CYTOLOGY 101: PRINCIPLES, PRACTICES AND COMMON DIAGNOSES OF CYTOLOGY IN SMALL ANIMAL MEDICINE

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PREPARING & SCREENING SAMPLES TO MAXIMIZE YIELD FROM YOUR CYTOLOGY

Cytology offers a low-invasive rapid diagnostic test for veterinary patients. **Advantages** of cytology include: rapid results, inexpensive (compared with a surgical biopsy), often provides a definitive diagnosis or can narrow the list of differentials, and causes minimal patient discomfort.

**OBTAINING YOUR SAMPLE**

The goal of preparing slides is to have a **cellular, thin preparation of intact cells**.  
**NOTE:** If a lesion is cystic, it is often beneficial to first aspirate off the fluid portion, and then aspirate any solid portion of the lesion left after fluid removal. Both portions should be examined.

**Fine Needle Aspirates**
Techniques vary, but tissue samples are most commonly collected using a 20, 22 or 25 **gauge** needle. If any glass dust/grit is present, dry wipe the slides prior to use. We will be showing you a video of Dr. Birkenheuer in action- he submits cytology that is consistently cellular, intact, and diagnostic.

- **The Aspiration Technique (typically the first approach):**
  - Attach a 22gauge needle to a 6cc or 12cc syringe, break the seal on the syringe.
  - Insert the needle into the tissue- pull back to 1-2cc of negative pressure.
  - Gently poke while redirecting and maintaining steady negative pressure.
  - Remove the syringe and needle, take the needle off and fill syringe to 6cc with air
  - Holding the needle 1/8-1/4” away from the slide expel the sample with one ‘reasonably forceful’ push.
  - Hold the sample slide and lay a second slide across perpendicular to the sample slide.
  - Do not apply pressure, allow gravity to disperse the material.
  - Keep the hand holding the sample slide flexible (like a springboard) and in one smooth motion, sliding forward and with a slight downward pressure slide the material across.

- **The Aspiration Technique with Pulsatile Negative Pressure (for lesions refractory to exfoliation)**
  - Use the technique above but after entry into the tissue apply repeated negative pressure in by pulling back on the plunger in a pulsatile fashion to no more than ~2cc of air.
  - Release the plunger prior to removal from the tissue and use the technique above to expel and spread your sample.

- **Non-aspiration technique (when the first attempt appears very bloody):**
  - Insert a 22gauge needle into the tissue and gently poke while redirecting.
  - Remove the needle, attach a 6cc syringe filled with 6cc of air.
  - Use the technique above to expel and spread your sample.
NOTE: lubricant material and ultrasound gel stain a bright magenta color and can obscure your sample and make diagnosis difficult- minimizing these on the surface of the skin before FNA will help.

ADDITIONAL SLIDE PREPARATION TECHNIQUES

- **‘Drawback and push forward’ or wedge technique:** This is similar to making a blood smear. It works well for all liquid specimens including tissue and mass aspirates if they are very fluid and do not contain visible flecks of tissue (in that case, use a squash technique).
- **Squash technique (slide sandwich technique with pressure applied):** This works well for samples clumps of mucus or cells are present. The sample is placed on one slide and a second slide is placed over it, gentle pressure is applied and the slides are pulled apart similarly to the techniques described above.
- **Touch imprints.** Touch imprints of biopsy specimens can provide preliminary findings and can confirm good sampling of the lesion. Note: touch imprints of ulcerated or draining lesions may contain surface contamination or infection may not be representative of the underlying lesion; if there is a discrete mass that is ulcerated, often an aspirate of the mass portion is needed. **The most common problems encountered with touch imprints are inadequate blotting of the specimen and ‘non-exfoliative lesions’.**
  - Blotting: The cells of interest will often only adhere to the glass if blood and serum are removed. Blot the tissue gently on a paper towel until the towel begins to stick to the tissue. If multiple slides are being made, you many need to reblot often, especially for very bloody tissues such as liver or spleen.
    - For delicate or small biopsy specimens, you may not have enough sample and gently touching a glass slide to the tissue several times may still be useful, especially for bone marrows because they tend to exfoliate very well, even without blotting.
    - For ‘firmer’ samples that may not exfoliate well you can gently roughen the surface by scraping a scalpel blade over it, then making touch imprints

ARE YOUR SLIDES WORTH SENDING OFF FOR CYTOLOGY?

1. **Do they have enough (or any) cells? Are the cells are broken? Is only blood is present?** Is it the ‘right’ tissue?

    **Examine the slide on low power (4x or 10x)** Look for areas with intact cells that are spread out adequately for evaluation- you can use red blood cells if they’re there as a reference- if they’re piled up or condensed you’re cells of interest will be also.

2. **Examine several areas at high power (40 or 50x).**

    If you are using a 40x objective, remember to place a dry coverslip on top of the cytology specimen before you view it- this objective is designed to be used with a coverslip and without one things will look blurry.
A LOGISTICAL APPROACH TO DIAGNOSING LYMPH NODE ASPIRATES

The nice thing about lymph nodes is there are really a very limited number of diagnoses available so it is easy to develop a systematic approach to lymph node evaluation. The three basic findings for lymph tissue are 1) normal or unremarkable 2) reactive/hyperplastic and 3) lymphoma. Additional lymph node findings include inflammation, metastatic neoplasia or non-diagnostic specimens (acellular, aspiration of perinodal fat, salivary tissue).

Step 1: Sizing lymphocytes
The real key to diagnosing normal, reactive and neoplastic lymph tissue is being able to accurately size lymphocytes, so your first step is always to find a measuring stick and then try to identify a small lymphocyte. To do this, you will first need to find a thin area with intact cells – don’t bother looking in the thick regions to size your lymphocytes, you won’t be accurate. There are few helpful measuring sticks- the best measuring stick is a neutrophil. A small lymphocyte will be smaller than a neutrophil, will have coarse, dark chromatin and just a tiny amount of cytoplasm, often visible on only one side of the cell. Can’t find a neutrophil? In a pinch, you can use a well spread out red cell. In dogs, RBCs are approximately the same size or slightly smaller than a small lymphocyte (this won’t hold true in cats or other species who have smaller RBCs). One more measuring stick; the nucleus of a plasma cell is approximately the size of a small lymphocyte. Intermediate sized lymphocytes are approximately neutrophil sized to slightly larger, they have a little more cytoplasm and the chromatin is a little more ‘open’ (dispersed, stains paler and is less clumped). Large lymphocytes are up to 4 times the diameter of a red cell with pale lacy chromatin, a thin rim of cytoplasm and, depending on the level of maturity, may or may not have nucleoli.

