# **Understanding Avian Laboratory Tests**

Peter S. Sakas DVM, MS
Niles Animal Hospital and Bird Medical Center
7278 N. Milwaukee Ave. Niles, IL 60714
847-647-9325 FAX 847-647-8498
www.nilesanimalhospital.com

Because the hands-on examination can be much less revealing in birds than in other animals, clinical pathology (laboratory testing) plays an important part in the avian physical examination. A comprehensive physical examination should include a complete blood count (CBC), a fecal examination, and a choanal or oropharyngeal smear. Some veterinarians also include cloacal and choanal cultures, blood chemistries, serology, radiographs, and other specific tests.

This discussion will attempt to demystify avian laboratory work and give you a better understanding about each of the tests that are routinely performed on pet birds. In this way you can also be able to interpret the results of these tests that are performed on your avian companions.

#### **Avian CBC**

Everyone has heard the term CBC, but what does it actually mean? The term CBC is an abbreviation for "Complete Blood Count," a test to evaluate certain blood parameters. Another term used associated with blood work is Hematology, which is a general term referring to the study of blood.

Blood counts should be performed as part of any routine yearly physical examination, new bird examination, and evaluation of a sick bird, if the condition of the bird will allow it. The CBC enables rapid diagnosis and treatment. The CBC is probably the most useful of all diagnostic tests.

## Hematocrit/Packed Cell Volume

The hematocrit or packed cell volume is an essential part of the CBC. It is the measurement of the percentage of red blood cells (RBCs) in the circulatory system. Evaluation of the hematocrit is essential for determining the state of health of a bird and can be easily accomplished. The blood is collected in small, specialized tubes called microhematocrit capillary tubes. The tubes are then placed in a special centrifuge, which spins rapidly and separates the blood into the component parts, the packed down red blood cells, a small packed down layer of white blood cells (also termed the buffy coat) and the liquid portion of the blood called plasma.

Now some of you are familiar with the term serum and know that it also refers to the liquid portion of the blood. So what is the difference between plasma and serum? The plasma contains all elements of the liquid portion of the blood including the "clotting factors." Serum is formed by allowing the blood to clot so that the clotting factors are consumed. The liquid portion of the blood is removed, leaving the clot, so that all that is left in serum is the liquid portion of the blood with no clotting elements. Special tubes

can be used called serum separators which can be placed in the centrifuge and through spinning can separate out the serum without waiting for it to clot.

A normal avian hematocrit/packed cell volume (PCV) ranges from 40 to 60%, with the percentage varying for different species of bird. The hematocrit, as a measurement of the RBCs, can show if there is an increase or decrease in the percentage of RBCs.

A low percentage of RBCs is termed anemia (without blood). Anemia in birds can be due to several factors including, blood loss (through hemorrhage/bleeding), consumption of RBCs (RBC rupture due to some sort of poisoning or autoimmune condition), or lack of production (a metabolic or infectious condition suppressing RBC production or a problem with the bone marrow itself, where RBCs are produced).

An increase in the percentage of RBCs, termed hemoconcentration or polycythemia (many cells), can indicate certain conditions. If a bird is dehydrated the liquid portion of blood is reduced, leading to a relative increase in the percentage of RBCs. Abnormal increases in the production of RBCs can be due to a bone marrow problem which can lead to dramatic increases in the hematocrit.

### Plasma Color

The plasma color is evaluated and can give indications as to the state of health. The plasma should typically be clear, however, certain varieties of birds have plasma that normally is a faint yellow color, such as cockatiels.

If the plasma is yellow (also termed icterus or jaundice) it can be an indicator of liver disease, which bears further investigation through blood chemistries. However, certain factors which are not related to liver disease may cause a color change, such as increased carotene levels in the diet, which may lead to yellow-colored plasma, caused by an increased intake of vitamin A foods.

Fat in the plasma, termed lipemia (fat in the blood), is visualized by a cloudiness to the normally clear plasma, caused by the suspension of fat cells in the plasma. This can range from a small amount to significant levels which almost make the plasma look like butter in severe cases. As fatty liver disease (hepatic lipidosis) is a common and severe disease condition in birds, evaluation of the plasma is a useful adjunct in the diagnosis and monitoring of the condition.

