A Field Manual for Collection of Specimens to Enhance Diagnosis of Animal Diseases

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A Field Manual for
Collection of Specimens
to Enhance Diagnosis of Animal Diseases

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This book is intended to serve as a guide for animal health workers in agriculture. These are dedicated people who are in closest contact with the animals and who are always working to maintain optimal well-being of the animals in their care. All too often, in every corner of the world, their efforts do not yield full benefits because of a gap in understanding between these specialists in the field and the laboratory specialists who analyze their samples. This book describes standard operating procedures to help bridge that gap.

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CHAPTER 1

INTRODUCTION
CHAPTER 1 - INTRODUCTION

Maintaining animal health is a team effort. It is also a chain event, starting with the animal in the field, and coming back to the animal in the field, but with many essential links in between, including veterinarians, paraveterinarians, veterinary assistants, and laboratory workers. A key element of this chain event is diagnosing disease in the laboratory. Although a few diseases can be diagnosed at the field level, most require some form of laboratory testing.

Laboratorians depend on field personnel to submit samples that will enable them to make a diagnosis. The quality of the samples and the information accompanying the samples greatly facilitate the ability to pick the best test and get results that will be useful for the field diagnostician.

The purpose of this manual is two-fold. First, it is to provide background on proper specimen sampling and packaging to ensure that high quality specimens arrive at the laboratory. The second purpose is to take some of the mystery out of the laboratory procedures to give the field workers greater insight into what happens to the samples after they are submitted.
Collection of Specimens / INTRODUCTION

Samples submitted to lab → Laboratory testing → Visit by animal health professional → Samples taken → Sick animal → Treatment → Report back to veterinarian

"Report back to veterinarian" and "Visit by animal health professional" are connected to indicate a cyclical process.
1.1 VALUE OF DIAGNOSTICS AND SURVEILLANCE

**Diagnostics** are important beyond the field level and the individual animal, herd, or flock. While awareness and ability to diagnose disease helps the animals affected and the producer, it is also critical in establishment of a national database. This database is used to make fact-based decisions for the best nation-wide control programs to finance and support, leading to greater animal health for the national herd and flock.

**Surveillance** is the act of monitoring disease and taking action based on findings. It is at the core of all national animal health programs. Central to surveillance is submission of samples to the diagnostic laboratory. Without diagnostic activities, there is no surveillance. Without surveillance, there is no overall assessment of animal health.
There are two categories of surveillance - active and passive. Both are important for the national animal health program.

- **Active surveillance** targets a specific disease. Samples are collected proactively to determine if a specific disease is present and to what extent. A good example might be rinderpest, where, during the eradication program, serum samples were collected specifically for the purpose of determining the extent of disease distribution in order to enhance eradication procedures.

- **Passive surveillance** refers to those samples that might be collected spontaneously from diseased animals. It is what “bubbles up” from field observations, and depends to a large extent on the awareness of the field veterinarians and the overall capacity of the laboratory.

For a country to maintain international recognition for its ability to monitor disease, and so participate in global trade, diagnostic and surveillance activities are essential. Everyone in the animal health chain is part of the overall system that allows for optimal disease control and economic success.
1.2 LABORATORY TESTING – AN OVERVIEW

Specimens are submitted to the laboratory to help determine what might be causing illness in the field or to determine the extent of infection in animals in the field. A myriad of different tests exists. These can be categorized according to two different questions:

1. Are we looking for the agent or the antibody?
   And, if agent, what type of agent – bacteria, virus, fungus, parasite, toxin?
2. What is the test technique?

*Beginning with the first question, is the purpose to look for agent or antibody?*

- Are we testing for the presence of the agent (organism or toxin)?
- Are we testing for evidence that the animal has HAD the disease, i.e., by looking for a specific immune response to it that we can identify?

If we are testing for the presence of the agent, we will try to visually identify, grow, or detect the organism or its nucleic acid. Material for this test has to come from an animal that IS sick, that is harboring the organism at the time the samples are collected. There are a number of possible samples that can be submitted - swabs, blood, tissues. Serum is NOT a useful sample for detecting presence of an agent.
For detection of the agent (infectious organism or toxin), the following kinds of techniques are possible:

You can visualize it, for instance seeing the organism under the microscope. In some rare cases, the organism is found just by making a smear and looking under the microscope. Here are some examples:

- Fungal hyphae in an impression smear
- Bacteria in an impression smear
- Whipworm egg in a stool sample
- Trypanosomes in a blood smear
You can grow it, for instance growing the bacteria on agar or the virus in cell culture. In most bacterial infections, the bacteria can be grown and identified. This is usually done on agar plates, as seen below. Viruses will also grow in the lab, but these need specialized conditions and have to be grown inside of cell cultures.

Samples are streaked onto agar and bacterial colonies grow

Specialized media can detect *Salmonella*

Biochemical reactions in panel form identify bacteria
You can detect it through immunologic means, for instance through Agar Gel Immunodiffusion (AGID) or Fluorescent Antibody (FA) or Enzyme Linked Immunosorbent Assay (ELISA).

Fluorescent antibody testing - labeled antibody highlights antigen in tissue or smear

Agar gel immunodiffusion (AGID) - antigen and antibody diffuse toward each other and if they meet, there is a line of precipitation in the agar

ELISA - antibody is bound to the well, antigen binds, and then a secondary antibody with a color label highlights the presence of antigen
**You can detect the nucleic acid of the organism** through polymerase chain reaction (PCR). Sometimes, especially for microorganisms that can be difficult to grow, finding the DNA or RNA that is specific for the infectious agent is the solution. This requires some expensive equipment and dedicated laboratory rooms.

**You can find the toxin** through chemical assays. Most of these tests require some sophisticated equipment.

If we are testing for evidence that the animal has HAD the disease, we will try to detect specific antibodies to the agent. Because antibodies develop as the disease progresses, usually animals are clinically normal by the time the sample is taken. When animals are clinically recovered, there usually is no more agent, so tissues aren’t helpful. Where are antibodies? - In the blood, or more specifically, in the serum. So the only sample to submit here is serum.

**Tests for detection of the response (antibody production) to the agent are all serologic tests.**

Animals infected with a microorganism will make antibodies specific to that organism, so it is possible to see, by testing for that antibody, whether or not the animal has been infected with that agent. For this, the test sample is not tissues or swabs, but rather serum. Antibodies are molecules produced to fight the infection and they are present in the blood of an animal, or more specifically in the non-cellular portion of the clotted blood, which is referred to as serum.

In an acute disease situation when animals are clinically ill, the tests for the agent are much more accurate. Later in the course of the disease,
when animals are recovering, the agent may no longer be present and, at this point, the test for the immunologic response (or antibodies) is preferred.

**What is the difference between testing for agent and antibody?**

With testing for the agent, if the test is positive, it means that the animal was infected at the time the sample was taken. But the same is not true for antibody testing. Antibodies to a specific disease will continue to circulate for months or years. So, just finding the presence of antibodies to a particular agent does not mean that the disease most recently experienced was due to that agent. It could be due to a disease that happened last year, or it could be due to vaccination. So, when using serology to diagnose a very recent infection, it is necessary to take two samples for serology – one when the animal is clinically ill, and then another one 3-4 weeks later. A four-fold increase in titer means it was a recent infection.
**Testing for ACUTE disease and CHRONIC disease**

For an acute illness, testing for antibodies at the time the animal is ill will NOT tell you if the animal has that disease. For an acutely ill animal, the ONLY valid test is to test for the agent. Only after two weeks will the animal have enough antibodies to measure.

![Graph of ACUTE DISEASE](image)

However, the situation for CHRONIC illnesses is considerably more complicated. Animals with chronic illnesses should have antibodies. But if you find antibodies to the chronic illness, does it mean that this chronic illness is responsible for their current problem? Not necessarily.
Of course, then there is the whole complicating problem with vaccination. Vaccinated animals will have antibodies, it doesn’t mean they have had the disease.
Here are two examples to work through:

1. Some chickens are dying and you suspect Newcastle disease. You submit tissue and serum to the lab. The lab uses the serum, finds antibodies to Newcastle disease and sends you results – “Newcastle positive”. Does this mean the acute disease was due to Newcastle? No! Perhaps the birds were vaccinated and they have died of a different disease. Perhaps the birds WERE dying of Newcastle (despite the vaccination), but you would still need to confirm the presence of the AGENT in the tissue because this is an acute disease.

