Chapter 2

Specimen Collection and Preservation

Specimens are used to provide supporting information leading to the diagnosis of a cause of disease or death. A specimen may be an intact carcass, tissues removed from carcasses, parasites, ingested food, feces, or environmental samples. The specimen should be as fresh and undamaged as possible.

Choosing a Specimen

An entire, fresh carcass is the best specimen to submit to the laboratory for diagnosis. This allows the diagnostician to assess all of the organ systems and to use appropriate organs for different diagnostic tests. Obtain the best specimens possible for necropsy; decomposed or scavenged carcasses are usually of limited diagnostic value. A combination of sick animals, animals that were euthanized after clinical signs were observed and recorded, and some of the freshest available carcasses compose an ideal specimen collection. The method of euthanasia should not compromise the diagnostic value of the specimen (see Chapter 5, Euthanasia). More than one disease may be affecting the population simultaneously, and the chances of detecting multiple diseases will be maximized if both sick and dead animals are collected. Specimens submitted should be representative of the species involved. If more than one species is affected, collect several specimens of each species; try to obtain a minimum of five specimens per species.

Tissue Collection

The primary consideration when collecting carcasses or tissues for diagnosis should be personal safety. Some wildlife diseases are transmissible to humans, and every carcass should be treated as a potential health hazard. Wear disposable rubber or plastic gloves, coveralls, and rubber boots. If gloves are not available, inverted plastic bags may be used (Fig. 2.1). Before leaving an area where carcasses are being collected, double-bag used gloves and coveralls, and disinfect boots and the outside of plastic bags with a commercial disinfectant or a 5 percent solution of household chlorine bleach. Also, double-bag specimens in plastic before removing them from the area. These precautions will help protect the people in the field and minimize transmission of disease to unaffected wildlife populations.

If it is impossible to submit an entire carcass for diagnosis, appropriate organs must be removed from specimens. If possible, do not dissect carcasses in the field without first consulting disease specialists about methods of dissecting and preserving tissues or parasites or both. Assistance can be obtained from a variety of sources (Appendix B). It is

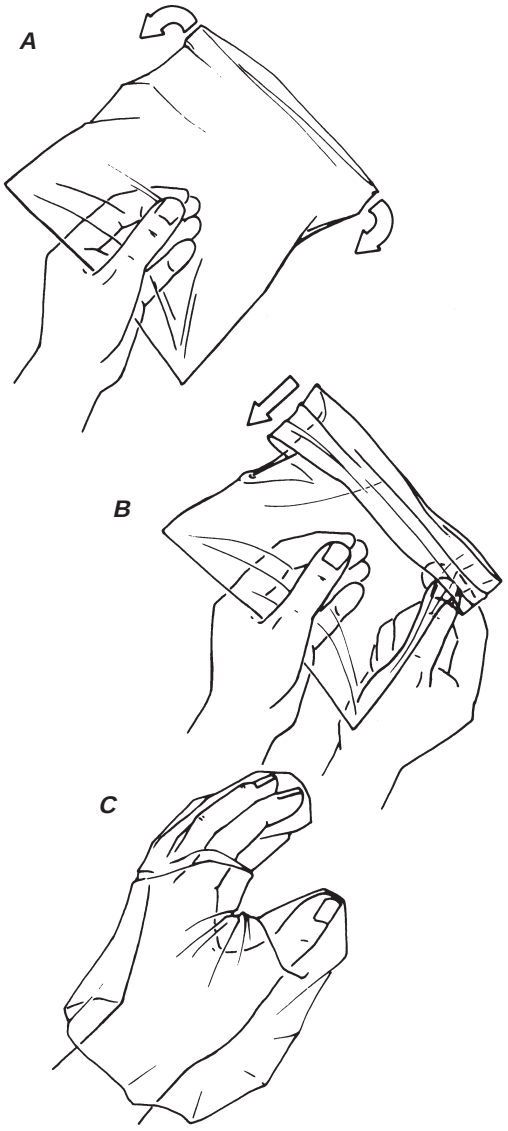


Figure 2.1 *Use a plastic bag to protect hands from direct contact with animal tissues during the collection of specimens if plastic or other waterproof gloves are not available.* ***(A)*** *Grasp bag at the bottom and* ***(B)*** *with other hand pull open end down over hand holding bag* ***(C)****. Repeat for the "unbagged" hand. Reversing this process when handling small specimens will automatically place specimens in the bag, which then need only be sealed and put into a second bag for packaging and shipment.*

best to become familiar with these sources and their ability to provide specific types of assistance before an emergency arises. The basic supplies and equipment that should be included in a field kit for specimen collection will vary with the species being sampled and the types of analyses that will be conducted. Keep a small kit packed in a day pack for ready use (Fig. 2.2). Sources of supplies used for collecting, preserving, labeling, and shipping specimens are listed in Appendix C.