Another change that can be seen in the plasma is hemolysis, the rupture of RBCs. When hemolysis occurs the plasma has a reddish color. Hemolysis can occur due to a disease condition or a toxicosis (poisoning), causing cell rupture, however, the most frequent cause of hemolysis is related to the collection of the blood sample. If there was difficulty in collecting the sample, either through a toenail clip or venipuncture (through a blood vessel), rupture of RBCs can occur, hence hemolysis. It is important to determine if the blood sample is hemolyzed, for if the RBCs have ruptured, certain chemicals can be released into the blood, altering some test results.

#### Plasma Protein

The final step in the evaluation of the centrifuged tubes is to determine the plasma protein levels. A special device called the refractometer is used. The plasma is placed on a special section of the refractometer, as light passes through the plasma; the total solids interfere with the light passage through the sample, thus the more solids, the more interference, hence the higher number. Typically plasma protein makes up the bulk of the total solids so the value obtained is considered an indicator of the total protein. However, certain material in the plasma that interferes with the passage of light through the sample can falsely elevate the 'total protein' value. One of the more common interfering substances is fat (lipemia). Therefore it is essential to determine if there is fat so that it can be taken into account when the total protein is determined. If the fat level is too high, evaluation of the number is not reliable for protein evaluation.

The normal plasma protein range is 3.0–5.5 mg%, with the percentage varying for different species of bird. Smaller birds tend to have values on the low end of the range. Baby birds also tend to have lower protein values than adults, which increase as they mature. Low protein levels can also indicate a poor diet, malnutrition or disease conditions causing protein loss, which can sometimes occur in chronic gastrointestinal disease. Dehydration can cause an increase in the protein levels.

## White Blood Cell (WBC) Count

White blood cells, also called leukocytes, are an important part of the body's defense against disease. In response to infection the WBC count typically increases. Therefore measure of the WBC count can give an indication if there is infection or inflammation occurring. Increases in WBCs can also occur in some cancer conditions such as leukemia. A bird under stress may have a WBC that is doubled, but greater elevations are a definite indication of disease. Reduced numbers of WBCs can occur due to bone marrow disease, severe acute disease and other conditions. It is very important to know the normal range of WBC numbers so increases or decreases can be properly evaluated.

White blood cell counts can be determined by various methods either through estimation or varied counting techniques. At this time, there is no automated system, such as is used with humans or dogs and cats that has proven effective for determining avian white blood cell counts. The complicating factor is that birds have nucleated RBCs in addition to nucleated WBCs. Most automated systems count nucleated cells, which are WBCs, as humans, dogs cats and other mammals have non-nucleated RBCs. The nucleated avian RBCs interfere with most automated counting methods. A new system that shows promise is laser flow cytometry which may be able to perform automated WBC counts in birds. Veterinarians who do not feel comfortable performing hematology procedures send the blood samples to one of the commercial laboratories that perform avian clinical pathology.

There are different varieties of WBCs and changes in their numbers can indicate particular disease conditions. The cells include heterophils (equivalent to the mammalian neutrophil or PMN), lymphocytes, monocytes, eosinophils, and basophils. A differential count can then be performed to evaluate the percentages of each type of cell. The normal

distribution of leukocytes is approximately 50% heterophils and 50% lymphocytes with small percentages of the other cells, but different factors will change the distribution. Some species respond to stress with a lymphocytosis (increased lymphocytes), while others do so with a heterophilia (increased heterophils). Allergic or parasitic conditions may show increased numbers of eosinophils. Chronic disease conditions may display increased numbers of monocytes (monocytosis). Monocytosis can occur in cases of chlamydiosis (psittacosis).

#### **Fecal Examination**

Another important component of the avian physical examination is the microscopic examination of droppings. Evaluation of both wet mounts and Gram stains is useful.