NORMAL LYMPH NODE
In normal lymph node, approximately 75-95% of cells are small, mature lymphocytes. The remainder of cells consists primarily of intermediate sized lymphocytes with low numbers of large lymphocytes. Rare plasma cells may be seen; nuclei are round, similar in size to small lymphocytes, are eccentrically located in the cytoplasm and have very coarse chromatin. The cells have moderate amounts of deeply basophilic cytoplasm with a perinuclear clear zone (golgi apparatus). Mott cells are plasma cell distended with large vacuoles of immunoglobulin (Russell bodies). Rare mast cells may be seen. These are large round to oval cells with round, central nuclei and abundant basophilic to purple cytoplasmic granules which often obscure the nuclear detail. Granules may not stain with Diff-Quik. You may see a few histiocytes, other inflammatory cells should be proportionate to the degree of hemodilution present. Lymphoglandular bodies are present in the background. These are platelet sized basophilic fragments of cytoplasm which pinch off of proliferating lymphocytes. While not specific for lymphocytes, they are highly characteristic of lymph tissue.

HYPERPLASTIC/REACTIVE LYMPH NODE
Lymphoid hyperplasia (proliferation of the lymphoid population secondary to antigenic stimulation) and reactivity (expansion of the plasma cell population) can be localized or generalized. Localized reaction can be due to any source of inflammation draining to that lymph node including skin disease,
an abscess, injury or even an inflamed neoplasm. Generalized reactions can occur with rickettsial diseases (RMSF, ehrlichiosis / anaplasmosis), FeLV and FIV or any systemic inflammatory condition.

*One of the first clues that you are dealing with a reactive/hyperplastic lymph node is your observation from low power.* What you will notice is that there is a lot of variation in cell size and chromatin texture/color (dark and coarsely clumped to pale and open) whereas normal and lymphoma nodes are more monotonous appearing from low power. Specifically, *small lymphocytes still predominate but intermediate to large lymphocytes are increased and there may be increased number of plasma cells +/- Mott cells.* Increases in other cell types are variable; you may see increased numbers of macrophages and mast cells. Neutrophils and eosinophils may also be increased but represent less than 5% and 3% of the nucleated cell count respectively (if greater, then node is inflamed). A normal cytologic appearance in a lymph node that grossly is increased in size can also be consistent with hyperplasia.

*Atypical hyperplasia* can occur. In this condition, the predominant cell type is intermediate in size rather than small, and this can be difficult or impossible to distinguish from small / intermediate cell lymphoma (see below). Atypical hyperplasia is seen most often in dogs with Ehrlichiosis but has also been reported in other disease such as Leishmaniasis. *Atypical hyperplasia of the peripheral lymph nodes in young FeLV positive cats is similar and can look like lymphoma – be cautious about diagnosing lymphoma in the peripheral lymph node of a cat, it is very uncommon.*

**LYMPHADENITIS**

This is characterized by *increased numbers of neutrophils (>5%), eosinophils (>3%), macrophages or MNGC* (no set number for these). Name the lesion by the predominant inflammatory component. Reactive hyperplasia is usually present as well. Presence of suppurative or pyogranulomatous inflammation means a thorough hunt for etiologic agents is needed – bacteria, protozoa, fungi, algae. Some neoplasms can induce suppurative inflammation if metastatic to the lymph node (SCC).

**LYMPHOMA**

Lymphoma affecting the peripheral lymph nodes of dogs (ie multicentric lymphoma) is the most common form in dogs (80% of cases). Other forms of lymphoma exist in dogs including craniomediasinal, gastrointestinal, cutaneous, and primary extranodal forms. *Lymphoma of the canine lymph node can be confidently diagnosed when lymphoblasts represent greater than 50% of the cell population.* In reality most canine lymphoma cases have something more like 90% or greater blast cells, which makes it even more straightforward. Lymphoma can also occur in the internal lymph nodes and organs of dogs (see below). *Lymphoma is very uncommon in feline peripheral lymph nodes- be cautious about diagnosing lymphoma in the peripheral lymph node of a cat* (consultation with a pathologist for these samples is recommended).

Blast cells are intermediate to large sized round cells with large round nuclei, delicate chromatin, distinct nucleoli. Nuclei are surrounded by a thin of basophilic cytoplasm that may have fine vacuoles. Cells may have a prominent perinuclear clear zone. These cells look very distinctive from low power: Large pale nuclei surrounded by a dark blue rim of cytoplasm. *Keys to correctly identifying cells as*
blasts are the features of immaturity: visible nucleoli and dispersed/active chromatin (pale, lacy, delicate). Don’t get too hung up on absolute size; even if the cell is not much bigger than a neutrophil, if it has these features, it counts as a blast. Normal intermediate or even slightly larger lymphocytes should have condensing chromatin and should not have prominent nucleoli. Remember that cells other than plasma cells can have a perinuclear clear zone, it only indicates that the cell is actively producing proteins (plasma cells are one of the few fully differentiated cells with a prominent golgi area because they are very active making immunoglobulin). Do not mistake lymphoma for a plasma cell tumor – few plasma cell tumors will have an overtly blastic appearance and plasma cell neoplasms are infrequently found in lymph nodes. Unlike many malignant neoplastic populations, lymphoma tends to be relatively uniform in size (less anisocytosis and anisokaryosis). Lymphoglandular bodies often are increased in the background and may be larger and more basophilic. Mitotic figures (normal or abnormal) may be increased in number.

There are some potential difficulties in the diagnosis of lymphoma. Small / intermediate cell lymphomas are characterized by small to intermediate sized cells that are not overtly blastic.

Alimentary lymphoma in cats is one of the most common examples of small/intermediate cell lymphoma and one of the most commonly occurring cancers of the cat. Cytologic findings of a small to intermediate cell population on FNA of intestines or intestinal lymph nodes in the cat do not rule out indolent lymphoma. Dr. Suter will speak to you about a great solution to help with low-invasive diagnosis using a test called PARR (PCR for antigenic rearrangement) that can be performed on the slides submitted for cytology. Otherwise, small to intermediate cell lymphomas an infrequently occur and may be difficult to differentiate from hyperplasia based on cytology (need histopathology, immunocytochemistry, flow cytometric evaluation, receptor gene rearrangement PCR, etc.).

Immunophenotype (B or T cell) cannot be accurately predicted based on morphology and provides useful information for you and your client as an often important prognostic factor in dogs. This can be determined using monoclonal antibodies specific to B and T cell lineage on formalin fixed tissues, unstained cytology slides or via flow cytometry. Lineage and clonality can also be determined by PCR evaluation for antigen receptor gene rearrangement (PARR) from tissue aspirate or biopsy, or from cells scraped off of a cytology preparation. Hypercalcemia is classically associated with T-cell lymphomas. Most lymphomas affecting the peripheral lymph nodes of dogs are B-cell origin.

