocol.

All trash and debris will be removed from around the exterior of poultry houses or buildings inhabited by or exposed to poultry. If possible, trash and debris should be disposed of on the affected premises; if it is not possible to accomplish disposal on-site, the movement and disposal off-site must be approved by the TAHC.

All poultry litter will be removed from the poultry houses or buildings inhabited by or exposed to poultry. Manure and litter from outside roosting areas will be removed as thoroughly as possible. If possible, poultry litter and manure should be disposed of on the affected premises; if it is not possible to accomplish disposal on-site, the movement and disposal off-site must be approved by the TAHC.

The interior walls, roosting areas, ceilings and equipment (including feeders, waterers, fans, heaters, and cages) will be power washed to remove all organic material. Exterior roosting areas, fences, building roofs, machinery and other items exposed to poultry will be power washed to remove as much organic material as possible. After cleaning, these areas will be disinfected with a virucidal agent approved by the TAHC. Flaming of metal surfaces with a propane torch may also be used in the disinfection process where appropriate.

Following final disinfection, any soil surface exposed to poultry manure (buildings with dirt floors, the ground under outside roosting areas) will be treated with lime or a similar product.

Following final disinfection, the premises will remain free of poultry for a minimum of 21 days prior to restocking. Restocking will be subject to approval by the TAHC.

Repopulating premises

Previously infected commercial and non-commercial premises

1. The premises must remain empty for 21 days following cleaning and disinfection.
2. The premises must be inspected by task force to determine that the cleaning and disinfection was adequate before repopulation can occur.

Permitting movement of birds and bird products under hold order

Birds and bird products under hold order may be moved under the following conditions.

Movement of birds

1. Permits are issued by the Permitting Unit of the Task Force.
2. The birds must be accompanied by a VS Form 1-27, Permit for Movement.
3. The birds show no clinical signs of illness.

4. The premises must have been sampled during Round 1 and 2 of the surveillance plan with negative results for AI.
5. At least twenty-one days (one OIE defined incubation period) have passed since potential exposure to AIV; if the birds are being shipped to a terminal destination (slaughter), fourteen days must have elapsed.
6. The task force permitting staff must give notice, by phone call and/or fax, to an authorized State animal health official of the movement and when a permit (VS Form 1-27) has been issued.

Movement of hatchery and table Eggs

1. The eggs must be accompanied by a VS Form 1-27, Permit for Movement.
2. The originating premises must have been sampled at least once for AI and is submitting samples according to the surveillance plan requirements.

Movement of birds to shows or exhibitions

All restrictions listed above apply. Movements from the affected zone are not encouraged and must be considered on a case-by-case basis.

Processing paperwork

1. The electronic records in the EMRS are organized such that each premises has an open “investigation” containing contact information. Nested within are electronic “forms” that represent each of the hardcopy forms and control actions. The data entry group will enter all information as soon as possible as it is obtained.
2. All Task Force personnel will place all originals forms, notes, etc into the permanent files. Files will not be kept in personal folders.
3. Epidemiologists will not modify EMRS records. Instead, they will submit a data correction form so that all forms that would be affected can be changed.
4. The Survey Form (one page) contains contact information, bird information, and health commentary (generally from Surveillance field teams). Anyone learning of new premises with birds can complete this form. **The minimum requirement for filling out this form is an address.** The Survey Form is used by the data entry group to create a new electronic “investigation” of a premises. All other electronic forms will be nested under this investigation. Other information on the Survey Form is used to create a “herd exam.” This is a second electronic form nested within the “investigation.” SURVEY will be the reason selected.
5. Quarantine and hold order forms represent state enforced actions. The surveillance or diagnostics groups issue these forms to isolate animals.
6. A hardcopy of the Laboratory Submission Form will be completed and sent with specimens to the laboratory. An electronic Laboratory Submission form will be created by the data entry group under which separate sample forms will be created for each type of sample. Once the results are received, the DRO enters the laboratory results into the EMRS.
7. Appraisal Forms will be completed by appraisers. Hardcopies will be placed in the master file and electronic versions will be created by the data entry group.
8. The Data Correction Form will be completed to request data modifications in the EMRS.

Appendices

AI SURVEILLANCE STANDARDS FOR TEXAS, 2004 (AKA THE SURVEILLANCE PLAN)

Introduction

Whenever an H5 or H7 AIV is determined to have infected a flock of domestic birds in Texas, depopulation of the index flock will be completed on an expedited basis and cleaning and disinfection of the premises will begin. Epidemiological tracing of all movements (personnel, vehicles, eggs, birds) will begin even while the laboratory is attempting to isolate the virus.

