The collection and interpretation of cytologic specimens are complicated by several factors including: poorly exfoliating lesions, blood contamination, breaking/distortion of cells, and sampling bias. The complications may be minimized by preparing more slides from multiple sites/aspiration attempts to reduce sampling bias and increase the likelihood of preserving intact cells; using less suction to reduce blood contamination; making scrapes or impression smears of biopsy specimens; and preparing adequate smears.

1. Sample Collection

The collection technique used depends on the type of lesion being sampled. Below are descriptions of the most common collection techniques and indications for their use:

A. Fine needle aspirate
Aspirates are appropriate for the vast majority of lesions however some lesions contain more fragile cells (e.g. lymph nodes) that may be lysed by this technique, in which case a non-aspirate technique (see B. below) may be attempted. Fine needle aspirates are most successful when a small gauge needle (</= 20 gauge) of appropriate length for the lesion being aspirated is attached to a 12 or 20 mL syringe. The needle is then inserted into the mass/organ/lesion and approximately 6-8 mL worth of suction is applied to the syringe 3 or 4 times. The needle should be redirected in the lesion 2-3 times (without releasing the suction). Release suction prior to removal of the needle. This helps to both prevent/limit blood contamination and to avoid suctioning of air and displacement of the sample into the barrel of the syringe such that it cannot be expelled onto a slide. Remove the needle, draw air into the syringe then reattach the needle. The appropriate volume of sample can be gently expelled onto the glass slide and smeared (see below for smear making techniques).

B. Fine needle core, non-aspirate technique
Vascular masses and masses that contain fragile cells (e.g. lymph nodes) may require a non-aspiration technique. In such instances, normal aspiration

(Continued on page 2)
attempts may result in marked hemodilution and/or cellular lysis. This is particularly true for fragile lymphoid tissue/lymphocytes (especially if they are neoplastic). Care must, therefore, be taken to gently collect and smear slides in order to prevent cell lysis. To do this, a small gauge needle (≤ 20g), with or without an attached air-filled syringe, is inserted and removed from the tissue/lesion rapidly and several times in succession without applying suction. The needle may be redirected during this procedure. An air-filled syringe is then attached to the needle and the contents are expelled onto a glass slide near the frosted-edge. Pull/slide-over-slide smears are then made (see below).

C. Impressions/scrapings
Impression smears are collected by pressing a slide directly onto the lesion. They generally represent only the superficial pathology of a lesion, such as superficial inflammation and/or infection while the underlying, primary pathology may be missed. Whenever possible, superficial crust and exudates should be removed from the lesion prior to impressions and scrapings. Nonetheless, if the lesion is flat or not amenable to aspiration, impressions of the mass/lesion may be considered. Scraping such lesions will sometimes allow exfoliation of deeper pathology. Scrapings are collected by using a scalpel blade to scrape the surface of the lesion. Scraped material is then transferred to a glass slide and smeared using a pull/slide-over-slide technique. Impression smears and/or scrapings of the cut surface of biopsy specimens allow rapid preliminary cytologic assessment of lesions. This is often done intraoperatively or when time may be of the essence. The tissue should be blotted on a clean absorbent material prior to making impressions in order to prevent significant blood contamination.

D. Swabs
Certain areas such as the vagina and ear canals are not amenable to aspiration techniques and swabs of the area are required. Samples are collected by inserting a sterile cotton swab (this may be premoistened if the area is not itself moist) into the lesion/area to be swabbed, removing, and gently rolling (not sliding/smearing) the swab over the surface of a glass slide.

Table 1: Indications for the use of different cytologic sample collection techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine needle aspirate</td>
<td>• Useful for most mass lesions</td>
</tr>
<tr>
<td>Fine needle core/non-aspirate</td>
<td>• Useful for vascular lesions or lesions</td>
</tr>
<tr>
<td></td>
<td>containing fragile cells (e.g. lymph nodes)</td>
</tr>
<tr>
<td>Impressions/scrapings</td>
<td>• Consider using for flat lesions not</td>
</tr>
<tr>
<td></td>
<td>amenable to aspiration</td>
</tr>
<tr>
<td></td>
<td>• May prepare impressions/scrapings of surgical</td>
</tr>
<tr>
<td></td>
<td>biopsies</td>
</tr>
<tr>
<td>Swabs</td>
<td>• Use for areas not amenable to aspiration</td>
</tr>
<tr>
<td></td>
<td>(e.g. ears, vagina)</td>
</tr>
</tbody>
</table>

**Technical Tidbit:** Pools of blood/cells should be smeared either using a blood-smearing technique (push smears) or with a slide-over-slide technique (pull smears) so as to allow a more dispersed distribution of erythrocytes and tissue cells. This permits much better visualization of cell morphology than allowing large pools of blood and cells to dry without dispersion.

