Technical Update

# PROPER COLLECTION AND HANDLING OF DIAGNOSTIC SAMPLES

## *PART ONE: SEROLOGY AND BLOOD COLLECTION*

Diagnostic samples are used to determine health status or identify specific pathogens in pullet, layer and breeder flocks. Routine samples include whole blood, serum, formalin-fixed tissue and swabs: tracheal, choanal, oropharyngeal, cloacal, organs and joints. For specific investigations, Fast Technology for Analysis of nucleic acids (FTA) cards can be used to collect feather pulp, whole blood or isolates from any type of swab.

### SAMPLE SUBMISSION

When submitting samples to a diagnostic laboratory, it is important to provide thorough and relevant flock information on the laboratory submission form. Critical information that should accompany all diagnostic sample submissions includes:

- Flock identification and location
- Age of flock
- Date of sample collection
- Vaccination program
- Flock history, including pertinent health or production problems

This information is vital to the flock veterinarian and diagnostician to make a meaningful interpretation of serological or diagnostic results and provide recommendations to improve flock health and/or production.

### *Summary of Guidelines for Proper Serum Collection*

- Select normal representative birds (10 to 20 sera samples), unless working up a diagnosis.
- Collect 2.0 to 3.0 mL of blood from each bird.
- Samples collected with a needle are cleaner than with a scalpel.
- Do not damage samples by forcing the blood sample back through the needle into the clot tube.
- Ensure blood runs down the side of the clot tube and position the tubes nearly flat until the clot is formed.
- Leave blood in the clot tube for 10 to 12 hours at about 80°F (27°C).
- Do not shake, roughly handle or freeze the blood while the clot is forming or hemolysis will occur.
- Remove clot gently, or pour off serum.
- Do not mail samples without first removing the clot.
- Keep the serum samples cool and send immediately to the laboratory on wet ice or cold pack.

**Ages for blood collection in breeder flocks:**

1. 10 to 12 weeks
2. At time of transfer (grow to lay farm)
3. Every 10 to 12 weeks during egg production

**Ages for blood collection in commercial layer flocks:**

1. One time prior to transfer (grow to lay farm)
2. Every 10 to 12 weeks during egg production

## SEROLOGY

Serology is the study of serum antibody levels, also known as titers. The immune system develops antibodies that circulate in the blood after a bird is exposed to an antigen, whether by vaccination or exposure to a wild-strain pathogen. Antibodies are found in the serum portion of blood (the liquid portion after the clot develops). Serum is free of all blood cells and clotting factors.

The flock's serum antibody titers are used to monitor efficacy of vaccination programs, evaluate field challenges or diagnose disease. The value of this information depends on the quality of the serum samples received by the laboratory. Poor quality samples lead to erroneous and misleading results. Selection of birds for blood collection, techniques used to collect blood, and handling of blood samples and serum all influence laboratory results.

### *Selection of Birds*

For routine serological monitoring, serum samples should be collected from normal, healthy birds. Do not use cull birds that are sick or appear distressed, as their antibody titers are not typically representative of the overall flock health status. During a potential disease investigation, however, blood samples should be collected from birds that are exhibiting the clinical signs or lesions of the suspected pathogen or syndrome.

In caged housing systems, it is important to select birds from various locations throughout the house. When a flock is enrolled in a routine serology program, collecting blood from the same birds (or same cages) is recommended. This will reduce the variability of results when compared to collecting blood from different birds at each time of testing. In floor housing systems, identifying the same birds is difficult. Large plastic wing bands or feathers marked with dye can be useful to allow consistent collection.

### *Number of Samples*

Twenty good quality serum samples should be collected for routine flock profiling and for disease investigation; however, a minimum of 10 samples may be sufficient to estimate flock antibody titers.

### *Ages for Sampling*

For routine monitoring, the first blood collection should be 10 to 12 weeks of age. By this age, a pullet flock has an opportunity to respond to early live vaccinations and maternal antibodies are absent. Antibody titers from this age group can be used to assess the overall immune status of a young flock and priming effect of live vaccines used in vaccination programs. This early serology assessment can screen for potential disease challenge in the grow house.

Another important time for antibody titer evaluation is immediately prior to transfer of the pullet flock to the laying house. This is a good time to check the pullet's immune response against *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), Newcastle disease (NDV), infectious bronchitis (IB), avian encephalomyelitis (AE), and avian influenza (AI). In breeders, transfer is also an ideal time to assess adequate sero-conversion for chicken anemia virus (CAV) and avian encephalomyelitis (AE). Collecting serum before transfer establishes a baseline titer level for a flock moved to a multi-age complex. Titer response from inactivated (killed) vaccines will peak at 3 to 5 weeks post-vaccination. When monitoring flocks during the egg production period, a 10 to 12 week interval is sufficient to monitor changes in antibody titer levels.

During a disease outbreak investigation, blood should be collected when clinical signs of the disease are first observed, followed by an additional blood collection from the same birds 3 to 5 weeks later. This collection time frame allows for specific antibody production against a potential disease agent. The comparison of titers from these paired sera samples may demonstrate significant changes in titers for a suspected pathogen.

Reserving the first serum sample (by freezing) to be run at the same time as the second sample reduces lab test variance due to external factors or changes in reagent lots. A similar tactic can be used to monitor the efficacy of killed vaccines given in a pullet program.

### *Volume of the Blood Sample*

With proper collection and handling technique, 2.0 to 3.0 milliliters (mL or cc) of whole blood will yield 1.0 to 1.5 mL of serum. This volume of serum is sufficient for routine ELISA testing for Newcastle disease, infectious bronchitis, infectious bursal disease (IBD or Gumboro), AE and AI by agar gel immunodiffusion (AGID), as well as for MG, MS and pullorum-typhoid (PT) by plate agglutination testing. Sufficient serum should be kept frozen in reserve, in case additional testing is required in the future.

### *Equipment Used for Blood Collection*

Disposable, sterile 3 or 5 cc syringes are used, depending on the size of the sample to be obtained. The size of needle depends on the anatomical site used for blood collection.

| Blood Collection Site | Needle Length | Needle Gauge |
|---|---|---|
| Wing vein | 0.5–1.0 inch (1.25–2.54 cm) | 20–22 gauge |
| Cardiac puncture | 1.5 inch (3.81 cm) | 18–20 gauge |

Always use disposable needles and replace needles every 5 to 10 birds. Dull needles cause tissue trauma and make accurate punctures of veins more difficult. All blood collection equipment must be changed between flocks to eliminate the potential for disease transmission. Rinsing the needle and syringe between birds with distilled water prevents blood from clotting within the needle. Sterile 3.0 mL plastic or glass blood tubes with leak-proof tops are ideal for blood collection and storage, as they allow for proper clotting of samples. Similar tubes are ideal for storing separated serum as well.