The Texas Poultry Federation has an established "Memorandum of Understanding Implementing the Assessment for Emergency Avian Influenza and Exotic Newcastle Disease" to conduct surveillance for avian influenza (<http://gallus.tamu.edu>). Participants in this plan are required to conduct active serological surveillance in their commercial poultry operations. The plan also includes guidelines for enhanced surveillance of non-commercial poultry operations following the disclosure of evidence of the presence of H5 or H7 types of avian influenza virus in the state. These guidelines include requiring testing of all non-commercial poultry operations within 5 miles of a confirmed positive operation.

The TAHC will identify all poultry operations (non-commercial and commercial) and other entities with domestic birds within a 10-mile (16 km) radius of any infected flock. Serologic and PCR testing of birds on all identified operations will be conducted.

The objectives of these surveillance standards are to describe the approach the TAHC will employ to establish freedom from avian influenza within the control zones.

Three distinct zones, with varying intensities of surveillance will be established around each infected flock: (1) Affected Zone includes the area within 8 km (5 miles) of an infected flock; (2) Surveillance Zone includes the area between 8 km (5 miles) and 16 km (10 miles) of an infected flock

Affected Zone

The Affected Zone includes the area within 8 km (5 miles) of an infected flock. The target population includes all commercial and non-commercial poultry operations.

Commercial poultry operations

Personnel from the commercial poultry operations located in the affected zone will initially collect serum samples and tracheal swabs from birds in each biosecurity unit (house) located on the operation for testing by AGID and real-time reverse transcriptase polymerase chain reaction (RRT-PCR).

The total number of birds to be sampled in each unit is presented in Table 1. A second round of sampling will occur 14 to 21 days after the initial sampling and will again include both serum and tracheal swab collection. Sick and freshly dead birds will be targeted for sampling. Swab samples will be assayed by the RRT-PCR at either the Texas Veterinary Medical Laboratory in College Station, Texas, or the National Veterinary Services Laboratories in Ames, Iowa. Assay procedures briefly include:

- Extract RNA from tracheal swab pools using approved methods, i.e. Qiagen RNeasy extraction columns for swabs and Trisol LS extraction for tissue.
- Screen specimens with the AIV RRT-PCR matrix primer/probe set (this is the most sensitive assay). Specimens found to be positive by the matrix assay should then be tested by the AIV H5 and H7 primer/probe sets.
- Specimens positive by the matrix primer/probe set that cannot be confirmed by either the H5 or H7 specific primer/probe sets should be considered a suspect and additional samples collected for further testing or the samples should be tested by virus isolation.
- RRT-PCR-positive specimens and suspect positive specimens (original specimen and extracted RNA) should be shipped to the NVSL for confirmation.

Serological monitoring (AGID) results for each commercial operation in this zone will be documented for the 3-month period prior to the identification of the index flock and for at least the 90-day period of this targeted surveillance.

Non-commercial poultry operations

An inventory of at-risk of non-commercial operations will be developed following surveys of the affected zone. At-risk operations are defined as those with poultry, waterfowl, game fowl, or ratites. All at-risk non-commercial operations will initially have serum and swab samples collected. Serum samples will be assayed by the AGID and swab samples will be assayed by RRT-PCR. Tracheal swabs will be collected from gallinaceous birds and ratites. Only cloacal swabs will be collected from any waterfowl. Swab samples will be assayed by RRT-PCR at either the Texas Veterinary Medical Laboratory in College Station, Texas, or the National Veterinary Services Laboratories in Ames, Iowa (procedures provided above). The total number of birds to be sampled on each operation is presented in Table 1. A second round of sampling will occur 14 to 21 days after the initial sampling and will include both serum and swab collection.

Movement Controls

Movement controls will be implemented within the affected zone. The TAHC will place state hold orders on all commercial and non-commercial poultry operations within the Affected Zone. These orders will remain in place until surveillance is completed in all zones. In addition, permits will be required for movements of poultry or the movement of poultry related equipment or materials, such as litter, originating within the affected zone. Movement permits will be required until surveillance is completed within the affected and surveillance zones.

