

Cytology for Vet Techs 2011 SAVT Conference

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Interpretation of cytological specimens is dependent on the quality of sample submitted and the history provided. Therefore, it is imperative that the proper, appropriately processed sample is submitted and that an adequate history is provided. The following steps should help to maximize the diagnostic yield of cytologic specimens.

Step 1: Deciding what is the appropriate sample

Collection of the appropriate sample depends on the type of lesion. Superficial soft tissue masses/inflammatory lesions, intraabdominal masses, peripheral and mesenteric lymph nodes, internal organs, and body cavity effusions lend themselves well to cytologic evaluation. Firm lesions however are frequently poorly exfoliative for cytologic evaluation, often requiring biopsy and histologic evaluation for definitive diagnosis. This does not however imply that firm lesions should not be aspirated, only that you and your clients' expectations should be appropriate.

Deciding upon whether to aspirate or biopsy is dependent upon several factors. Table 1 outlines the pros and cons of each.

Table 1: Pros and cons of cytology vs. biopsy

	Pros	Cons
Cytology	<ul style="list-style-type: none"> ▪ Non-invasive, relatively atraumatic ▪ Quick, immediate (in-house) to 24-48 hour turnaround time (send-out) ▪ Relatively inexpensive ▪ Anesthesia/analgesia often not required ▪ May forego the need for surgical biopsy 	<ul style="list-style-type: none"> ▪ Screening test ▪ More susceptible to sampling bias ▪ Unable to evaluate tissue architecture ▪ Cytologic criteria of malignancy overlap with hyperplasia ▪ Few immunostains available ▪ Possible transplanted of tumor cells
Histology	<ul style="list-style-type: none"> ▪ Often more definitive ▪ Less susceptible to sampling bias ▪ Tissue architecture can be evaluated ▪ Ability to evaluate metastatic potential based on nearby tissue/vessel invasion ▪ Many immunostains available to help categorize lesions definitively 	<ul style="list-style-type: none"> ▪ Invasive ▪ Slower, 48-72 hour turnaround time ▪ More costly ▪ Anesthesia/analgesia required

Step 2: Sample collection

Once you have determined to collect a cytologic specimen, you must decide which technique to use. The collection and interpretation of cytologic specimens are complicated by several factors including: poorly exfoliating lesions, blood contamination, cell disruption/distortion, 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.

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 of suction is applied to the syringe 3 or 4 times. The needle is then redirected in the lesion 2-3 times (without releasing the suction). Suction is then released 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. The needle is then removed, air is drawn into the syringe and the syringe is reattached to the needle. The appropriate volume of sample is then squirted 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 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 (≤ 20 g), with or without an attached air-filled syringe, is inserted and removed from the tissue/lesion rapidly and several times in succession. 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. They often reveal 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 into the lesion/area to be swabbed, removing, and gently rolling (not sliding/smearing) the swab over the surface of a glass slide. The swab may be premoistened if the area is not itself moist.

Step 3: Sample processing

Of utmost importance is the proper labeling of slides. Whenever possible slides should be labeled with the patient name and specimen source prior to collection of the sample because once slides are made, they can easily be mixed up and confused with aspirates of the same or different lesions. 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.

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 at a right angle to the slide on which the sample has been placed. The top slide is then gently pulled across the 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 cytology non-

diagnostic. Both the top and bottom slides will contain cells and can be submitted for cytologic evaluation.

iii. Rollback/Stop Flow 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 other than the spreader slide is stopped and lifted abruptly approximately $\frac{3}{4}$ of the way down the sample slide rather than continuing off of the slide. By lifting the spreader slide abruptly, a concentrated 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 full fluid analyses including total solids, WBC count, and RBC count. 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 as enumeration of nucleated cells and proteins does not offer any additional information as there are no “normal” values to compare them to. 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.

Fluids should be submitted in an EDTA tube to prevent sample clotting. 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 anticoagulated 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. 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 prepared and packaged separately from formalin fixed tissue biopsies.

Step 4: Evaluation of sample quality

Screening of samples prior to submission to ensure that a diagnostic sample has been obtained is always recommended. Below 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.