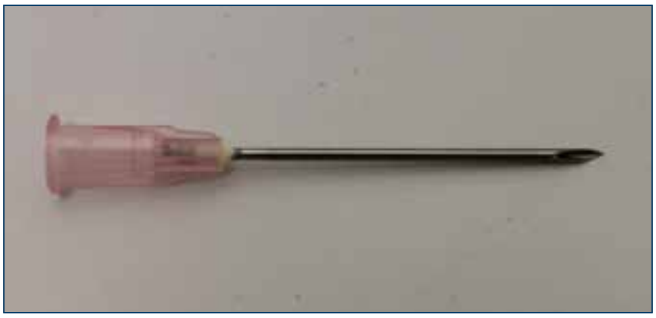

*Figure 1. Disposable needles should be changed every 5 to 10 birds to prevent tissue trauma and cross-contamination.*

*Figure 2. Disposable 5 mL syringe and 22 gauge needle ideal for collecting blood from the wing vein of adult birds.*

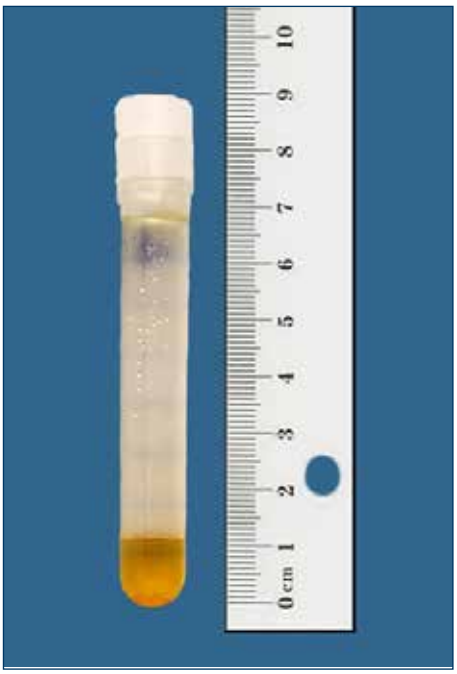


*Figure 3. Plastic blood collection tube with cap, ideal for separating serum and transporting sample. Note proper volume (1.0 mL) of golden colored, transparent serum.*

## METHODS USED FOR COLLECTING A BLOOD SAMPLE

### 1. Wing (brachial) vein method using a needle

The brachial vein of the wing is an acceptable site for blood collection for birds 4 weeks and older. In younger birds, this vein is too small for efficient blood collection.

**STEP 1**

*Figure 4. Hold bird by both legs.*

**STEP 2**

*Figure 5. Place legs under elbow of non-dominant hand.*

**STEP 3**

*Figure 6. Free both hands to gain access to underside of wing.*

**STEP 4**

*Figure 7. Remove feathers to better view the brachial vein.*

**STEP 5**

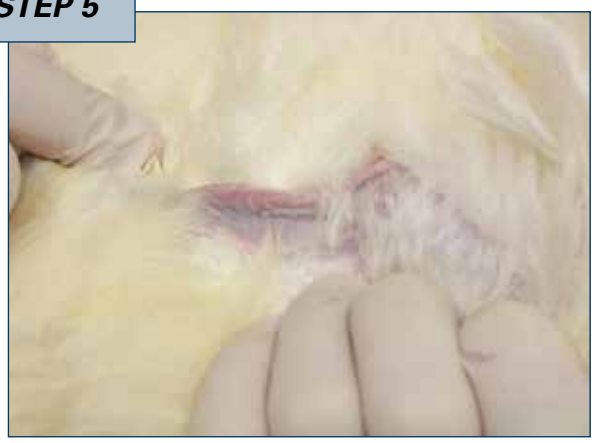

*Figure 8. Visualize the brachial vein.*

**STEP 6**

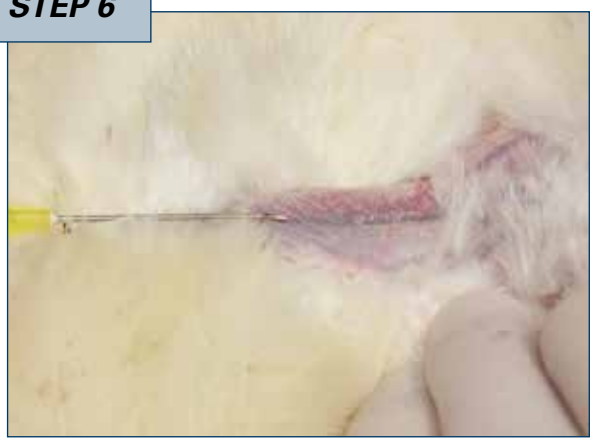

*Figure 9. Orient needle in alignment with vein, bevel pointed up, with tip of needle pointed toward wing tip.*

**Wing (brachial) vein method using a needle (continued)**

**STEP 7**

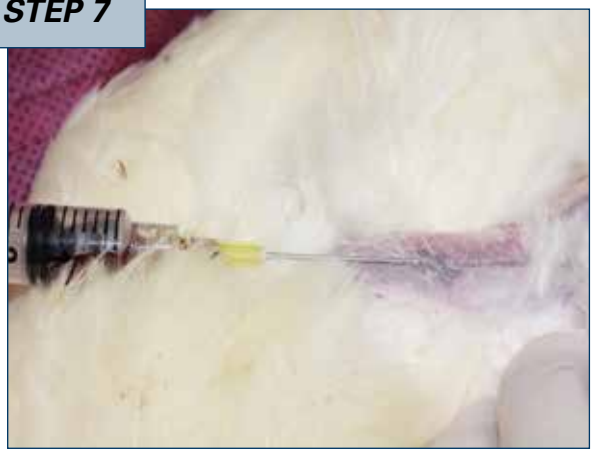

*Figure 10. Needle should be inserted first under the skin and then into the vein mid-way between elbow and shoulder joints.*

