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.

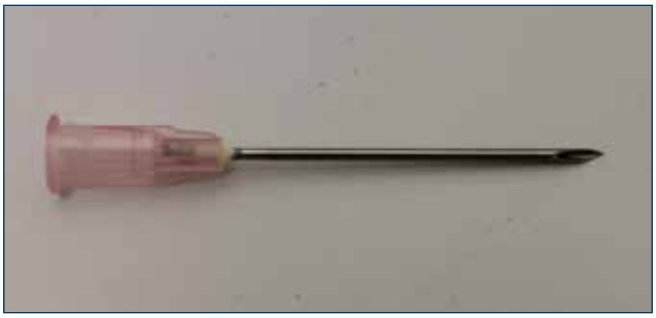

Figure 1. Disposable needles should be changed every 5 to 10 birds to prevent tissue trauma and cross-contamination.

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

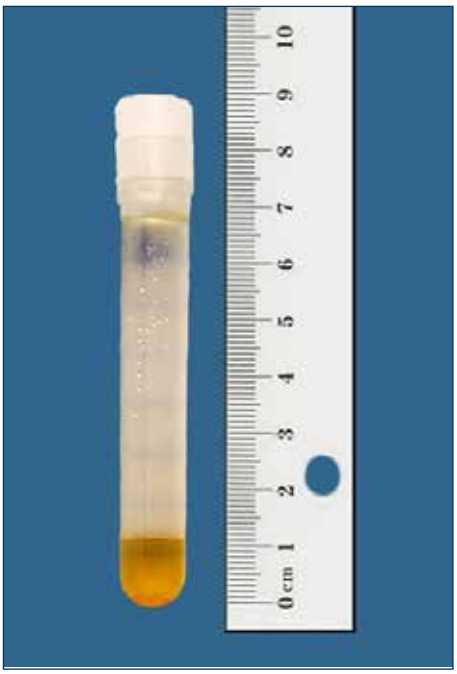

Figure 3. Plastic blood collection tube with cap, ideal for separating serum and transporting sample. Note proper volume (1.0 mL) of golden colored, transparent serum.

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

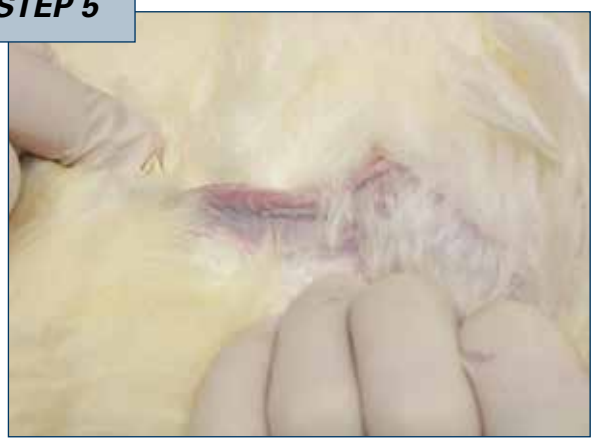

Figure 8. Visualize the brachial vein.

STEP 6

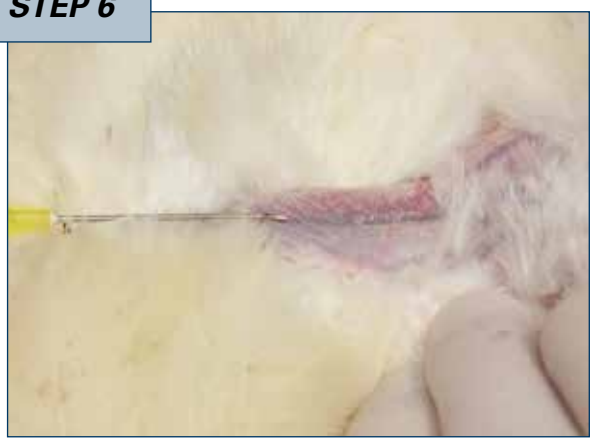

Figure 9. Orient needle in alignment with vein, bevel pointed up, with tip of needle pointed toward wing tip.