(Continued on page 3)
2. Sample Processing

A. Preparing Smears

   i. Push smears

   Push smears are smears prepared as you would a peripheral blood smear. These types of smears are ideal for very bloody or liquid aspirates. To prepare a push smear, place a new, clean slide on a level surface. (As a right-handed person, I typically place the slide in front of me, facing right to left, with the frosted edge of the slide facing right). Expel a small amount of sample near the frosted edge of the slide. Place a second spreader slide in front of the sample at a 30° angle. Back the spreader slide into the sample allowing it to disperse by capillary action along the edge of the spreader. Once the sample has dispersed to nearly the edge of the bottom slide, the spreader slide is pushed rapidly, in one smooth motion, without putting any downward pressure, towards the end of the slide and off of the edge. Ideally, the smear should be a nice “thumb-print” shape and should not extend beyond the edge of the smear.

   ii. Pull/slide-over-slide smears

   Once needle contents are expelled onto a glass slide, a second slide is used as a spreader by placing it gently on top of the sample slide, either at a right angle or parallel to the sample slide. The spreader (top) slide is then gently pulled across the sample (bottom) slide to thinly smear the sample. Do not press down with the top slide; let the weight of the top slide distribute the sample. Excessive pressure applied during this process may rupture cells, rendering the cytologic specimen non-diagnostic. Both the top and bottom slides will contain cells and can be submitted for cytologic evaluation.

   iii. Roll preparations

   Roll preparations are typically used on poorly cellular fluid samples. They provide a means to concentrate cells without having to sediment or centrifuge the sample. Roll preparations are prepared identically to push smears, however the spreader slide is stopped and lifted abruptly approximately ¾ of the way down the sample slide rather than continuing off of the slide. By lifting the spreader slide abruptly, a sharp line of fluid and tissue cells will be left on the sample slide.

B. Processing fluids

Fluids collected from body cavities (with known cellular and protein content) should be submitted for a full fluid analysis, which includes total solids, WBC count, and RBC count (Test code: VFAC). Enumeration of the cell counts and total solids allows more precise classification of the effusion and in some cases, allows comparison of fluid cell and protein content over time. Fluids collected from cystic masses or from washings (e.g. tracheal wash) should be submitted for cytologic evaluation (Test code: VCYTO) as enumeration of nucleated cells and proteins does not offer any additional information as there are no “normal” values to compare.

(Continued on page 4)
them to; furthermore for washings, the amount of fluid added/retrieved causes an unknown dilutional effect to the cellularity. When sampling urine, keep in mind that cells are very susceptible to lysis upon prolonged contact with urine, either in vivo or in vitro. Thus, allowing the patient to void first with subsequent urine collection 2-3 hours later via cystocentesis is most desirable for urine samples to be submitted for cytologic analysis.

Clear transparent fluids that are unlikely to clot can be submitted in a sterile red-topped serum tube. If the fluid has any blood-contamination or is turbid, an aliquot of the fluid should additionally be submitted in an EDTA anticoagulated tube. If a culture is being submitted, aliquot a separate portion of the fluid into a sterile non-anticoagulated container. Whenever possible, fresh direct smears, +/- roll preparations, +/- fresh smears of a sedimented portion of the fluid should be submitted along with the fluid.

C. Staining slides

Staining of at least one slide is recommended prior to submission of slides. This allows evaluation of sample quality prior to submission. Heat fixation/other fixation techniques are not required prior to staining. Any commercial Romanowski type stain (e.g. Diff-Quick) is typically acceptable. Slides/samples stained with vital dyes (e.g. methylene blue, Sedi-Stain) cannot be reevaluated.