Table 1. Number of birds to sample from each house on the premises*	
Number of birds in	Minimum number of birds to
10 or less	Sample all
20	15
30	15
40	15
50 or greater	20
* Assuming a 95% or greater sensitivity and 99% specificity for the diagnostic testing system used, sampling the indicated number of birds will result in a 95% certainty that at least one positive bird will be detected if at least a 25% prevalence of AI virus shedding exists among birds on the premises	

Surveillance Zone

The surveillance zone includes the area between 8 km (5 miles) and 16 km (10 miles) of an infected flock. The target population includes all commercial and non-commercial poultry operations. Surveillance activities in this zone may be conducted simultaneously with those in the affected zone.

Commercial poultry operations

Personnel from the commercial poultry operations located in the surveillance zone will initially collect serum samples from birds in each biosecurity unit (house) located on the operation for testing by AGID and tracheal swabs for real-time reverse transcriptase polymerase chain reaction (RRT-PCR) testing. The total number of birds to be sampled in each unit is presented in Table 1. A second round of sampling will occur 14 to 21 days after the initial sampling and will again include both serum and tracheal swab collection. Sick and freshly dead birds will be targeted for sampling. Swab samples will be assayed by RRT-PCR at either the Texas Veterinary Medical Laboratory in College Station, Texas, or the National Veterinary Services Laboratories in Ames, Iowa.

Serological monitoring results for each commercial operation in this zone will be documented for the 3-month period prior to the identification of the index flock and for at least the entire 90-day period of this targeted surveillance.

Non-commercial poultry operations

An inventory of at-risk non-commercial operations will be developed following surveys of the surveillance zone. At-risk operations are defined as those with poultry, waterfowl, game fowl, or ratites. All at-risk non-commercial operations will initially have serum and swab samples collected. Serum samples will be assayed by the AGID and swab samples will be assayed by RRT-PCR. Tracheal swabs will be collected from gallinaceous birds and ratites. Only cloacal swabs will be collected from any waterfowl. Swab samples will be assayed by RRT-PCR at either the Texas Veterinary Medical Laboratory in College Station, Texas, or the National Veterinary Services Laboratories in Ames, Iowa (procedures provided above). The total number of birds to be sampled on each operation is presented in Table 1. A second round of sampling will occur 14 to 21 days after the initial sampling and will include both serum and swab collection.

Dangerous Contacts and Sick Calls

Flocks determined epidemiologically to be dangerous contacts will have both serum and swab samples collected initially. If results of the initial sampling are negative, a second set of serum and swab samples will be taken 14 to 21 days after the initial sampling. Any flocks in which sick birds are reported will have both serum and swab samples collected. Tracheal swabs will be collected from gallinaceous birds and cloacal swabs will be collected from waterfowl. All dangerous contact and sick call samples will be submitted either to Texas Veterinary Medical Diagnostic Laboratory or the National Veterinary Services Laboratories in Ames, Iowa. The total number of birds to be sampled is presented in Table 1.

Criteria for declaring premises positive for AI H5 or H7 virus

A recent H7 infection in an index flock had been confirmed by serology, but no virus was isolated for the appropriate pathogenicity studies. As observed with that particular virus in the field, clinical signs may not be a reliable indicator of infection. Flocks/birds showing clinical signs of respiratory disease, drop in egg production, or with lesions consistent with AI (edema of the head, comb, wattles, subcutaneous hemorrhage of non-feathered areas, hemorrhage/necrosis of comb, wattles, trachea, heart, and gut) should be considered suspicious until confirmed or ruled out by laboratory testing.

Premises inside any of the three surveillance zones with clinical signs and/or gross lesions consistent with avian influenza virus may be declared positive with one of the following laboratory tests:

- Isolation and identification of an H5 or H7 AIV
- Positive RRT-PCR with an H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies

Premises inside any of the three surveillance zones without clinical signs and/or gross lesions:

Two of the following conditions must be met to declare a premises as positive.

- Isolation and identification of an H5 or H7 AIV
- Positive AIV RRT-PCR with an H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies
- Directigen Flu A or other approved antigen capture positive test (cannot be only criterion even with an epidemiologic link to designate a premises as positive)
- Epidemiologic link

Protocol for surveillance sampling

According to the Avian Influenza Surveillance Standards, two distinct zones, with varying intensities of surveillance will be established: (1) Affected Zone includes the area within 8 km (5 miles) of an infected flock; (2) Surveillance Zone includes the area between 8 km (5 miles) and 16 km (10 miles) of an infected flock.

The tables below provide the timeline and necessary samples to be collected in the three zones.

