

## **COMPLETE BLOOD COUNT**

**As a general rule, I do not recommend laboratory tests on clinically healthy “pet birds” that are brought to me for routine physical examination and grooming on a regular basis. In my experience, if a bird is acting normal at home, if I see nothing abnormal on the exam, then in all likelihood laboratory tests will be normal as well. That being said, there are two basic tests (which can be requested) that are good, additional indicators of health; the fecal Gram stain and the complete blood count. The Gram stain is discussed in a separate paper.**

**The complete blood count (CBC) is a good, sensitive indicator of the bird’s general health. A change in the hemogram can be noted when no other abnormalities are detected. It is the single most important blood test that can be performed. If all parameters are normal on the CBC, then this is a good indication that the bird is not currently fighting some type of generalized infection or inflammatory disease process. However, a normal CBC does not rule out the possibility that the bird is an asymptomatic carrier of a bacterial or viral disease or has a localized infection.**

**There are three types of cells which are evaluated on the CBC; red blood cells (RBC’s or erythrocytes), white blood cells (WBC’s) and thrombocytes. In addition, the plasma (non-cellular part of the blood) is examined for color, protein, and the presence of parasites.**

**The following is a list of parameters measured on the CBC with a range of normal values. Values can vary greatly because there are so many different kinds of psittacine birds. There are also differences between juveniles and adults.**

## CBC PROFILE

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PARAMETER	RANGE OF NORMAL VALUES
RBC (millions of cells/cu mm)	2.5 – 4.5
PCV % (packed cell volume)	42 – 55
Polychromasia	slt (slight)
Anisocytosis	slt
MCV	100 – 200
WBC (cells/cu mm)	5000 – 15000
Heterophils %	40 – 75
Lymphocytes %	20 – 50
Basophils %	0 – 5
Eosinophils %	0 – 2
Monocytes %	0 – 3
Buffy coat %	0 – 1
COMMENTS	none
Thrombocytes	present
Plasma	
Color	clear or pale yellow
Total protein (gm/dl)	2.5 – 5.5
Parasites	none

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## **RED BLOOD CELLS**

Examination of the erythrocytes provides the veterinarian with information concerning anemia-related problems and the bone marrow response (which is where RBC's are produced). In a healthy bird, most RBC's should be mature, but a small percentage of immature erythrocytes is expected which indicates a normal replenishment from the bone marrow. Immature RBC's are slightly larger than mature RBC's and the cytoplasm is lightly stippled which produces a difference in color. ANISOCYTOSIS (size variation between RBC's) and POLYCHROMASIA (color variation between RBC's) are terms used to indicate the presence of immature RBC's. On the form used to indicate the results of the CBC, a slight (slt) response is normal while moderate or maximum indicates a strong bone marrow response to some type of anemia problem. If a bird is anemic and shows slight to no polychromasia or anisocytosis over a period of several weeks, this can indicate that the bone marrow is depressed or non-regenerative which is a grave situation.

Mean corpuscular volume (MCV) is a measurement of the average size of RBC's. The value rises in anemic conditions that are regenerative because newly produced red blood cells increase in numbers and these cells are larger than mature cells. If the value is below normal and the bird is anemic, then this indicates a non-regenerative state.

Anemia means a decrease in the normal amount of red blood cells. It can be due to either blood loss or lack of production of new RBC's from the bone marrow. The ratio of the volume of RBC's to the volume of whole blood is called the PACKED CELL VOLUME (PCV). This number is expressed as a percentage. It is determined by putting a sample of blood in a tiny collecting vial called a microhematocrit tube. At the laboratory this tube is spun in a centrifuge which separates the red blood cells from the plasma.

In a normal, healthy bird, the PCV ranges between 42 – 55%. Small birds such as budgies and cockatiels average 50% or higher while the values in larger species are usually lower, between 42-45%. Any value less than 35% indicates anemia and obviously the lower the value, the more critical the condition. Values higher than 55% are most often caused by dehydration.