## Wet Mounts

With this procedure, a small amount of fresh dropping is placed on a slide and mixed with one or two drops of saline. A coverslip is added, and the slide is examined under low and then high power. With experience, one will be able to recognize normal background bacteria, undigested food material, and other normal components.

Normal avian droppings should not contain an abundance of bacteria, and the normal population is generally cocci (small round bacteria). If there is a predominance of bacilli (rod shaped bacteria), Gram staining should be done to determine whether the bacilli are gram negative and potentially pathogenic. Large amounts of gram-positive bacilli are sometimes noted, but generally they are simply the result of the bird being fed lactobacillus as dietary supplementation, either as an additive or as a component of a commercial diet. Motile bacteria are almost always pathogenic (disease-causing).

An occasional nonbudding form of Candida (variety of yeast) is normal, but budding yeast and mycelia (branching form of yeast) are indicative of invasive, infective forms. Birds fed bread products will frequently have nonpathogenic yeast in their droppings.

Parasitic infections can also be diagnosed from the evaluation of wet mounts. Protozoa (one-celled organisms) can be detected in fresh samples or in samples preserved in 5% formalin for staining and dark-field phase-contrast microscopy. Examination of wet mounts can also reveal the presence of the protozoan parasites, Giardia, Hexamita, and Trichomonas. Occasionally, helminth (roundworms) eggs may be detected.

#### **Gram Stains**

Gram staining is an important means of determining the character of the bacterial population in the droppings, but it is only a screening tool. In general most bacteria can be differentiated by their Gram staining characteristics, bacteria that take up the "positive" blue stain are called Gram-positive and those that take up the "negative" red stain are called Gram-negative. There are some groups of bacteria that do not take up the Gram stain and require special staining techniques (such as acid fast bacteria-which cause tuberculosis).

Gram-positive bacteria constitute the normal alimentary tract flora in birds; the pathogens most commonly isolated from birds are Gram-negative. However, the presence of a few Gram-negative bacteria in psittacine droppings is normal. A population of 10% Gram-negative bacteria is typically considered within normal limits. Caution must be exercised when interpreting the presence of Gram-negative bacteria, since most studies have failed to correlate the presence or absence of Gram-negative bacteria with culture results. Gram staining should be just one aspect of a diagnostic screening. It is important to also evaluate the bird and its clinical signs and, for final confirmation of the bacteria and pathogenicity, to perform culture/sensitivity testing.

### **Choanal and Oropharyngeal Smears**

Choanal, nasal, and oropharyngeal (throat) swabs and flushings are valuable diagnostic tools that are often overlooked. They can be collected for Gram staining, cytology, or culture.

Since the choana receives flora from the upper respiratory tract and the oral cavity, a choanal smear will provide a good indication of the flora present in those areas. Sampling can be accomplished with moistened swabs.

Bacterial, Candida, and Trichomonas populations can be detected with a wet mount and then further characterized with Gram staining. Trichomonas is best seen on a fresh wet mount; however, it is often present intracellularly, making diagnosis difficult.

An oropharyngeal smear is useful for detecting pharyngitis (throat inflammation), which is extremely common in pet birds due to the prevalence of vitamin A deficiencies. Pharyngitis may present as excessive mucus in the mouth, abscessation, congested breathing, and/or poor eating habits. Smears may also show increased numbers of epithelial cells. Other indications for sampling include rhinitis (nasal inflammation) and periorbital sinusitis (a sinus infection characterized by swelling around the eyes).

## **Sampling Other Sites**

Impression smears or needle aspirates should be taken at any site that exhibits abnormality, and the samples should be hematologically stained to identify cellular response and Gram stained to characterize the microbial flora. Samples from the choana, crop, and cloaca can be easily obtained with moistened swabs. Samples from the trachea can be obtained while the bird is under anesthesia. Surgical techniques, such as endoscopy, are required for obtaining samples from the periorbital sinuses and internal organs.

## **Culture and Sensitivity**

Bacterial culture and sensitivity are extremely valuable for confirming diagnoses of bacterial diseases. Because treatment regimens should be started as quickly as possible, especially for seriously ill birds, in-house microbiology is ideal. The sooner results are generated; the sooner proper therapy can begin.

