CBC from A to Z

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Introduction
A complete blood count (CBC) is commonly performed in veterinary patients because it provides useful diagnostic information on the circulating blood cells. The automated portion of the CBC provides objective, numerical information about white blood cells (WBC; total concentration and differential), platelets (concentration and average size), and red blood cells (RBC; hemoglobin concentration, concentration, hematocrit, average size, and average cellular concentration of hemoglobin). Microscopic examination of a blood smear is a critical part of a CBC to confirm the validity of the automated data, assess cellular morphology, and look for cellular inclusions (e.g., organisms, Heinz bodies).

Blood Collection and Handling
An overnight fast is recommended in dogs and cats to minimize the risk of lipemia that can interfere with hemoglobin measurement and affect RBC morphology. Atraumatic venipuncture is important to minimize platelet clumping and iatrogenic hemolysis that will falsely lower RBC concentration and alter calculated RBCs indices. Blood should be collected directly into a syringe or a vacutainer tube containing some type of anticoagulant. Collection into a syringe followed by transfer into tube containing an anticoagulant is at increased risk for clot formation or platelet clumping. EDTA is the anticoagulant of choice for mammals because it provides the best cellular preservation and staining. If necessary, citrate or heparin can be used. Because there is significant dilution when blood is collected into citrate, calculations must be performed to compensate for the dilution. Heparin is associated with altered WBC staining (making it difficult to assess morphology) and increased frequency of cell clumping. Because cell clumping with all anticoagulants tends to increase with storage, prompt preparation of smears following collection is recommended. If there are clots in the sample, the blood should be discarded and another sample collected. Clots will falsely decrease in the measured concentration of all cell types and may alter the WBC differential.

It is important to properly fill collection tubes. Tubes that are overfilled are prone to clotting due to a relative deficiency of anticoagulant. Tubes that are underfilled contain an excess of anticoagulant which may cause dilution of blood and associated lowering of measured cell concentrations. In the case of EDTA, the excess concentration of anticoagulant causes a hyperosmolar environment that will cause RBCs to shrink.

Cells begin to deteriorate after collection and prolonged storage can alter measured cell concentrations and morphology. Quality blood smears should be made as soon as possible after collection to maximize preservation of cellular morphology. This is also important when looking
for erythrocyte parasites because some external organisms (e.g., Mycoplasma sp.) will drop off cells with storage. The quality of the blood smear will directly impact the interpretations that can be made from microscopic review of the smear. The goal is to produce a smear that extends 1/2 to 2/3 of the length of the slide. To make a blood smear, a drop of blood is placed at one end of a slide and a clean pusher slide is backed into the drop and used to drag the blood across the slide. A well prepared smear will be thick at the point of application of blood and will get progressively thinner until it reaches the feathered edge. To be of diagnostic quality, it must contain a sufficient monolayer region where the RBCs are close together not overlapping and the WBCs are spread thin enough to optimize nuclear and cytoplasmic detail. The length and thickness of smear will be altered by the size of the drop of blood, the speed of the pusher slide (faster causes shorter, thicker smears), and the angle of the pusher slide (more upright causes shorter, thicker smears). Streaks in the smear can be avoided by using clean pusher slides and avoiding downward pressure on the pusher slide.

Blood smears should be air dried. A hair dryer set on low can be used to speed up the drying process. Excess heat should be avoided because it will cause cells to deteriorate. Improper drying can result in irregular refractile inclusions on RBCs following staining that make it difficult to evaluate morphology. Unstained smears can be safely stored at room temperature for days if they are protected from scratching and dust. Unstained smears should not be refrigerated because the water condensation that occurs when smears are brought back to room temperature may damage the cells. Unstained smears should be kept away from formalin because the fumes will cause poor staining of the smears.

If blood will not be analyzed within several hours of collection, it should be stored in the refrigerator at 4°C. Storage up to 24 hours in the refrigerator will likely have minimal effects on the concentration of blood cells. The morphology of erythrocytes and platelets is not generally affected until the cell counts begin to decline. Storage related morphologic changes can occur in leukocytes within 1 to 2 hours of collection and include cytoplasmic vacuolization, membrane blebbing, nuclear swelling, pkynosis, karyorrhexis or cell lysis. Freezing will cause cell lysis and should be avoided.

**Hematology Analyzers**

Blood cells (WBC, RBC, and platelets) can be counted using a hemocytometer or an automated analyzer. Manual counting requires a minimal investment in equipment (e.g., hemocytometer, microscope, diluent system) but is less reliable than automated cell counting, prone to operator error, and labor intensive. Manual RBC counts are not recommended and a properly performed spun hematocrit is a reliable method to determine if a patient is anemic or has erythrocytosis. Automated hematology analyzers that have been validated for use in veterinary species are more reliable and less time consuming. Most of the analyzers currently on the market also provide a full or partial WBC differential. However, automated analyzers are more expensive to purchase and require adherence to proper quality control and a quality assurance program.