The actual number of RBC's can also be counted, using either automated cell counting machines or by manual techniques which provide good estimates. The number of RBC's is recorded as a number times 10 to the sixth power which means millions of cells per cubic millimeter (cu mm) of whole blood. The normal number of RBC's varies depending on whether the bird is a juvenile or adult and the kind of bird being examined. In general, most adult, healthy psittacines average around 2.5 – 4.5 million RBC's/cu mm of blood. As a bird becomes anemic, the number of RBC's decreases.

## **WHITE BLOOD CELLS**

White blood cells (WBC's) are part of the body's defense or immune system. There are five types of WBC's found in birds. Heterophils, eosinophils, and basophils are known as granulocytes because they all contain colored granules (tiny spindle shaped bodies) in their cytoplasm. These cells are all produced in the bone marrow. Lymphocytes and monocytes are known as mononuclear WBC's. These two types of cells have a single nucleus and no granules in their cytoplasm. They originate in the spleen and in tiny collections of lymphoid tissue found throughout the body as opposed to the bone marrow.

During the collection of blood for a CBC, a tiny drop is placed on a glass microscope cover slip. A second cover slip is placed on top and the two are pulled apart creating a blood smear one cell layer thick. Back at the laboratory, this smear is stained with a blue dye which colors the different cells so they can be identified. By looking at the smear under the microscope, the total white blood cell count can be estimated as well as determining the number and relative proportions (%) of the five different types of WBC's. In addition, the WBC's can be examined for abnormal characteristics. All these findings are significant in determining whether a bird is fighting some type of disease and how serious it is.

## **WHITE BLOOD CELL COUNT**

**The total WBC count can also be determined by physically counting all of the granulocytes in a dilution of whole blood that is stained and put in a cell counting chamber called a hemacytometer. The cytoplasmic granules of these cells are specifically highlighted by the dye which makes them easy to count. By also knowing the percentage of mononuclear WBC's present from the blood smear, one can arrive at a fairly accurate total WBC count.**

**The normal WBC count can vary from laboratory to laboratory. It is dependent on the staining quality of the smear and the proper identification of cells. In the lab we use, the normal WBC count in adult psittacines varies between 5000-15000 cells/cu mm of blood. In smaller species (i.e. budgies, cockatiels) the normal range tends to be lower (5000-10000 cells/cu mm) than in larger species such as Amazons and macaws (12000-15000 cells/cu mm). Juvenile parrots usually have higher WBC counts than adults. It is not uncommon to see counts in the 20000-25000 cells/cu mm range in large psittacines that are near weaning age.**

**A crude, rapid technique for estimating if the WBC count is high is by measuring the BUFFY COAT. When the microhematocrit tube is spun at the laboratory there is a tiny layer of white cells that separates out at the junction between the red cells and plasma. This is called the buffy coat. In a normal, healthy psittacine bird, the buffy coat should be 1% or less of the total volume of blood. A buffy coat of 2% or higher indicates a high WBC count. 6% is the highest amount our lab has ever measured.**

**An increase in the WBC count is called leukocytosis. A common cause of leukocytosis is the actual transporting and handling of a bird at the clinic. This is a physiological change due to stress. Disease causes include bacterial and fungal infection, psittacosis, avian TB, trauma, toxicities, and certain cancers, such as leukemia. A decrease in the WBC count is called leucopenia. The most common causes of this include acute viral infections and the end stages of overwhelming septicemia (blood poisoning) in which the body literally runs out of WBC's with which to fight with.**

The percentage of the different types of WBC's is called the differential. As stated before, alternations of the differential from the normal range and changes in cell characteristics are significant for various disease conditions.

## **HETEROPHILS**

The heterophil is the most common granulocyte found in psittacine birds. The normal percentage of heterophils is between 40-75%. It is considered the "window to the bird's state of health" since even subtle changes in numbers and characteristics can occur with problems such as stress, low-grade infection, and mild inflammation. These cells are instrumental in the body's defense with incredibly large numbers being available to destroy and engulf bacteria or other infectious agents seen in acute or overwhelming infection and inflammation

Heterophils exhibit toxic changes in stress or disease conditions that can be seen microscopically. A low degree of toxicity is called degranulation. This is seen as a decrease in the number and size of cytoplasmic granules and a change in color of the cytoplasm from colorless or light blue to a muddy pink or red color. If degranulated heterophils are seen on the blood smear, this will be noted under the COMMENTS section. If nothing is written, then this means all the heterophils appear normal. Mild degranulation of heterophils can be considered normal in birds under stress. However, this finding should alert the veterinarian that something is going on, especially if the total WBC count is higher than normal.