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

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

Step 5: Filling out the requisition

Interpretation of all cytologic specimens occurs in the context of the signalment, history, and clinical findings. When filling out requisition forms, consider what information might help the pathologist interpret the sample. For example, the history “Mass aspirate” is not particularly helpful as compared to “Fine needle aspirate of a 2 x 3 x 2 cm subcutaneous mass on the ventral abdominal wall. Mass is soft, freely moveable. Suspect lipoma.” The following pieces of information allow a more thorough and thoughtful cytologic interpretation:

1. Signalment including: species, breed, age, sex
2. Clinical signs
3. Clinical history including the duration of the lesion, any previous treatment and its effects, previous cytologic or histologic evaluation of lesions, and pertinent laboratory data
4. Gross appearance of the lesion including:
 - a. Specific location
 - For example, we often receive descriptions such as “2 cm abdominal mass”. This description could describe an intraabdominal mass, a cutaneous or subcutaneous mass
 - b. Size
 - c. Freely moveable or fixed?
 - d. Firm or soft?
 - e. Painful or warm?
 - f. Haired or not haired?
 - g. Ulcerated or inflamed?
 - h. Cystic or not?
 - i. Any other pertinent information (e.g. the mass bled significantly when aspirated)
5. Clinical differentials/impressions

Evaluation of a Cytologic Specimen

Once you have determined that the slide is adequately cellular and representative of the lesion aspirated, determining what pathologic processes are represented on the slide can be a daunting task. Important points to remember when evaluating cytologic specimens are: 1) aspirate everything and 2) always correlate cytologic findings with the signalment of the patient and clinical presentation of the lesion. For example, some “fatty/lipomatous” lumps are actually mast cell tumors. Likewise, if all you find on an aspirate are large aggregates of adipocytes (cytologically consistent with a lipoma) but your lesion is firm, cutaneous, and ulcerated...you likely just aspirated normal subcutaneous fat and not the lesion. A methodical, step-by-step approach to evaluating the slide constituents will help to decipher what pathological process(es) is/are present so that you can present this information to the veterinarian to aid in making a diagnosis.

In general, most cytologic specimens can be grouped into three broad categories: normal, inflammatory, or neoplastic (benign or malignant). This brings up two important points: 1) you must know what normal cytologic appearance of any tissue is before you can consider interpreting abnormal tissues and 2) lesions may contain one, two, or all three of these cell populations (normal, inflammatory, and neoplastic).

Inflammatory conditions are classified based on the predominant cell type: neutrophil, macrophage, lymphocyte, plasma cell, and eosinophils. Again, you may have one or more of these populations present.

Neoplastic tissues are generally classified into four categories: epithelial, mesenchymal/spindle, round cell, and “other” and each of these categories can further be subdivided into benign or malignant. In-depth discussion of these categories can be found in the references listed below and is beyond the scope of these proceedings.

The following approach and the algorithm in Figure 1 will help you to thoroughly evaluate cytologic samples:

- 1) Describe what you see at low power (4-10x).
 - a. Cells: Degree of cellularity?
 - i. Variability in cell type: >1 cell type, variation within cell populations?
 - ii. Are cells alone or in clusters?
 - iii. Shapes of cells: round, spindle, oval?
 - b. Background material: mucus, protein, blood, matrix material
 - c. Organisms: large ones may be seen at this magnification.

- 2) Describe what you see at high power (40-100x).
 - a. Are the cells inflammatory cells, tissue cells, or both?
 - i. Inflammatory cells:

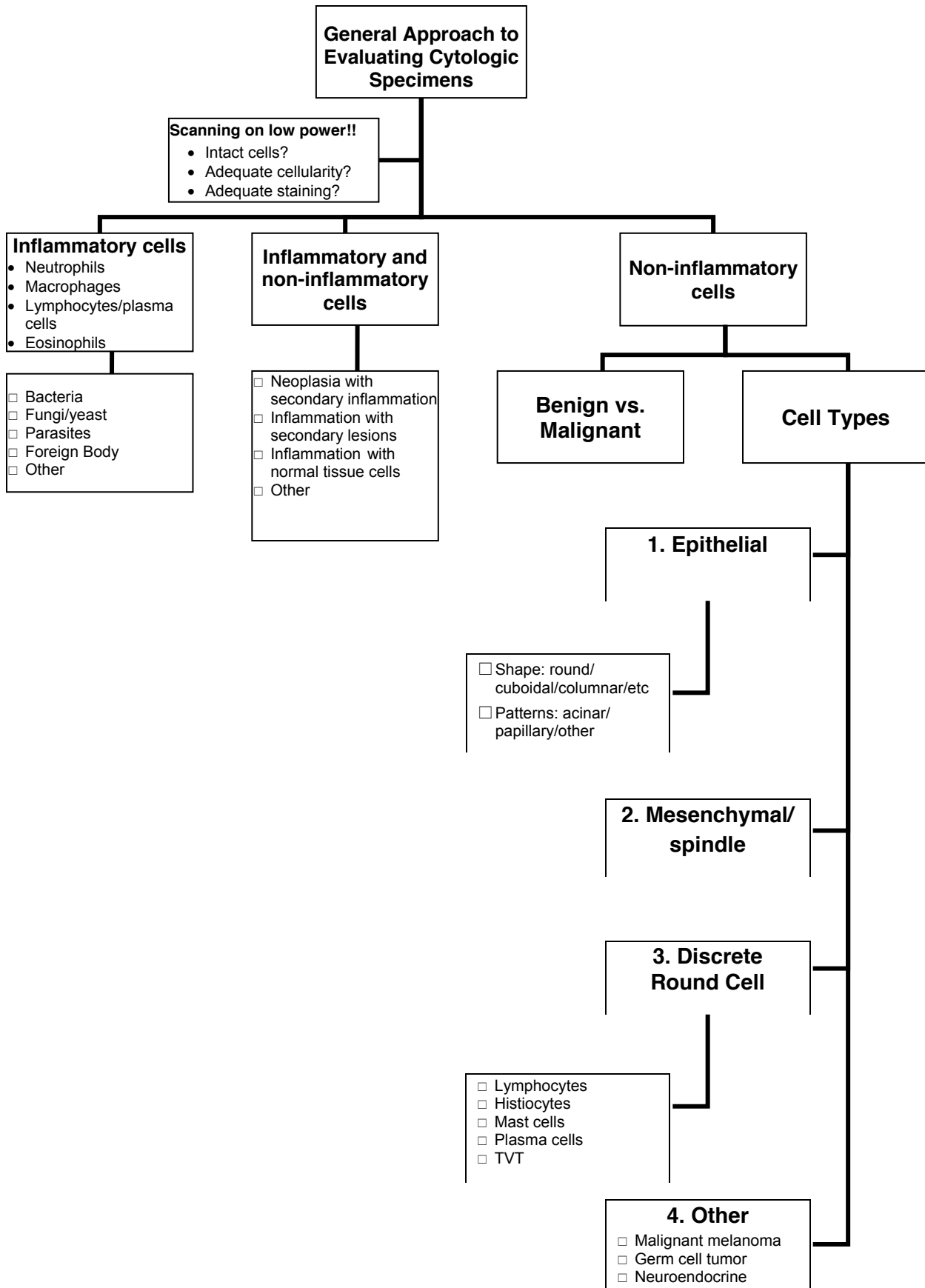
- Identify the cells and characterize the reaction: neutrophils, macrophages, lymphocytes, plasma cells, and eosinophils
- Think about etiologic agents that could cause this type of reaction
- Look for etiologic agents and attempt to identify them if found

ii. Tissue cells

- Attempt to characterize cells as epithelial, mesenchymal, discrete/round cell, or “other” and identify them if possible.
 1. Epithelial: larger cells, tendency to cluster, round to caudate shape, usually highly cellular
 2. Mesenchymal: small to medium sized, exfoliate singly, spindle to stellate shape, poorly cellular
 3. Discrete/round cell: small to medium sized, discrete, typically highly cellular
- Evaluate everything you can about the cells:
 1. Shape and degree of pleomorphism
 2. Cytoplasm: color, amount, texture, borders (indistinct, defined)
 3. N:C ratio
 4. Nucleus: location in cell, shape, size, number, staining
 5. Nuclear chromatin: pattern, abnormal mitotic figures
 6. Nucleoli: number, shape, size, variability
- Evaluate each population of tissue cells for criteria of malignancy
 1. General Criteria: anisocytosis/macrocytosis, hypercellularity, pleomorphism, monomorphism (in the case of lymphoid cells)
 2. Nuclear Criteria: karyomegaly, increased nuclear to cytoplasmic ratios, anisokaryosis, multinucleation, increased/abnormal mitotic figures, coarse chromatin pattern, nuclear molding, macronucleoli, angular nucleoli, anisonucleoliosis

3) Compose a morphological diagnosis.