Whirl-Pak® bags are very effective containers for tissue specimens. These bags have a sterile interior, are easy to carry in the field, and can be used to hold a variety of samples (Fig. 2.3). Specimen identification should be written directly on the bag with an indelible marker.

If lesions are noted, collect separate tissue samples for microscopic examination, microbiology, toxicology, and other analyses. With a sharp knife or scalpel cut a thin (l/8–1/4 inch, 3–6 millimeter) section of tissue that includes all or portions of the lesion and adjacent apparently healthy tissue (Fig. 2.4). Take care not to crush tissue in or around the lesion. Place the tissue sample in a volume of l0 percent buffered formalin solution equal to at least 10 times the tissue volume to ensure adequate preservation. Formalin is classified as hazardous; take appropriate measures to prevent skin contact or vapor inhalation. Jars, such as pint or quart canning jars, are convenient containers for preservation of tissues, but wide-mouth plastic bottles (Fig. 2.5) eliminate the potential breakage problems. After 2 or 3 days in 10 percent formalin, tissues can be transferred to Whirl-Pak® bags that contain enough formalin to keep the tissues wet. Write the specimen identification with indelible marker or pencil on a piece of index card, place the card inside the bag, and write the information directly on the bag with indelible marker. Pack the bags for shipping so as to prevent tissues from being crushed. Check with the courier regarding current requirements or restrictions for shipment of formalin.

If it is necessary to collect a blood sample from a live bird (if, for example, botulism is suspected), and syringes and needles are not available, sever the bird's head from its neck and collect the blood in a wide-mouth plastic jar.

Photographing external and internal lesions provides a record of the color, location, and appearance of lesions when appropriate camera equipment is available. Use a macro lens, high speed film, and a fast shutter speed to achieve maximum depth of field and sharply focused photographs with a hand-held camera. Include in the photograph for scale a coin or another readily recognized indicator of actual size. Explain on the history form submitted with the specimens what photographs were taken.

Photo by James Runningen

Figure 2.2 *A basic necropsy kit that can be packed into a small day pack. Clockwise, from top of photo: Data recording: field notebook, tags, pencils, markers. Protective apparel: rubber gloves, disposable shoe covers and coveralls, mask. Necropsy equipment: disinfectant for cleaning instruments, scrub brush, heavy shears, forceps, scissors, scalpel handle and blades. Measuring equipment: hanging scale and ruler. Sampling materials: microscope slides, syringes and needles, swabs, blood tubes, aluminum foil, Whirl Pak® bags, plastic bags, wide mouth plastic jars. Preservatives: ethanol for parasites, formalin for tissue samples.*