METASTASTIC NEOPLASIA
This is characterized by the presence of a foreign cell population (carcinoma cells for example) or high numbers of a cell type that should only be present in very low numbers (mast cells in the lymph node). Metastatic cells may be present in low numbers anywhere on the slide so it is vital that all areas of all slides are examined on low power; a diagnostic cluster can be present anywhere. I have found that metastatic squamous cell carcinoma can be particularly tricky; it is easy to miss a few small clusters or individualized tumor cells if you are not thorough or try and go too quickly. Negative findings do not exclude the possibility of metastatic disease.
OTHER

**Artifacts.** Ruptured cells can appear as a uniform population of intermediate sized lymphocytes; be sure you are evaluating intact cells before making interpretations. Cells in thick areas take up stain poorly making nuclear morphology difficult to assess. Dense/thick regions prevent cells from spreading out making sizing difficult.

**Perinodal adipose tissue** appears similar to a lipoma or normal SQ fat (see lipoma in cutaneous lumps and bumps).

**Salivary tissue.** Salivary gland is commonly aspirated instead of the mandibular lymph node. On cytology, it has a streaming, pink mucinous background with prominent *windrowing of RBCs* (rowing up of cells, due to a thick or mucinous material, commonly seen in salivary aspirates and normal joint fluid, named after the appearance of windrows of field crops such as hay). This is often much easier to appreciate on low power. The glandular epithelial cells *appear as foamy cells with small, round nuclei and abundant pale cytoplasm*. They are often arranged in small clusters (glandular epithelium), but because the cells are delicate, they can easily rupture resulting in ‘naked nuclei’ – don’t mistake these for small lymphocytes! It is important to not mistake salivary tissue for metastatic neoplasia.

**COMMON MISTAKES OR DIFFICULTIES WITH LYMPH NODES**

1. **Selecting too thick of an area for examination.** Similar to examining a peripheral blood smear, you need to choose an area where the cells are spread out enough so you can clearly see individual cells. The individual cells also need to be spread out – sometimes the background in a region is thick and the cells can’t adequately spread and instead appear like little dark bullets; you need to be able to see good nuclear and cytoplasmic detail. If cells are balled up or not spread out, find another region. Unlike a peripheral blood smear, there is no formula for finding the best area – it can be anywhere on the slide so you need to spend time on low power hunting for those regions.

2. **Attempting to interpret ruptured cells.** Make sure you can see both the nucleus and the cytoplasmic border to be sure you are evaluating intact cells. When cells rupture, they swell; the nucleus will stain a lighter purple and will be smudgy.

3. **Not critically evaluating cell size.** To classify lymphoid populations, you need to be able to gauge size, using the measuring sticks mentioned above will help and eventually this becomes second nature.

4. **Trouble distinguishing a predominately blastic population (lymphoma) from a reactive population.** Pay close attention to size AND maturity of the cells (see lymphoma section). Pick good areas – blast cells are delicate and may be easily damaged, they also stain poorly when in too thick of a region making identification of nucleoli difficult.

5. **Differentiating nucleoli from clumped chromatin.** Clumped chromatin is similar in color to the rest of the chromatin but is darker and often is not a distinct shape with a sharp margin. Nucleoli are slightly to significantly different in color (usually more blue vs. chromatin’s purple color) and are a distinct shape. Some lymphomas have very delicate nucleoli, you need to pick good areas to examine.

6. **Rushing to oil.** Once you go to oil, you have to stop and clean the slide in order to go back to 40x – this typically dissuades people from further evaluation. It’s even worse with ‘real’ cytology preps.
because you can’t simply wipe the oil off, you need to either clean the slide in zylene or put a coverslip on top of the oil. For these reasons, oil should be the last step in the process. Utilize the 10x and 40x objectives to identify and examine multiple areas of the slide.

FLOW CYTOMETRY & IMMUNOPHENTYPING
Jonathan E. Fogle, DVM, PhD, DipACVIM

HOW DOES FLOW CYTOMETRY WORK?
Flow cytometry provides a way of analyzing cells based on their size, granularity, and cell surface markers. The table below lists the antigens we generally stain for and the cells they are found on. Using flow cytometry, we can immunophenotype lymphoid cell populations to determine the range of cell types within the population. The diversity of cell types within the population can help determine if the cells are neoplastic or reactive. A population that expresses only one phenotype suggests neoplasia rather than a reactive process. Aberrant surface marker expression (e.g. CD3 but not CD4 or CD8, loss of CD45) also suggests neoplasia. In cases of leukemia, expression of CD34 suggests an acute, not a chronic disease. Canine and feline leukocytes typically display CD45, which is used in conjunction with FSC and SSC characteristics to segregate the lymphocytes, monocytes and granulocytes.

When submitted for flow cytometry (immunophenotypic analysis) a sample is divided into multiple tubes for various controls and subjected to analysis using a “panel” comprised of different combinations of anti-receptor antibodies (e.g. CD4, CD21 etc.). Samples analyzed are typically reported as either a percentage of positive cells (dot plots) or as the mean fluorescence intensity (MFI). The results of this analysis are then interpreted by the flow cytometrist. **We will present several of the most useful clinical examples for you and discuss the immunophenotyping results.** It should be noted that flow cytometry should not be used as the only method of determination of neoplasia. History, clinical signs, and cytology are important and necessary for the laboratory to correctly interpret the flow cytometry results. Additionally, difficult cases may also need analysis for clonality by PARR.

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>ANTIGEN GENERALLY FOUND ON</th>
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<tbody>
<tr>
<td>CD3</td>
<td>T cells</td>
</tr>
<tr>
<td>CD4</td>
<td>T-helper cells</td>
</tr>
<tr>
<td>CD8</td>
<td>T-cytotoxic/suppressor cells</td>
</tr>
<tr>
<td>CD21</td>
<td>Mature B cells</td>
</tr>
<tr>
<td>BS</td>
<td>Some but not all B cells (CD molecule unknown)</td>
</tr>
<tr>
<td>CD14</td>
<td>monocytes/macrophages</td>
</tr>
<tr>
<td>CD34</td>
<td>Hematopoietic progenitor cells; acute leukemias</td>
</tr>
<tr>
<td>CD79a/b</td>
<td>Part of the B cell receptor; Ab stains intracellular component; seen on immature B cells</td>
</tr>
<tr>
<td>CD11d</td>
<td>Macrophages of spleen and bone marrow; LGL CLls</td>
</tr>
<tr>
<td>CD45</td>
<td>All leukocytes (lymphs – bright; PMNs – dim; blasts – dim to negative)</td>
</tr>
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'PARR’- PCR FOR ANTIGEN RECEPTOR REARRANGEMENT