2. Sheep are aborting and you send fetus, placenta and serum from the dam to the lab. The lab uses the serum, does an antibody test for *Brucella* and reports back – *Brucella* positive. Did *Brucella* cause the abortion? Well, you don’t know because *Brucella* is a chronic disease so infected animals will have antibodies for life and maybe some other agent caused the abortion. In order to confirm that *Brucella* caused the abortion, you would have to find the AGENT in the fetal tissues or placenta.

*Then, what about the second question from the beginning of this chapter, what type of test will be done?*

There are many kinds of diagnostic tests, and sometimes it might seem like an alphabet soup - FA, PCR, AGID, ELISA. It can get confusing though, because some of the same laboratory TECHNIQUES, or standard types of tests, such as FA, ELISA, AGID - are used, in differing configurations of reagents, to detect antigen OR antibodies.
Here are the basics of how these techniques work:

- **Fluorescent antibody technique** –

There are two kinds of fluorescent antibody (FA) tests – the Direct (DFA) and the Indirect FA (IFA).

Fluorescein isothiocyanate (FITC) is a molecule that can easily be attached to an antibody. FITC appears colorless under normal light, but when ultraviolet light hits it, it gives off a bright green color that you can see.
The Direct (DFA) Test

The DFA test is often used to detect antigens in tissues or smears. For instance, for rabies, the brain from an animal suspected of having rabies is smeared onto a slide. Then a fluorescent antibody specific for rabies is put onto the slide, incubated, and then the slide is washed. Any rabies antigen in the smear will bind with the rabies antibody and won’t be washed off. You can then put the slide under a fluorescence microscope, and if there are antibodies there, they will appear as bright green spots, showing you that rabies virus was in the brain of the animal sampled.
Indirect / Direct (DFA) Testing

The IFA technique is a variation of the DFA. Whenever an “indirect” is in a test name, it means that there is another layer of antibody involved. So, the test will have a “primary” antibody, which is usually the test antibody, and then will have a “secondary” antibody, which is made to bind to the primary antibody, and this secondary antibody has a label we can detect.
Agar gel immunodiffusion –

Agar gel immunodiffusion - abbreviated as AGID, is based on the fact that when antibodies meet antigen in a solid phase, they will bind and form a line of precipitate. When liquids are put into a hole in a gel (solid phase), they will diffuse out from that hole in a radial fashion. AGID can be used to detect antigen or antibody.

For detection of antigen, the test material is put into one well and the known antibody is put into another well. As they diffuse toward each other, if there is antigen there, a line of precipitate will form.

Similarly, AGID can be used to detect antibody. Known antigen is put into one well and the test serum is put into another well. If there is antibody present, a line of precipitate will form.
Antigen was placed in the central well, and test and control sera in the peripheral wells. Lines of precipitation indicate positive serum samples.
ELISA –

ELISA is an acronym for Enzyme-Linked ImmunoSorbent Assay. The basis of the ELISA test is that antibodies with a color label can specifically bind antigen or antibodies and the color can be measured very exactly in a machine.

Step 1 – coat plate with antigen

Step 2 – add test antibody, e.g. chicken

Step 3 – add anti-chicken antibody with label

ELISAs use a specially-made plate where the plastic wells will bind proteins. In the case of antibody detection, the bottom of the plate is bound with antigen. Then the test serum is added, often in differing dilutions, incubated and washed. After that, a detector antibody is added, usually an anti-species antibody that has an enzyme tag. After incubation and washing again, a substrate is added. This substrate will take on a color if the enzyme is present. So color seen there means that the antibody specific to the antigen was present in the animal.
Here reagents are being loaded into an ELISA plate.

A colored reaction which can be read visually.
Polymerase chain reaction (PCR) –

Extremely low amounts of DNA or RNA specific for the agent can be multiplied to result in a product that is detectable.

(Step 1)

The material to be amplified, in a small tube along with some specific nucleotides and enzymes, is placed in a Thermocycler that goes through repeated cycles of heating and cooling
(Step 2)

The resulting products are put into a gel and electrophoresed.

(Step 3)

The results are read on a special transilluminator.
CHAPTER 2

TAking SAMPLES
TAKING SAMPLES

This chapter will focus on how to best collect the samples in the field, and so optimize the key element of sample collection, as seen in the circle diagram below:
There are seven sections in this chapter:

- **2.1** How to necropsy a mammal
- **2.2** How to necropsy a bird
- **2.3** How to use gross pathology to inform diagnostics
- **2.4** How to take samples for toxicology
- **2.5** How to take samples from a live animal
- **2.6** How to take environmental and feed samples
- **2.7** Personal protection when sampling for disease
2.1 HOW TO NECROPSY A MAMMAL

Recognizing and recording abnormalities are enhanced by developing a consistent routine in the dissection and collection of tissues. There is often a tendency to move quickly to the suspected lesion or body system, which risks missing important information. A good necropsy involves paying attention to ALL the clues that can be provided, so the routine has to be followed, with attention to detail at every step.

THE CONSISTENT ROUTINE ENTAILS 6 STEPS:

1. Obtain history
2. Examine the animal externally
3. Open the body
4. Remove the organs and set aside for detailed examination and sampling
5. Examine and sample the organs
6. Write the report

1. Obtain the history

A complete individual animal and herd history should be obtained. This history should include: age, breed, and sex of affected animals, husbandry conditions (including housing, feed), clinical signs, and any treatments administered and whether the animal died or was euthanized. How many animals are affected? Have there been any recent additions to the herd or flock?
2. External examination

Many people believe a necropsy begins when you start using the knife. But if you neglect to look at many external aspects of the carcass, some key findings can be missed.

- Examine the site where the animal was found for clues: predators (dogs), lightning, poisons and poisonous plants, signs of trauma.

- Evaluate the general body nutritional and hydration status.

- Look at the external orifices for any discharges and for color of mucous membranes. Look at teeth for age and abnormal wear.

- Are there any skin lesions or external parasites?
Collection of Specimens / TAKING SAMPLES

- Sunken eyes are an indication of dehydration.
- Check the teeth to determine the age of the animal.
- Examine all mucous membranes - mouth, nares, conjunctiva, anus, vulva or prepuce - for any discoloration or other abnormalities. What is the color of the mucous membranes? Is there pallor? Icterus? Congestion? Cyanosis? How are the teeth - determine age and wear.
- Look in the external ears - any exudates? Parasites?
- How are the joints? Are any swollen?
- How about the feet - any lesions here? Look between the claws.

Look carefully at the skin for any evidence of skin lesions or external parasites.
3. Open the body

The next step in the necropsy is to expose the internal organs. Place the animal on its left side.

Pull the right front leg up, insert knife into skin in the axilla and cut from the inside out to reflect the fore limb. For the hind limb, lift the limb, insert knife, cut through soft tissues and then insert the knife at the coxofemoral junction, cutting through the teres (or round) ligament, so that the hind limb can be reflected also. It is always a good idea, when cutting skin, to cut from the inside out because cutting through hair will dull the knife very quickly. Dull knives make the necropsy more difficult and also dangerous as you are more likely to cut yourself with a dull knife because of the increased effort needed to cut. A stronger push will cause the knife to go into your hand by accident much more easily.
Close-up of the cut teres ligament, with head of femur exposed. You must cut this ligament in order to completely reflect the hind limb.

Now connect the two openings by slicing along the ventrum (once again from the inside out) to cut through the skin along the ventral abdominal wall.
Now it is time to complete the cut in the skin by extending it to the ramus of the mandible. Insert knife and cut down to connect. Peel back the skin.

Here is what the carcass looks like now. Ballotte the abdomen gently - is there any fluid? How do the subcutaneous tissues “feel”? If they are a little “sticky”, it means the animal is dehydrated.
Now that all the skin has been removed on this side of the body, the next step is to expose the viscera. First, cut a window in the abdominal musculature and remove the abdominal muscle. Begin by making a cut that goes parallel to and just behind the last rib (see the arrows in the lower left picture). It is a good idea to “tent” the muscle a little as you make your first cut, to avoid cutting through the viscera beneath. Once an opening is made, continue to pull the muscle away from the viscera as you cut. If you nick the intestine, the peritoneal cavity can become a mess and is harder to evaluate.
The peritoneal and pleural cavities are very separate spaces. The pressure in the pleural cavity should be negative. To ensure that it was still negative when the animal died, lift up the diaphragm and insert your knife through. You should hear a slight “whoosh” of air move into the pleural cavity. You may now cut more of the diaphragm because you are about to open the pleural cavity by cutting through the ribs.
Using rib cutters, cut through the ribs at both the ventral and dorsal aspects, and remove the ribs. Alternatively, in young animals, the ribs are soft enough that you can just cut through the ribs ventrally and then push back the ribs, breaking them at the dorsal portions (photo at right). 