In severe, generalized illnesses, the WBC count may rise above normal with a varying number of the heterophils appearing dark and shrunken. In addition to degranulation, these cells may appear foamy (the presence of vacuoles in the cytoplasm), or to have ruptured. The presence of bacteria may also be evident in the cytoplasm. Such heterophils are described as being toxic. The degree of heterophil toxicity usually increases with the severity of the bird's condition. Birds having a high degree of toxicity in most or all of their heterophils often have a grave prognosis. This is especially true if leucopenia is present.

## **EOSINOPHILS AND BASOPHILS**

**Eosinophils are similar in appearance to heterophils but can be differentiated by the color and shape of their cytoplasmic granules and the darker staining nucleus. Eosinophils occur in very small numbers with the normal range considered to be 0-2%. The function of the avian eosinophil is unclear; however, increased numbers are typically associated with parasitic infections, allergic reactions, and significant tissue damage. Changes in cell morphology have not proven helpful.**

**Basophils are easy to identify because of their dark-staining (basophilic) cytoplasmic granules. They also occur in small numbers with a normal range of 0-5%. The exact function of the basophil is not known. Increased numbers are often associated with chronic, long term illnesses. They also appear to play a role in the early stages of inflammation.**

## **LYMPHOCYTES**

**Lymphocytes and heterophils are the most numerous WBC's seen in psittacine birds. The normal proportion of lymphocytes is 20-50% but it varies between species. Some species such as cockatiels and Amazon parrots are naturally lymphocytic which means more lymphocytes than heterophils are seen normally. Lymphocytes are a very important part of the bird's immune system.**

**Lymphocytes increase in numbers (lymphocytosis) in certain types of chronic infections and lymphoid leukemia. They decrease in numbers in acute viral infections. In a disease state, lymphocytes will become reactive.**

**Microscopically, the normally pale blue cytoplasm becomes multi-colored with light blue, clear, and pink areas and the cytoplasm becomes vacuolated (the presence of tiny bubble-like structures). In chronic conditions lymphocytes with azurophilic (purple) granules in the cytoplasm have been noted. A few reactive lymphocytes on a blood smear is normal but if many are reactive then this is a significant finding that the body is responding to an infection of some sort. If reactive lymphocytes are present, this will be noted under the COMMENTS section. Reactive lymphocytes synthesize antibodies and other agents involved in the host immune system.**

**Birds with a marked lymphocytosis in which most or all of the lymphocytes appear abnormal or immature is consistent with leukemia. The WBC count in such birds can be greater than 100,000 cells/cu mm of which most are lymphocytes.**

## **MONOCYTES**

**These are the largest WBC's found in avian blood and they are very similar in appearance to lymphocytes. They occur in small numbers with a normal range of 0-3%. An increase in the percentage of monocytes (up to 10%) is seen with certain chronic diseases such as psittacosis, systemic fungal infections, TB, and massive tissue destruction. Changes in cell morphology are not significant.**

**The evaluation of the WBC count, the differential, and cell morphology is very useful in the assessment of the progress of an avian patient. For example, a bird with an initial leukocytosis, increased heterophils which appear toxic and reactive lymphocytes would be showing a favorable response to therapy if the total WBC and heterophil counts were decreasing, the toxic heterophils had disappeared, and the reactive lymphocytes returned to normal.**

## **THROMBOCYTES**

**Thrombocytes are the third type of cells found in avian blood and these are active participants in blood coagulation or clotting. Birds do not have platelets (cell cytoplasmic fragments) which perform this function in mammals.**

**Thrombocytes are produced in the bone marrow, are slightly smaller than erythrocytes, and are often clumped together in small numbers when viewed on a blood smear under the microscope. In addition to blood clotting, these cells may have the ability to phagocytize (or eat) bacteria and foreign material (like some WBC's) and they may be able to carry oxygen (like RBC's) if an extreme anemic condition exists.**



During the CBC evaluation, the presence of thrombocytes is noted on the form and if the numbers seem decreased or increased, this is also indicated. Increased numbers of reactive thrombocytes can indicate a chronic disease condition.