Steven Suter, VMD, PhD, DipACVIM (Oncology)

WHAT IS PARR?
Canine/feline PARR is a PCR-based assay to determine if a population of cells is the result of the clonal expansion of B or T cells, which usually, but not always, implies lymphoid neoplasia. The test utilizes genomic DNA and PCR primers that are specific to the canine V(D)J splice junctions of B and T cell receptor gene segments in lymphocytes. Because a clonal expansion of a population of neoplastic lymphocytes can be PARR positive for both B and T cell rearrangements, PARR should never be used for the lineage assignment of canine lymphoma. Additionally, PARR should never be used as the sole assay to determine if an animal has lymphoma or leukemia. Instead, PARR can be one of the tests used to interpret difficult cases in addition to cytology, immunocytochemistry, flow cytometry, history, and clinical signs. The main advantage of PARR is the ability to differentiate a monoclonal/oligoclonal or neoplastic lymphoid proliferation from a reactive and polyclonal or pseudoclonal proliferation. The PARR assay can be run on fresh lymph node aspirates, bone marrow aspirates, cellular effusions, blood, cells scraped from cellular cytology slides, formalin fixed tissues (25 micron sections), and fresh frozen tissues (25 micron sections kept at -80 degrees).

WHEN IS PARR MOST USEFUL?

Feline PARR: An aid in the diagnosis of irritable bowel disorder versus low grade alimentary lymphoma in cats.

Alimentary lymphoma is one of the most commonly occurring cancers of the cat and the incidence of this disease has increased significantly over the past decade. Despite the prevalence of this disease, appropriate diagnosis and treatment can present a major challenge to veterinarians. There are two main forms of feline alimentary lymphoma which include the most common small-cell lymphocytic, well-differentiated, low-grade phenotype and the large-cell, lymphoblastic, high-grade phenotype. It can be difficult to differentiate low grade lymphoma from inflammatory bowel disease, even in the face of full thickness biopsies. The algorithm for distinguishing between alimentary inflammatory versus neoplastic disease in the cat often includes abdominal ultrasound, fine needle aspirates/cytology, endoscopic or full thickness biopsies/histopathology, and immunophenotyping (with lymphocyte markers on tissue or flow cytometry samples obtained from aspirates). There has been increased recognition of the value to add the Feline Polymerase Chain Reaction for Antigenic Rearrangement (PARR) to this algorithm. Feline PARR is a PCR test for a clonal population of cells which is supportive of a neoplastic population. The sensitivity of feline T cell PARR is reported to be ~89%. This offers an extremely sensitive means of detecting a clonal population of T cells that is supportive of alimentary lymphoma. Feline B cell PARR is approximately 60% sensitive. Cats presenting with an indolent disease course with clinical symptoms of irritable bowel disorder are the largest target population that PARR may benefit. PARR can be performed on cytology samples offering an initial low invasive diagnostic tool. PARR can also be performed on histopathology samples when more invasive diagnostics are required. Increasing awareness about which cases this test is likely to help as a diagnostic tool, which samples to submit and how to process them will aid in the diagnosis of irritable bowel disorder versus low grade alimentary lymphoma in cats.
Although immunophenotype of feline gastrointestinal lymphoma varies in the literature a recent publication characterizing feline gastrointestinal lymphoma reported a majority of T-cell origin. (Moore PF, Rodriguez-Bertos A, Kass PH. Vet Pathol. Feline Gastrointestinal Lymphoma: Mucosal Architecture, Immunophenotype, and Molecular Clonality. Vet Pathol 2011Apr 19) 89% of 103 cases were of T-cell origin (predominantly small cell), 11% were of B-cell origin (predominantly large cell/lymphoblastic) and PARR in that study detected 90% of the T-cell lymphomas.

**CANINE PARR**

In dogs, PARR has a reported sensitivity and specificity of approximately 90%.

Useful applications include:

- Immunophenotyping* when flow cytometry is not accessible or definitive
- Supportive evidence of lymphoid origin when a cancer is ‘anaplastic’ (ie difficult to tell who the cell really is)
- Distinguishing histiocytic disease from LSA
- Confirming a neoplastic plasma cell population when cytology is not definitive

**IMMUNOPHENOTYPE OF CANINE LYMPHOMA IS IMPORTANT**

Most lymphomas affecting the peripheral lymph nodes of dogs are B-cell origin. Prognosis of lymphoma in dogs depends on several factors, clinically the following parameters are commonly used to assess prognosis: Phenotype, B-cell phenotype has a better prognosis than T-cell (B is bad, T is terrible). Stage of disease. The stage of lymphoma in dogs has a modest association with prognosis. Staging is based on tissues involved and is given a Roman number (I-V). The stage is followed by the letter a or b to indicate absence (a) or presence (b) of clinical signs. Stage Ia is the best, stage Vb is the worst. Most dogs are diagnosed at stage III, IV or V. Stages are as follows:

- Stage I: Involvement is limited to a single node or lymphoid tissue in a single organ (not bone marrow)
- Stage II: Involvement of many lymph nodes in a regional area (with or without tonsils)
- Stage III: Generalized lymph node involvement
- Stage IV: Liver and/or spleen involvement (with or without stage III disease)
- Stage V: Manifestation in the blood (neoplastic cells in circulation) and involvement of bone marrow and/or other organ systems (with or without stages I, II, III or IV disease)

Initial response to treatment. Dogs that respond positively to initial induction have a better prognosis than those who do not respond well. Many dogs who respond well show significant improvement of clinical signs within 3-5 days of induction. The median survival time for stage IV or V B-cell lymphoma in dogs on multiagent chemotherapy protocol is 12-14 months, while the mediate survival for dogs with stage IV or V T-cell lymphoma on multiagent chemotherapy protocol is 6-9 months.

**Prognosis in cats** is more difficult because of the wide variation in tissues affected. Overall, compared with dogs, cats are less likely to respond to treatment, are less likely to have high remission rates and tend to have shorter survival periods. In general, important factors in prognosis for lymphoma in cats include: 1) Response to treatment- cats that have a complete response have a better prognosis vs. those that have a partial response, 2) FeLV status- positive FeLV status has a worse prognosis vs.
negative status, and 3) **Clinical stage-** early clinical stage is better than late clinical stage. Stages are as follows:

- **Stage 1:** A single extranodal tumor or one nodal area
- **Stage 2:** A single extranodal tumor with regional lymph node involvement OR two or more nodal areas on the same side of the diaphragm OR two extranodal tumors on the same side of the diaphragm with or without regional lymph node involvement OR a resectable primary GI tumor with or without associated mesenteric lymph node involvement.
- **Stage 3:** Two single extranodal tumors on opposite sides of the diaphragm OR two or more nodal areas above and below the diaphragm OR any primary, extensive, unresectable, intra-abdominal disease OR any paraspinal or epidural tumor
- **Stage 4:** Stage 1, 2, or 3 with liver and/or spleen involvement
- **Stage 5:** Stage 1, 2, 3 or 4 tumor or tumors with initial involvement of the CNS and/or bone marrow.