Note the color, position, and size of all organs and look for any adhesions or accumulations of fluid within cavities. In the case of exudates at any of the surfaces, now is the time to take samples, using swabs or syringes.
4. Remove the organs

Examination of the organs within the body is not very efficient. The following organs are removed and placed next to the carcass: respiratory tract with heart, tongue and esophagus (“pluck”), rumen/abomasum/intestines, liver, urogenital tract, reproductive tract, brain.

We’ll go through how to remove each organ system.

_First, the “pluck” -_

Insert the knife between the tongue and the medial surfaces of the mandible. Gently tug to pull the tongue ventrally. You will have to cut some muscle attachments of the tongue to the area of the mandibular symphysis. Once the tip of the tongue has been pulled through the mandible insert pressure to pull the tongue backwards.
Cut the hyoid bones to free the caudal tongue, esophagus and trachea from the pharynx. This is not as easy as it looks and you may have to palpate a bit to find the hard hyoid connection. The hyoid makes an inverted “V” from this perspective and it is best to cut through the very top of that inverted “V” because this is cartilage and will cut more easily than the rest of the structure which is bone. Connective tissue surrounding the esophagus and trachea are teased away as the pluck is pulled ventrally and caudally.

Pull the tongue/trachea/esophagus caudally and cut where the pericardium is attached to the ventral body wall.
While still applying gentle tension toward the thoracic inlet, the heart and lungs are removed by severing or tearing all attachments holding them in place.

At the level of the diaphragm, it is necessary to cut the three attachments (aorta, esophagus, caudal vena cava.) holding the pluck to the abdominal viscera.

Set the “pluck” – tongue, esophagus, trachea, heart, and lungs – aside, in a clean area.
Now it is time to remove the intestinal tract

But this involves separating the liver from the intestines. So, before we remove anything, let’s make sure that the bile duct is patent. Make a small slit in the duodenum, and then gently squeeze the gall bladder. You should see bile move into the lumen of the duodenum.

Find the end of the colon and cut it.
Insert your hand up above the intestines and rumen to find the kidney and the mesenteric attachments of the intestine. The right adrenal is also in this location, so before you cut too much, see if you can identify the right adrenal and sample it if needed.

Cut the intestines at their connections with the liver (bile duct) and pull away from the body cavity.
Remove the rumen (spleen is tightly adherent to rumen). Cut the attachments of the spleen to the rumen and set the spleen down in a clean area.

Remove the liver and set aside.
Now that the abdominal cavity is free of the large digestive organs, identify the left adrenal, remove it and set it aside.

**Now it is time to remove the urogenital tract**

You may remove it in its entirety, or remove it in pieces, i.e., kidneys, then bladder, then uterus/ovaries (or testes), etc.
Now for the brain

Pull the head dorsally to extend the neck. Cut through the musculature to expose the atlanto-occipital junction. Begin cutting through this junction while moving the head back and forth to facilitate the knife moving through the junction without having to cut through the cartilage.

After the head is disarticulated, peel the skin dorsocranially over the skull. Once the calvarium is exposed, make three cuts with a saw. Then you can pry the calvarium off to expose the brain.
Hold the skull upside down and gently tear (or cut) all the cranial nerves that are attaching the brain through the floor of the skull.
The brain should fall into your hand. Look inside the skull - the pituitary and some ganglia remain. Sample these if needed.

Before we are finished with the carcass, there are four more tissues/organs to examine.

Cut through some *skeletal muscle* to examine color and consistency.
Identify some **peripheral lymph nodes** and note size and color. Cut through to see the inner surface - is there edema, reactivity? What is the consistency?

How are the **joints**? Cut through some large joints - carpus, stifle - what is the consistency of the fluid? Are the membranes clear?
Bone marrow is usually taken from the femur, as follows.

5. Examine and sample the organs

You should now have a collection of organs that might look like this.

In examining and sampling, we usually go from the “cleanest” to the “dirtiest”. Usually this order is: lymphoid tissue, brain, lungs, heart, kidneys, reproductive tract, liver, intestinal tract. Note any abnormalities for each (color, size, shape, consistency, exudates). Be sure to examine both capsular and cut surface. Make several cuts in each organ. Collect specimens for further diagnostic work.
A Note on Sampling of Tissues -

Tissues should be collected in duplicate, with half going into 10% formalin for histopathology and half as unfixed tissues that will be used for bacterial culture, virus isolation, fluorescent antibody testing, toxicology, etc.

The following tissues should be collected in all cases: lung, liver, spleen, heart, brain, kidney, intestines. Additionally, any other tissue that might have lesions (skin, adrenal, ovary, etc.) should also be collected.

Crushing should be avoided when taking samples since this may cause histological artifacts. This can be avoided by using a sharp blade and a hard surface.

When examining any organ with a mucosal surface (trachea, esophagus, intestine, etc.) care should be taken not to damage or destroy the mucosal surface by rubbing the surface with fingers or instruments.
Lymphoid system

The spleen should be relatively flat, not turgid, and will not fracture when folded. On cut surface, it should be uniform.

Lymph nodes should not bulge on cut surface and should have a uniform homogeneous, white-tan appearance. An exception is the mesenteric lymph nodes which usually have dark (brown-black) pigment at their centers. This is normal in ruminants.
Brain

Often the brain is sliced down the middle to create symmetrical halves for frozen and formalin specimens.
Heart

The **heart** can now be examined. Identify the heart within the pluck and gently open the pericardial sac to see the fluid within. Then remove the heart from its attachments to the rest of the pluck.

It is important to examine all chambers of the heart. This can be confusing. The best way is to open the left ventricle first. You can use a knife to make a vertical cut from apex to base over the left ventricle, then use scissors or a knife to extend the cut up through the left atrium and into the aorta. This will allow you to see both left chambers and both left valves on the left side (left a-v valve and aortic valve).
To open the right side of the heart, make a C-shaped cut, following the outline of the right ventricle. Extend the cut so that all of the right ventricle and right atrium are open and exposed. Now you can see the inside of both right-sided chambers and both valves on the right (right a-v valve and pulmonic valve).
Respiratory system

Begin by cutting down the trachea and into the bronchi, making note of any mucus, froth, or petechiae.

Palpate all lobes of the lung. Lungs should be uniformly pink and slightly spongy. Make several cuts through the lungs - be sure to look in all lobes.
Before finishing with the pluck, be sure to examine the two digestive system organs that are part of the pluck. Make several cuts through the tongue to look at the musculature. Cut down the esophagus to see the mucosa and look for abnormalities.

**Urinary tract**

For each kidney, peel the capsule back. It should peel easily. Slice through the kidney to examine the cut surface.

Open the bladder. Examine the mucosal surface. In this photo, you can also see one horn of the uterus and an ovary.
Reproductive tract

At left is the female reproductive tract. Slice through the ovaries. Also cut into the uterine horns, uterine body, and cervix, observing mucosal surfaces.

Cut into the testes to look at the parenchyma.
Liver

The liver’s surface should be examined for any abnormalities. It should be palpated for any nodules, friable areas, or other abnormal changes. Several slices are made into the liver in order to examine the deeper structure of the liver.

Open the gall bladder and look at the mucosal surface.
Intestinal tract

Spread out the intestines and determine the various parts. Begin with the duodenum and follow down the jejunum, to the ileum, which empties into the cecum at the ileo-ceco-colic junction. The large intestine slowly narrows to become the spiral colon, which then progresses on down to the terminal colon.

At this point, if you need to collect feces, you can get them from the terminal colon.

Also, now is the time to take a segment of small intestine for sampling. It is best to tie off a loop to prevent intestinal contents from contaminating your other tissue samples.
Look at the mesenteric lymph nodes. Cut through several to see the cut surface.

Open segments of the intestinal tract, beginning with the duodenum and working caudally. Be sure to examine the mucosal surface carefully and open numerous segments. If the history indicates an intestinal problem, you may need to open the entire intestinal tract.
Now for the forestomachs

Because of the way you removed the tract, the abomasum is still attached to the forestomachs. Open the abomasum first. Remember this is the only glandular part of this mass. Look at the contents of the abomasum and examine the mucosal surface. You may need to run some water lightly over the mucosa to get a good view. Then open the reticulum and the omasum.