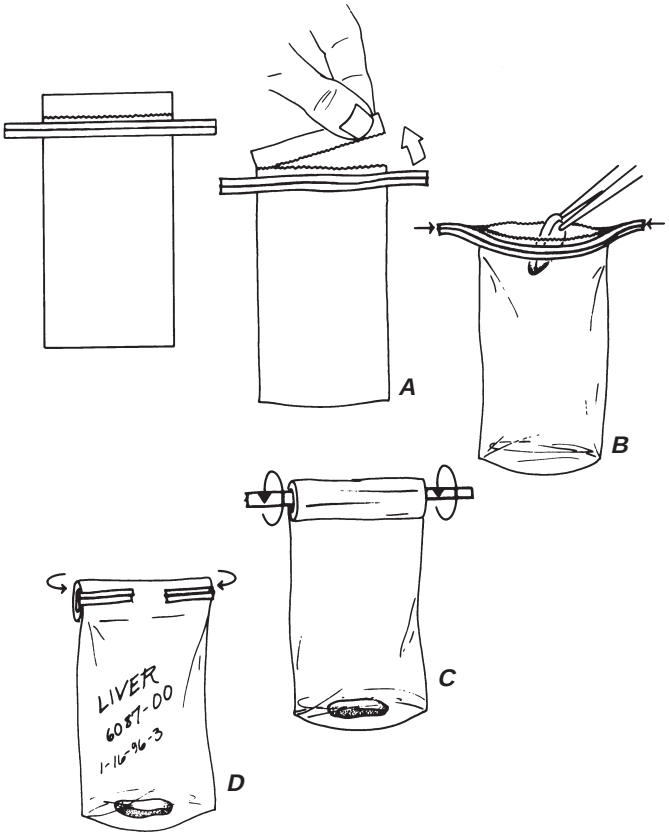


Figure 2.3 *Using Whirl-Pak® bag for specimen collection.* ***(A)*** *Remove top at perforation.* ***(B)*** *Open bag by simultaneously pushing the protruding wire-reinforced tabs toward the center to insert the specimen and any appropriate preservative.* ***(C)*** *Close bag by pulling on tabs and then twirling bag while holding tabs.* ***(D)*** *Secure the closure by folding tabs around bags and label bag with type of specimen, date, and any identifying numbers.*

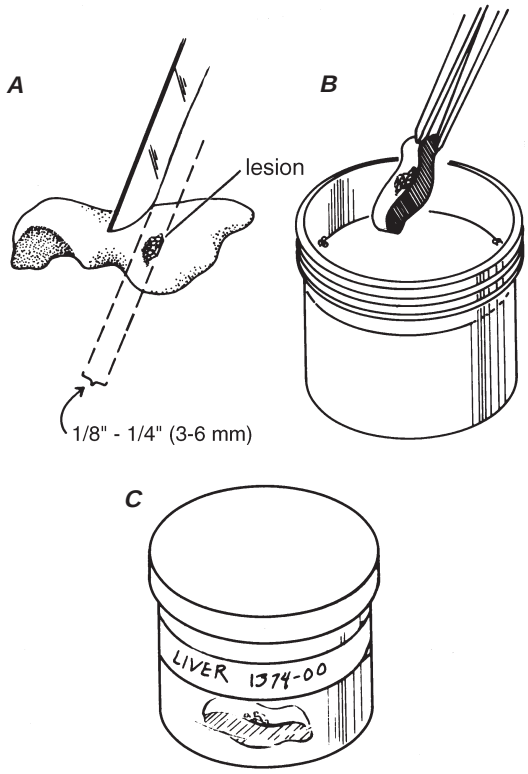


Figure 2.4 *Tissue sample collection for microscopic examination.* ***(A)*** *Tissue sample should include lesion, such as spots in liver, plus some apparently healthy tissue. The sample must be no thicker than 1/4 inch to ensure adequate chemical fixation by preservative. Use as sharp an instrument as possible (scalpel, knife, razor) for a clean cut.* ***(B)*** *Place tissue sample into container of 10 percent buffered formalin or other suitable fixative or preservative. The volume of formalin in the container should be about 10 times the amount of tissue sample.* ***(C)*** *Complete the process by securing the lid and properly labeling the container.*

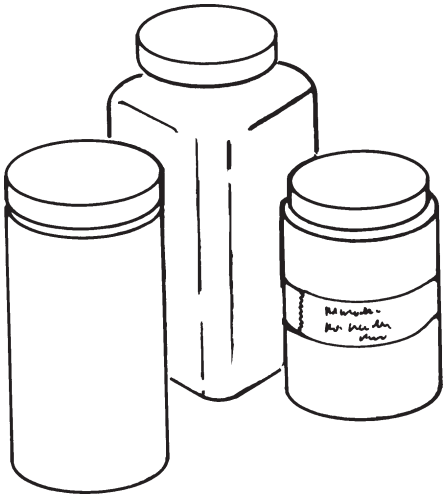


Figure 2.5 *Plastic bottles used for tissue specimens. Regardless of size or shape, specimen bottles should have a wide mouth and threaded caps for secure closure.*