Non-commercial Farms:

Zone	Round 1		Round 2*	
	Serum	Swab**	Serum	Swab***
Affected Zone	YES	YES	YES	YES
Surveillance Zone	YES	YES	YES	YES

* Sampling for Round 2 will begin 14 to 21 days after Round 1 sampling.

**Tracheal swab samples will be collected from all gallinaceous birds and cloacal swab samples will be collected from all waterfowl during Round 2.

Commercial Farms*:

Zone	Round 1		Round 2**	
	Serum	Swab***	Serum	Swab***
Affected Zone	YES	YES	YES	YES
Surveillance Zone	YES	YES	YES	YES

* Samples from commercial farms may be collected by trained commercial farm employees rather than task force personnel.
**Sampling for Round 2 will begin 14 to 21 days after Round 1 sampling.
***Only tracheal swab samples will be collected from commercial farms.

Dangerous Contacts and Sick Calls

Flocks determined epidemiologically to be dangerous contacts will have both serum and swab samples collected initially. If results of the initial sampling are negative, a second set of serum and swab samples will be taken 14 to 21 days after the initial sampling. Any flocks in which sick birds are reported will have both serum and swab samples collected. Tracheal swabs will be collected from gallinaceous birds and cloacal swabs will be collected from waterfowl. All dangerous contact and sick call samples will be submitted either to Texas Veterinary Medical Diagnostic Laboratory or the National Veterinary Services Laboratories in Ames, Iowa. The number of birds to be sampled from each house is presented in Table 1.

Surveillance Teams will require the following supplies:

- Biosecurity supplies - Tyveks, boot covers, gloves, hair net, dust mask, protective eyewear, disinfectant spray bottle, disinfectant, waterless hand cleaner.
- Blood tubes and blood collection instruments
- Brain Heart Infusion broth (BHI) - must be stored in cooler located in "clean" area with ice packs (should be clear amber color, if discolored or cloudy discard).
- Sterile Dacron swabs
- Cooler, ice packs or wet ice
- Zip lock bags, large trash bags
- GPS unit
- Forms: Avian Influenza Surveillance

Procedures before arriving at a premises:

1. Surveillance Teams will receive their assignments each morning from the operations chief or his or her designate.
2. The Surveillance Teams should prepare the vehicle for the day's assignments by re-stocking all needed supplies and forms.
3. Maintain a clean and dirty area of the vehicle at all times.

4. The Surveillance Teams will attempt to notify premises owner that their arrival is imminent. Blood tubes, BHI tubes, and zip lock bags should be labeled with waterproof marker with premises identification number.

5. The vehicle should be parked in an area near the premises but in a "clean" area. Windows should be kept closed and the vehicle should be kept locked when personnel are away from it.

6. One team member is identified as the sampler and the other is the catcher.

Procedures upon arrival at a premises:

1. Prior to exiting the vehicle, each team member should put on one pair of boot covers.

2. Team member will ask the owner to come to the "clean" area or near the vehicle to complete the necessary paperwork. The "clean/dirty" line should be strictly adhered to.

3. Team members will put on Biosecurity equipment (refer to Biosecurity SOP) and gather necessary supplies to collect samples. The sampler will duct tape a large plastic bag and two zip lock bags to the Tyvek suit. The necessary number of blood tubes and/or BHI tubes and swabs will be put into one of the two zip lock bags. The other bag is for the blood tubes and/or BHI tubes once they are ready for laboratory submission. Team members will wear two pairs of gloves.

Procedures while on the premises:

1. The Surveillance Team will observe all of the birds to get an accurate inventory of the flock to record on the Avian Influenza Survey Form. They will also determine the health status of the birds.

2. If dead or sick birds are observed, exit the bird housing area immediately and contact a supervisor.

3. Refer to the table to determine which samples need to be collected for each time period and zone. The following are guidelines for serum sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- Blood is collected from the wing vein into a 3 ml red top tube. Collect at least 1 ml of blood from

each bird using a sterile blood collection tool (scalpel or needle (22g) and syringe (3cc)).

- Place tubes in zip lock bag taped to Tyvek suit. Place blood collection instruments in trash bag.

The following are guidelines for swab sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- The sampler will use dry swabs to collect an oropharyngeal swab from each gallinaceous bird. Collect only cloacal swabs on waterfowl species.
- Put up to 5 birds' samples in each BHI tube, keeping species separate and cloacal and oropharyngeal swabs separate. Write the premises identification number, the species and the location on each tube.
- Swirl each swab in the media and wring out against the side of the tube then remove the swab. DO NOT LEAVE SWABS IN THE TUBE.
- Dispose of used swabs in trash bag taped to the Tyvek suit of the sampler.