**STEP 8**

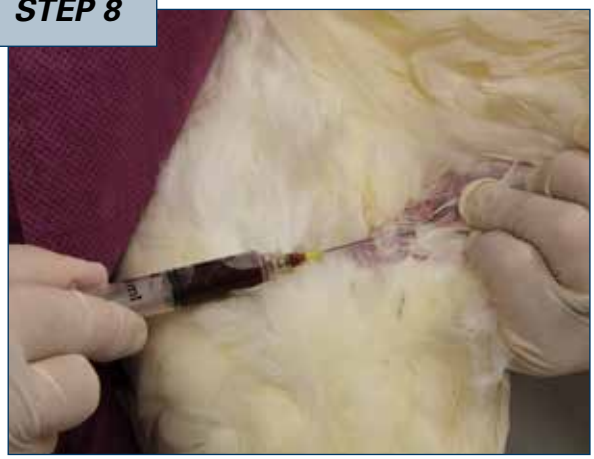

*Figure 11. If needle is within the brachial vein, blood will fill syringe with minimal pull on syringe plunger. Pulling back on plunger with too much force will create high negative pressure, causing the vein to collapse and stopping the flow of blood into the needle.*

**STEP 9**

Once the needle is removed from the vein, the application of slight pressure with a finger over the injection site will promote more rapid clotting. Formation of a hematoma or blood clot in the injection area is common.

All needles should be discarded in a designated sharps container.

***Needles should never be recapped.***

*Figure 12. If a hematoma forms before a sufficient quantity of blood has been obtained, it may be necessary to stop and attempt collection from the bird's opposite brachial vein. Once a hematoma has formed, it is nearly impossible to visualize the vein and thus impossible to collect blood.*

**If blood is not flowing into the syringe:**

1. Needle is not in the vein.
2. Needle is plugged with a clot.
3. Vein has been punctured and a hematoma is forming.

**2. Wing vein puncture using a scalpel blade**

Although this method can provide more rapid blood collection, it does have the potential to induce more trauma than using a needle and syringe.

a. A #11 scalpel blade inserted into a #3 or #4 scalpel blade holder is used to puncture the brachial vein just above the elbow joint.

b. A blood tube is used to collect the blood as it hemorrhages from the cut. This method is more likely to result in sample contamination with bacteria, mold, etc. Wiping the skin with rubbing alcohol prior to the cut may limit contamination.

c. Depending on the size of the cut, this method can cause significant trauma (blood loss, stress, etc.) to the bird and involves risk of severing the brachial artery and nerve.

**3. Cardiac puncture methods**

Collecting blood directly from the heart can provide rapid blood collection, and allow for collection of larger volumes of blood (4 to 10 mL). Additionally, cleaner blood samples can be collected compared with wing vein method. Cardiac puncture methods should only be practiced by trained personnel. Poor technique in needle placement and repeated attempts to locate the heart can result in fatal hemorrhage; however, this risk is minimized with practice. If fatal hemorrhage is suspected, the bird should be humanely euthanized promptly.

***a. Anterior (thoracic) cardiac approach***

This is a one-person technique where the bird is restrained by holding both legs in one hand while operating the syringe with the other hand. The proper position of the bird is flat on its back with the bird's head extended downward over the edge of a table (or cage or handler's knee). Using the index finger as a guide, the needle is inserted into the thoracic inlet at the highest point of the inverted V formed by the clavicle (wishbone). The needle is kept in the same plane as the keel bone and angled back toward the tail. The entire length of the needle (1.5 inch or 3.81 cm, 18 gauge) is usually inserted with little resistance into the heart. While inserting the needle, a slight negative pressure is applied. When the needle enters the heart, the blood will flow easily into the syringe. When the needle is positioned incorrectly, usually not in the same plane as the keel bone, it can enter the respiratory tract and air will flow back into the syringe. Hemmorhage into the lungs or airsacs can result from needles positioned incorrectly. Should incorrect needle insertion occur, and evidence of respiratory distress is observed, the bird should be humanely euthanized in an appropriate manner.

***b. Lateral cardiac approach***

A lateral approach is practiced by inserting a needle through the left thoracic wall. This is usually a two-person procedure where the bird handler lays the bird flat on a table on its right side, holding both legs in one hand and both wings in the other. The landmark is the groove formed by the edge of the breast (pectoral) muscle, where the ribs can be felt. A 1.5 inch (3.81 cm), 18-gauge needle is used. The point of needle insertion is about 2 inches (5.0 cm) vertical from the point of the keel bone. The needle is held at a 90° angle to the plane of the keel bone. Proper bird positioning is essential for consistent results with this method. As in the anterior approach, incorrect needle insertion may require the bird to be humanely euthanized before fatal hemorrhage occurs.

## PROPER BLOOD SAMPLE HANDLING

Once a blood sample has been collected into a syringe, the sample should be carefully transferred to a tube to promote clot formation. Clotting occurs when all the cells in the blood are drawn together by the coagulation process and separates from the fluid portion of the blood (serum).

- The needle should be removed from the syringe before the blood is pushed into the clotting tube (Figure 13). Forcing the blood back through the needle will rupture red blood cells (hemolysis), resulting in a poor quality sample.
- Slowly inject the blood into the clot tube, allowing it to run down the side of the tube, which encourages clot formation. Blood must be placed in the clot tube before the coagulation process begins.
- Do not disturb the blood tubes while the clotting process is occurring. Tubes should be allowed to stay positioned nearly flat (horizontal) to maximize surface area of the clot as it forms (Figure 14). The amount of serum yielded from clotted blood depends on the surface area of the clot. Tubes held upright in the vertical position have little surface area and produce only a small quantity of serum. Use a test tube holder for keeping tubes in this flat position. If a test tube holder is not available, then a block of wood drilled with appropriate sized holes or a wire rack can be used.
- The time required for a clot to form depends on the ambient temperature where samples are kept. The ideal temperature for clot formation is 80 to 100°F (27 to 38°C). At this temperature, serum separation will take approximately 12 to 18 hours for completion. At cooler temperatures, the clotting process is slower and the serum yield is reduced.

Blood samples can be damaged and are subject to bacterial contamination if exposed to higher temperatures for longer periods of time. This can occur when blood samples are left in a hot car or in direct sunlight.

Bacteria or mold contamination will cause serum to appear slimy with solid cheese-like particles. Opportunistic microorganisms feed on the antibodies in the serum and lower the amount of antibody measured by the laboratory.