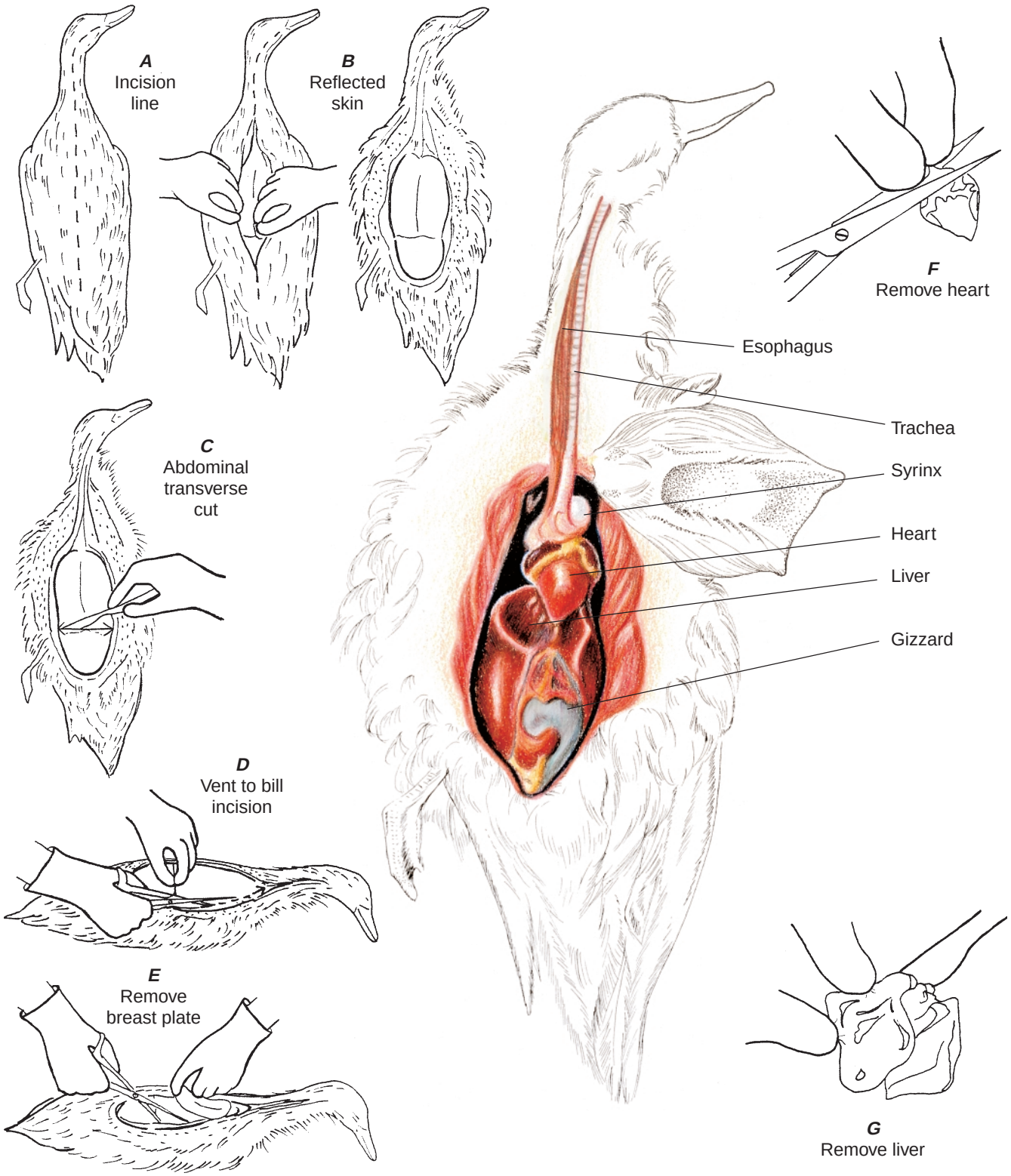
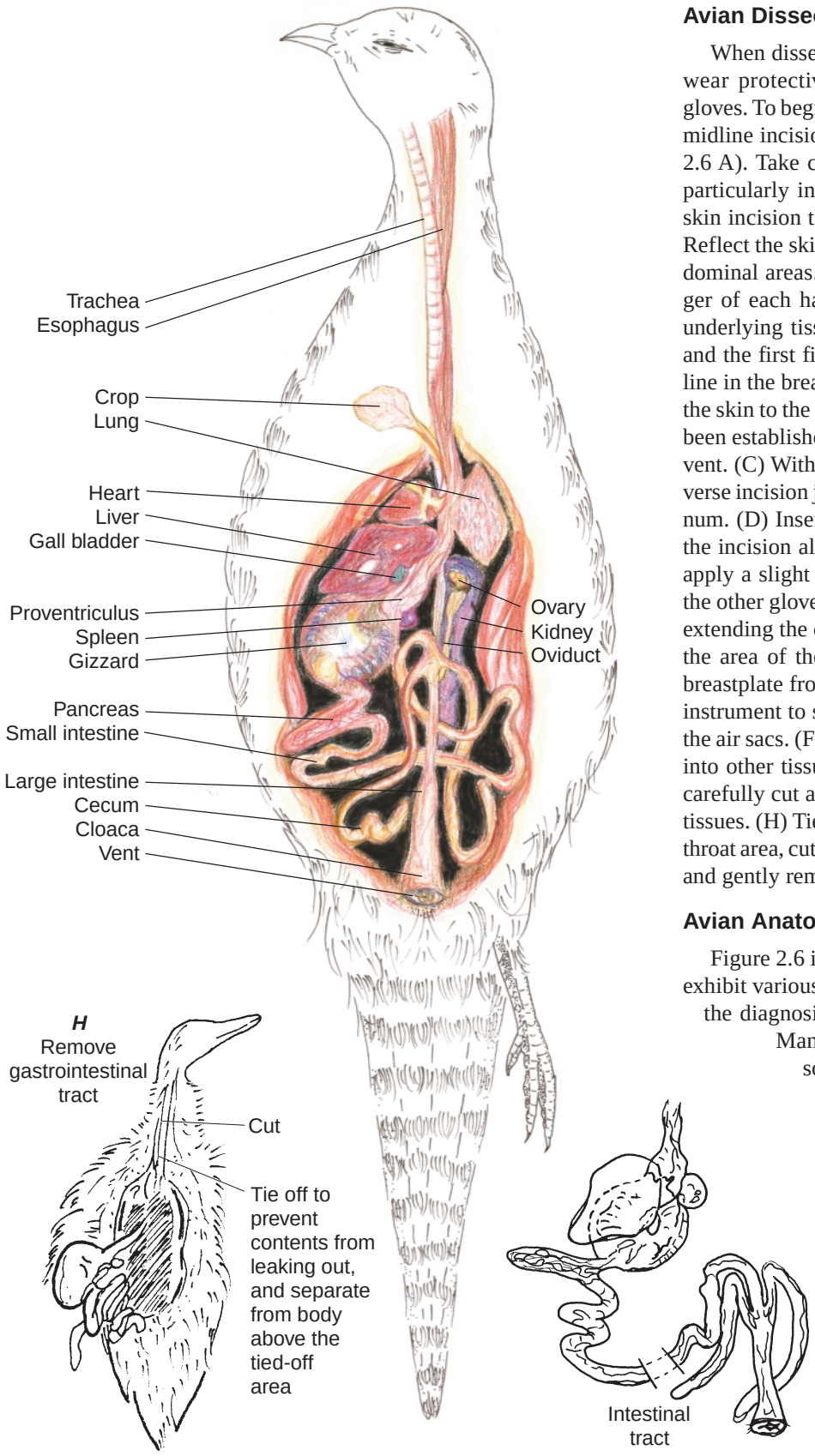