Last will be the rumen itself. Remove some of the contents and look at the pillars and the papillae. Also note the consistency of the contents.
A Note on Sampling of organs -

Tissues should be collected in duplicate, with half going into 10% formalin for histopathology and half as unfixed tissues that will be used for bacterial culture, virus isolation, fluorescent antibody testing, toxicology, etc.

As a review, the following tissues should be collected in all cases: lung, liver, spleen, brain, kidney, intestines. Additionally, any other tissue that might have lesions (skin, adrenal, ovary, etc.) should also be collected.

For specific problems, you may want to take additional samples from key organs. For instance, if it is a skin disease, take more samples of skin and draining lymph nodes. If the animal has pneumonia, take additional lung samples and tracheobronchial lymph nodes.
6. Write the report.

No necropsy is complete until all findings have been recorded in written form. The report should include at least the following information:

- Species, breed
- Age, sex
- History
- Died or euthanized?
- Nutritional, hydration status
- Findings from external examination
- Findings by organ system:
  - Lymphoid (spleen, lymph nodes)
  - Respiratory
  - Digestive
  - Urogenital
  - Musculoskeletal
  - Nervous

**When describing tissues, keep these four qualities in mind – color, consistency, distribution, and size.**

See p.86 for sample Necropsy Report Form.
Recognizing and recording abnormalities are enhanced by developing a consistent routine in the dissection and collection of tissues. There is often a tendency to move quickly to the suspected lesion or body system, which risks missing important information. A good necropsy involves paying attention to ALL the clues that can be provided, so the routine has to be followed, with attention to detail at every step.
The consistent routine entails 6 steps:

1. Obtain the history
2. Examine the animal externally
3. Open the body
4. Remove the organs and set aside for detailed examination and sampling
5. Examine and sample the organs
6. Write the report

STEP 1. Obtain the history

A good individual animal and flock history should be obtained. This history should include:
1. Bird’s age
2. Sex
3. Breed
4. Clinical signs
5. History of trauma or disease
6. History of any treatments administered
7. Any other information that may be relevant to the case such as type of feed and water
8. If the bird is a member of a flock the following is also required:
   • Number of birds in the flock
   • Number of birds in the affected group
   • Number of affected birds
   • Clinical signs of the flock should also be noted

A proper history can help in determining what samples should be taken and what tests are necessary for making the correct diagnosis.
STEP 2. Examine the bird externally

- Examine the bird for any signs of trauma and evaluate the bird’s general body condition.
  - If possible the bird should be weighed.
  - The keel bone should be felt to determine if there is any pectoral muscle atrophy.
- The skin, feathers, eyes, ears and beak should be examined for any abnormalities. Take a close look at the comb and wattles - any swelling? Discoloration?
- Look at the back of the bird for evidence of feather picking.
- If any skin lesions are noted, they should be sampled.
- Examine all mucous membranes (mouth, nares, and conjunctiva) for any discoloration or other abnormalities. How about the cloaca? Any diarrheal staining? Urates present? Trauma?
- Look at the joints - any swelling?
Prior to opening the body, you might want to swab the trachea or cloaca. When swabbing the trachea, insert the swab up in the choana, as depicted in the photograph on the left below, prior to inserting in the trachea.

Dip the whole carcass into a bucket of soapy water to thoroughly wet all the feathers - this will decrease the dander that might aerosolize from the skin and will also keep your instruments free of small feathers as you do the dissection.
STEP 3. Open the body

- The body should be placed on its back with its feet facing you.
- Reflect the wings back.
- Cut through the skin between the legs and the breast so the legs can be fully abducted and lie flat against the table.
- Remove the skin from the ventral surface of the bird by cutting across at the caudal edge of the keel and then pulling skin cranially and caudally - peel away from the muscle to expose the muscular body wall.
- Make a small cut into the body cavity using scissors or a scalpel blade - just behind the breast bone, and then pull the abdominal muscle caudally to expose some abdominal viscera.
- Extend the cut up through the cervical area and cut open the beak at the angle of the jaw. Now oral cavity, esophagus, trachea and crop are all visible.
The keel bone and breast muscles are then removed by incising the pectoral muscles on each side of the keel and cutting through the ribs. Use the heavy poultry shears. Remove the keel and breast muscles entirely - you should now be able to see the internal organs from oral cavity to rectum.
• At this point, all internal viscera should be examined in situ for any abnormalities before removing any organs. Note the color, position and size of all organs and look for any adhesions.

• Examine the air sacs for increased thickness or cloudiness (caudal thoracic air sac is at the end of the forceps).
STEP 4. Remove the organs and set aside for examination

It is probably easiest to remove the abdominal viscera first and then go back and remove the thoracic organs.

Remove the liver - in birds the liver takes up a big portion of the abdomen.

The spleen can be a difficult organ to find once everything is removed so it is a good idea to locate it now and set it aside. It is spherical in shape and located on the right side at the junction of the proventriculus and ventriculus. Pull the proventriculus aside and it should pop into view. Take it out now and set it in a clean dry spot.

Find the junction of the esophagus and stomach, cut here and pull the digestive tract out, all the way to the cloaca. The digestive, urinary, and reproductive tracts come together at the cloaca.
Located in the cloaca is the light cream-colored saccular organ, the **bursa of Fabricius**. The bursa of Fabricius contains lymphoid follicles and can be easily found in young birds. Once the bird reaches sexual maturity, it undergoes involution and therefore becomes smaller as the bird ages.

Cut at the end of the large intestine, but leave the bursa in the bird.

Here are two views of the bursa - undisturbed (left) and incised (right).
Kidneys are nestled up against the body wall - there are three portions - cranial, middle and caudal poles. The reproductive tract lies on top of and at the cranial end of the kidneys. See photos – top photos below are from a male, (left immature; right mature); lower photos are an immature female (left) and a mature female (right). In females only the left side of the tract persists, the right is vestigial and too small to be identifiable.
Adrenal glands are often difficult to find. Here they are lying just cranial to the testes in an immature male.

Pull the heart and lungs away - the lungs are closely adhered to the dorsal body wall, and therefore, careful teasing of the lungs away from the ribs may be necessary to remove them.

Open up the nasal cavity to take a close look at the sinuses.
Collection of Specimens / TAKING SAMPLES

Observe the sciatic nerve which is an important location where Marek’s disease can be seen.

Open some joints to observe the fluid and synovial membranes.

The brain can now be removed. Using the smaller scissors (not poultry shears), chip away at the skull, beginning from the foramen magnum, and remove the calvarium covering the cerebral hemispheres and the cerebellum. Take the brain out - use care, this organ is quite soft.
STEP 5. Examine and sample the organs

It is a good idea to go from the “cleanest” organs to the “dirtiest”. Usually this order is: lymphoid tissue, brain, lungs, heart, kidneys, reproductive tract, liver, intestinal tract.

Note any abnormalities for each (color, consistency, distribution, and size). Be sure to examine both capsular and cut surface. Make several cuts in each organ. Collect specimens for further diagnostic work.
Lymphoid system

The spleen in birds is a small round organ that should be a uniform mahogany color on capsular and cut surfaces.

The bursa changes dramatically with age. Birds older than 10 weeks will have a bursa that may even be difficult to locate. A normal bursa in a young bird has an accordion-like structure and is a homogeneous tan color.

Brain

Often the brain is sliced down the middle to create symmetrical halves for frozen and formalin specimens.
Heart

The heart can now be examined. Make one incision into each ventricle, and examine muscles and valves.
**Respiratory system**

Begin by cutting through the larynx, trachea, and syrinx making note of any mucus, froth, or petechiae. Lungs should be pink, “spongy”, and free of any fluid.

**Kidneys**

These should be smooth and homogeneous. A reticular pattern is an indication of dehydration.
Reproductive tract

Testes are homogeneous on capsular and cut surfaces.

Ovaries should be free of inflammation. Sterile egg yolk peritonitis is a common finding in “spent” layers.
Liver

The liver’s surface should be examined for any abnormalities. It should be palpated for any nodules, friable areas, or other abnormal changes. Several slices are made into the liver in order to examine the deeper structure of the liver.