## **PLASMA**

Plasma is the fluid portion of blood in which particulate components are suspended. Plasma is distinguished from serum which is the cell-free portion of the blood from which fibrinogen (blood clotting factors) has been separated in the process of clotting.

When blood is drawn for a CBC, the cell components need to be evaluated; therefore, the blood must not clot. Heparin is the anticoagulant of choice in birds. The microhematocrit tubes or larger vials used to collect blood for a CBC are heparinized to prevent coagulation.

## **Color**

After the microhematocrit tube is spun at the laboratory the plasma is visually examined for color. In most birds it should be clear or pale yellow in color. The yellow color is due to the presence of carotenes which are yellow pigments found in a variety of plant materials, including some seeds. Yellow colored plasma should not be misinterpreted as icteric plasma. In mammals, icterus or jaundice is due to increased amounts of bilirubin pigments in plasma as a result of liver disease. Birds do not have bilirubin; therefore they do not become icteric if they have liver disease.

Sometimes the plasma appears pink in color. This is due to hemolysis which is the breaking apart of RBC's and the subsequent release of red hemoglobin molecules into the plasma. Hemolysis is most commonly caused by improper handling of blood samples, such as forcibly expelling blood through the needle of the syringe, shaking rather than simply inverting the tube to mix blood with the anticoagulant, overcentrifugation, or freezing and thawing of the sample. Some toxicities can cause lysis of blood cells, the most common example being lead poisoning. Occasionally an overwhelming infection causing septicemia will also cause hemolysis.

The plasma can also appear white or milky in color which is due to the presence of fat. This is called lipemia. Lipemic plasma is most commonly seen if a blood sample is drawn shortly after the bird eats a meal high in fat (i.e. oily seeds). Sometimes if the bird is excessively overweight, the serum will always appear mildly lipemic. Liver or pancreatic disorders can cause lipemia. Lastly, in female birds that are reproductively active, the plasma will be lipemic due to yolk globules (fats and protein) which are synthesized in the liver and transported via the plasma to the ovary where they are incorporated in the oocyte.

### **Total Protein**

While plasma is mainly water, it does contain a substantial amount of protein. Most plasma proteins, with the exception of antibodies and hormones, are synthesized in the liver. Proteins form the basis of organ and tissue structure, operate as catalysts (enzymes) in biochemical reactions, are regulators (hormones), and are the transport and carrier compounds for most of the constituents of plasma. The total protein level in the plasma is often used as an indicator for the health status of the patient.

The measurement of total protein is a simple test to run in the laboratory. Most practitioners use an instrument called a refractometer which gives a good estimate of the protein level as long as the plasma is clear. Hemolysis or lipemia will give a falsely elevated level. The normal value for psittacine species is in a range between 2.5-5.5 grams/deciliter (g/dl). Low total protein can reflect chronic disease (especially liver and kidney disorders), cancer, parasitism, long term stress, and starvation or malnutrition. Increased values may indicate dehydration, chronic infection, or leukemia.

### **PARASITES**

Blood parasites were often found in the blood smears of wild-caught, imported birds in years past. However they are extremely rare in domestic-raised pet birds that are kept indoors.

Blood parasites are transmitted by bloodsucking insects (flies, mosquitoes, or mites) and whether they cause clinical signs of disease is determined by the

**type of parasite found and the species of bird involved. In general, most blood parasites found in psittacine birds are incidental, will cause no clinical disease, and treatment is usually not recommended.**

**Parasites that invade red blood cells include Hemoproteus, Plasmodium (avian malaria), Leukocytozoon, Atoxoplasma, and Aegyptianella. Of these, only Hemoproteus was commonly seen in wild-caught psittacines, mostly in cockatoos. Microfilarias are microscopic, immature forms of filarial worms (related to heartworms in dogs) that are found in plasma. They have been seen in a variety of psittacines, especially cockatoos. The adult filarial worms are usually undetected and may occur in the air sacs, thoracic and abdominal cavities, or joints. Surgical removal of adult worms and Ivermectin are the treatments of choice.**