3. Evaluation of sample quality

Screening of samples prior to submission to ensure that a diagnostic sample has been obtained is always recommended. Table 2 is a list of commonly encountered problems with cytologic specimens that result in a low diagnostic yield. Whenever possible these scenarios should be avoided. When screening a slide prior to submission, make sure that the sample is adequately cellular, that cells are intact, and that cells are evenly and adequately dispersed.

Table 2: Common sample collection and slide preparation problems that result in low diagnostic yield

<table>
<thead>
<tr>
<th>Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only submitting one slide</td>
</tr>
<tr>
<td>Staining slides with a vital dye prior to submission</td>
</tr>
<tr>
<td>Marked blood contamination</td>
</tr>
<tr>
<td>Poor cellularity sample; often due to inadequate negative pressure during collection or slow/shallow needle passes during the non-aspiration technique</td>
</tr>
<tr>
<td>Sample is too thick</td>
</tr>
<tr>
<td>Drops of blood/cells are not dispersed on the slide</td>
</tr>
<tr>
<td>Cells are lysed during collection and processing of the sample</td>
</tr>
<tr>
<td>Sample dries out or clots prior to/during slide preparation</td>
</tr>
<tr>
<td>Exposure to formalin fumes</td>
</tr>
<tr>
<td>Lubricating gel/ultrasound gel obscure the cytologic specimen</td>
</tr>
</tbody>
</table>

*Technical Tidbit: If you are submitting both cytologic specimens and formalin fixed tissue specimens, be aware that any exposure of unstained cytologic specimens to formalin fumes will result in an irreversible formalin artifact that limits staining of cells and interpretation of the sample. Cytologic specimens should always be packaged separately from formalin fixed tissue biopsies.*
Canine hyperadrenocorticism (HAC) can be manifest by a variety of clinical signs and laboratory abnormalities. Polyuria, polydipsia and polyphagia in a dog with alopecia and a pot-bellied appearance point toward a presumptive diagnosis of canine HAC. Characteristic laboratory abnormalities include increased serum alkaline phosphatase in the absence of hyperbilirubinemia and increased serum cholesterol. Unfortunately, not all dogs with HAC display characteristic clinical and laboratories findings. A number of ancillary laboratory assays are used to help diagnose canine HAC, including the urine cortisol to creatinine ratio (UCCR), the low dose dexamethasone suppression test (LDDS) and the adrenocorticotropic hormone (ACTH) stimulation test.

The UCCR is an excellent screening test for canine HAC. In the diagnostic assessment of a dog with clinical signs and physical examination findings suggestive of HAC, the UCCR is recommended as the first screening test following minimum data assessment (i.e., CBC, chemistry profile and urinalysis) (figure 1). The UCCR has significant negative predictive value (i.e., a good rule out test). If the UCCR results are within the reference range, HAC is unlikely. The positive predictive value of the UCCR is poor. Many dogs with nonadrenal illness and other forms of stress yield abnormal results similar to that of dogs with hyperadrenocorticism.

If the UCCR test is abnormal, more specific endocrine tests are needed to establish a diagnosis of HAC, such as the LDDS or ACTH stimulation test (figure 1). The sensitivity of the UCCR in ruling out HAC is reported as 85 – 90% but the specificity is low (20% or less).

Because stress can cause a false positive result in the UCCR assay, urine samples obtained by the client in the non-stressful home environment may yield more specific results than a urine sample obtained from a dog in a hospital examination room.

References:


Figure 1

Diagnostic Evaluation of the Dog with a Clinical History and Physical Examination Consistent with Cushing's Syndrome

- Database (CBC, chemistry profile, urinalysis)
- Consistent with diagnosis
- Low dose (0.01 mg/kg) dexamethasone screening test
- 4 and 8 hour cortisols (μg%)
  - Low, high: <1, >1
  - High, high: >1, >1
  - High, low: >1, <1
  - Low, low: <1, <1

Cushing's

Abdominal ultrasonography by excellent radiologist

- Bilateral adrenomegaly
  - PDH
  - ACTH stimulation as baseline study
  - Treat 1 choice: o,p'-DDD

- Normal adrenals

One normal/enlarged adrenal: other not visualized or normal-sized

- Adrenocortical tumor?
  - Endogenous ACTH or high dose dexamethasone suppression test
  - ACTH = N or HDDS = suppression
  - Treat as pituitary dependent hyperadrenocorticism
    - 1st choice: o,p'-DDD
    - 2nd choice: trilostane
    - No metastases
    - Metastases

