PRACTICAL CYTOLOGY FOR THE VETERINARY PRACTIONER

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Cytology in Practice

Cytology (the microscopic evaluation of cells) is a valuable tool for practitioners, and is commonly used for diagnosis, staging, and monitoring of treatment of companion animals with cancer. Cytology is quick, safe, inexpensive, and simple to learn. While cytology does not provide the same information as histopathology, it is often diagnostic, and results may affect further diagnostic plans, treatment recommendations, or client decisions. If in-house diagnosis cannot be performed, evaluating the quality of the sample before submitting it to an outside lab is essential. In general, it is the author's recommendation that cytologic samples of any suspected malignant neoplasm be submitted to a clinical pathologist for confirmation. The most common method of obtaining cytology samples is fine needle aspiration (FNA). Palpation is not a substitute for aspiration – there is no particular "way" pathology feels. **All lumps, bumps, and masses should be aspirated!**

Sample Collection

Sampling a mass via FNA is rapid and requires few supplies. 22 gauge needles are most commonly used in the author's practice, however gauges from 18 to 25 may be considered. 6mL syringes will facilitate expelling needle contents onto clean, dust-free glass slides—the use of smaller syringes may not provide the necessary force to completely expel needle contents. Before aspirating, prepare by setting out at least 4-6 clean glass slides, preparing a syringe by filling it with several milliliters of air, and, if aspirating multiple things, labeling the slides in pencil. Both the **bare needle or "woodpecker" technique**, as well as the **"syringe" or aspiration technique** may be used. In the "woodpecker" technique, stabilize the mass with the non-dominant hand and introduce the needle into the mass. Rapidly introduce the needle into various parts of the mass while not exiting the skin, using a pecking motion. Then withdraw the needle, attach the air-filled 6mL syringe, and expel the contents onto prepared slides. For the aspiration technique, the needle is attached to an empty 6mL syringe and introduced into the mass. The plunger is then drawn back and released several times, and the needle may be redirected within the mass and aspiration repeated if necessary, then withdrawn. The needle is then briefly removed, several milliliters of air are drawn into the syringe, the needle is reattached, and the contents are expelled onto a glass slide. The bare needle technique works well for most masses, however the aspiration technique may provide better results in poorly exfoliating neoplasms or very small masses where there is little room to redirect and "peck" with the bare needle.

Slide Preparation

After poor sample cellularity, poor slide preparation is one of the biggest barriers to cytologic evaluation of masses, and so good slide preparation is key. This is accomplished in part by preparing slides and syringes before beginning aspiration, so that needle contents can be expelled quickly before

clotting occurs. Samples should be smeared in a thin layer for best evaluation, and **all** samples should be smeared, even if only a very small amount of sample is expelled onto the slide. Most samples may be prepared by utilizing the "**pull**" technique. After expelling the sample, place a second clean glass slide onto the first either horizontally or vertically, and pull gently away from the first slide's frosted edge. The second slide should be flat against the first. The blood smear or "**push**" technique may also be used, especially where the aspiration sample is not very viscous. Either the "pull" or "push" technique may be used (the author prefers the "pull" technique for nearly all samples) as long as the result is a **monolayer** of intact, nucleated cells that may be evaluated.

After smearing the sample, slides should be air-dried. In general, heat fixation of slides is not recommended as it can distort cellular morphology. Once dried, standard stains such as Diff Quick or Wright's stain are preferred. If submitting samples to an outside laboratory for evaluation, stain one or two slides to ensure their diagnostic quality. These stained slides may be submitted, but should be accompanied by several unstained, air-dried slides. An important note is that cytology slides should be prepared and shipped well away from any formalin containers. The fumes can distort and degrade cytologic samples, and so air-dried cytology slides and formalin containers should **never** be shipped together.

<u>Microscopic Evaluation of Samples</u>

After staining, the slide is ready for microscopic evaluation. The two most important questions the be answered as a practitioner are:

1) Was the intended tissue sampled?
    - For example, lymph node vs. salivary gland
2) Is the sample adequate for evaluation?
    - Are there enough intact, nucleated cells for evaluation?

Begin by scanning the slide at low-power (10x) magnification to assess the overall cellularity and quality of the sample. This initial scan will help answer the above questions. After ensuring the sample quality, areas of interest such as cell clumps or clusters can then be centered and assessed at higher power to identify their type and cellular characteristics. The following diagnostic algorithm is helpful in evaluating suspected neoplastic cytology samples. Its main benefit is that it is not necessary to answer all four questions in order to obtain helpful case information. In order:

1) Is the sample adequate? (If not, resample.)
    If YES, then:
2) What general class of disease does the sample fall into? (More than one process may be present)
    a. Normal or hyperplastic
    b. Inflammatory
    c. Neoplastic
    If NEOPLASTIC, then:

3) What is the tissue of origin?
    a. Epithelial
    b. Mesenchymal
    c. Round cell (discrete)
4) Does the lesion appear benign or malignant?