- **Anatomic location.** Mediastinal lymphoma in FeLV positive cats has the worst prognosis, only 2-3 months with chemotherapy. Nasal lymphoma has the best prognosis with median survival times approaching 1.5 years with local radiation treatment. Renal lymphoma has a shorter survival time of 3-6 months with chemotherapy. Doxorubricin. Addition of Doxorubricin to the chemotherapy protocol is associated with better survival times. NOTE: Phenotype (B or T) is NOT a prognostic indicator in the cat.


- **103 (89%) T-cell lymphoma**
  - 84 mucosal (small cell)- median survival 29mos.
  - 19 transmural- median survival 1.5mos.
    - 9/19 = lymphoblastic
  - all largely confined to SI
  - **PARR detected 90% of T-cell cases**

- **19 (11%) B-cell lymphoma**
  - 19 transmural- median survival 3.5mos.
  - most lymphoblastic (a cytologic diagnosis!)
  - stomach, jejunum, ileoceco-colic junction

**CYTOLOGY SLIDE SAMPLES SUBMITTED TO NCSU-VHC CYTOLOGY SERVICE MAY BE SUBMITTED DIRECTLY FOR PARR. DETAILED SUBMISSION INFORMATION IS AVAILABLE AT THE END OF THE NOTES SECTION OR AT:**

**CYTOLOGY:** [http://www.cvm.ncsu.edu/vhc/csd/Cytology.html](http://www.cvm.ncsu.edu/vhc/csd/Cytology.html)

**PARR & FLOW CYTOMETRY:** [http://www.cvm.ncsu.edu/dphp/labs/clinicalimmunologylab.html](http://www.cvm.ncsu.edu/dphp/labs/clinicalimmunologylab.html)

Googling ‘NCSU Cytology’ or ‘NCSU PARR’ works too!
COMMON LUMPS & BUMPS IN SMALL ANIMAL PRACTICE

Epidermal and follicular cysts
These cysts are very common, non-neoplastic lesions located in the dermis of dogs or cats and may represent up to a third of all non-neoplastic tumor like masses from dogs. They can be fluctuant or firm, and are typically smooth and well circumscribed. The dorsum and extremities are common locations. The cyst is lined by mature squamous epithelial cells; keratinized epithelial cells and cellular debris accumulates in the center. **On cytologic examination, they consist of mature, fully keratinized squamous epithelial cells and cell fragments.** Mature keratinized squamous cells are angular, flat, and basophilic cells that have lost their nuclei during maturation. **From low power, these aspirates have been described as looking like ‘blue cornflakes’ scattered on the slide.** The cytoplasm of the cells and cell fragments exhibit evidence of keratinization which is characterized by thick/dense or ‘waxy’ appearing cytoplasm and a distinct pale to deep ‘robin’s egg’ or ‘sky’ blue color. A background of keratin debris may be seen. Cholesterol crystals may also be present. **Rupture or trauma will induce intense pyogranulomatous inflammation because keratinized cells induce a foreign body type response.** Whenever I have pyogranulomatous inflammation from a mass-like lesion in a dog, I always look for clumps of keratin to determine if it likely a ruptured follicular or epidermal cyst. **Cytologically these lesions appear similar to epithelial tumors with follicular differentiation thus both differentials are applicable. They are all typically benign and excision is curative.**

Tumors with follicular differentiation
**Similar to epithelial and follicular cysts, tumors with follicular differentiation can have large accumulations of keratinocytes and keratin debris.** There are several different types of follicular tumors (some people call them adnexal tumors) with trichoepithelioma and pilomatrixoma being most common. Typically, they are benign although malignant forms do rarely occur. These tumors are usually hairless, raised, well circumscribed and may be ulcerated. **Tumors and cysts can have a similar cytologic appearance and many clinical pathologists will bottom line all of them as ‘epithelial inclusion cyst’, but it should be understood that histopathology is necessary to distinguish cysts from tumors and to accurately identify tumor type.** The primary feature is the same as for cysts; the presence of mature squamous epithelial cells and cell debris.

You may also see ‘ghost cells’; keratinized epithelial cells with an empty hole in the center where the nucleus used to be. This indicates a special type of keratinization that epithelial cells of the hair bulb undergo as they form the hair shaft (matrical keratinization), so finding lots of these indicates you either have a follicular cyst or a tumor with follicular differentiation. When I see large numbers of ghost cells, I favor tumor. Ghost cells can be individualized or they can pile up together forming crude linear structures like an attempt at forming a hair bulb, often they have a sort of honeycombed appearance because of the empty nuclear spaces. With follicular tumors, you may also see small amounts of uniform basilar epithelial type cells or sebaceous cells if the wall of the tumor is aspirated.

**Rupture or leakage of follicular tumors or follicular/epithelial cysts will produce intense suppurative to pyogranulomatous inflammation.** Look for islands of mature squamous cells amongst the inflammatory cells.
**Basal cell tumor**

The tumor that for years been called ‘basal cell tumor’ has now been reclassified; ‘basal cell tumor’ as a specific tumor type is no longer recognized in dogs (but is still recognized in cats). Instead, what was previously called basal cell tumor on cytology is now recognized to be a group of different tumors with overlapping features including, trichoblastomas in dogs (tumor of the hair bulb) formerly basal cell tumor in dogs), sweat gland tumors, basal cell tumors in cats (benign tumor of basal cells in cats), and less commonly basal cell carcinoma (basal cell carcinomas are low grade tumors – locally invasive but with low metastatic potential, good prognosis with removal).

Differentiating these tumor types typically requires histopathology. This is why most clinical pathologist will bottom line a cytology composed primarily of basilar appearing cells as “cutaneous basilar neoplasia” (or similar terminology), but some will still use ‘basal cell tumor’ as a more general term. **They are all typically benign and excision is curative.**

Basal cell tumors (cats) and trichoblastomas (dogs) are the most common skin tumor of cats and are also common in dogs. These tumors are usually benign or very low grade. Typically, they present as a solitary dome shaped, firm, hairless mass. They are freely movable but firmly adhered to the overlying skin. Common sites in dogs and cats include the head, neck and shoulders.

