Clinical Pathology, Parasitology, Cytology in Birds, Reptiles and Small Mammals

Claudia Hochleithner, DVM
Manfred Hochleithner, DVM, Dipl. ECAMS

Tierarztpraxis Strebersdorf

Mühlweg 5, 1210 Vienna
Austria

INTRODUCTION

When working through a clinical scenario, it is important to maximize the amount of information obtained from small samples. One drop of blood smeared properly onto a coverslip or slide will provide a white blood cell estimate, cell differential, cell morphology, anemia evaluation and hemoparasite evaluation.
A second drop can be used for a PCV. If it does not fill the capillary tube properly, because this value is a percentage of red cells, the hematocrit tube can be read at 50% rather than 100%, or a lesser percentage as necessary. In addition, with a minimal amount of whole blood or plasma, the practitioner can perform a chemistry panel or PCR testing.
The goal of this presentation is to present clinical patients, create a diagnostic plan, demonstrate data interpretation, and correlate histopathologic findings. We will also provide some physiologic explanation of why the specific disease induces the changes in the laboratory data, as well as discuss treatment options.

BIOPSY AND NECROPSY SUBMISSIONS

Proper collection and handling of both biopsy and necropsy samples is important for better representing the results of the cause of the disease. The purpose of this overview is to describe handling of the samples, appropriate collection of tissues, and some explanations to aid in reading the pathology report.

HANDLING

It is important to handle tissue biopsies very carefully, as artifactual changes can easily be introduced, which will complicate interpretation of the results. Gentle tissue handling includes collection by sharp incision either by the endoscopic biopsy cup, scissors, or scalpel blade for a clean edged specimen. Any crushing from dull cutting edges will distort the cells and in small biopsies can result in a non-diagnostic sample. Careful forceps work is also important as pulling on the tissues will distort the cellular architecture.
Scoop the specimen up with forceps and be careful not to squeeze.
If the sample is extremely small or friable, place it in a separate container or in a tissue cassette. This will prevent these small pieces from being overlooked and protects friable samples from becoming the unexamined amorphous debris at the bottom of the jar. Do not crush or force these samples into tissues cassettes. This

can result in compression artifact if the sample is not completely fixed. In addition, do not force large samples in the formalin jar.
For tissue samples, the size of the tissue collected and preserved in formalin does matter. Formalin only penetrates ~ 0.2 cm in 24 hours so your tissue sections should not be larger than 0.5 cm. Opening a tubular organ enhances formalin penetration. Formalin preservation is slower in very bloody, dense tissue such as a congested spleen or liver. It is fine to remove completely fixed tissue samples and transport them in smaller amounts of formalin. Be sure that the tissues are completely fixed, as if unfixed samples are pressed into the walls of the containers, those areas will not be exposed to the formalin and will start degrading during the time between collection and reaching the histology lab.
In winter months, allow samples to fix for 24 hours prior to shipping. This avoids freeze-thaw artifact if temperatures fall below 0° C during shipping.

If you have collected culture swabs, swabs for PCR, or exfoliative cytology slides, remember to prevent their exposure to formalin. Even formalin fumes can cause havoc with samples such as cytology slides. The exposure to formalin fumes 'fixes' the cellular material on the slide resulting in a uniform blue haze when the slides are then stained for evaluation. Formalin will also sterilize the culture swabs if it leaks into the samples.

EVALUATION OF CYTOLOGY SAMPLES

The goals of cytologic evaluation include categorization of the lesion into one or more of the five cytologic categories, interpretation and diagnosis of pathological processes, identification of infectious agents, and recommendation(s) for additional diagnostics. The five cytological categories include inflammation, normal tissue/hyperplasia/neoplasia, mixed cell population, cyst formation and hemorrhagic lesion.

BLOOD FILM PREPARATION AND HANDLING

The quality of the blood film is essential for the ability to get accurate diagnostic information. The goal is to produce a blood film with a monolayer of cells. The most practical way for blood film preparation is the push smear (wedge) technique using two glass slides in a 30- to 45-degree angle. Avoidance of excess pressure is key to prevent cell lysis during smearing. The monolayer is an area of even cell distribution and is the best area for evaluation of cell morphology, white blood cell (WBC) estimate, and WBC differential. After blood film preparation, quick drying of the blood film to avoid drying artifact can be achieved by waving slides rapidly in the air or by using a blow dryer with appropriate distance. Excess heat or heat fixation needs to be avoided. Romanowsky type-based stains, such as Diff-Quik™ or Wright-Giemsa provide excellent results. It is important to change the stain set regularly as per manufacturer's recommendations and environmental regulations. If fecal cytology samples are also processed in the in-clinic laboratory, it is crucial to keep a separate set-up designated for fecal samples to avoid bacterial contamination of blood films and other cytology specimens during staining. For archiving purposes, slides are best saved stained but can be fixed in high quality methanol for 5 minutes for staining at a later time.