A complete blood count (CBC) provides useful diagnostic information. Automated bench-top hematology analyzers can provide numerical data including white blood cell (WBC) concentration and differential, platelet concentration, and red blood cell (RBC) parameters. However, a CBC should never be considered complete without microscopic examination of a blood smear to: (1) verify that the automated cell concentrations and WBC differential are correct; (2) assess cell morphology; and (3) look for organisms. Currently available analyzers cannot always reliably differentiate every leukocyte in all animals. Analyzers often have difficulty identifying leukemic cells, immature or toxic neutrophils, reactive or atypical lymphocytes, hypogranular eosinophils, basophils, and nucleated RBCs.

When examining a blood smear, it is best to start by scanning on low magnification (10x objective) to assess staining quality, cell distribution, and accuracy of measured cell concentrations. A new smear should be made if the smear is of poor quality (e.g., no monolayer region, leukocytes unevenly distributed at the feathered edge). The feathered edge should be examined for large structures (e.g., platelet clumps, microfilaria, neoplastic cells). Platelet, leukocyte and erythrocyte appearance should be evaluated in the monolayer region, which is often one to two 10x objective field widths away from the feathered edge. This is where the cells are neither balled up too thick nor flattened so thin that they are damaged. A common mistake is to assess cells in areas of the smear that are too thick.

If the automated WBC differential does not appear representative of cell populations noted microscopically (Figure 1), a manual WBC differential should be performed (typically using 40x or 100x objectives) and used to calculate the absolute concentrations of each cell type. A minimum of 100 intact leukocytes should be consecutively counted, taking care not to include cells twice. Leukocytes are evaluated for immaturity, toxic changes, reactivity, inclusions or organisms. Nucleated RBCs are not included in the differential, but should be counted to correct the automated WBC concentration. Damaged and distorted cells should not be included in the differential; they indicate a problem with smear making technique or a fragile cell population when present in significant numbers.

Erythrocytes are assessed for changes in size, color, or shape and the presence of inclusions or organisms (100x objective). In anemic animals, polychromasia is used to determine if the bone marrow is responding appropriately. Specific morphologic changes are suggestive for certain disease processes (Figure 2).

If platelet clumps are present on the smear, the automated platelet concentration may be inaccurate and should be considered a minimal value. If platelet clumps are not present, platelet concentration can be estimated by multiplying the average platelet number per field (100x objective) by 15,000/μL or 20,000/μL, depending on the microscope used.

At the DCPAH, every CBC includes a microscopic examination. To send samples, ship EDTA-anticoagulated blood overnight on a cold pack. Include two unstained blood smears (made from fresh blood and isolated from the cold pack) to allow evaluation of cells without artifacts associated with shipping.
Diagnostic Handling Tip #4 - Proper Forms Mean Faster Results

In a busy practice, it’s easy to get sidetracked by the multiple demands of paperwork, patients, phones, and other duties. One thing that will save you time in the long run is to make sure your laboratory submission forms are properly filled out. Omitting critical information can cause unnecessary delays in results, time-consuming follow-up phone calls with the laboratory, and unhappy clients. Be sure your submittal forms include all requested information about your clinic, your patient, and the requested laboratory procedures. Work with your laboratory to understand informational requirements, and your results can be on time every time.

Making Sense of Leptospirosis Titers in Dogs

By: Carole A Bolin, DVM, PhD

Canine leptospirosis has recently received increased attention as a cause of hepatic and renal disease. *Leptospira* serovars Canicola, Icterohaemorrhagiae, Grippotyphosa, Pomona, and Bratislava are identified as significant causes of canine leptospirosis in North America.

Serology, using a microscopic agglutination test, is the most commonly used diagnostic test. Serum samples are usually tested for antibodies against leptospiral serovars Canicola, Icterohaemorrhagiae, Grippotyphosa, Pomona, Hardjo, and Bratislava—sometimes Autumnalis is also included. The report received by the veterinarian will contain antibody titers of the serum against each of the leptospiral serovars tested.

Interpretation of leptospiral serologic results is sometimes considered an ancient mystical art form and is complicated by a number of factors including:

1) Antibodies produced in response to infection with a given serovar of *Leptospira* often cross-react with other serovars of leptospires. Therefore, a dog infected with a single serovar is likely to have antibodies against more than one serovar. However, in general, the infecting serovar is assumed to be the serovar to which the dog develops the highest titer.