If birds are dehydrated (in especially hot weather or due to stress), they produce poor serum samples that are gelled. Additionally, serum from birds after a recent meal appear cloudy due to excess fat in the serum. Lipemic (fatty) samples are not ideal to run in the laboratory, as the fat will interfere with any optical based test or antibody fixing test such as ELISA.

Blood in the process of forming a clot should not be frozen. The samples should not be shaken or allowed to roll around. Roughly handled blood samples will yield serum containing the pigments of ruptured red blood cells. This process is called hemolysis and makes serum appear red or pink in color. Hemolysis interferes with laboratory tests measuring antibody levels. Samples containing blood clots should not be mailed, as significant hemolysis can occur en route to the laboratory.

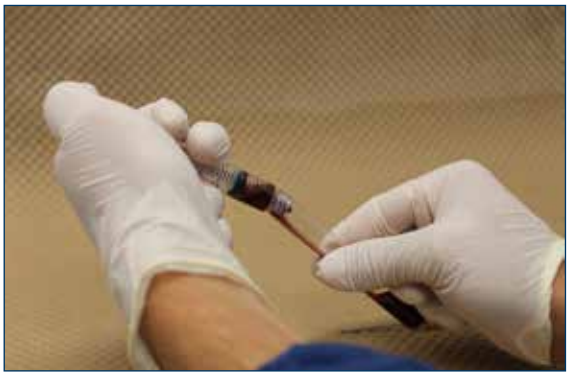

*Figure 13.*

*Figure 14.*

## SERUM COLLECTION AND HANDLING

After serum has separated from clotted blood, pour serum out of the clot tube into another collection tube, or tease the clot out of the tube with a wooden stick (such as a toothpick), leaving only serum in the tube. A clot must be handled gently during the process of separating the serum. A good quality serum sample will appear clear to pale yellow in color. Cloudy, slimy, or hemolyzed samples should not be sent to the laboratory.

### *Sending Serum Samples to the Laboratory*

Once serum has successfully separated from a clot, it should be kept cool (45°F or 7°C) and sent immediately to the laboratory. Serum should not be frozen if intended for use within 3 to 5 days. The tubes containing individual bird serum samples should be tightly capped, organized by flock into sealed plastic bags and clearly identified with labels or indelible ink. Styrofoam insulated containers with at least one cold pack should be used for mailing. It is best to avoid mailing serum samples to the laboratory on Thursdays or Fridays, as they will arrive at the laboratory over the weekend. Serum that must be stored for longer periods of time should be frozen at +14°F to -40°F (-10°C to -40°C).

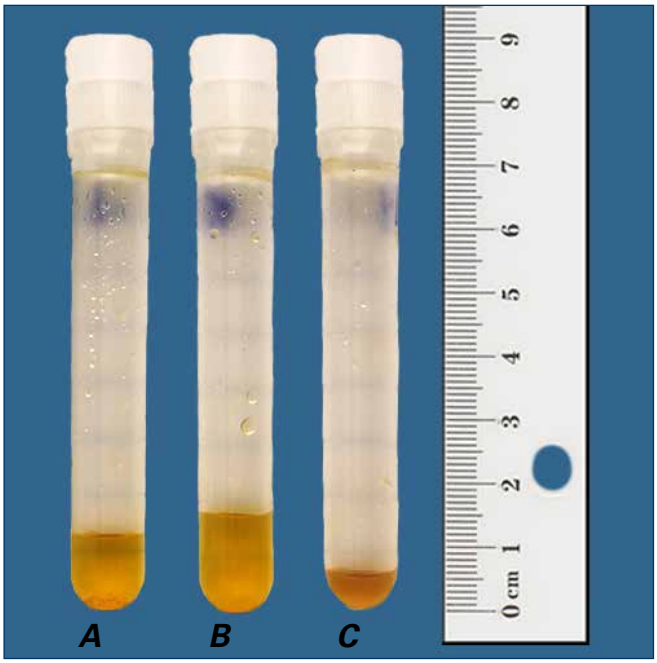


*Figure 15. Examples of good serum samples. Note the transparent, golden color and adequate volume (>0.25 mL).*

***Do NOT send serum samples to the lab that:***

- Contain less than 0.25 mL of serum
- Are excessively hemolyzed (red)
- Are excessively lipemic (fatty)
- Contain clots
- Are gelled, slimy or contain cheese-like particles

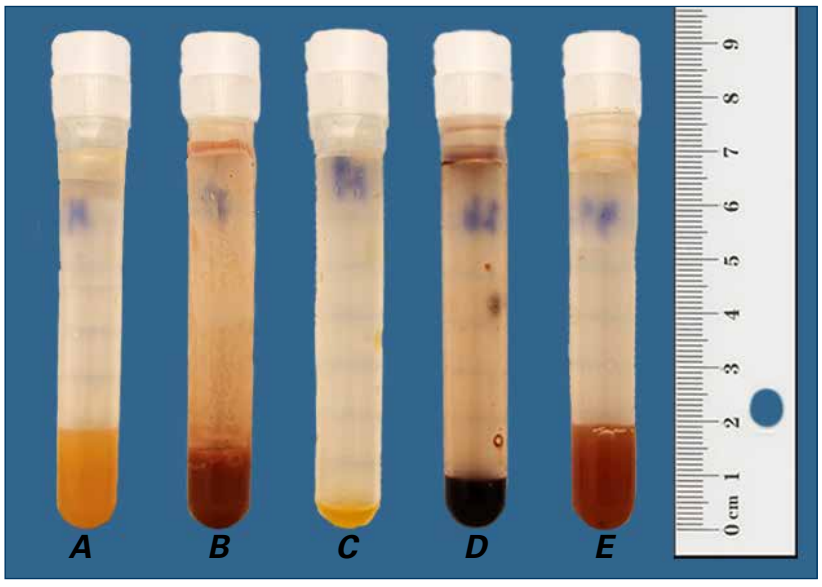


*Figure 16. Examples of poor quality serum samples. A: cloudy, turbid sample; B: lipemic sample; C: too little volume (<0.25 mL); D: clot and no serum; E: hemolyzed sample.*

# PROPER COLLECTION AND HANDLING OF DIAGNOSTIC SAMPLES

## *PART 2: FTA CARDS*

### FTA CARDS

Fast Technology for Analysis of nucleic acids (FTA) cards are made of specialized paper with chemicals that protect nucleic acids (RNA or DNA) from degradation, while removing the infectious capability of pathogens. Samples on FTA cards can be used for molecular genetics to identify and analyze specific chicken DNA segments, and for diagnostic purposes to detect DNA or RNA from pathogenic bacteria or viruses. An FTA card's compact size provides an ideal means to transport samples domestically or internationally with proper import approval. FTA cards also provide a stable means to store diagnostic samples for extended periods of time at room temperature.