**CYTOLOGICALLY:**

These tumors are classically epithelial in appearance and arranged in **tight clusters with almost no individualized cells seen.** Clusters are often extremely dense and very basophilic. Cells have a relatively **uniform** appearance, cuboidal shape, and **scant amounts of deeply basophilic cytoplasm** that may occasionally be pigmented. **Some can have cystic portions.** Aspiration of these regions results in a basophilic background with scattered cellular debris. Often cholesterol crystals or melanin granules are present, and you may or may not have scattered clusters of basilar epithelial cells. **If you find you have aspirated fluid, you should remove as much of the fluid as possible, then try aspirating any remaining tissue mass.** You may see small regions of sebaceous or squamous differentiation – individualized or small clusters of sebaceous cells or foci of keratinized epithelial cells. Meibomian gland adenoma of the eye may look like a basal cell tumor with sebaceous differentiation

**Difficulty: Other tumors can, on cytology, appear similar to basal cell tumors (i.e. appear to be composed of uniform clusters of basal epithelial cells), so it is important to keep in mind other potential tumors as well. Location should help tip you off as to when to be more cautious- ie we have seen sebaceous epitheliomas (a kind of sebaceous tumor with a lot of basal cells) and sweat duct tumors which both typically benign) that look ‘basilar’. And we have also seen salivary ductular carcinomas and mammary tumors (that can be malignant) that look ‘basilar’ but location should tip you off to be cautious (ie associated with a particular structure like the mammary gland or salivary gland).**

**Sebaceous hyperplasia**

This is a common condition in older dogs and can also be seen in cats (females more than males). It represents a hyperplastic response of the normal sebaceous epithelium. Classic locations are on the
head and dorsum. In cats, it’s common to find lesions at the tail base. Typically the growths appear as a raised, hairless lesion with a cauliflower appearance or as an intradermal multilobulated mass. Grossly, the sample may appear oily or greasy when unstained. **Cytologically, this lesion is composed of uniform and tightly cohesive clusters of sebaceous epithelial cells.** Cells have moderate to abundant, highly vacuolated/foamy cytoplasm and small central nuclei. There may be small numbers of more basal-appearing cells (reserve cells). These are the cells which are waiting to differentiate into sebaceous cells. The background of the slide is often very vacuolated from sebaceous material released by ruptured cells. Cytology alone can’t differentiate sebaceous hyperplasia from sebaceous adenoma – this is generally of little significance as adenomas have similar clinical features and a benign behavior Meibomian gland adenoma of the eye may look similar cytologically.

**Sebaceous adenoma**
Behavior and cytologic appearance are similar to sebaceous hyperplasia. Cytology alone can’t differentiate sebaceous hyperplasia from sebaceous adenoma – which is usually of little significance as adenomas have similar clinical features to hyperplasia and usually a benign behavior. Meibomian gland adenoma of the eye may look similar cytologically. Finding high numbers of basilar type cells may indicate the tumor is a sebaceous epithelioma or one of a number of other tumors featuring basal cells with regions of adnexal or squamous differentiation. These are also still typically benign and excision when needed is curative.

**Lipoma**
Lipomas are particularly common in older dogs who may develop multiple tumors, but can also be seen in cats. Liposarcomas do occur but can be difficult to distinguish from other types of sarcomas or even neuroendocrine tumors; these tumors should have clearly malignant features. Lipomas present as soft to firm, well-circumscribed, freely movable subcutaneous masses; occasionally they can be in deeper tissues and be less movable, more fixed. **Dogs can also develop infiltrative lipomas.** These are also composed of well differentiated adipose tissue, but can infiltrate and cause destruction of muscle and connective tissue. NOTE: other tumors can be grossly mistaken for a lipoma, I’ve seen this most often with MCT and hemangiopericytomas. Grossly, with lipomas the slides appear greasy and do not dry. **The adipocytes appear as clusters of very large signet ring shaped cells with abundant pale pink cytoplasm.** These cells are easy to see on 10x because of their size. **Normal subcutaneous or perinodal adipose tissue looks the same.**

**Alcohol fixatives used in Romanowsky-type stains will dissolve away any free lipid and may clear away cells as well. You can skip the fixative and stain the slides with only the red and blue portions of quick stains** – but this can lead to contamination of your stain solution.

**Squamous cell carcinoma**
In dogs, common sites of SCC include the head, distal extremities (nail beds), ventral abdomen, and perineum. It’s the second most common oral tumor of dogs (first is melanoma). In the oral cavity, a rostral location has a better prognosis (usually benign), whereas a caudal location is grave, especially if sublingual or tonsillar. SCC of the nasal planum is very bad. In cats it is associated with chronic solar
injury in animals with white hair. Common sites include ear pinnae, frontal ridges, eyelids, nose, and lips. It’s the most common oral tumor in this species and carries a poor prognosis in this location.

Often these tumors are ulcerated and accompanied by suppurative inflammation +/- a secondary bacterial infection. Cells can be found in sheets or, quite commonly, as individualized cells or small clusters of cells. Sometimes you will see angular shaped cells that are reminiscent of squamous cells of the normal skin. You may find a few tad pole shaped cells – cells with a ‘tail’ of cytoplasm giving them a tadpole-like shape. Well differentiated tumors will show evidence of keratinization – dense or waxy cytoplasm with a characteristic ‘robin’s egg’ or ‘sky’ blue color of varying intensity.

Remember, not all cells within a tumor will be noticeably keratinized. Fine, perinuclear vacuolization is common. Emperipolesis is often seen - when one cell crawls into another cell’s cytoplasm. Here it is neutrophils migrating into the cytoplasm of neoplastic squamous cells. This is not specific for SCC, but it is one of the tumor types where I most often see it.

**There are some difficulties with diagnosing SCC.** Cells of this particular tumor type may be highly individualized and can be mistaken for mesenchymal or round cell tumors. The key is to search for small clusters with definitive cell-cell junctions and recognizing evidence of keratinization. Well differentiated tumors with inflammation can be difficult to distinguish from dysplastic changes induced in non-neoplastic cells secondary to chronic inflammation or irritation. If you have a lot of inflammation without really strong criteria of malignancy, be cautious. Other types of tumors can also keratinize (thyroid, mammary, transitional cell, papillomas, lung tumors, etc.), so don’t jump on SCC as a diagnosis if it is in an atypical location or the presentation is atypical.

**Perianal gland adenoma**

This tumor is most common in intact, male dogs (may actually be hyperplasia in these guys – over 90% of intact dogs are cured by castration plus mass removal, castration alone can result in partial tumor regression) and is rare in cats. It is also rare in intact females as estrogens tend to suppress tumor growth while androgens support it. It is sometimes referred to as a ‘hepatoid tumor’ or ‘hepatoid cells’ because they bear a remarkable resemblance to hepatocytes. Malignant tumors are uncommon and usually are associated with greater cellular pleomorphism, although well differentiated, malignant or invasive tumors have been reported.