In a culture, the sample is placed on special plates or media which promote the growth of bacteria. There may be one or more types of bacteria growing in the culture media, which are then isolated in pure culture and identified. The pure culture of each bacteria isolated is then subjected to special testing techniques to determine their susceptibility to various antibiotics, termed sensitivity.

Culture has its limitations, however. Some pathogens (disease causing organisms) fail to grow on conventional media, such as Chlamydophila (psittacosis organism), mycobacteria (tuberculosis organism), megabacteria, and anaerobes (bacteria that do not require oxygen for growth), and others are present only in low numbers or are intermittently shed, such as Salmonella.

Overall, the normal alimentary tract microbial flora in pet birds consists of anaerobic and Gram-positive bacteria. The anaerobic flora is poorly characterized. The Gram-positive bacteria can be described as follows: The normal aerobic bacilli include Lactobacillus, Corynebacterium, and Bacillus. The normal aerobic cocci include nonhemolytic Streptococcus, Micrococcus, and many Staphylococcus species, although not Staphylococcus aureus. Aspergillus and most other hyphael fungi that are isolated from the cloaca are usually transient microflora passing through the gut. They are seldom pathogenic in the gut but may indicate environmental or food contamination.

Canaries and finches often have sparse normal flora, and cultures that lack aerobic growth can occur in normal birds of these species. Gram-negative bacteria are generally considered to be abnormal in all pet variety birds; however, their presence does not always indicate that treatment is required. Gram-positive bacterial infections are more commonly encountered in canaries and finches than in psittacines. Birds can tolerate small numbers of Gram-negative bacteria, and certain strains of Escherichia coli and Enterobacter may be harmless. However, the presence of Gram-negative bacteria in large numbers is indicative of illness.

Determining which antibiotics should be part of the avian sensitivity panel can be problematic. If in-house microbiology is not conducted, commercial laboratories can perform avian cultures and sensitivities and provide a range of appropriate antibiotics, all within a good turnaround time.

## **Blood Chemistry**

In general, serum is preferred over whole blood or plasma for chemistry analysis. However, there are some commercial laboratories that prefer to run chemistries on plasma. Serology is the general term used to describe evaluation of the serum portion of the blood. This can include chemistries or other specialized tests. Collection and storage of blood should be done carefully, as mishandling can damage the cells and lead to inaccurate results. The following basic tests are recommended for any avian chemistry profile. Their results will provide a good evaluation of the bird's state of health.

## Protein

Normal serum protein values are typically from 3.0-5.5 mg%. Low protein levels may indicate malnutrition, malabsorption, chronic disease, renal disease, liver disease, parasitism, or stress. Elevated values indicate dehydration, shock, or infection. Hemolysis and lipemia will also produce elevated values.

## Calcium

Normal serum calcium values range from 8.0 to 13.0 mg%. Low calcium levels, which are frequently seen as the cause of seizures in birds, can result from poor calcium supplementation in the diet, renal disease, and other metabolic conditions. Ovulating birds have elevated calcium levels, apparently related to the calcium needed for eggshell formation. Oversupplementation with vitamin D3 will increase serum calcium and lead to renal mineralization. Neoplasms (cancers) will also elevate serum calcium.

#### Glucose

Normal serum glucose for most birds ranges between 200 and 500 mg%. Hypoglycemia (low blood sugar) occurs with malnutrition, liver disease, fasting, and systemic disease. Hyperglycemia (high blood sugar) may occur during breeding, stress, egg yolk peritonitis, and pancreatitis (inflammation of the pancreas). Diabetes mellitus is commonly seen in budgies and cockatiels but has also been described in several other species of birds. Diabetic birds usually have glucose values that are higher than 700 mg%; frequently the value is over 1,000 mg%. A diagnosis of diabetes is facilitated by repeated serum glucose testing; persistently high glucose levels over time will rule out the other causes of transient hyperglycemia (such as stress or eating).