Figure 2.6 *Dissecting a duck carcass:* ***(A)*** *incision line;* ***(B)*** *reflect the skin to expose the underlying anatomy;* ***(C)*** *make a transverse abdominal cut below the breast muscle;* ***(D)*** *extend cut through the ribs and wishbone;* ***(E)*** *remove breast plate;* ***(F)*** *dissect out heart;* ***(G)*** *remove liver; and* ***(H)*** *tie off and remove the gastrointestinal tract.*



Avian Dissection

When dissecting a bird, it is always advisable to wear protective clothing, particularly disposable gloves. To begin, insert a scalpel or a knife to make a midline incision through the skin of the breast (Fig 2.6 A). Take care not to penetrate the body cavity, particularly in the abdominal region. Continue the skin incision to the vent and to the base of the bill. Reflect the skin away from the neck, breast, and abdominal areas. (B) Use the thumb and the first finger of each hand to reflect the skin to expose the underlying tissues. It is easiest to place the thumb and the first finger of each hand along the incision line in the breast area and then push and gently pull the skin to the side. When an opening in the skin has been established, work towards the bill and then the vent. (C) With a sharp blade, make a shallow transverse incision just below the breast muscles and sternum. (D) Insert the thumb of one gloved hand into the incision along the midpoint of the sternum and apply a slight pressure upwards. With a scissors in the other gloved hand, carefully cut through the ribs extending the cut on each side of the breast through the area of the wishbone. (E) Gently separate the breastplate from the carcass; use a scissors or other instrument to sever any connections and push aside the air sacs. (F) Dissect out the heart without cutting into other tissues. (G) Gently remove the liver and carefully cut away its area of connection with other tissues. (H) Tie off the gastrointestinal tract near the throat area, cut the esophagus above the tied-off area, and gently remove the entire gastrointestinal area.