The number of birds to sample from each house on the premises is given in the table below.

Table 1. Number of birds to sample from each house on the premises.	
Number of birds in each house	Minimum number of birds to be sampled
10 or less	Sample all
20	15
30	15
40	15
50 or greater	20

Procedures for exiting the premises:

1. Pick up all samples collected, bag(s) of trash and take to clean/dirty line established earlier.
2. At this area the sampler will remove all biosecurity clothing using appropriate procedures (see Biosecurity SOP). The sampler will return to the vehicle to obtain trash bags and zip lock bags in order to double bag samples. At the clean/dirty line the catcher will hold the bags with serum and swab samples so the sampler can spray with an approved virucidal disinfecting spray. The samples will then be

placed in the second bag, handed over the line and the second bag will be disinfected with an approved virucidal disinfecting spray. All nets or other equipment should be disinfected at this time.

3. The catcher will remove all Biosecurity clothing using appropriate procedures and cross the clean/dirty line. Double bagged samples will now be placed in a third bag and labeled with the premises identification number, owners name, and species of birds sampled. The third bag is then sprayed with virucidal disinfecting spray and placed in the dirty cooler. The trash bag of used biosecurity clothing and trash is sprayed with virucidal disinfecting spray and placed in a second bag which is then sprayed and placed in the dirty area of the vehicle.

4. Both team members will spray their hands with virucidal disinfecting spray and then wash their hands with waterless hand cleaner.

Procedures for disinfecting the vehicle and surrounding area:

1. One Team member will use the sprayer to disinfect the vehicles tires, the ground where the trash had been placed and any other areas of the vehicle that may have been contaminated.

2. Both team member will spray their shoes with virucidal disinfecting spray and will disinfect their hands again with waterless cleaner just prior to entering the vehicle.

Procedures upon returning to the ICP:

1. Place bags containing trash in the designated dumpster.

2. Place ice-packs from the dirty cooler, soiled nets, and any other items to be cleaned and disinfected in the designated C&D area.

3. Deliver samples to be transported to the laboratory for testing to the designated sampled receiving and processing area.

4. Vehicles should be washed a commercial car wash that night or in the morning before going out on any other assignments.

5. Team members will shower and change clothes before the next days assignments.

Procedures for transporting samples to the laboratory:

1. A designated courier will transport samples to the laboratory once per day.
2. Make copies of laboratory submission forms and include with other paperwork for each premises. The original forms are to accompany the samples to the laboratory.
3. Seal plastic bags to be transported to the laboratory with security tape to ensure integrity of sample tracking and chain of custody.
4. Deliver sealed bags containing samples to laboratory personnel at curbside along with laboratory submission forms.

PROTOCOL FOR SURVEILLANCE SAMPLING IN ANY NEW AFFECTED AND SURVEILLANCE ZONES CREATED IN RESPONSE TO NEWLY INFECTED FLOCKS BEING FOUND DURING THE INITIAL ROUNDS OF SURVEILLANCE

According to the Avian Influenza Surveillance Standards, distinct zones, with varying intensities of surveillance will be established: (1) Affected Zone includes the area within 8 km (5 miles) of an infected flock; (2) Surveillance Zone includes the area between 8 km (5 miles) and 16 km (10 miles) of an infected flock. All flocks located within the Affected Zone will be placed under movement restrictions.

The tables below provide the timeline and necessary samples to be collected in the affected and surveillance zones. Baseline sampling will occur in the new affected and surveillance zone around the new (third) infected premises. Those premises that have been previously sampled as part of a preexisting affected or surveillance zones may require re-sampling for baseline testing if they are also located within the newly described affected or surveillance zone.

Commercial and Non-commercial Farms:

Zones	Baseline (Round 1)		Round 2*		Round 3**	
	Serum	Swab***	Serum	Swab**	Serum	Swab**
New Affected	YES	YES	YES	YES	YES	YES
New Surveillance	YES	YES	YES	YES	YES	YES

* Sampling for Round 2 will begin 7 days after Baseline sampling.
 **Sampling for Round 3 will begin 7 to 10 days after the Round 2 test date.
 ***Tracheal swab samples will be collected from all gallinaceous birds and cloacal swab samples will collected from all waterfowl.