Intestinal tract

Look in the mouth for any abnormalities. Cut down the esophagus and see how much food is in the crop.
Open the **proventriculus** and make note of the lining which is normally bumpy due to the presence of digestive glands. Note any abnormalities.
The ventriculus, or gizzard, should be examined next. Because the gizzard is responsible for grinding ingested material, it has a thick external muscularis layer and contains small stones or grit. The ventricular glands secrete a thick protective gel, known as koilin, which has a yellowish color. The gizzard thickness should be examined and the surface examined for erosions, ulcerations, discoloration, or other abnormalities. Peel the koilin back to look at the mucosa.

Proventriculus-ventriculus The junction (arrow in photo, previous page) is an area with abundant lymphoid tissue and should be examined carefully for lesions.
The **small intestine** of birds is typically arranged into several loops before entering the colon. The first loop is the **duodenum**. It is easily identified by the location of the **pancreas** within the duodenal loop mesentery.

The remaining loops make up the **jejunum and ileum**. The **colon** is relatively short with two long **ceca** and connects to the **cloaca** via the **colorectum**.

In this picture you see the two ceca and the large colon in between. There are large lymphoid patches in the proximal portions of the ceca.
Sampling of organs –

- Tissues should be collected in duplicate, with half going into 10% formalin for histopathology and half as unfixed tissues that will be used for bacterial culture, virus isolation, fluorescent antibody testing, toxicology, etc.

- The following tissues should be collected in all cases: lung, liver, spleen, bursa, brain, kidney, intestines. Additionally, any other tissue that might have lesions (skin, adrenal, ovary, etc.) should also be collected.

- Label the tissues that are collected fresh so that the technician performing the tests can determine what each tissue is.

- Crushing should be avoided when taking samples since this may cause histologic artifacts. This can be avoided by using a sharp blade and a hard surface.

- When examining any organ with a mucosal surface (trachea, esophagus, intestine, etc.) care should be taken not to damage or destroy the mucosal surface by rubbing the surface with fingers or instruments.
In order to improve fixation of tissues, samples should not exceed 5mm in thickness and volume of fixative should be at least 10 times the volume of tissue.

Fresh samples should be packaged so that they remain cool and to minimize possibilities of leaking. Be sure to submit the proper paperwork to accompany the samples.

**STEP 6. Write the report**

No necropsy is complete until all findings have been recorded in written form. The report should include at least the following information:

- **Species, breed, age, sex**
- **History**
- **Died or euthanized?**
- **Nutritional, hydration status**
- **Findings from external examination**
- **Findings by organ system:** Lymphoid (spleen, bursa), Respiratory, Digestive, Urogenital, Musculoskeletal, Nervous

*See p.86 for sample Necropsy Report Form.*
Necropsy is not just about the collection of samples to send to the diagnostic laboratory. A veterinarian performing a necropsy can gain a significant amount of valuable information through closely observing gross lesions, describing carefully, formulating accurate morphologic diagnoses, and creating a list of differential diagnoses. In this way, the veterinarian in the field acts as a full team member with the laboratory personnel.

A complete necropsy report entails recounting the signalment fully, describing any and all lesions that are present in each of the body systems completely (in such a way that the laboratorian can visualize what the prosector saw), creating morphologic diagnoses, and then listing potential rule-outs. All too often, the necropsy report only lists the presumed diagnosis in the field. This hampers the diagnostician’s ability to more fully explore the case and makes it difficult for the laboratory and field to function as a team.

On the following page, take note of all the headings which make up a comprehensive Necropsy Report...
<table>
<thead>
<tr>
<th>Necropsy Report</th>
</tr>
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<tbody>
<tr>
<td><strong>Date:</strong></td>
</tr>
<tr>
<td><strong>Signalment (species, breed, sex, age):</strong></td>
</tr>
<tr>
<td><strong>Degree of autolysis:</strong></td>
</tr>
<tr>
<td><strong>History:</strong></td>
</tr>
<tr>
<td><strong>External appearance:</strong></td>
</tr>
<tr>
<td><strong>Appearance of body cavities:</strong></td>
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<tr>
<td><strong>Digestive System:</strong></td>
</tr>
<tr>
<td><strong>Respiratory System:</strong></td>
</tr>
<tr>
<td><strong>Cardiovascular System:</strong></td>
</tr>
<tr>
<td><strong>Immune organs (spleen, lymph nodes, bursa in avian):</strong></td>
</tr>
<tr>
<td><strong>Urinary tract:</strong></td>
</tr>
<tr>
<td><strong>Reproductive tract:</strong></td>
</tr>
<tr>
<td><strong>Nervous System:</strong></td>
</tr>
<tr>
<td><strong>Muscles, bones, joints:</strong></td>
</tr>
<tr>
<td><strong>Other findings:</strong></td>
</tr>
<tr>
<td><strong>Morphologic diagnoses:</strong></td>
</tr>
<tr>
<td><strong>Diagnostic rule-outs:</strong></td>
</tr>
</tbody>
</table>
Description of lesions

Every single body system is assessed. If no lesions are present, it is acceptable to write “No abnormal findings” or NAF. In the case of gastrointestinal and reproductive systems, however, even if there are no lesions, NAF is not acceptable. For the gastrointestinal tract, it helps to note how much ingesta was present in the tract. For the reproductive system, what is the breeding/maturity status? Is the animal neutered? If female and not neutered, is she pregnant?

Each lesion should be described in full, including color, consistency, distribution, and size. For each of these categories, there are brief descriptions below and the relevance of each.

**Color**

Color is an important descriptive term used for macroscopic lesions. The rainbow of colors usually seen at necropsy includes: red, yellow, white, black, brown, green, and translucent. Each can be correlated with certain pathogenic processes and help veterinarians understand the underlying process responsible for the lesion.
RED is seen predominantly in processes involving circulatory problems such as congestion and hemorrhage.

Multiple red foci in the liver of chicken. Hemoperitoneum in horse that was kicked.

YELLOW is a key finding in acute inflammation, as in fibrin, but also occurs with disorders of bilirubin, which can occur because of hemolytic disorders or hepatic problems.

Piglet intestine – there was acute damage to the mucosa, with resulting fibrin exudation (yellow). Cat with liver tumor – many body surfaces (including foot pads) have become yellow due to excess circulating bilirubin.
indicates one of several processes – perhaps the animal is very anemic, or it can indicate lack of blood flow to a certain area as in an infarct. The third possibility is fibrosis, which is present in chronic lesions.

Goat with very pale conjunctiva – the abomasum of this goat had many blood-sucking *Haemonchus* nematodes

Dog with thrombotic problem, here are some white heart infarcts

Pig with pleuritis, it started out as fibrin strands (yellow) which now have been replaced by fibrosis (white)
is less common but serves as an indicator of oxidized (old) blood or melanin.

A segment of small intestine of a horse, entrapped by a pedunculated lipoma. Section is filled with oxidizing blood and appears black.

Melanin discoloring large portions of the meninges in a lamb. This is melanosis, and can be considered normal.
**BROWN** is seen in exudate, especially in cases where pus is mixed with erythrocytes.

Here is the opened thorax of a horse – filled with brown fluid – this is pus mixed with some old hemorrhage.

**GREEN** discoloration is seen in severe autolysis, as pigment-producing bacteria proliferate and pour out their products, or in organs adjacent to the gall bladder, when bile leaks out postmortem to stain adjacent areas.
is observed in cysts, mucus, or fibrin-poor edema.

A chicken intestine with abundant mucus exudate in the intestine.

Translucent edema fluid distending the mesentery of a cow.
Consistency

We generally refer to the consistency of lesions as being SOFT, FIRM, or HARD. To decide which applies to your lesion, think in this way:

Most lesions are **SOFT**. Within this category, there are additional features, such as liquid, semi-liquid, and friable. *Pus is usually liquid or semi-liquid. Also, pus can take various colors – it may be white, tan, slightly green, or pink, depending on any pigments produced by the offending bacteria and/or mixing with blood.*

A **FIRM** lesion usually indicates that the lesion has been ongoing for some time, as it takes a while for fibrous tissue to accumulate.

**HARD** lesions are rare, and mean that there is some bone or at least abundant mineral present within. That takes a long time so these lesions are always chronic.
Distribution

The terms generally used to describe distribution include:

**FOCAL** means just one area – if the area is very large, it can be termed “focally extensive”

**MULTIFOCAL** means many focal areas within an organ.

**DIFFUSE** is reserved for use when an entire organ or surface is affected.
Size

How big is the lesion? How much extra fluid is in a cavity? It is not enough to say that it is BIG or there is a LOT – because big to one person may mean the size of a large coin, to another it might mean the size of a football. Measuring (even if only in a very rough way), is quite helpful. Oftentimes we don’t have access to a measuring device in the field. Here are some helpful guides when no measuring devices are nearby!