Avian Anatomy

Figure 2.6 illustrates organs and tissues that may exhibit various lesions and that may be sampled for the diagnosis of disease agents described in this Manual. Species variation may result in some differences in the appearance and relative size of particular organs and tissues, but their location will be similar among species. Notable differences between the types of species illustrated are the small flat spleen in normal ducks and the larger oval spleen in pheasants. Also, pheasants have a crop and ducks do not; instead, the area just forward of the gizzard (the proventriculus) is more prominant in waterfowl.

Labeling Specimens

Proper labeling, maintaining label readability, and preventing label separation from specimens are as critical as proper specimen selection and preservation. The label should be as close to the specimen as possible; for example, a label should be attached to a carcass, attached to a tube of blood, or placed within the vial of preservative with a parasite. Double labeling, or placing a label on the outside of a plastic bag holding the specimen whenever practical, is worth the effort. The double labeling prevents confusion and potential

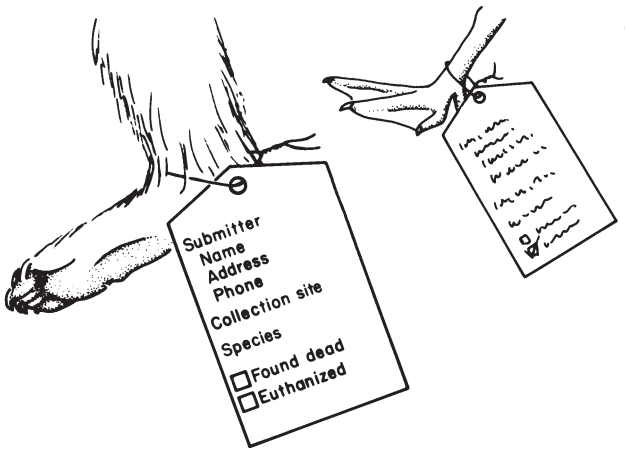


Figure 2.7 *Proper tagging of specimen. History of the specimen (see text for details) should be placed on back of tag.*

errors in specimen records at the diagnostic laboratory when specimens are received from multiple carcasses. Manila tags can be used, but take care to prevent their exposure to large amounts of fluids that may destroy the tag; tag destruction can be reduced by using tags with high rag content or even linen tags. Use soft lead pencil or waterproof ink on these tags; do not use ballpoint pen, nonpermanent ink, or hard lead pencil. The most durable tag is made of soft metal, such as copper or aluminum, and can be inscribed with ballpoint pen, pencil, or another instrument that leaves an impression on the tag.

Carcass

Identify each carcass with a tag fastened with wire to a leg (Fig. 2.7). If tags are not available, use a 3- by 5-inch card placed inside a plastic bag within the bag holding the carcass. Information on the tag should include the name, address, and telephone number of the submitter, collection site, species; whether the animal was found dead or was euthanized (indicate method); and a brief summary of any clinical signs. Place each tagged carcass in a separate plastic bag and seal the bag.

Tissues and Organs

When a specimen is in a plastic bottle, jar, or tube, wrap a piece of adhesive or masking tape entirely around the container and use an indelible marker to write on the tape. List the type of animal from which the sample was taken, the kind of tissue, and the date the sample was taken. When plastic bags are used as the first containers for tissues, they should be labeled with the same information directly on the bag. Do not insert tags inside containers with tissues and organs collected for microbiological or chemical analyses because the tag or the ink on it may contaminate the specimen. When chemically resistant tags are available, insert the tags into containers with preservatives such as formalin or alcohol.

Specimen Preservation

Chill or freeze all specimens, depending on how long it will take to ship to a diagnostic laboratory. Freezing reduces the diagnostic usefulness of carcasses and tissues, but if specimens must be held for 2 or more days, freezing the specimens as soon as possible after collecting them minimizes their decomposition. Formalin-fixed tissues should not be frozen. See Chapter 3, Specimen Shipment, for detailed instructions for packing and shipping specimens.

J. Christian Franson

(All illustrations in this chapter are by Randy Stothard Kampen, with the exception of Figure 2.6)

Supplementary Reading

Roffe, T.J., Friend, M., and Locke, L.N., 1994, Evaluation of causes of wildlife mortality, *in* Bookhout, T.A., ed., Research and Management Techniques for Wildlife and Habitats (5): Bethesda, Md., The Wildlife Society, p. 324–348.

Wobeser, G.A., 1997, Necropsy and sample preservation techniques, *in* Diseases of wild waterfowl (2nd ed): New York, N.Y., Plenum Press, p. 237–248.