Perianal gland adenomas usually appear as slow growing (months to years), raised, smooth to lobulated masses near the anus but may also be found anywhere along the mid-line of the dog, from nose to tail with the most common non-perianal regions being the underside of the tail, perineum, prepuse, and caudal thighs. They may ulcerate and become infected if traumatized.

These tumors are distinctly epithelial and are characterized by very cohesive clusters of relatively uniform, large, oval to polygonal cells with round, somewhat eccentric nuclei. Individualized cells are uncommon. They have very characteristic cytoplasm: it is abundant and finely granular/mottled, pink and blue. NOTE: overstaining with the blue portion of Diff Quik can result in loss of the pinkish granular/mottled quality. You may see a small numbers of reserve cells (basilar type cells) flattened around the periphery of the large ‘hepatoid’ cell clusters. These tumors are well
vascularized, you will usually have a background of blood. These are benign tumors but can be difficult to control locally and excise completely in the perianal/perineal region—early excision is helpful.

**Apocrine gland adenocarcinoma.**
This tumor is most commonly seen in older dogs but is rarely seen in cats. It is a **highly malignant tumor that arises from the apocrine glands of the anal sac and they often metastasize to regional lymph nodes even when the tumor is grossly small (<1cm); anywhere between 46-96% have already metastasized at the time of clinical detection.** Grossly, it is a firm subcutaneous tumor firmly attached to the anal sac, rectal exam may be necessary to detect it, especially as many have inward, rather than outward, growth. Metastasis to the regional lymph nodes (sublumbar or inguinal) is common; more distant metastasis can occur late in disease. Hypercalcemia is found in 25-50% of cases due to PTHrP production. Initial clinical signs (polyuria and polydypsia, constipation) are often related to the hypercalcemia rather than the tumor mass. Monitoring for the return of hypercalcemia after surgical resection can be used to screen for recurrence. Prognosis is generally poor, but with proper clinical management and early detection, affected animals may live several years.

Although considered a carcinoma, this tumor has a **classically neuroendocrine appearance** on cytology—loosely cohesive clusters of cells with round nuclei and indistinct cell margins. Cells are easily ruptured resulting in numerous ‘naked nuclei’ in the background. Loose acinar or rosette-like arrangements may be seen within clusters. **Often apocrine gland adenocarcinomas can look deceptively uniform with little anisocytosis or anisokaryosis seen.** They have round nuclei with delicate chromatin and may have small indistinct nucleoli. Typically they have a small amount of pale blue cytoplasm which may be finely vacuolated.

**Soft tissue sarcoma**
Sarcomas will have features of mesenchymal cells and varying criteria of malignancy. Some tumors will aspirate well, other will follow the rules for mesenchymal cells and aspirate poorly. A soft tissue sarcoma is simply a sarcoma arising from the soft tissues—this is an umbrella term covering a variety of tumors that can arise in the tissues. Although there are a variety of different sarcomas that can be grouped in the soft tissue sarcoma camp, distinguishing between them is difficult cytologically as various tumors can have similar features—**some soft tissue sarcomas warrant histologic evaluation to better determine tumor type and grade.** Tumor grade is directly correlated with prognosis. Grading is done on histopathology sections and depends heavily on mitotic index (more mitotic figures is bad). Grading of soft tissue sarcomas is on a scale from 1-3. Approximately 10-15% of grade 1 and 2 tumors and 40% of grade 3 tumors will metastasize. Grades 1 and 2 carry a good prognosis with surgical and/or radiation treatment.

In dogs, the most common soft tissue sarcomas (in order of frequency) are **hemangiopericytoma** (not related to hemangiosarcomas), **fibrosarcoma** and **peripheral nerve sheath tumors**, but there are many other types. Definitive identification requires biopsy and histopathology.
In cats, the most common soft tissue sarcomas are fibrosarcomas and injection site sarcomas, although other types of sarcomas do occur. Fibrosarcomas tend to develop on the head and neck. In young cats, they are associated with FeLV and FSV. Injection site sarcomas develop in cats primarily at sites of previous vaccination, but development following other types of injections (steroids) has been reported. Some studies incriminate the aluminum adjuvant in the FeLV and rabies vaccines, but whether or not vaccines without adjuvant are safer is still unclear. Current guidelines for vaccination are to use the rear legs of cats since amputation of the limb is possible if a sarcoma develops. Current recommendations for treating vaccine reactions as given by the Vaccine-Associated Feline Sarcoma Task Force follows the 1, 2, 3 rule; treat if 1) the mass is increasing in size more than 1 month after vaccination, 2) the mass is greater than 2 cm in diameter, or 3) the mass is still present more than 3 months after vaccination.

It is important to be able to distinguish a vaccine reaction from an injection site sarcoma. Vaccine and other injection site reactions typically have a mix of inflammatory cells predominated by neutrophils and macrophages. Vaccine reactions, in particular, can have a prominent lymphoplasmacytic inflammatory component as well. Reactive fibroblasts, endothelial cells and histiocytes can mimic neoplastic sarcoma cells; be cautious when you see suspicious cells on a background of inflammation. Acute vaccine reactions may also contain adjuvant material. This material is pink to magenta, and coarsely clumped. It can be found extracellularly or within macrophages and looks similar to ultrasound gel or lubricant material.

Diagnosis sarcomas can have some difficulties. Sarcomas can be confused with granulation tissue or granulomatous inflammation – be cautious with inflammation and look for very strong criteria of malignancy or biopsy. Sarcomas and poorly differentiated carcinomas can appear similar – a tumor this poorly differentiated should be overly malignant in appearance and behavior. Sometimes with cytology all you can say is ‘malignant neoplasia’ and you may not be able to tell what kind. Inflammation can induce fibroblast and endothelial cell proliferation, be cautious interpreting pleomorphic mesenchymal cells present in inflammatory lesions unless multiple strong nuclear criteria of malignancy can be established. Benign tumors such as hemangioma or fibroma can be mistaken for a well differentiated sarcoma or reactive cells. Some carcinomas induce a marked scirrhous response – if only this portion of the lesion is aspirated it could be confused with a mesenchymal neoplasm.