The spread of your hand, from thumb to the tip of the small finger is about 20cm. So this mouse, from nose to tail base is about 10cm in length.

The end of your index finger, from the last joint to the end of the finger, is about 2.5cm. That would make the hole in the stomach approximately 7cm diameter.
Fluid Measurements

And for fluid measurements, think of some common measures you already know:

- One teaspoon is 5ml
- A small bottle of water is usually 500ml
- Cyst in a cow’s ovary; probably contains about 5ml
- This thorax of a small dog may contain almost 500ml of purulent fluid

Be sure to use all these descriptors (color, consistency, distribution, size) as you record the lesions in the necropsy report. Paint a picture with words, this will be very helpful for the laboratory personnel.
Morphologic diagnosis

Morphologic diagnoses are our “code words”, our special medical language for communicating the key aspects of pathology. There is a standard format for writing morphologic diagnoses, using the following criteria:

**COMPONENTS OF A MORPHOLOGIC DIAGNOSIS**

...Think... **S-T-D-P-O**

**Severity** – is it just a little problem? Did it cause serious compromise? We usually use the words *mild* / *moderate* / *marked*.

**Time course** – is it acute, subacute, chronic? Fibrin and hemorrhage indicate an acute reaction; fibrosis takes longer, so the lesion is chronic.

**Distribution of lesion** – is it focal, multifocal, diffuse?

**Process** – is it hemorrhage, necrosis, or if it is inflammation, we use the term for exudates - serous, fibrinous, catarrhal, etc.

**Organ** – If it is inflammatory, it is the name of the organ followed by *-itis* at the end - pleuritis, splenitis, hepatitis, etc.

We write the morphologic diagnoses in the report only after all the descriptions are completed. It is a way of communicating to the laboratory what you, as the field veterinarian, feel are the most significant
changes in the carcass. A morphologic diagnosis consists of up to 5 words, and here are some examples:
- Severe acute diffuse fibrinous enteritis
- Mild chronic focal granulomatous pneumonia
- Moderate subacute multifocal necrotizing hepatitis

However, often with non-inflammatory lesions, the morphologic diagnosis is shorter and here are some examples:
- Diffuse hepatic necrosis
- Intestinal torsion
- Multifocal degenerative arthropathy
- Thoracic trauma with hemorrhage

For each of these morphologic diagnoses, there can be several different causes of disease. It is now the laboratory’s job to try to determine which disease, using your description, morphologic diagnoses, and the excellent set of samples you have provided.

**List of rule-outs**

This is the last box to fill in on the report and it is your best guess, as a field veterinarian, as to what could be causing the problem you see on the farm. The laboratory may subsequently think of additional diseases to investigate, but this is the very first list of possible disease diagnoses. This serves as a guide for the laboratorians to begin their work.
2.4 TAKING SAMPLES FOR TOXICOLOGY

Analyses for the presence of toxicants or drugs can be done on body fluids like blood or urine, or on tissues collected at necropsy. The suspected source of the toxicant, such as feed or water, can also be analyzed. Many diagnoses involving a suspected toxicant rely not only on finding residues of the material in tissues but also on the presence or absence of compatible lesions in the animal.

As a general rule, when a poisoning is suspected a wide variety of tissues should be collected cleanly and put into individual plastic bags, which should then be either refrigerated or frozen for shipment to the analytical laboratory. A good range of toxicology samples to collect at necropsy would include stomach or rumen contents, feces, brain, liver (without the gall bladder), kidney, body fat, skin, and urine.

The minimum sample size to collect varies with the suspected toxicant. For example, for analysis for heavy metals such as lead, mercury, and cadmium, the laboratory would prefer at least 1g each of liver and kidney shipped chilled or frozen. Ideally, a submission for an organic toxicant screen using gas chromatography/mass spectrometry would include 25g each of gastrointestinal contents, liver, and kidney, as well as urine if available.
To test for nitrates, the best sample is aqueous fluid from the eye of a dead animal.

When sending samples for toxicologic analysis, it is important to also send a full history and to indicate what poison or groups of poisons that you suspect. It is not economically feasible, for example, to ask the lab to “check for any toxicants”. Rather, you should provide a narrower list of possibilities based on clinical and post-mortem findings.
2.5 TAKING SAMPLES FROM A LIVE ANIMAL

Blood

This is the most common sample collected from a live animal. The jugular vein is the preferred location for small ruminants and horses. The wing vein is the site for birds. The tail vein is the easiest site for sample collection in cattle.

For small ruminants and horses, apply digital pressure at the lower end of the jugular to fill it. Insert the needle, bevel up, and draw back on the syringe. When finished withdraw the needle and hold a finger over the site for a few seconds. ➔
In adult cattle, the tail vein is the easiest site for blood sampling. Elevate the tail, palpate for the junction of the vertebrae, and insert the needle here.

For poultry, blood can be taken from the wing vein in small birds or from the jugular in larger birds. The wing vein (left photo) can be found on the underside of the wing. Pluck some of the feathers for better visibility. Hold off the end of the vessel to fill it. Insert the needle, parallel to the skin; be sure to have the bevel up. Go through the skin first, then go into the vein. In larger birds, the jugular is easy to use (photo at lower right). Again, it might be helpful to pluck some feathers to more easily visualize the vessel. Hold it off below to help fill it, and then insert needle with the bevel up.
To make a blood smear, do the following with the blood you have collected. This smear can be packaged and sent to the laboratory. No fixation is necessary unless the transit time to the lab is prolonged.

Put a drop of whole blood onto one end of a glass slide. Using a second glass slide, make contact with the drop of blood and then pull the slide across.

The resulting blood smear should look something like this. The “feathering” at the end is the best place to examine for individual cells.
Swabs

Swabs are often used to collect exudates from lesions, for example taking a swab from an abscess.

Additionally, swabs are used to collect tracheal and oropharyngeal fluid samples from birds to test for avian influenza.
Feces

Preferably, fecal samples should be taken directly from the rectum or just after defecation. This is particularly important for the diagnosis of lungworms and protozoans such as *Giardia* and trichomonads.

Urine

In cattle, massaging the area under the escutcheon should result in a flow of urine within one minute. In sheep, occluding the nares for a short period sometimes precipitates urination. In all other cases, catheterization is necessary unless the animal can be confined and monitored constantly to catch the next instance of urination.
Parasites, skin scrapings

Larger external parasites can simply be picked off and placed into a container. Ticks and fleas should be submitted for identification or stored in 70% alcohol.

For smaller parasites, such as skin mites, scrape with a razor blade to be sure you go deep enough to get the parasite.

Scrape the skin with a razor blade and put the collected material onto a slide with some mineral oil. Then you can put the slide under a microscope to see the mites.
Impression smears

Take the tissue and touch gently to a glass slide. Allow to air dry.

If the tissue is very bloody, you might want to blot a few times on a paper towel prior to making the smear.
2.7 TAKING ENVIRONMENTAL & FEED SAMPLES

In cases when a toxin or mineral deficiency is suspected in the environment or in the feed, samples can be collected for laboratory analysis.

**Feed** -
Collect a minimum of 1 liter of feed, making sure it is representative of what the animal has been consuming.

**Dried hay and bales** -
Sample a minimum of 8 bales or piles, collecting a minimum of ten (200g each) samples. Combine and mix samples and place a 300-350g subsample into an airtight plastic bag.

**Dried grains** -
Collect "hand-grab" samples (100g each) from ten separate locations within the bags, piles or bins. Combine, mix and place 300-350g subsample into an airtight plastic bag.

**Fresh forages (plants)** -
Collect fresh plants (stems and leaves only) into a paper bag to prevent it from growing mold. Avoid contamination by soil or animal manure.

**Labeling:** Each bag needs to be clearly labeled with owner name, type of sample, and date collected.

If samples cannot be delivered to the laboratory within 2-3 days, it is recommended to freeze the materials.
- Pack in plastic, air-tight bags
- Remove all air before sealing
- Freeze
2.7 PERSONAL PROTECTION AND HEALTH CONCERNS

Many animal diseases are zoonotic. It is important to protect the operator and any people in the vicinity from these hazards.