#### Cholesterol

Normal serum cholesterol values in birds have not been well documented, but normal seems to range between 100 and 300 mg%. Elevated levels can be seen in birds on high-fat diets, obese birds, and birds with hypothyroidism (underactive thyroid gland). Low levels can be seen in birds with liver and kidney disease.

#### Uric Acid

Uric acid is the primary nitrogenous waste product of the avian kidney, and its level in the serum is an excellent indicator of renal function. Normal values vary depending on the measurement technique but generally range between 2.0 and 10.0 mg% (up to 15.0 mg% in some species). Uric acid values greater than 10.0 mg% are considered elevated in most species and are most often due to renal disease. However, starvation, dehydration, tissue trauma, and aminoglycoside (a class of antibiotics) therapy may also be responsible. Even with advanced renal disease, the uric acid level may remain within normal limits but on the high end of the range. In end-stage liver disease, the value may decrease.

## Aspartate Aminotransferase (AST)

Previously termed serum glutamic-oxaloacetic transaminase (SGOT), AST is considered one of the more reliable indicators of liver disease in caged birds. Serum values greater than 350 IU/L are considered abnormal and are often indicative of liver disease. Liver,

heart, or muscle damage can also result in elevated values. This test is not of benefit in diagnosing chronic or end-stage liver disease as the values will be low or decrease due to loss of hepatocytes (liver cells).

#### Bilirubin

Although serum bilirubin is not an important test for liver disease in birds, since icterus is due to biliverdin, the main biliary pigment in birds, elevations of bilirubin may be seen in severe liver disease. A word of caution: Often, yellow plasma is due to elevated carotene levels in the blood and not elevated biliverdin or bilirubin levels. When yellow plasma is observed, the nature of the bird's diet should be evaluated, since high consumption of carrots, sweet potatoes, squash, and other carotene-rich vegetables can raise blood carotene levels.

## Lactate Dehydrogenase (LDH)

Normal serum LDH levels range between 70 and 400 IU/L. Elevated values are most common with liver disease in birds, and the values are thought to rise and fall more quickly than AST levels in birds with this condition. These differences may provide information on the chronicity of the liver disease. Elevations may also occur with heart or muscle damage. If values are increased when creatinine phosphokinase (CPK) values are normal, liver disease is probably indicated. As with uric acid and AST, decreased values are seen in end-stage liver disease.

#### Bile Acids

Bile acids have been found to be the most sensitive indicator of liver disease in birds. Their concentration indicates the clearing capacity of the liver. Normal values range between 6.0 and 100 Kmol/L. When liver function is compromised, bile acids are not reabsorbed from the blood by the liver, so the proportion in the peripheral circulation increases. Elevated values have been correlated with liver disease in many avian species and therefore suggestive of hepatobiliary (liver) disease. With cirrhosis of the liver, serum bile acid levels decrease due to the reduction in bile acid production.

## Creatinine Phosphokinase

Measurement of serum CPK is a useful aid in distinguishing between muscle and liver disease in birds. Since CPK is found primarily in cardiac and skeletal muscle, an elevation usually indicates a muscular condition. Normal CPK values range between 100 and 550 IU/L. Elevations occur with damage to skeletal muscle (e.g., from injections, trauma, and feather picking) and with myocardial (heart muscle) disease. Elevations also often occur with advanced proventricular dilatation disease.

#### **Amylase**

Normal serum amylase values range between 100 and 600 IU/L. Elevated levels, as high as three times the upper limit of the normal range, may be seen with acute pancreatitis. In some cases of enteritis, even in the absence of pancreatic lesions, amylase levels may be almost twice the upper limit of normal. Amylase elevations may also indicate proventricular dilatation disease, but in most cases of this disease amylase activity is normal or only slightly elevated.

## <u>Lipase</u>

Although serum lipase levels are poorly established in pet birds, elevations may indicate severe cases of acute pancreatitis.