There are two types of FTA cards, each with a specific purpose, which can be obtained from GE Healthcare:

**FTA "Classic" (WB120205)** bind DNA/RNA and release proteins when treated with the appropriate buffers.

**FTA "Elute" (WB120410)** bind proteins and release DNA/RNA following treatment.

Consult with the receiving laboratory prior to sample collection to ensure the correct type of FTA card is used.

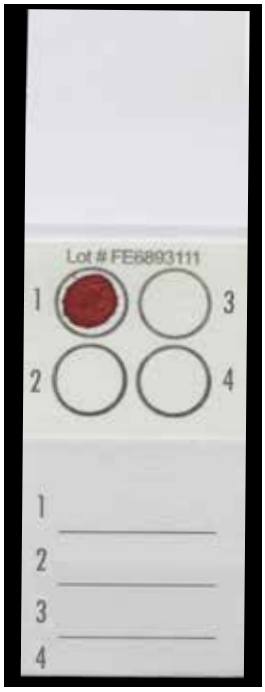


*Figure 1. FTA card.*

Samples appropriate for placement on an FTA card and their diagnostic purpose:

- Whole blood – disease diagnostics, genetic analysis
- Feather pulp – pathogen identification
- Swabs from choanal cleft, cloaca, joints and organs – pathogen identification
- Tissue impression smears – pathogen identification

**Steps for blood sample collection on an FTA card:**

1. A sample is applied to the FTA card in the designated field by needle and syringe (Figure 2) or by blood loop (Figure 3) and allowed to air dry. The circle does not need to be filled entirely.
2. Up to four samples can be applied on one FTA card. Samples placed on the same card should not be allowed to mix (Figure 4), or cross-contamination of individual samples will occur.
3. Place the dry card in a sealed plastic bag for transport.
4. Once completely dry, the sample on the FTA card poses no risk of transporting infectious material. Check local regulations for importation requirements of FTA samples.
5. The laboratory receiving the FTA card will process the individual samples and extract the DNA or RNA to perform the appropriate assays requested.
6. All samples sent to Hy-Line International require prior approval and appropriate import permits.


*Figure 2. Application with needle and syringe.*

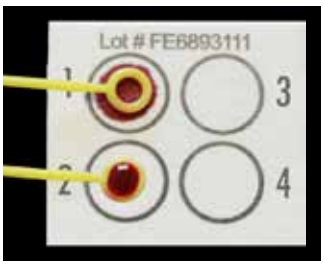


*Figure 3. Application with blood loop.*


*Figure 4. Cross-contamination.*

**Steps for making a tissue impression smear on an FTA card:**

1. Cut a small piece (5 $mm^3$) of tissue to make the impression smear (Figure 5).
2. Apply the tissue in the designated field by pressing the tissue onto the FTA card by using a finger (Figure 6).
3. Remove excess tissue to facilitate drying.
4. Place the FTA card on its side to allow cards to air dry (Figure 7). After the sample has completely dried, place in a sealed plastic bag for transport.
5. Up to four samples can be applied on one FTA card. Samples placed on the same card should not be allowed to mix to prevent cross contamination of the individual samples (Figure 8).

*Figure 5. Use a small sample of the tissue of interest. Larger samples will spread beyond the designated area.*

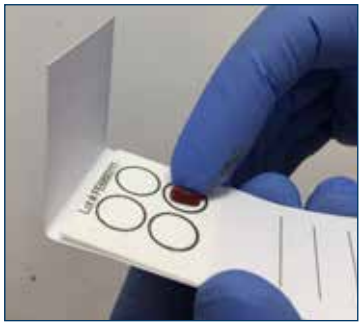

*Figure 6. Lightly press the tissue onto the FTA card and remove excess tissue.*


*Figure 7. Completely dry the sample on the card by placing the card on its side.*

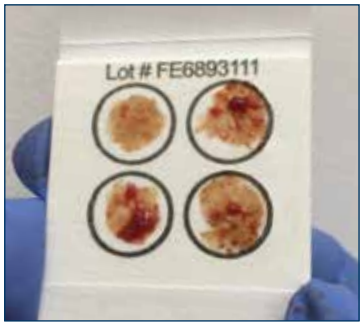


*Figure 8. Up to four samples can be placed on one card.*

When submitting samples to a diagnostic laboratory, it is important to provide thorough and relevant flock information on the laboratory submission form.

Critical information that should accompany all diagnostic sample submissions:

- Flock identification and location
- Age of flock
- Date of sample collection
- Vaccination program
- Flock history, including pertinent health or production problems
- This information is vital to the flock veterinarian and diagnostician to make a meaningful interpretation of serological or diagnostic results and provide recommendations to improve flock health and/or production.

**Benefits of FTA cards:**

- Sample Collection – easy to perform
- Safety – sample is stable and non-infectious
- Transportation – small size, although international permits required
- Storage – room temperature

# PROPER COLLECTION AND HANDLING OF DIAGNOSTIC SAMPLES

## PART 3: SWABS

Diagnostic samples are used to determine health status or identify specific pathogens in pullet, layer and breeder flocks. Routine samples include whole blood, serum, formalin-fixed tissue and swabs: tracheal, choanal, oropharyngeal, cloacal, organs and joints. For specific investigations, Fast Technology for Analysis of nucleic acids (FTA) cards can be used to collect feather pulp, whole blood or isolates from any type of swab.

### MOLECULAR DIAGNOSTICS

The advent of molecular diagnostics such as PCR and rt-PCR has provided new tools for rapidly and accurately diagnosing poultry diseases. It is now possible to sequence the genome of many pathogens. Sequencing allows comparing isolates to better understand the disease epidemiology. Tissues, swabs and FTA cards can be submitted for molecular diagnsotics.