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

STEP 7

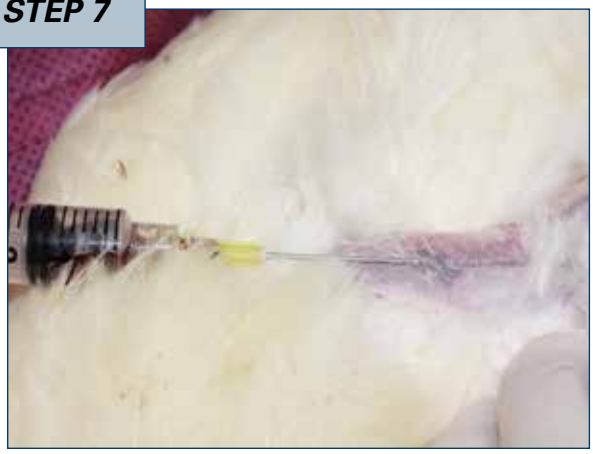

Figure 10. Needle should be inserted first under the skin and then into the vein mid-way between elbow and shoulder joints.

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).

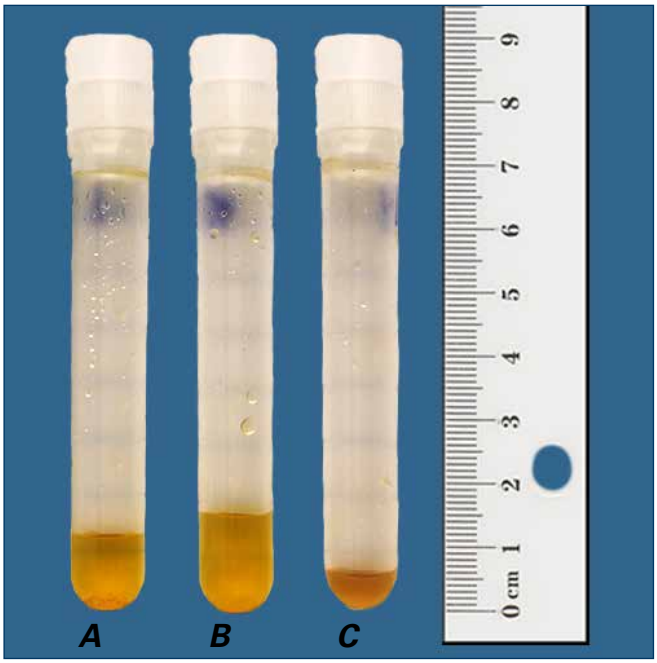

Figure 15. Examples of good serum samples. Note the transparent, golden color and adequate volume (>0.25 mL).

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

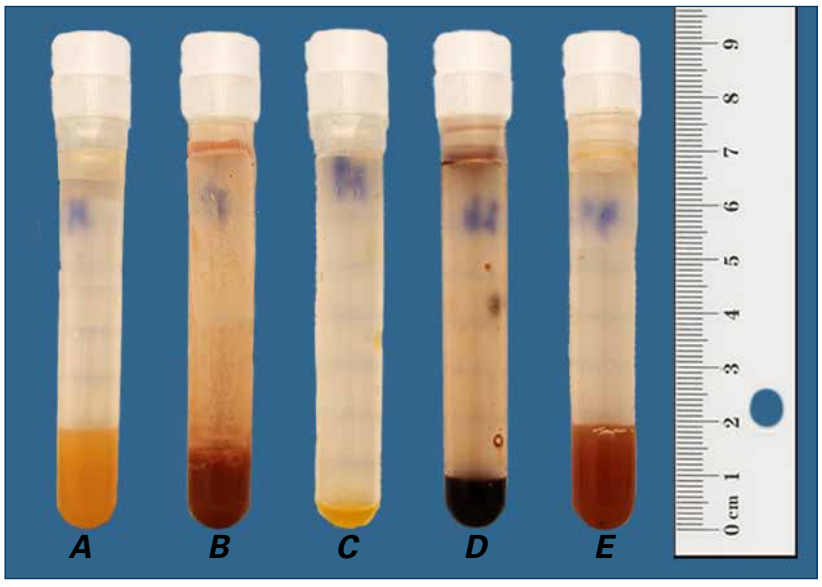

Figure 16. Examples of poor quality serum samples. A: cloudy, turbid sample; B: lipemic sample; C: too little volume (<0.25 mL); D: clot and no serum; E: hemolyzed sample.

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