Figure 1: Algorithm for the General Approach to Evaluating Cytologic Specimens



Cytologic Samples Amenable to In-House Evaluation

The limitations of cytology including sampling bias, the presence of more than one cell population, the lack of ability to evaluate tissue architecture, and the poorly exfoliative nature of some lesions make collection and interpretation of cytologic samples challenging. However, achieving proficiency in in-house evaluation of some commonly encountered cytologic samples is possible negating the necessity of sending every cytology to a referral laboratory. Several cytological samples are routinely evaluated in-hospital such as urine sediments and ear swabs. The following are lesions that you will see and aspirate with variable frequency in practice and are samples amenable to proficient in-house cytologic assessment:

1) *Inflammatory lesions (abscesses, draining lesions, etc.)*

Inflammation can be primary in the case of bacterial abscesses or cutaneous fungal infections but can also be secondary to underlying cystic accumulations, foreign bodies, immune- or hypersensitivity reactions, or neoplasia. Knowledge of the signalment, history, and gross appearance of the lesion you have aspirated should help in determining what differential diagnoses are most likely for any given mass. In general, the type of inflammation present can give you an indication of the cause and chronicity of the lesion. For example, primarily neutrophilic inflammation is often more acute and secondary to bacterial infections (particularly if the neutrophils are degenerate) whilst mixed neutrophilic-macrophagic (pyogranulomatous inflammation) typically accompanies causes of more chronic inflammation such as foreign bodies, fungal infection, and infection with higher order or atypical bacterial organisms (*Mycobacteria sp.*, *Actinomyces sp.*, and *Nocardia sp.*). Similarly, lymphoplasmacytic inflammation tends to accompany causes of antigenic stimulation such as vaccine/injection site reactions and eosinophilic inflammation tends to accompany insect bites, parasitism, hypersensitivity reactions, and mast cell tumors. If you have aspirated an inflammatory lesion, take the time to attempt to identify likely causes of the type of inflammation present. A general rule of thumb is to look at a smear for a minimum of 5-10 timed minutes before you can comfortably say that etiologic agents are not observed. Often times, reducing the magnification to the 10x objective and looking for areas of the slide with aggregates of cells will reveal organisms in the center of the inflammatory cell aggregate. Remember also that a single dose of antibiotic or antifungal therapy prior to aspiration can markedly reduce the number of organisms such that they are not appreciable on cytologic smears.

2) *Lipomas*

Aspiration of lipomas often produces many clear oleaginous lipid droplets and not many cells. Increased yield may be achieved by using the non-aspirate technique and rapidly inserting and redirecting a needle into the lipomatous mass. Adipocytes are typically large, lipid-filled, clear, balloon-like cells but may appear more basophilic and spindle-shaped if they contain less lipid. The presence of adipocytes on a slide does not necessarily warrant a diagnosis of lipoma as aspiration of normal subcutaneous or perinodal adipose tissue is indistinguishable. Diagnosis of a lipoma must only be made in the context of the knowledge of the gross characteristics of

the mass. For example, if a mass is described as firm and attached to a rib cage but adipocytes are aspirated, it is likely that the adipocytes represent perilesional adipose tissue and not the aspirated mass.

3) *Perianal gland tumors (also known as hepatoid gland tumors or circumanal gland tumors)*

These tumors arise from modified sebaceous glands (circumanal glands) that encircle the anus of dogs but few of these glands may be present on the tail and rump as well. Samples are typically cellular and contain numerous clusters of uniform, polygonal epithelial cells with distinct intercytoplasmic margins. They contain a moderate amount of grainy basophilic to amphophilic cytoplasm; round nuclei; finely stippled chromatin; and one or two small round nucleoli (similar to hepatocytes; hence the term “hepatoid”). These lesions are said to be hormone responsive and occur predominantly in intact male dogs however anecdotally, they seem to occur with equal frequency in both genders and states. The vast majority of these tumors are benign (adenomas) however well-differentiated perianal gland adenocarcinomas do occur and are cytologically indistinguishable from perianal gland adenomas. Thus, most clinical pathologists favor the generic term “perianal gland tumor” and recommend surgical excision to determine the presence or absence of tumor invasion and definitive diagnosis of benignancy vs. malignancy. Generally speaking, perianal gland tumors carry a favorable prognosis which is contrary to that of the major differential diagnosis for a perianal tumor in a dog: the anal sac (apocrine gland) adenocarcinoma.