Dangerous Contacts and Sick Calls

Flocks determined epidemiologically to be dangerous contacts will have both serum and swab samples collected initially. If results of the initial sampling are negative, a second set of serum and swab samples will be taken 14 to 21 days after the initial sampling. Any flocks in which sick birds are reported will have both serum and swab samples collected. Tracheal swabs will be collected from gallinaceous birds and cloacal swabs will be collected from waterfowl. All dangerous contact and sick call samples will be submitted either to Texas Veterinary Medical Diagnostic Laboratory or the National Veterinary Services Laboratories in Ames, Iowa. The number of birds to be sampled from each premises is presented in Table 1 below.

The number of birds to sample from each premises:

Table 1. Number of birds to sample from each premises.	
Number of birds on the premises	Minimum number of birds to be sampled
10 or less	Sample all
20	15
30	15
40	15
50 or greater	20

Surveillance Teams will require the following supplies:

- Biosecurity supplies - Tyveks, boot covers, gloves, hair net, dust mask, protective eyewear, disinfectant spray bottle, disinfectant, waterless hand cleaner.
- Blood tubes and blood collection instruments
- Brain Heart Infusion broth (BHI) - must be stored in cooler located in "clean" area with ice packs (should be clear amber color, if discolored or cloudy discard).
- Sterile Dacron swabs
- Cooler, ice packs or wet ice
- Zip lock bags, large trash bags
- GPS unit
- Forms: Avian Influenza Surveillance

Procedures before arriving at a premises:

1. Surveillance Teams will receive their assignments each morning from the operations chief or his or her designate.
2. The Surveillance Teams should prepare the vehicle for the day's assignments by re-stocking all needed supplies and forms.
3. Maintain a clean and dirty area of the vehicle at all times.
4. The Surveillance Teams will attempt to notify premises owner that their arrival is imminent. Blood tubes, BHI tubes, and zip lock bags should be labeled with waterproof maker with premises identification number.
5. The vehicle should be parked in an area near the premises but in a "clean" area. Windows should be kept closed and the vehicle should be kept locked when personnel are away from it.
6. One team member is identified as the sampler and the other is the catcher.

Procedures upon arrival at a premises:

1. Prior to exiting the vehicle, each team member should put on one pair of boot covers.
2. Team member will ask the owner to come to the "clean" area or near the vehicle to complete the necessary paperwork. The "clean/dirty" line should be strictly adhered to.
3. Team members will put on Biosecurity equipment (refer to Biosecurity SOP) and gather necessary supplies to collect samples. The swabber will duct tape a large plastic bag and two zip lock bags to the Tyvek suit. The necessary number of blood tubes and/or BHI tubes and swabs will be put into one of the two zip lock bags. The other bag is for the blood tubes and/or BHI tubes once they are ready for laboratory submission. Team members will wear two pairs of gloves.

Procedures while on the premises:

1. The Surveillance Team will observe all of the birds to get an accurate inventory of the flock to record on the Avian Influenza Survey Form. They will also determine the health status of the birds.
2. If dead or sick birds are observed, exit the bird housing area immediately and contact a supervisor.
3. Refer to the table above to determine which samples need to be collected for each time period and zone. The following

are guidelines for serum sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- Blood is collected from the wing vein into a 3 ml red top tube. Collect at least 1 ml of blood from each bird using a sterile blood collection tool (scalpel or needle (22g) and syringe (3cc)).
- Place tubes in zip lock bag taped to Tyvek suit. Place blood collection instruments in trash bag.

The following are guidelines for swab sample collections:

- The catcher will catch and remove birds from cages or use a net to catch loose birds. The owner may also assist with bird handling. Birds will be restrained so that the sampler can collect necessary samples.
- The sampler will use dry swabs to collect an oropharyngeal swab from each gallinaceous bird. Collect only cloacal swabs on waterfowl species.
- Put up to 5 birds' samples in each BHI tube, keeping species separate and cloacal and oropharyngeal swabs separate. Write the premises identification number, the species and the location on each tube.
- Swirl each swab in the media and wring out against the side of the tube then remove the swab. DO NOT LEAVE SWABS IN THE TUBE.
- Dispose of used swabs in trash bag taped to the Tyvek suit of the sampler.