Necropsy procedures pose the greatest risk of disseminating infectious agents to humans because of the large amount of tissues and body fluids exposed during dissection. Protection of the person doing the necropsy and protection of other animals in the vicinity are important. The following biosafety/biosecurity precautions should be taken during necropsy:

Wear personal protective equipment - gloves, apron, and boots.

For some diseases (such as rabies, echinococcusis, and avian influenza) a respirator is recommended as well.

Encourage those not adequately protected to keep a distance from the carcass.
Dispose of carcasses appropriately, away from scavengers that might drag parts to other locations and inadvertently expose people.

Burial is a method of disposal that can be used for all species. Incineration may also be an option. Composting has been shown to work for poultry, sheep and goat carcasses. Which method you choose depends on environmental regulations, familiarization with procedures, weather conditions, and location of site in relationship to the community.

Wear rubber boots during necropsy, and sanitize the boots by washing in disinfectant so as not to spread infectious agents beyond the site of the necropsy.

Necropsy uses sharp instruments. Take your time, don’t rush. Be sure your knife is sharp. Most accidents with knives happen because the knife is dull.

For some diseases, such as rabies, a vaccine is available for humans and only those people who are vaccinated should be opening the carcass and sampling tissues.

Collecting materials from live animals also entails some health risks. Collecting blood with a needle and syringe should be done carefully to avoid needle sticks. Be sure that the needles get capped again after the collection.

Restraint of animals for collecting samples should be done appropriately so that physical harm by kicking or biting cannot occur.
CHAPTER 3

PACKAGING AND TRANSPORT
CHAPTER 3 - PACKAGING AND TRANSPORT

In this chapter, we will cover how to best maintain the samples in between the time when they are collected and when they arrive at the laboratory. This is a critical period when deterioration of a sample can happen, rendering it useless for the laboratory.
There are two sections in this chapter:

- 3.1 How to preserve specimens
- 3.2 Important considerations in sending specimens
3.1 HOW TO BEST PRESERVE SPECIMENS FOR USE IN THE LAB

The importance of how samples are preserved prior to arriving at the laboratory must not be underestimated!

Some key concepts to keep in mind for TISSUES include:

Keep the tissues cool –

Bacteria are usually present in ALL collected samples. Unfortunately the non-specific bacteria will replicate even faster than any bacteria that might be causing a disease. So it is important to keep the tissue cool because the bacteria grow at warm temperatures and the non-specific bacteria will quickly outgrow any specific bacteria we might be trying to find. Also, the non-specific bacteria will generate toxic products that might kill any viruses or fungus we are looking for in the sample.

Try to keep the various tissues separated from one another –

As tissues get grouped together, bacteria from one can quickly overtake another. This is especially true if intestinal samples or fecal materials are included. Use plastic bags that can be sealed - “ziplock” or “whirlpak” bags are the best.
Samples should be kept moist –

If the sample dries out, any agents in there might dry out as well, making it difficult to isolate the infectious organism.

*For swabs*, immerse the swab in sterile saline or sterile water and keep at 4°C until it can be sent to the laboratory.

**For serum samples** – let the blood coagulate.

This is best done by taking the sample in a red-topped tube and leaving it at 37°C for 12-24 hours. The red top on the tube is a universal symbol that the tube contains no anticoagulant; consequently the blood will coagulate, and after coagulation, the serum can be removed and put into a clean tube.

If you have a centrifuge, you can get more serum, but if not, you should be able to get enough serum without having to spin the tube.

If you don’t remove the serum, the red cells in the clot will slowly break down, releasing their hemoglobin into the serum, causing the serum to take on a pink-red color and making many serologic tests difficult to undertake.
Once the serum is removed and in a separate tube, keep it cool until you can get it to the laboratory. If it will be more than one week before the sample can be shipped to the laboratory, freeze it, because antibody levels will slowly fall, even at 4°C.

*For external parasites*, mites, fleas, and ticks can all be kept in 70% alcohol indefinitely.

*For fecal samples*, keep them cool until they can be sent to the laboratory. Nematode eggs usually survive well at 4°C but can be destroyed by freezing, so DO NOT FREEZE.

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**At the time of collection of samples from a necropsy, have the following materials on hand:**

- Formalin in screwtop jar
- Syringe, needle and blood tubes
- Sterile swabs
- Sealable plastic bags for collection of tissue specimens
- String for tying intestine
- Tubes - for collection of fluid (urine, ocular fluid, body cavity effusions), placement of swabs
- Small plastic container (feces, stomach contents)
- Cold packs
- Cooler
- Waterproof marker
- Paper forms - necropsy report form, submission form
3.2 SENDING SPECIMENS TO THE LABORATORY

We have covered the importance of taking samples in an appropriate way in the field, and preserving them until they can be sent to the laboratory. The next important task is to get them to the laboratory in a condition that will be useful for the laboratorian.

Some key concepts here include:

1. Speed in getting the materials to the laboratory.
   The longer the package is in transit, the greater probability that bacteria will proliferate or the agent will degenerate, so that a diagnosis cannot be made. Package should be well-labeled to help ensure that it is not delayed in transit.

2. Keep the samples cool on the way to the laboratory.
   Pack with cold packs to help ensure that the package does not get warm and promote bacterial overgrowth. It is preferable not to use ice, because it will melt and may cause the package to leak.
3. **Use packaging that will prevent leakage and crushing.**

Leakage of contents will likely cause a package to be refused by the shipper. Also, the package should be sturdy because if it is crushed, it is more likely that samples will be ruined or that leakage will occur.

4. **Be sure that all your samples are well-labeled.**

The laboratorian needs to understand what the samples are. Is the swab from a trachea or the cloaca? Is the tissue in the bag liver or intestine? Are the serum samples from cattle or water buffalo or chickens?

5. **Be sure that appropriate paperwork is included with all of the samples.**

The diagnosis of disease is a group effort. Every stop along the chain has some information to contribute. By giving the laboratorian a complete history of the problem and any gross necropsy findings, he or she is likely to do a much better job with his/her part of solving the big puzzle. Be sure to package the paperwork in a waterproof way so that even if some of the samples happen to leak, the paperwork will still be readable.
6. **Alert the laboratory.**
If you let the laboratory know that a submission is coming, testing procedures can be prepared in time for when the sample arrives.

One of the most important things to keep in mind when sending specimens to the laboratory is to ensure that the laboratory gets AS MUCH INFORMATION AS POSSIBLE. The people who work in the laboratory need to know all of the history and gross findings so that they can help to choose the best tests.
CHAPTER 4

TESTS DONE
IN THE
LABORATORY
CHAPTER 4 - TESTS DONE IN THE LABORATORY

This chapter briefly describes what happens to the samples when they arrive at the laboratory. The various testing procedures are described in a general way, just to give the field animal health worker an overall impression of what is done with the submitted specimen.
There are six sections in this chapter:

- 4.1 Histopathology
- 4.2 Virology
- 4.3 Bacteriology
- 4.4 Mycology
- 4.5 Parasitology
- 4.6 Toxicology
4.1 WHAT’S DONE IN THE LAB – Histopathology

Pathology consists of looking at the gross findings in a necropsy and also at microscopic architecture of the tissues (histopathology). The advantage of doing histopathology (microscopic examination of tissue) is that it is a nonspecific test and can be used for a wide variety of diseases. An experienced pathologist can often supply many ideas about what might be causing the disease.

Tissue samples for histopathology should be submitted in formalin. Once the tissue is immersed in formalin, the formalin permeates the tissues and coagulates protein, thereby inactivating all infectious agents and stopping all biological processes. Once fixed, the tissue remains the same for weeks, months, years, decades.

You may be collecting samples for histopathology when you do a necropsy. Try to collect tissues of no more than 5mm thickness and collect into formalin in a ratio of 10:1, formalin:tissue. Then the container of formalin containing the tissues can be sent to the laboratory. Below is a depiction of what the pathologist will do with them:
The formalin-fixed tissues are “trimmed” to fit into a plastic cassette. Lids are put onto the cassettes and they are loaded onto a tissue processor.