4) *Sebaceous hyperplasia/adenomas*

These tumors typically present as cutaneous, wart-like growths. Cytologic differentiation between sebaceous hyperplasia and an adenoma is not possible but bears no clinical significance as the treatment and prognosis are the same for either lesion. Sebaceous adenomas are frequently found on breeds such as the Cocker Spaniel. They are benign lesions for which surgical removal is curative. When aspirated, small clusters of cohesive sebaceous epithelial cells exfoliate. Sebaceous epithelial cells are characterized by containing a large volume of cytoplasm engorged with many small, discrete, punctuate, clear sebum containing vacuoles and a small, central round nucleus with densely clumped chromatin. A variant of a sebaceous adenoma is a sebaceous epithelioma which contains many more basilar epithelial cells and only few fully differentiated sebocytes.

5) *Basilar epithelial neoplasms*

There are a number of tumors arising from basal cells of the skin, adnexa, and hair bulb. All of these tumors appear cytologically similar despite the ability to differentiate them histologically. Because cytologic differentiation of these tumors is not possible, the more general term “basilar epithelial neoplasm” is used as a cytologic diagnosis. The most commonly encountered in the dog is a trichoblastoma, a benign tumor of basal cells of the hair bulb. Basilar epithelial neoplasms exfoliate variable numbers of small, cuboidal, basophilic basilar epithelial cells in small clusters or ribbons. The cells are cuboidal with a small amount of moderately basophilic cytoplasm, which can contain melanin; round nuclei; clumped

chromatin; and indistinct nucleoli. The masses may be cystic and contain variable amounts of keratinized debris, melanin pigment, hemorrhage, and inflammatory cells in the background. The majority of these tumors are benign but as indicated above, they may rupture and become secondarily inflamed and infected.

6) *Keratin containing (+/-cystic) masses*

Similar to the basilar epithelial neoplasms, keratin containing cystic masses can arise from various portions of the hair follicle and adnexa. Some of the histologic categories of keratin containing cysts include the infundibular cyst (also known as an epidermal inclusion cyst), dermoid cysts, sebaceous duct cysts, pressure point comedones, and infundibular keratinizing acanthomas. The first three tend to be more fluid filled while the latter two tend to be less cystic. Aspiration of the cystic masses typically procures a brown chunky fluid. Cytologic evaluation of these lesions reveals a large amount of keratinized debris including nucleate and anucleate keratinocytes and keratin flakes/bars admixed none to variable numbers of neutrophils and macrophages. If the mass is truly cystic then a brown to basophilic background containing numerous vacuolated macrophages, few erythrocytes, and clear, rectangular cholesterol crystals (from cellular degeneration) will also be present. Most of these masses are benign, but secondary inflammation and infection commonly occur.

7) *Mast cell tumors*

Mast cell tumors tend to exfoliate well. Cytologic evaluation reveals a population of round cells that has exfoliated singly. They contain a moderate amount of blue-gray cytoplasm, with variable numbers of purple, coarse or fine, cytoplasmic granules. If highly granulated, the granules may obscure the nucleus. Mast cell tumors are often accompanied by variable numbers of eosinophils; few large, basophilic, plump spindle cells (reactive fibroblasts); and few collagen fibrils. Beware of poorly granulated or agranular mast cell tumors which can be mistaken for histiocytomas. Also be aware that mast cell tumors often present as a soft, fluctuant subcutaneous mass and be mistaken for a lipoma. Please endeavor to aspirate all soft tissue masses. Mast cell tumor grading cannot be done cytologically and requires histopathologic assessment of the mass.

8) *Histiocytomas*

Histiocytomas are benign tumors that will spontaneously regress within 2-3 months. They are most frequently diagnosed on the ears, head, and extremities of young dogs (<2 years old). They are described as "button" tumors and are domed, pink, cutaneous masses. Secondary ulceration is frequent. Histiocytomas, like other round cell tumors, tend to exfoliate well upon aspiration. Cytologically, a population of discrete round cells is seen. Cells are often described as having a "fried-egg" appearance with a small central round nucleus (the "yolk") and a moderate amount of light gray cytoplasm (the "egg-white"). As the tumor regresses, it becomes infiltrated with small lymphocytes and these may also be seen cytologically. Beware that agranular mast cells and some plasma cell tumors are cytologically similar to histiocytomas.