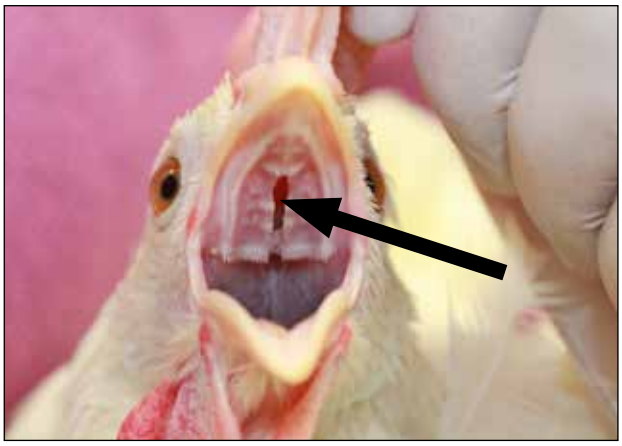

*Figure 1. Choanal cleft (arrow) is present in the upper beak.*

### SAMPLE SUBMISSION

When submitting samples to a diagnostic laboratory, it is important to provide thorough and relevant flock information on the laboratory submission form. Send the swabs to the diagnostic laboratory for immediate analysis. Do not freeze samples.

***Collection of Swabs***

Cotton or dacron tipped swabs are an effective, non-invasive method for sampling for Mycoplasmas, bacteria, and many viruses (examples: infectious bronchitis, avian influenza, infectious laryngotracheitis, Newcastle). Samples for PCR, virus isolation, bacterial isolation or other tests can be obtained from swabbing the oral/choanal cleft, trachea, cloaca, affected joints, and organs.

Critical information that should accompany all diagnostic sample submissions:

- Flock identification and location
- Age of flock
- Date of sample collection
- Vaccination program
- Flock history, including pertinent health or production problems

*Figure 2. Proper technique for restraining the bird while collecting oropharyngeal swabs.*

*Figure 3. Be sure to insert swab into the choanal cleft when collecting oropharyngeal swabs.*

| Pathogen(s) | Sample Pooling for PCR | Media Used | Location |
|---|---|---|---|
| Avian Influenza | pool of 11 swabs in 5.5mL media<br>pool 5 swabs in 3mL media | BHI (Brain heart infusion) | Tracheal, orорharyngeal |
| Newcastle Disease | pool of 5 swabs in 5.5mL media | BHI | Tracheal, oropharyngeal |
| Mycoplasma gallisepticum/synoviae | pool of 5 swabs per PCR reaction | Dry or BHI media | Tracheal, oropharyngeal |
| Bacteria and/or Viruses | only pool tissues from a single bird; pool by organ system (respiratory, enteric, reproductive) | media provided in the culturette tube | Affected organs |

See http://poultryimprovement.org/documents/WIAV0020.pdf for recommendations for collecting specimens from poultry for viral diagnostic testing.

It is advised to confirm the proper type of swabs and necessity for enrichment media for sample collection and transport. Prior communication with the diagnostic lab will ensure correct sample handling and expedite processing. When submitting swab samples in liquid media such as brain-heart infusion broth (BHI), many laboratories request that the actual swab is not included in the tube. The proper procedure in this case is to gently swish the swab in the media to wash off any material, and then roll the swab against the tube as it is pulled out to drain any excess fluid from the swab that may contain pathogenic material. Depending on the test, 5 to 11 individual swab samples can be pooled together without reducing the sensitivity of the PCR.

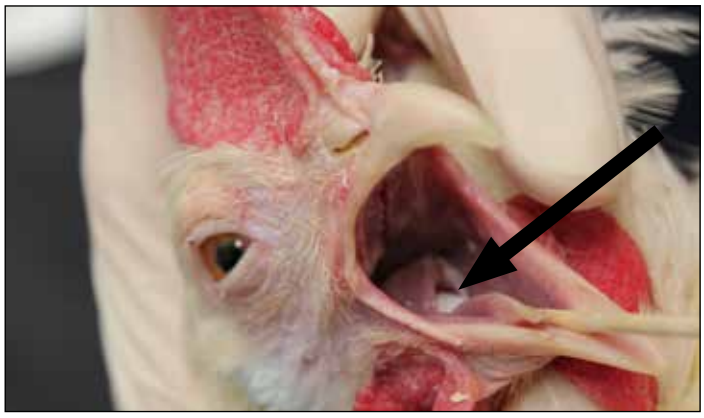

*Figure 4. The swab is inserted gently through the glottis into the trachea.*

*Figure 5. Remove feathers and clean the skin with an alcohol pad before cutting into the joint of an euthanized bird.*

*Figure 6. Swab the synovial surface of the affected joint.*

*Figure 7. Expose the cloaca and insert the swab gently into the cloaca and rotate the swab over the mucosal surface.*

# PROPER COLLECTION AND HANDLING OF DIAGNOSTIC SAMPLES

## PART 4: TISSUE COLLECTION AND SUBMISSION FOR HISTOPATHOLOGY

### HISTOPATHOLOGY

Histology refers to the evaluation of cells and tissues using a microscope. As a follow-up to the post-mortem exam, histology can be a valuable tool in assessing flock health. Some poultry diseases can only be diagnosed by histopathology. For example, the clinical presentation of infectious laryngotracheitis virus or wet pox within a flock can be virtually identical, but the diseases cause distinctly different and characteristic histopathologic changes that allow a definitive diagnosis.

Successful use of histopathology as a diagnostic practice requires the availability of appropriately selected and preserved samples.

*Figure 1. After the tissue samples have been processed and histological sections placed onto glass slides, a trained avian pathologist examines the tissue sections to look for evidence of disease.*

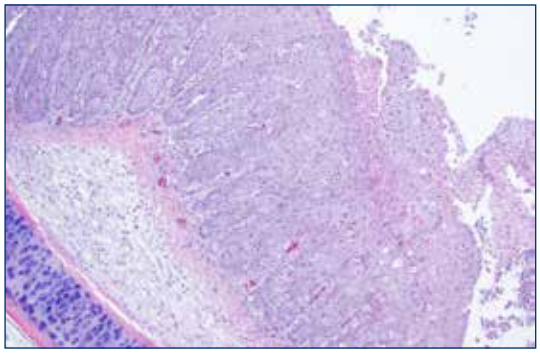

*Figure 2. Microscopic view of the tissue of the trachea.*

### *Sample Collection*

Collect specimens for histopathology as soon as possible after death to avoid deterioration of tissues. Fresh tissue samples from birds humanely euthanized immediately prior to post-mortem examination provide the best quality slides. If mortality must be used for tissue collection, they should be determined to be fresh as possible, and not decomposed.