There are two common types of automated analyzers: impedance counters and laser cell counters. Because of the significant variation in cell sizes between species, analyzers should be validated for the species being tested. Impedance analyzers determine cell number and size as cells pass single file through a small opening through which a small current flows. Because cells
are poor electrical conductors, they will disrupt the electrical field when they pass through the aperture and cause a voltage pulse. The size of the pulse is proportional to the cell size. The number of pulses in a set period is indicative of the cell concentration. RBCs and platelets are separated based upon size. Leukocytes are counted after lysis of RBCs. Impedance analyzers may have difficulty separating large platelets and small erythrocytes. Laser counters evaluate cells as diluted blood passes single file through a laser light beam. Cell types are separated based upon light scatter patterns that are determined by cell size and internal structure.

While automated analyzers generally perform well in healthy animals, problems may arise in ill animals. Agglutination of RBCs will alter RBC indices and falsely decrease RBC concentration and automated hematocrit. Platelet clumping will falsely decrease platelet concentration, increase mean platelet volume and may falsely increase WBC concentration. None of the analyzers currently available can reliably differentiate all of the different cell types found in blood. Analyzers often have difficulty identifying leukemic cells, immature neutrophils, toxic neutrophils, reactive lymphocytes, eosinophils with non-staining granules, basophils, mast cells, and nucleated erythrocytes.

**Blood Smear Evaluation**

High quality blood smears should have an even distribution of cells and an adequate monolayer region where the erythrocytes are close together but not overlapping and the leukocytes are optimally spread. Smears should be stained with a Romanowsky-type stain (e.g., Diff-Quik®, Wright’s) that provides both nuclear and cytoplasmic detail.

Smears should be scanned on low magnification to assess staining quality, determine adequacy of the monolayer region, verify that the measured cell concentrations are accurate, and assess cellular distribution. Agglutination of RBCs indicates increased antibodies on the RBC surface and support immune-mediated disease. RBC concentration may be falsely decreased and RBC indices altered. While automated hematocrit is not reliable due to RBC agglutination, spun hematocrit can be used to determine the severity of an anemia. Leukoagglutination of WBCs is generally not of pathologic significance but may alter WBC concentration and differential. Platelet clumping is usually an artifact of platelet activation during blood collection. Platelet concentration may be falsely decreased. Smears have three recognizable regions: the thick region that extends from the application drop, the monolayer and the feathered edge. Platelet, leukocyte and erythrocyte morphology should be evaluated in the monolayer region. If an adequate monolayer is absent or the cells are not evenly distributed, a new blood smear should be prepared. With erythrocytosis, leukocytes tend to be distorted and difficult to evaluate. Blood can be diluted with equal parts of serum or plasma and a new smear made to assess WBC morphology. The feathered edge should be carefully evaluated for abnormal large structures (e.g., platelet or leukocyte clumps, mast cells, microfilaria, neoplastic cells, cells containing phagocytized organisms) that may be absent in the monolayer; however, cells are often too distorted and disrupted in this region to assess morphology. In the thick area of the smears, erythrocytes are stacked on each other and leukocytes are rounded up, small and darkly staining. Cellular morphology cannot be reliably assessed in these regions but overall cell density can be assessed.
If the analyzer provides flags indicating a problem with the WBC differential or if the percentages of the individual types of leukocytes (i.e., neutrophils, monocytes, lymphocytes, eosinophils, basophils, and others) appear different than the automated differential upon scanning the smear, a manual differential is performed and used to calculate the absolute concentrations of each cell type. The WBC differential is usually performed using high dry (40x) or oil (50x) objectives. A minimum of 100 cells should be consecutively counted while moving across the smear in an organized manner, making sure to not evaluate an area more than once. If the WBC concentration is significantly increased, a 200 or 300 cell differentials is recommended. Optimal evaluation of morphology of WBCs, RBCs and platelets is performed using the 100x oil objective. Damaged and distorted cells should be ignored. Erythrocytes are assessed for changes in size, degree of polychromasia or hypochromasia, abnormal shapes, inclusions or organisms. Leukocytes are evaluated for the presence of immature cells, toxic changes, reactivity, inclusions or organisms. If platelet clumps are present on the smear, then the automated platelet concentration should be considered inaccurate and a minimal value. If platelet clumps are not present on the smear, platelet concentration can be estimated based upon the average platelet number per field using the 100x objective. This number is multiplied by 15,000/μL (for microscopes with a wide field ocular) to 20,000/μL (for microscopes with a smaller field ocular). Platelets are also evaluated for size, morphologic changes, and organisms.

Suggested References