9) *Lymph nodes: reactive vs. lymphoma vs. metastatic neoplasia*

Normal lymph nodes contain primarily small lymphocytes (nucleus = diameter of erythrocyte), few plasma cells, macrophages, neutrophils, and rare eosinophils and mast cells. Aspiration of normally sized or slightly enlarged lymph nodes is difficult and perinodal adipose tissue or salivary gland (in the case of submandibular lymph nodes) are frequently inadvertently aspirated.

A reactive node will contain a more heterogeneous population of lymphocytes including small, medium, and few large immature lymphocytes along with variably increased numbers of plasma cells.

Lymphoma is a heterogeneous group of lymphoid diseases and may be composed of large immature lymphocytes (~85% of all canine lymphoma) or populations of small or medium sized lymphocytes. Diagnosis of lymphoma consisting of large immature lymphocytes (2-3x the diameter of an erythrocyte) can be made in-hospital with some practice. Diagnosis of small or medium-cell lymphomas is difficult to make cytologically and should be sent out for evaluation. A diagnosis of lymphoma is typically reserved for nodes in which a monomorphic population of large immature lymphocytes comprises >50% of the cells with few plasma cells and many cytoplasmic fragments in the background. Be aware that neoplastic lymphocytes are fragile and frequently lyse. Lysed and free nuclei may appear large and immature with prominent nucleoli so make sure to always interpret only those cells with an intact cytoplasm around the nucleus. Also be aware that previous treatment with corticosteroids or an early infiltration of the node with neoplastic cells may result in a cytologic specimen that contains <50% lymphoblasts but that is still suspicious for lymphoma.

10) *Joint fluid*

Joint fluid is not frequently aspirated but when it is, it is amenable to in-house evaluation. Normal joint fluid is highly viscous and contains only scattered, individual, small, round mononuclear cells containing a small amount of lightly basophilic cytoplasm. Joint fluid can become either reactive or inflamed. Reactive joints contain increased numbers of mononuclear cells, occasionally found in aggregates that display increased cytoplasmic volume, vacuolation, and/or basophilia. Mononuclear reactivity occurs secondary to joint instability (e.g. ruptured cranial cruciate ligament) or cartilage lesions (e.g. osteochondrosis dessicans) that result in degenerative joint disease. Inflamed joints contain a variable number of neutrophils. There are both septic (e.g. bacterial arthritis) and non-septic (e.g. immune-mediated polyarthritis) causes of inflammatory joint disease. Septic arthritis will often contain large numbers of mildly degenerate appearing neutrophils (pale, swollen nuclei) but organisms are rarely seen (particularly in small animals). Non-septic arthritides will often contain low to moderate numbers of non-degenerate neutrophils. Clinical history and ancillary diagnostics (e.g. tick-borne disease serology) will help to prioritize potential etiologies for neutrophilic inflammatory arthritis.

There are obviously many more lesions than the 10 listed above that could be sampled on a day to day basis. However, other commonly encountered lesions often require a significant amount of additional training for proper interpretation. For example, reactive, benign, and malignant spindle cells have cytologic features that overlap significantly. It is very easy to overinterpret and misinterpret the presence of spindle cells in a sample. The same holds true for differentiation of reactive mesothelial cells from neoplastic epithelial cells in body cavity effusions. Other frequently aspirated but complex lesions that may pose diagnostic dilemmas include mammary tumors and lesions composed of mixed populations of inflammatory and tissue cells such as squamous cell carcinomas since inflammation often results in dysplastic changes that are misinterpreted as neoplastic changes.

If you are particularly interested in cytology, make sure to open a dialogue with your Clinical Pathologist so that they can discuss interesting cases with you and help you hone your skills.

As a closing remark, remember the adage: "When in doubt...send it out!"

The following references are excellent cytology resources:

1. Diagnostic Cytology and Hematology of the Dog and Cat. 3rd Ed. 2008. Rick L. Cowell, Ronald D. Tyler, James H. Meinkoth, and Dennis B. DeNicola, eds., Mosby Elsevier.
2. Canine and Feline Cytology: A Color Atlas and Interpretation Guide. 2nd Ed. 2010. Rose E. Raskin and Denny J. Meyer, eds., Saunders Elsevier.
3. Avian & Exotic Animal Hematology and Cytology. 3rd Ed. 2007. Terry W. Campbell and Christine K. Ellis. Blackwell Publishing.