Do not collect samples from birds that have been previously frozen. The freeze and thaw processes can disrupt cellular features, leading to poor quality slides.

Samples should be collected using a scalpel or razor blade that is sharp and sterile (Figure 5). Avoid using scissors, as they can crush tissue and destroy microscopic details.

An individual sample should be no larger than 1 $cm^3$ (1x1x1 cm) to allow for adequate penetration of the tissue with fixative. Larger pieces of tissue will decompose in the center before adequate penetration by the fixative (formalin).

*Figure 4. Equipment used for collection of samples for histopathology.*

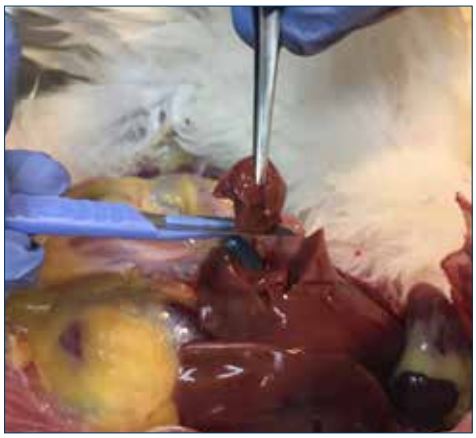

*Figure 5. Using a scalpel blade to cut the tissue sample.*

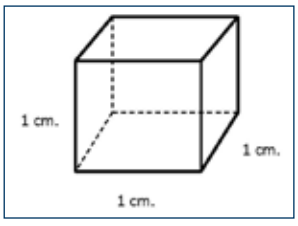


*Figure 3. Cubic cm.*

*Figure 6. For complete and rapid preservation of tissue, the sample should be no larger than 1 $cm^3$.*

### *Sample Selection*

Samples for histopathology should be collected at the time of post-mortem analysis. The selection of samples depends on observations made during the examination. Tumors and other masses, focal discolorations, and organs that are enlarged, atrophied, or otherwise abnormal should be sampled. When a particular disease is suspected based on flock history, tissues associated with that disease may be collected, even if they appear normal (see Table 1). A cross-section of all parts of the affected organ being sampled should be harvested whenever possible.

Tissue cut from the margin of the lesion, collecting both affected and normal tissues, is preferred. Whenever possible, collect healthy-appearing tissue of the same for comparison.

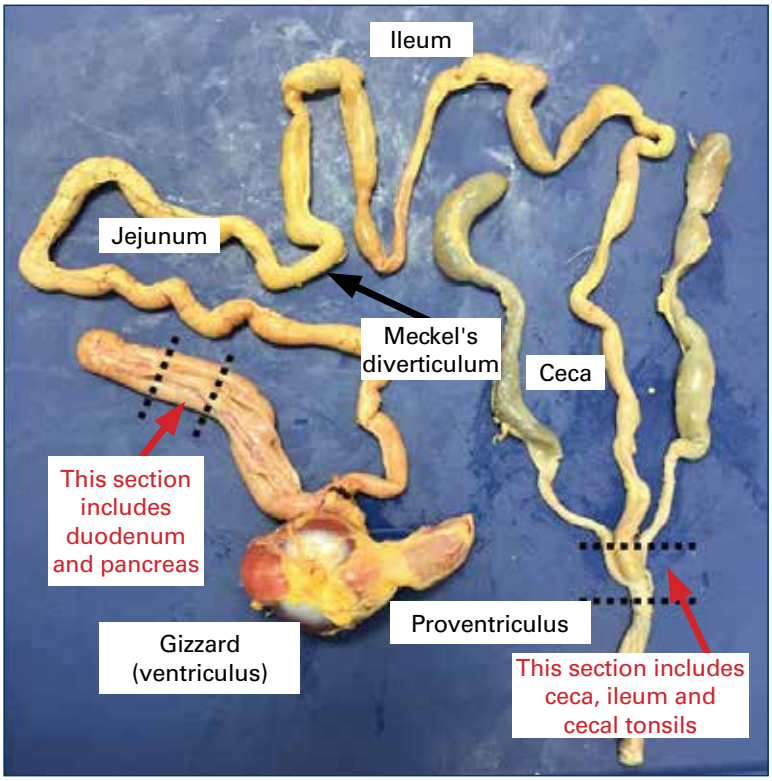


*Figure 7. Routine sample sites from the gastrointestinal tract. Cut 2–3 cm sections of intestine in the area of gross lesions or other areas of interest.*

### *Sampling for Specific Diseases*

When there is concern for a particular disease based on regional risk, a suspicious result on surveillance testing, or clinical signs in the flock, specific tissues should be collected. Table 1 provides examples of some diseases of concern and the special samples that should be taken.

| Disease of Concern | Samples Needed |
|---|---|
| Gumboro (IBD) | • Bursa of Fabricius, Thymus |
| Infectious Laryngotracheitis | • Trachea<br>• Larynx<br>• Conjunctiva |
| Marek's Disease | • Sciatic Nerve<br>• Brain<br>• Eye<br>• Tumors |
| Wet pox | • Trachea<br>• Larynx |
| Enteritis (coccidia, focal duodenal necrosis) | • Portions of the gastrointestinal tract affected |

*Table 1.*

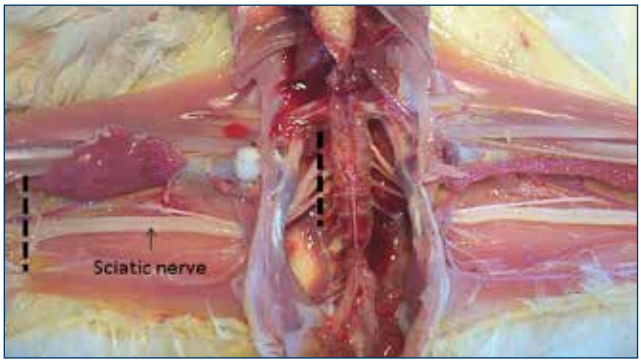


*Figure 8. The sciatic nerve of the leg is a sample frequently used for Marek's disease diagnosis. Cut the entire length of the sciatic nerve and place in formalin.*