2) Widespread vaccination of dogs with leptospiral vaccines complicates interpretation. In general, dogs develop relatively low titers (100 to 400) after vaccination and these titers persist for 1 to 3 months. However, some dogs develop high titers (1,600) after vaccination, and in these dogs, vaccination titers may persist for 6 months or more. Vaccination tends to produce antibodies against the leptospiral serovars in the vaccine but cross-reactivity is common.

3) There is a lack of consensus as to what titer is significant or indicative of leptospiral infection. Dogs tested soon after clinical signs develop often have low titers—therefore, low titers do not necessarily rule out a diagnosis of leptospirosis. In these cases, a significant rise in antibody titer is found when the dog is retested 5 to 7 days later. Diagnosis of acute leptospirosis based on a single serum sample must be made with caution and with full consideration of the clinical picture and vaccination history of the dog. With a consistent clinical picture and vaccination ≥ 3 months ago, an antibody titer of 1:800 to 1:1,600 is good presumptive evidence of leptospiral infection—but the pattern of titers is critical to consider. Antibody titers often persist for months following infection and recovery.

Are you confused yet? Here are my real-life recommendations regarding interpretation of leptospiral serology:

1) Send your sera to a lab that provides individualized result interpretation. A report with numbers or “positive” and “negative” is not sufficient.

2) Be sure to know the vaccination history for the dog—and provide this information to the lab.

3) Begin to pay attention to titers at the level of 1:400 to 1:800. Unless vaccination has been very recent, these titers are more likely to be associated with infection (current or past) than they are with vaccination. A titer in this range, particularly if associated with clinical signs, should be repeated to see if the titer is increasing.

4) In acute leptospirosis, the titers may be low on the first test; however, with a compatible clinical presentation, a titer of as low as 1:100 to 1:200 may be indicative of infection—particularly if the dog has not been vaccinated. In this circumstance, a titer recheck in 5 to 7 days is likely to reveal high titers.

5) Diagnosis on a single titer is often possible. If the dog had been infected (incubation period + duration of clinical signs) for 10 days to 2 weeks, titers may be diagnostic in the first sample.

6) Most importantly, if you have questions, ask the lab for help! If they are not willing or able to help with titer interpretation, find a new lab.
Cytology: A Valuable Diagnostic Tool
By: Dr. Jennifer Thomas

Animals frequently present with masses, effusions or organomegaly. It is often impossible using physical examination alone to determine the cause. In the hands of an experienced person, cytology is a valuable tool that may contribute to making a diagnosis, providing prognostic information, or determining the next diagnostic or therapeutic step. Samples are inexpensive to collect and special equipment is not required. In most cases, risks are minimal. DCPAH has four experienced board certified veterinary clinical pathologists to interpret your cytology samples; however, proper collection and slide preparation are critical to provide an adequate sample. Most samples are collected by fine needle biopsy using a needle (usually 20- to 22-gauge) and syringe (6 to 12 cc). The needle is inserted into the tissue. With the nonaspiration technique, the needle is redirected several times in the tissue and no suction applied. With the aspiration technique, gentle negative pressure is applied to the plunger of the syringe and the needle is redirected in the mass. Negative pressure is released prior to removing the needle from the tissue. Material does not have to be visible in the syringe to have an adequate sample. Immediately after removal from the tissue, the material in the needle should be expressed onto clean microscope slides, ideally only one drop per slide. All samples (no matter how small the drop appears) must be quickly spread before they clot or dry. Spreading is critical to provide a monolayer that is thin enough to allow evaluation of cellular morphology. Slides should be carefully labeled and allowed to air dry. Keep prepared slides at room temperature in a proper slide holder to protect them from scratching and from dust. Do not allow the slides to come in contact with formalin fumes.

Figure 1: Examples of nondiagnostic smears. Fine needle biopsy from the lymph node of a dog with peripheral lymphadenomegaly. A. Cells are lysed from overly aggressive collection techniques. Scratch artifact. B. Smear is too thick to be able to evaluate cellular morphology.

Fig. 2: High quality smear with an adequate monolayer region. Fine needle biopsy from the lymph node of a dog with peripheral lymphadenomegaly. The smear contains primarily large lymphocytes with prominent nucleoli and was diagnostic for lymphoma.
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