Inflammatory processes most often reveal a mixed population of inflammatory and tissue cells, and are generally characterized by the cell type(s) present. When predominantly neutrophils are seen (>85%), inflammation is termed **suppurative**. Evaluation of the maturity of the neutrophils (segmented vs. band neutrophils) as well as their morphology should be conducted. Degenerate changes in neutrophils are often indicative of a bacterial infection and may include karyolysis (the "pale, puffy" nucleus), vacuolization, and toxic granulation. Cells should also be closely examined for intracellular microorganisms. **Pyogranulomatous** inflammation consists of a mixture of neutrophils and macrophages, and is commonly observed in response to fungal infections or foreign material. **Granulomatous** inflammation consists predominantly of macrophages. Primarily **eosinophilic** inflammation should be considered in any specimen containing greater than 12% eosinophils, and is commonly seen in allergic and hypersensitivity responses, parasite infestation, and as a paraneoplastic response (for example, mast cell neoplasms).

The classic **criteria of malignancy** are used to identify suspected malignant cellular phenotypes, and should be interpreted in light of the behavior of the mass in question and the patient characteristics (i.e. signalment, health, etc). These criteria include:

1) Low-power indications
    a. Inappropriate cell type for anatomic location sampled (i.e. non-lymphoid cells in lymph node, non-epithelial cells in skin)
    b. Uniform population
    c. Pleomorphism (variation in cell size and shape within cell population)
2) Nuclear changes
    a. Anisocytosis (variation in cell size)
    b. Anisokarysosis (variation in nuclear size)
    c. Prominent and/or multiple nucleoli, especially of abnormal shapes (i.e. teardrop, polygonal)
    d. Nuclear molding
    e. Increased mitotic activity, especially bizarre mitoses
    f. Bi-, tri-, or multinucleated cells (interpret carefully in the face of inflammation!)
3) Cytoplasmic changes
    a. Vacuolization ("foamy" cytoplasm)
    b. Basophilia

Neoplastic samples may then be classified based upon their tissue of origin:

- **Epithelial** cells are found lining the respiratory, digestive, and urogenital tracts, in the skin and mucous membranes, and in secretory organs (the pancreas, thyroid, adrenal glands,

prostate, mammary gland, etc). Benign squamous epithelial lesions are **papillomas**, while benign glandular epithelial lesions are termed **adenomas**. In contrast, malignant squamous epithelial lesions are **carcinomas**, and while their malignant glandular counterparts are **adenocarcinomas**. Epithelial cells are generally round to polygonal, with a round nucleus that may be centrally or apically/basally placed depending on the particular origin (i.e. respiratory epithelium vs. skin) and degree of meta- or anaplasia. They tend to exfoliate well (though not always), resulting in moderately to highly cellular samples. Their major defining feature is orderly clustering or clumping of cells, which can be observed on low-power magnification. Be sure to distinguish this ordered clustering, where distinct cell-to-cell junctions may be observed and cells are often arranged in sheets or rafts, from the disordered aggregation of clumped mesenchymal cells or the high cellularity of round cell samples, which may give the appearance of clumping. This is most easily accomplished by examining less cellular areas of the slide and determining whether cells are still tightly associated or appear individually.

- **Mesenchymal** cells are cells of connective tissue origin and may be found in such tissues as cartilage, bone, smooth and skeletal muscle, nervous tissue, and blood vessels. Benign mesenchymal neoplasms are designated by their cell of origin, followed by the suffix **"-oma"** (i.e. fibroma) while malignant mesenchymal neoplasms are **sarcomas**. Mesenchymal cells are generally spindle or fusiform in shape. Nuclei are often eccentrically placed, with wispy, poorly defined cytoplasm streaming the direction the sample was smeared. Because mesenchymal cells inherently are tightly affixed to their environment, they generally exfoliate poorly, resulting in moderately to poorly cellular samples. Also, while they tend to appear as individual cells, loose and disorganized clusters or aggregates may be noted – be sure to distinguish this from ordered epithelial cell clustering as described above. The sample background is particularly important in assessing mesenchymal cytology, as these cells may produce extracellular matrix material that may be associated with cell aggregates or be smeared loose across the slide. This material may be eosinophilic or basophilic, sometimes with a smooth appearance and other times displaying a finely granular appearance.
- **Round (discrete)** cell neoplasms include lymphoma, mast cell tumors, histocytomas and histocytic sarcomas, transmissible venereal tumors (TVT), plasma cell neoplasms, and melanomas. They, as the name suggests, are characterized by their cytologic appearance as discrete, round cells with distinct cytoplasmic margins. Round cells generally have round or oval nuclei that may be centrally (as in lymphoma) or eccentrically (as in plasma cell tumors) placed. Neoplastic mast cells and melanomas may contain variable numbers of granules. Cells obtained from round cell tumors (especially lymphoma samples) are often fragile so care should be taken during slide preparation. While round cells are discrete and do not cluster or aggregate, these tumor types typically exfoliate abundantly, leading to highly cellular samples and tightly packed cells that may appear clustered. Care should also be taken to ensure a monolayer is achieved to ensure accurate cellular evaluation, and slides used to smear samples across other slides may be useful to find areas of lower cellularity for assessment of cell clustering.