### **Electrophoresis**

Electrophoresis has received much attention recently as a valuable diagnostic tool in avian medicine. A serum sample is placed on a special "plate" and an electrical charge is applied. The various components of the serum migrate across the plate and are separated by their response to the charge. Once separated electrophoretically, each of the components can be evaluated and quanitated. Most frequently, electrophoretic methods are used in birds to determine the percentage of five major protein fractions in serum: pre-albumin/albumin, alpha-1 globulins, alpha-2 globulins, betaglobulins, and gammaglobulins—in serum protein.

In healthy birds, albumin is the largest protein fraction, constituting up to 40% of total serum protein in some species. An inflammatory process will result in a decrease in albumin concentrations and an increase in total protein due to elevations in the alpha, beta-, and gammaglobulin fractions. The end result of these changes is a decrease in the albumin/globulin (A/G) ratio. Other conditions that result in a decrease in the A/G ratio include egg yolk peritonitis and chronic infectious diseases, such as aspergillosis, chlamydiosis, and tuberculosis. Frequently, the total protein concentration will be within normal range while the A/G ratio is decreased. This occurrence indicates that the A/G ratio is of greater clinical importance than total protein concentration.

A decrease in albumin level can develop due to reduced albumin synthesis in chronic liver disease or chronic infection. Albumin loss also occurs with renal disease, parasitism, gastrointestinal disease, and overhydration. Albumin increases occur with dehydration.

An increase in alpha- and betaglobulins can result from acute nephritis, severe active hepatitis, systemic mycotic (fungal) infections, and nephrotic (kidney) syndrome. An increase in gammaglobulins, which are composed primarily of immunoglobulins, will occur with antigenic stimulation, such as in cases of acute or chronic inflammation, infection, chronic active hepatitis, vaccinations, and immune-mediated disorders.

#### **Specialized Laboratory Services**

Some specialized laboratory services will be required for routine new bird examinations and for the identification of particular disease conditions. Such services include chlamydiosis testing, virology studies (such as testing for psittacine beak and feather disease and polyomavirus screening), Aspergillus titers, and DNA blood sexing.

#### Serological Analyses

The evaluation of serum is useful in the diagnosis of many disease conditions and specialized tests have been developed to aid in their diagnosis. Certain tests detect the presence of antibodies, which are specifically produced by the body in response to a specific antigen (antibody generating material), such as bacteria, virus or fungal organisms. Such testing has been used extensively in the diagnosis of Chlamydiosis with

various types of tests used through the years to detect the specific antibodies produced to fight the disease. Techniques in the past have included latex agglutination, complement fixation and elementary body agglutination. A bird that has not been exposed to the disease would not have Chlamydophila antibodies in their system, while a bird that has the disease or was exposed to it would have the antibodies present which could be detectable with these tests. Serology has also been used in the detection of Aspergillus, where specialized testing can be used to detect anti-Aspergillus antibodies and the actual organism or antigen.

## DNA Polymerase Chain Reaction (PCR) Probes

This test is designed to detect the specific nucleic acid sequence of the disease organism in blood samples or samples from a combination choanal/cloacal swab. It is the most sensitive of the tests available today for detecting many diseases in currently infected birds. It does not rely upon an antibody response and has the potential of detecting infected birds before they are positive by serologic methods. The major disadvantage of PCR is its extreme sensitivity in detecting small quantities of contaminating and irrelevant target nucleic acid. It does not differentiate between nucleic acid from viable or nonviable organisms, so that a positive result might indicate environmental exposure. It has been used in the detection of many important avian diseases, including Chlamydiosis (psittacosis), Polyoma, and Psittacine Beak and Feather Disease (PBFD). The samples have to be sent to a specialized laboratory.

## Biopsy/Histopathology

A biopsy is when a sample is taken from a living animal for diagnostic evaluation. This usually refers to tissue samples which are taken from abnormal areas or growths. These samples are placed in special solutions to preserve them, such as formalin. The sample is then sent to a pathologist who can then section the samples and evaluate them in a process called histopathology. The sectioned samples may be specially stained and evaluated microscopically so that a diagnosis can be made.