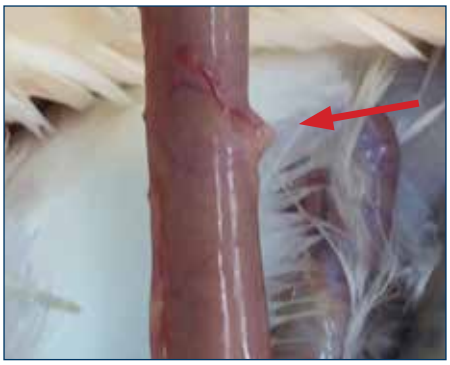

*Figure 9. Meckel's diverticulum is the physical landmark dividing the jejunum and ileum.*

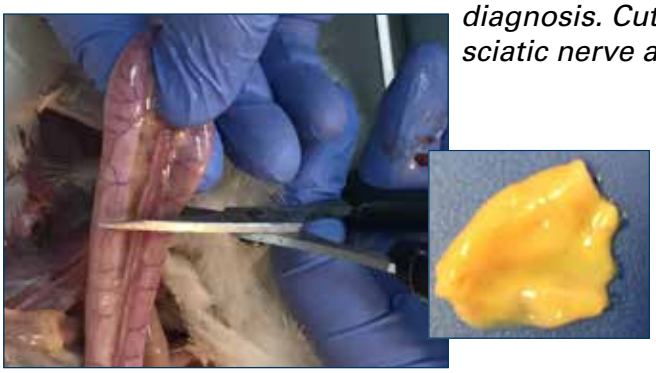

*Figure 10. Take a 2–3 cm section of intestine in the area of interest. When collecting intestine sections, gently open lumen of intestine (inset).*

*Figure 11. Submit the trachea in full; carefully open the trachea along its length.*

*Figure 12. Carefully remove cranium over the brain with scissors.*

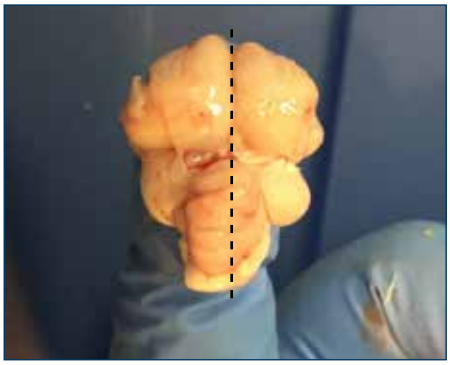

*Figure 13. Carefully remove brain and divide longitudinally.*

### *Sample Preservation*

Samples should be promptly submerged in a solution of 10% neutral buffered formalin for preservation. The volume of formalin solution in a single container should be at least 10 times the volume of all tissues. Samples must be fully immersed in the solution to be adequately saturated by fixative to prevent deterioration. Lung tissue and other air-containing tissues may be wrapped gently in absorbent cotton to aide immersion. Gently open the lumen of trachea and intestine samples to release trapped air.

After 48 hours in formalin, the tissues are adequately fixed. If necessary for shipping, the formalin can be decanted at this point. Decanted samples should be shipped immediately to minimize the risk of damage to the sample from drying.

*Figure 14. Immediately place the tissue into 10% neutral buffered formalin for preservation.*


*Figure 15. After 48 hours in formalin, tissues are adequately preserved.*

If samples may be subject to sub-freezing temperatures during shipping, already fixed samples can be decanted, and re-submerged in an "alcoholic formalin." This will protect against freeze-thaw damage to tissues. For a simple alcoholic formalin mix, combine and pre-mix 6.5 parts pure ethyl alcohol, 2.5 parts distilled water, and 1 part 37% formalin.

*Figure 16. After 48 hours, the formalin can be decanted and samples kept in sealable, leak-proof plastic bags.*

The formalin-fixed samples can be kept in sealable plastic bags (e.g. Whirl-Pak® bags), or remain in a securely sealed jar with formalin.

If the samples are to be mailed to the laboratory, double-bag the sample to prevent leakage. Remember that formalin is a poison and exposure to the liquid or vapor is harmful to humans.

*Figure 17. Whirl-Pak® type bags are sealable, leak-resistant, and can be used for storing and transporting samples.*

### *Sample Submission*

When submitting samples to a diagnostic laboratory, it is important to provide thorough and relevant flock information on the laboratory submission form. Critical information that should accompany all diagnostic sample submissions includes:

- Flock identification and location
- Age of flock
- Date of sample collection
- Tissue(s) collected
- Vaccination program
- Flock history, including description of any clinical signs, production problems, and the present level of mortality
- Special shipping regulations may apply for formalin filled containers
- Locally appropriate biohazard labelling on all transport containers
- Appropriate permits for international shipping (e.g. USDA-APHIS permits) if appropriate

This information is vital to the flock veterinarian and diagnostician to make a meaningful interpretation of diagnostic results and provide recommendations to improve flock health and/or production.

*Figure 18. Double bagging samples will prevent leakage of the formalin during transportation.*

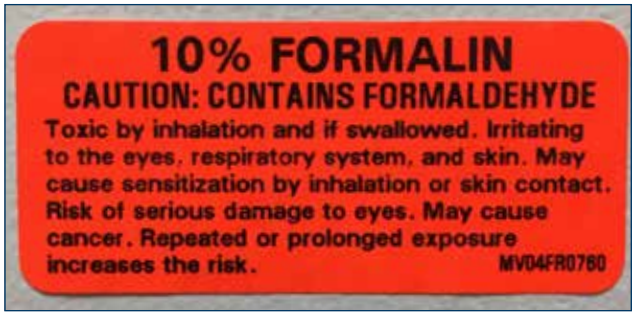


*Figure 19. Formalin is harmful to human health. Appropriate biohazard warning labels should be on all containers.*

### *Sample Processing*

After arrival at the diagnostic laboratory, the formalin preserved tissues are embedded into a paraffin block, then sectioned with a microtome into thin slices. Tissue slices of this thickness (4 micron) are thin enough to be examined by the pathologist under a light microscope. These slices are fixed on glass slides and stained. Various stains can be used to highlight different cell types, or other aspects of the tissue. The most frequently used stain for disease diagnosis is hematoxylin and eosin (H&E) stain.

## REFERENCES

1. Bermudez, Alex J. and Bruce Stewart-Brown. Chapter 1: Principles of Disease Prevention: Diagnosis and Control, "Disease Prevention and Diagnosis". Diseases of Poultry. 13th edition. Ames: Wiley-Blackwell, 2013.
2. USDA-APHIS. United States Veterinary Permit for Importation and Transportation of Controlled Materials and Organisms and Vectors. U.S. Department of Agriculture. 2016.