Procedures for exiting the premises:

1. Pick up all samples collected, bag(s) of trash and take to clean/dirty line established earlier.
2. At this area the sampler will remove all biosecurity clothing using appropriate procedures (see Biosecurity SOP). The sampler will return to the vehicle to obtain trash bags and zip lock bags in order to double bag samples. At the clean/dirty line the catcher will hold the bags with serum and swab samples so the sampler can spray with an approved virucidal disinfecting spray. The samples will then be placed in the second bag, handed over the line and the second bag will be disinfected with an approved virucidal disinfecting spray. All nets or other equipment should be disinfected at this time.
3. The catcher will remove all Biosecurity clothing using

appropriate procedures and cross the clean/dirty line. Double bagged samples will now be placed in a third bag and labeled with the premises identification number, owners name, and species of birds sampled. The third bag is then sprayed with virucidal disinfecting spray and placed in the dirty cooler. The trash bag of used biosecurity clothing and trash is sprayed with virucidal disinfecting spray and placed in a second bag which is then sprayed and placed in the dirty area of the vehicle.

4. Both team members will spray their hands with virucidal disinfecting spray and then wash their hands with waterless hand cleaner.

Procedures for disinfecting the vehicle and surrounding area:

1. One Team member will use the sprayer to disinfect the vehicles tires, the ground where the trash had been placed and any other areas of the vehicle that may have been contaminated.

2. Both team member will spray their shoes with virucidal disinfecting spray and will disinfect their hands again with waterless cleaner just prior to entering the vehicle.

Procedures upon returning to the ICP:

1. Place bags containing trash in the designated dumpster.

2. Place ice-packs from the dirty cooler, soiled nets, and any other items to be cleaned and disinfected in the designated C&D area.

3. Deliver samples to be transported to the laboratory for testing to the designated sampled receiving and processing area.

4. Vehicles should be washed a commercial car wash that night or in the morning before going out on any other assignments.

5. Team members will shower and change clothes before the next days assignments.

Procedures for transporting samples to the laboratory:

1. A designated courier will transport samples to the laboratory once per day.

2. Make copies of laboratory submission forms and include with other paperwork for each premises. The original forms are to accompany the samples to the laboratory.

3. Seal plastic bags to be transported to the laboratory with security tape to ensure integrity of sample tracking and chain of custody.

4. Deliver sealed bags containing samples to laboratory personnel at curbside along with laboratory submission forms.

Case definition for AI – Texas

OFFICIALLY RECOGNIZED LABORATORY PROCEDURES:

Virus isolation (gold standard) (takes 2-12 days)

- Inoculate suspensions of tissues or tracheal and cloacal swabs into 9-to-11-day-old embryonated chicken eggs
- Candle eggs daily (4 days) for embryo deaths and collect the allantoic-amniotic fluid (AAF)
- Perform hemagglutination (HA) test on AAF from dead embryos; test live embryos at the end of 4 days incubation.
- Test HA-positive AAF for avian Paramyxovirus type-1 (APMV-1) by the hemagglutination-inhibition (HI) test. Alternatively, HA-positive AAF can be identified as type A influenza using an antigen capture immunoassay, e.g. Directigen[®] or by agar gel immunodiffusion (AGID) with influenza type A reagents. HA-positive AAFs and original clinical specimen should be shipped to the NVSL for confirmation and pathotyping (when needed).
- A second egg passage is made for specimens lethal for embryos on the first passage and negative by the HA test.

Real-time reverse-transcription polymerase chain reaction (RRT-PCR)

(same-day results)

- Extract RNA from tracheal and cloacal swab pools using approved methods, i.e. Qiagen RNeasy extraction columns for swabs and Trisol LS extraction for tissue.
- Screen specimens with the AIV RRT-PCR matrix primer/probe set (this is the most sensitive assay). Specimens found to be positive by the matrix assay should then be tested by the AIV H5 and H7 primer/probe sets.

- Specimens positive by the matrix primer/probe set that cannot be confirmed by either the H5 or H7 specific primer/probe sets should be considered a suspect and additional samples are collected for further testing or the samples should be tested by virus isolation.
- RRT-PCR-positive specimens (original specimen and extracted RNA) should be shipped to the NVSL for confirmation.
- RRT-PCR is not reliable for establishing a negative status for cloacal swabs from waterfowl. Cloacal swabs from waterfowl must be tested by virus isolation.

Directigen Flu A Kit (Beckton-Dickenson)

- The Directigen test (20 minute test) is a pen-side test that can be used to quickly identify AI-positive birds/flocks. The test is most useful for testing birds with an acute virus infection, i.e. birds with clinical disease or dead birds. Compared to virus isolation, the test has a sensitivity of about 70% on an individual sample basis and about 80% on a flock basis. Samples with a heavy bacterial load may give false positive reactions. Positive samples must be tested by another test to confirm presence of H5 or H7 virus/RNA.