Adrenal tumor

- 1. Radiographs (chest and abdomen)
  - 2. Ultrasound (repeat if necessary)
  - 3. CT scan

Adrenal tumor not conclusive, site of tumor not conclusive

Adrenal tumor not confirmed, Cushing's is confirmed

Surgery

NEW URINE CORTISOL ASSAY FOR CANINE URINE CORTISOL TO CREATININE RATIOS

BY DALE T. WHIPPLE MT(ASCP)

Marshfield Labs has validated a new methodology for canine urine cortisol analysis for performance of urine cortisol/creatinine ratios. Historically, radioimmunoassay (RIA) methods have been utilized for measurement of urinary free cortisol (UFC) with or without an extraction procedure. RIA was convenient, but gave concentrations that were 2–6 fold greater than the actual amounts of cortisol present (1,2). RIA methods have been mostly replaced with more sensitive and specific enzyme-immunoassay (EIA) and chemiluminescent-immunoassay (CLIA) methods; however, these methods are still susceptible to interferences from the multitude of endogenous steroids, glucocorticoids, and their metabolites that are normally present in the urine. This has led some to say that a more precise definition of UFC should really be “urinary free corticoids” (3).

Our test, a liquid chromatography-tandem mass spectrometry (LC MS/MS) methodology will greatly reduce analytical interferences and improve accuracy compared to immunoassay methodologies.

The LC MS/MS, a hyphenated technique, is a highly sensitive, specific, accurate, and rapid diagnostic tool of the clinical laboratory. Hyphenated techniques represent a new genre of analytical methods, which combine multiple separation technologies to enable high analytical sensitivity and specificity. LC MS/MS measures the mass to charge ratios (m/z) of molecules and their fragments and is therefore much more specific for cortisol than are RIA, EIA, and CLIA assays that rely on an antibody with varying degrees of cross-reactivity with other endogenous compounds normally present in the sample.

In LC MS/MS compounds are first separated by liquid chromatography (LC) and are then ionized as they pass from the LC into the tandem mass spectrometer (MS/MS). The first MS selectively sorts the compounds by their m/z. The selected compounds are then fragmented, and the fragments are again selectively sorted in a second MS. This process eliminates many potential interferences and is much more specific because it is based on the molecular mass of a compound, not based on the reactivity of an antibody. Addition of an internal standard, stable isotope-labeled cortisol enables measurement of patient cortisol levels.

Because the LC MS/MS technique is much more specific for cortisol than immunoassay methodologies, the reference range has changed. A ratio of 5 nmol cortisol/mmol creatinine is used as the threshold for evaluating animals for hyperadrenocorticism.

Values of 5 or below suggest hyperadrenocorticism is unlikely. Results greater than the reference range can be found not only with canine hyperadrenocorticism but with many other disease states and in association with nonspecific “stress”. If hyperadrenocorticism is suspected on the basis of clinical signs and laboratory findings and the urine cortisol to creatinine ratio is above the reference range, other laboratory tests, such as a low dose dexamethasone suppression test or ACTH stimulation tests are indicated.

References:


How often do you consider venipuncture technique or the order in which you fill blood tubes? If you don’t often consider your technique or blood-draw order, be aware that both can have a significant effect on laboratory results.

Most veterinary techs and veterinarians are aware that traumatic venipuncture can result in platelet clumping/sample clotting due to the release of tissue factor but it can also result in aberrant clinical chemistry results such as increased creatinine kinase and AST due to muscle damage and hemolysis during sample collection.

Additionally, cross-contamination of additives can occur between tubes. A common scenario is markedly elevated potassium and markedly decreased calcium concentrations secondary to contamination of a serum sample with K+-EDTA from a lavender topped tube. To prevent this problem, there is a recommended blood draw order that avoids cross-contamination of additives between tubes.

The blood draw order for commonly used tubes are: blood culture tube (yellow top) → sodium citrate coagulation tube (light blue top) → red top serum tube → serum separator tube (gold top) → lithium heparin tube (green top) → EDTA tube (lavender top). If a routine coagulation assay is the only test ordered, a small portion of blood should be drawn into a non-additive tube and discarded prior to filling the light blue sodium-citrate tube. This will decrease contamination with tissue factor and prevent the initiation of the clotting process.