The tissue processor is a machine that moves the cassettes through sequential changes of fluid, starting with formalin, then moving to alcohol, then xylene, then hot paraffin. So, after 12 hours, the tissue in the cassette is no longer floating in formalin, but rather it is floating in hot paraffin. At this point, the hot paraffin is cooled, and we end up with blocks of wax, each containing a piece of tissue, such as liver, lung, brain, etc.
Technicians will cut a very thin slice of the wax block (containing the tissue), put it on a glass slide, then that glass slide is stained with special stains (known as hematoxylin and eosin), and the pathologist can examine the slide under the microscope to see all the details of the architecture of the tissue. There are other stains that can be used for specific purposes such as clearly identifying bacteria and fungi.
Here are some examples of the diagnoses that a pathologist could make by looking at the histology:

This is a histologic section of intestine from a cow that died with chronic diarrhea. There are many big pink cells with multiple blue spots around the periphery (nuclei). These cells are called multinucleate giant cells and when they occur in such large numbers in the intestine, we know the cow had Johne’s disease.

This is a histologic section of brain from a cow that had neurologic signs. Big open areas in a neuron (the neuron looks “spongy”) are an indication that this cow had bovine spongiform encephalopathy.
Here is a section of lung from a goat that was coughing a lot and then died. The big tubular structures are all nematodes - this goat had *Muellerius* lungworms.

Here is a section of intestine from a goat that died of diarrhea. The pink inclusions seen in the epithelial cell nuclei are diagnostic for peste des petits ruminants (PPR).
4.2 WHAT’S DONE IN THE LAB – Bacteriology

The bacteriologist will try to find a bacterial agent in the samples you submit. He or she will do this by taking the material submitted and streaking it onto a set of agar plates. If a swab is submitted, it can be streaked directly onto the agar. If it is tissue, it is treated differently as seen below:

The surface of the tissue will first be “seared” with a piece of hot metal. This kills all the surface bacterial contaminants, then the bacteriologist can cut into the tissue and take a piece from the inside and streak the plates.
Agar plates are incubated and checked every 24 hours for growth. If growth is seen, then there are a number of methods for identifying what the bacteria might be. A single colony might be picked and put into various biochemical test tubes. The reactions of different species can turn the media different colors, and this helps the bacteriologist to identify which bacteria is growing from the tissue.
Some bacteria require special media for inoculation. Here is a “stab” method of culture for some kinds of anaerobes, which have to grow in the absence of oxygen.

And there are specialized media for growing mycobacteria, which will not grow on the standard agar plates normally used for most bacteria.

For some bacteria that produce a toxin, the bacteriologist might test for the toxin instead. An example is enterotoxemia, where intestinal fluid can be tested for toxicity in an ELISA assay.

For bacteria that are hard to grow, or difficult to identify, other tests may be used. For instance, blackleg, caused by Clostridium chauvoei, is diagnosed by direct fluorescent antibody on a smear of the affected muscle. Leptospirosis is diagnosed by microagglutination with specific antibodies. Different strains of Salmonella can be distinguished by PCR.
4.3 WHAT’S DONE IN THE LAB – Virology

Identifying viruses is a little more difficult than identifying bacteria in the laboratory. Whereas most bacteria will grow freely on artificial media, viruses require living cells in order to replicate. This means that if virus is to be grown, there must be facilities to do cell culture, which requires a continuous supply of carbon dioxide, fetal calf serum, and sterile facilities for passing and maintaining cell lines.

Although many laboratories will try to grow viruses using cell cultures, there are also many laboratories that try to determine the presence of virus without actually growing it.

In some cases, the virus can be identified by taking the tissue and smearing it onto a slide, or freezing the tissue, cutting it on a cryostat, putting the section on a slide and then adding specific FITC-labeled antibodies to detect the virus. This is what is done for rabies. The following sequence of pictures demonstrates the technique:

Here are pieces of brain from an animal suspected of having rabies.

Each piece is smeared onto a glass slide.
The smear is “fixed” by immersing in acetone.

Then anti-rabies antibody with an FITC molecule attached is put onto the slide.

There is a short incubation period to allow any antibodies to attach to rabies antigen that might be on the smear.

Then the slide is examined with fluorescence microscopy. If there is any antibody bound, then the smear will have many bright green dots, indicating that rabies was present in the brain.
For some diseases such as PPR or avian influenza, an “antigen ELISA” is used. The ELISA plate is coated with antibody specific to PPR or avian influenza. Then the swab from the suspect animal is rinsed in saline and the saline is put into the ELISA plate. If there is antigen there, the antigen binds to the antibody. Then another antibody is added, that only binds if the antigen is bound, and that second antibody has a colored marker on it.

PCR is also used extensively for viral diseases.
4.4 WHAT’S DONE IN THE LAB – Parasitology

-- INTESTINAL PARASITES --

- *Fecal flotation is the best method for nematodes*

The feces are mixed with a saturated salt solution and then strained through cheesecloth or a tea strainer. The filtrate is collected into a centrifuge tube.
A coverslip is put on top of the full tube and after brief centrifugation, the coverslip can be removed and lowered onto a glass slide for examination. Nematode eggs will float to the top during centrifugation and can now be seen.

- **Direct Smear**
  Although this technique lacks sensitivity, it is a good tool for the diagnosis of *Giardia*, trichomonads, and amoebae. It consists of mixing a small amount of feces with a drop of saline solution and adding a drop of Lugol’s iodine to enhance internal structures.

- **Fecal Sedimentation**
  The sedimentation technique is used to isolate eggs that do not float in common flotation solutions, mainly flukes, tapeworms, and some nematodes. The procedure is very simple and consists of mixing feces with ample water and allowing the mixture to sit for several minutes before decanting the supernatant. This procedure is repeated several times prior to sampling the water left after decanting for microscopic examination. Centrifugation can be used to speed the process.
-- PARASITES OF THE INTEGUMENTARY SYSTEM --

• **Mites**
  Skin scrapings smeared in mineral oil are the most common technique used to diagnose mites such as *Sarcoptes* and *Demodex*. When submitting/shipping mites to the diagnostic laboratory, these should be stored in 70% alcohol. However, scabs from chronic infections, particularly from *Psoroptes ovis* and *P. cuniculi* can be also submitted. These are digested in 10% sodium hydroxide before microscopic evaluation.

• **Ticks and fleas**
  These can be collected into 70% alcohol and will be preserved for prolonged periods. Examination under a dissecting microscope will allow for identification of species.

-- HEMOPARASITES --

• **Protozoans**
  Blood protozoans are best visualized in blood smears stained with Giemsa or Wright’s stains. These are performed on thin blood smears that have been fixed in methanol.
4.5 WHAT’S DONE IN THE LAB – Mycology

Fungi are often difficult to grow. There are special media for growing fungus and getting a culture often requires weeks to months as these organisms tend to reproduce slowly. Sabouraud’s Dextrose Agar is the standard medium for recovery and maintenance of commonly isolated fungi.

Often the laboratory may try to make a diagnosis of mycosis by simply examining the submitted material to try and visualize fungal elements. This is usually done through impression smears or histopathology. There are many special stains that can be used that will help to highlight the fungus within the smear or in the histologic section.

For instance, here is a histologic section from a lesion in a bird’s lung. It has been stained with a special fungal stain (called Periodic Acid Schiff). You can see the tube-like fungal hyphae within the lesion. This is *Aspergillus*. 
4.6 WHAT’S DONE IN THE LAB - Toxicology

Analyses for the presence of toxicants or drugs can be done on body fluids like blood or urine, or on tissues collected at necropsy. Many diagnoses involving a suspected toxicant rely not only on finding residues of the material in tissues but also on the presence or absence of compatible lesions in the animal.

The type of test done when the sample reaches the laboratory will depend somewhat on the type of toxicant suspected. The most important analytical methods in routine use include chromatography and various types of spectrometry or spectrophotometry such as atomic absorption spectrophotometry or mass spectrometry. Most of these methods are complicated and require expensive equipment.

A mass spectrometer uses a magnetic field to separate ions according to their mass and charge, and thus identify a material by its mass spectrum.
Chromatography is a technique that makes use of the variable distribution of materials between a stationary and mobile phase to separate components. It can be conducted in a gas phase (GC), liquid phase (LC) or on a thin layer (TLC) of glass or paper coated with a solid adsorbent. TLC and GC can be used to identify and quantify a variety of pesticides, feed additives and mycotoxins in animal specimens or feeds.

Atomic absorption spectroscopy can be used to measure the quantity of heavy metals in tissues, and atomic absorption and fluorescence spectroscopy can be sued to assess various nutritional deficiencies including copper, selenium, manganese, iron and zinc.
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