Serologic Assays (Type-Specific)

- AGID (gold standard for serologic monitoring) (requires 24 hr) - a type-specific test that detects antibodies to all subtypes of influenza A virus. Not reliable for monitoring waterfowl. Reagents available from the NVSL.
- Enzyme-linked immunoassay (ELISA) (same day results) - a type-specific test that detects antibodies to all subtypes of influenza A virus. ELISA-positive samples must be confirmed by AGID. Commercial kits are available (IDEXX, Synbiotics) - label restrictions for chickens only or chickens and turkeys. Not suitable for monitoring waterfowl.

Serologic Assays (Subtype-Specific)

- HI (same day results) - used to identify subtype-specific antibody in serum or to identify the hemagglutinin subtype on the influenza virus (performed at the NVSL). At the NVSL, surveillance serums submitted for serology may be tested only for antibodies to H5 or H7 by HI.

Positive sera will then be tested for specific neuraminidase antibodies.

- NI (same day results)- used to identify subtype-specific antibody in serum or to identify the neuraminidase subtype on the influenza virus (performed at the NVSL)

VIRUS CHARACTERIZATION: (takes 2-4 days)

- Subtype determination of hemagglutinin (H) and neuraminidase (N) antigens
- Determination of the amino acid sequence at the hemagglutinin cleavage site
- Chicken pathogenicity test (takes 10 days, only performed on selected isolates and those from outside the specified quarantine zones)
- Phylogenetic analysis (only on selected isolates)

SAMPLE COLLECTION:

- Tracheal swabs from gallinaceous birds for virus/RNA detection and serum for antibody detection should be collected during the first round of surveillance. Collect swabs from 10% of the birds (5 birds minimum) up to a maximum of 20 birds/premises. Pool tracheal swabs (up to 5 per tube) in brain heart infusion (BHI) broth (available from the NVSL).
- Collect tissue (lung, trachea, spleen etc) **only** when a differential diagnosis is needed. Tissues are not needed for AIV isolation/detection and will delay RRT-PCR results. Do not pool tissue from different birds or bird species together.
- From waterfowl, collect only cloacal swabs. The cloacal swabs from waterfowl must be tested by virus isolation; however, the swabs may be tested by RRT-PCR but if found to be negative, the result should not be considered definitive. Do not collect serum or tracheal swabs from waterfowl.

WHERE TO SHIP SAMPLES:

Surveillance swabs and serum collected from non-clinical premises will be shipped to the TVMDL system. Serology and Directigen (when necessary) testing will be performed at the Gonzales or Center laboratory with daily shipments of swabs to the TVMDL, College Station, TX for RRT-PCR testing. Samples that test positive at TVMDL will be sent to NVSL for confirmation.

CRITERIA FOR DECLARING A PREMISES POSITIVE FOR AI H5 OR H7 VIRUS:

A recent H7 infection in a flock was confirmed by serology, but no virus was isolated for the appropriate pathogenicity studies. As observed in this particular virus in the field, clinical signs may not be a reliable indicator of infection. Flocks/birds showing clinical signs of respiratory disease, drop in egg production, or with lesions consistent with AI (edema of the head, comb, wattles, subcutaneous hemorrhage of non-feathered areas, hemorrhage/necrosis of comb, wattles, trachea, heart, and gut) should be considered suspicious until confirmed or ruled out by laboratory testing.

Positive premises inside quarantine zone with clinical signs and/or gross lesions consistent with low or high pathogenicity avian influenza virus plus one of the following laboratory tests.

- Isolation and identification of an H5 or H7 AIV
- Positive RRT-PCR with H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies

Positive premises inside quarantine zone without clinical signs and/or gross lesions, two of the following conditions must be met to declare a premises as positive.

- Isolation and identification of an H5 or H7 AIV
- Positive AIV RRT-PCR with H5 or H7 specific primer/probe set
- Presence of H5 or H7 subtype-specific serum antibodies
- Directigen Flu A positive (cannot be only criterion even with an epidemiologic link to designate a premises as positive)
- Epidemiologic link

Suspect premises inside affected or surveillance zone:

- Flock/bird with no clinical signs, no lesions compatible with AI, and no epidemiological link but positive by one of the following tests:
 - Virus isolation
 - AIV RRT-PCR with H5 or H7 -specific primer/probe assay
 - Directigen positive
- A suspect premises would be further investigated and additional samples collected for testing.

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