## **Avian Necropsy**

What is the difference between a necropsy and an autopsy? Let us look at the words themselves. "Necro" refers to "dead" and "psy" to study, so necropsy is the "study of the dead." "Auto" refers to "self" so autopsy is "self study." So an autopsy is technically a necropsy, but because a "human is performing it on a human" it is an autopsy.

Performing necropsies is an important part of avian medicine. Frequently avian veterinarians are presented with birds that had "died suddenly" at home. Birds are very sensitive to airborne toxins or other potential household hazards so they can sometimes die suddenly at home. However, because birds tend to hide their illnesses, quite often they are near death or dead by the time a client notices that there is a disease condition. Necropsies can help determine the cause of these 'mysterious' deaths. Necropsies are also useful as a training tool for avian practitioners due to the wide array of conditions seen in pet birds.

If a practitioner had a bird under their treatment for a disease condition die, it is good practice to recommend a necropsy, as both the veterinarian and pet owner will benefit

from the knowledge gained. Sometimes the cause of death may not be readily obvious so that tissues may need to be sent out for histopathologic evaluation by an avian pathologist.

Many times clients are hesitant to have a necropsy performed because they would like to take the bird home for burial or do not want the bird dissected. These fears can be alleviated by the option of a "cosmetic" necropsy. In a "cosmetic" necropsy few or no feathers or removed and an opening is made just large enough to adequately evaluate the internal organs and obtain whatever samples are necessary. The incision is then sutured or tissue glued so that minimal disturbance is evident. However, depending upon the circumstances a complete and thorough necropsy is preferred.

The necropsy should be performed as soon as possible, otherwise the bird should be refrigerated, as autolysis (tissue breakdown) can occur very quickly in birds due to their high body temperature. Wetting the bird with soapy water prior to refrigeration can facilitate cooling. The bird should never be frozen as artifactual tissue changes will ensue, making histologic interpretation difficult. If the bird cannot be brought in for a long period before a necropsy can be performed then it could be frozen. Freezing/thawing will make gross observations difficult and severely hamper histopathologic analysis. However, viral or bacterial isolations and some toxicologic analyses can be conducted on frozen samples.

The instruments used for mammalian necropsy can also be used for birds, but smaller sizes facilitate the procedure. Due to the risk of potential zoonoses, gloves, and a surgical mask should be worn, the feathers should be soaked with soapy water to prevent aerosolization of pathogens, and the work area thoroughly disinfected following completion of the necropsy.

A detailed description of the avian necropsy technique is beyond the scope of our discussion, however, a few important points should be made. Before performing the actual dissection, the bird should be carefully evaluated for overall condition and checked for any obvious external abnormalities such as wounds, swellings, discharges, and staining. A systematic approach should be followed when performing the necropsy. Remember the old adage, "You miss more by not looking than by not knowing." Over time a familiarity will develop with normal avian anatomical appearance, pathological and artifactual changes seen on necropsy, which will enable the practitioner to become more effective when performing the necropsy.

Multiple samples should be taken and saved in 10% buffered formalin solution. If viral, bacterial or toxicologic studies may be needed, some tissue samples should be frozen. Cultures can also be obtained. If performing a necropsy on a neonate or juvenile, the Bursa of Fabricius (on the dorsal aspect of the cloaca) should be checked and if present it should be submitted due to its involvement in many disease conditions. The necropsy findings and diagnostic impressions should be recorded. Finally, when submitting samples, it is of utmost importance to deal with a pathologist or laboratory that is skilled

in avian histopathology. Avian veterinarians are fortunate because due to the interest in pet birds many excellent diagnostic facilities and pathologists are available.

## Conclusion

This discussion will have hopefully given you a better insight into the "mysteries" of avian laboratory work. By understanding these tests it will assist you in better evaluating the diagnostic processes that are part of avian medicine. Do not be afraid to discuss your concerns with your avian veterinarian. Also if you do not understand any of the laboratory results generated do not hesitate to ask for an explanation. It is of utmost importance that you have a clear understanding of what has been done.

This material was adapted from Essentials of Avian Medicine: A Practitioner's Guide 2nd Edition by Peter S. Sakas DVM, MS. AAHA Press (2002).