Histiocytoma
This is a very common tumor of young dogs (12-14% of all skin tumors) and the origin is the Langerhan cells of the epidermis (histiocytic cells). They tend to form on the front half of young dogs, especially the head and ears, and many will spontaneously regress within 3 months. Although it is classic in young dogs, I have seen them in dogs of all ages and sometimes dogs will form multiple tumors. The classic appearance is a small, hairless, dome-shaped lesion which may be ulcerated, but I have seen them described as ‘plaque-like’. On cytology, the tumor is composed of individualized round cells with variably well defined cell borders (sharp to indistinct) with variably shaped nuclei: round, oval or slightly indented (like a bin of potatoes). Nuclei have fine chromatin, sometimes nucleoli are present but are usually indistinct. Cells have moderate amounts of pale staining.
cytoplasm; the outer edges of the cytoplasm often stain lighter than the perinuclear area, and the edges may be ruffled in appearance. NOTE: you will only see the ruffled, paler edge if you are in a thin region where cells can spread out. Another characteristic feature, which you may see, is the presence of small lymphocytes scattered in the background; this usually heralds the onset of regression. Histiocytoma cells are usually relatively uniform (little anisocytosis or anisokaryosis) and pale, and it is uncommon to find binucleated or multinucleated cells. Occasionally, it is difficult to distinguish a histiocytoma from a plasma cell tumor.

Mast cell tumor
This is a very common skin tumor of dogs and cats. The classic feature is finding many mast cells, in sheets or individualized. MCT represents 16 to 21% of all canine skin tumors. Approximately 50-60% form on the trunk and 25% on the limbs. Boxers, Boston terriers, Labrador retrievers, beagles and schnauzers are at increased risk, although boxes typically develop low or intermediate grade tumors. Tumor grade, as assessed by histopathology, is highly correlated with biologic behavior. There are three grades: high (undifferentiated, grade III), intermediate (grade II) or low (differentiated, grade I). In general, mast cells that are uniform in size with uniform nuclei, are well granulated and have small uniform granules are more likely to be low grade. The population is very uniform with no bi-, multinucleated or giant cells noted. Poorly differentiated cells can be highly pleomorphic with multiple criteria of malignancy (giant forms, bi or multinucleated cells, marked anisocytosis and anisokaryosis, visible nucleoli, etc.). They also tend to have fewer granules (and occasional no or very few granules) and the granules are larger and more variably sized. Although cytologic characteristics may suggest low or high grade, it’s important to remember that one of the criteria used in grading is invasion of surrounding tissues which can’t be assessed on cytology thus histopathology is always required. Less than 10% of low grade tumors metastasize while 55-96% of high grade tumors will metastasize. Preputial, scrotal, subungal, oral and mucocutaneous junction sites carry a higher grade and worse prognosis. Low grade tumors are slow growing, solitary, ‘rubbery’ and may be hairless but usually don’t ulcerate. Undifferentiated tumors are fast growing and more prone to ulceration. Subcutaneous MCTs appear similar to lipomas and can be mistaken for them. After palpation or manipulation, you may see Darier’s sign: Erythema and wheals which form due to release of vasoactive amines from the granules.

Clinical grade is also associated with prognosis. Grading is by Roman numeral from I to IV with designation of absence (a) or presence (b) of systemic signs. Stage Ia is best, stage IVb is the worst. Staging is as follows:
- Stage I: One tumor confided to the dermis without regional lymph node involvement
- Stage II: One tumor confided to the dermis with regional lymph node involvement
- Stage III: Multiple dermal nodules; large infiltrating tumors with or without regional lymph node involvement
- Stage IV: Any tumor with distant metastasis, including blood or bone marrow involvement.

Mast cell tumors are the second most common skin tumor of cats (20% of all skin tumors). Most cutaneous MCTs in cats are behaviorally benign. There is a grading system that is different from dogs which divides feline tumors into compact (well-differentiated) or diffuse (anaplastic); most are
compact. Visceral (splenic or intestinal) MCT is much more common in cats vs. dogs and is much more likely to result in widespread dissemination to liver, visceral lymph nodes, bone marrow, lung and intestines. With splenic forms, there may be long term survival with splenectomy, even in the face of widespread dissemination. The same is not true of intestinal forms – this carries a grave prognosis. Cutaneous MCTs appear as solitary, raised, hairless, well circumscribed dermal nodules or they can be flat and plaque-like and resemble eosinophilic plaques. Some ulcerate. They are most commonly seen on the head (often involving the pinna at the base of the ear) and neck followed by the trunk and limbs. In horses, cutaneous MCTs are considered benign.

On cytology, sheets of round cells are observed. Well differentiated cells are filled with fine purple (metachromatic) granules that often obscure the nucleus, nuclear staining is often poor (they often appear pale blue- the granules hog all of the stain..). Granule color depends somewhat on the stain used; Diff Quik tends to produce somewhat bluer granules, especially if you are heavy handed with the blue stain. Poorly differentiated cells can be highly pleomorphic with multiple criteria of malignancy and lower numbers of larger granules. Eosinophilic inflammation often, but not always, is present; it is common in dogs and horses and uncommon in cats. Reactive mesenchymal cells and collagen fibers (collagenolysis) may be seen. Fibroblasts can be quite prominent in some canine tumors. In cats, small well differentiated lymphocytes may be seen and in some cats with multiple tumors, the cells may resemble poorly granulated histiocytes.

Quick stains (Diff Quik) may not stain mast cell granules. If you suspect MCT but are not seeing granules, try a wet-mount prep with new methylene blue, it will sometimes show granules that fail to stain with quick stains. Occasionally, MCT and granular lymphoma can be difficult to distinguish from each other; both can occur in internal lymph nodes and organs, and MCT cells with scant cytoplasm and few granules can be confused with granular lymphoma. If you are concerned about this, have the slides evaluated by a veterinary clinical pathologist and consider doing additional diagnostic tests (biopsy, PCR for antigen receptor rearrangement [PARR] to detect clonal lymphoid populations, etc.).

Plasmacytoma
This is an uncommon tumor in dogs and rare in cats. In dogs, it tends to form on the lips, ears and digits. This is typically a benign tumor in dogs, but the literature suggests it may be a more aggressive tumor in cats. Cells may be well differentiated and easily recognized as plasma cells or can exhibit prominent atypia. Cells are round with deeply basophilic cytoplasm that tends to stain darker at the margins and paler in the perinuclear zone. The pale perinuclear clear zone is variably prominent. Nuclei are eccentric with coarse chromatin, and are usually round but rarely may be bean or sickle shaped. Binucleated cells are a common and consistent feature. Low numbers of multinucleated cells are also fairly common and typically correspond to aspiration of the center of the tumor where the more pleomorphic cells are found. Pleomorphism in canine cutaneous tumors has not been shown to correlate with metastatic potential.

Lymphoma
Cutaneous lymphoma may be primary or, rarely, part of disseminated disease. Cutaneous lymphoma may be solitary or generalized and is classified as epitheliotrophic or non-epitheliotrophic. Extracutaneous involvement can occur. In dogs, T-cell lymphoma is more common. Appearance of
the lesion can be highly variable and can appear as nodules, plaques, ulcers and erythemic or exfoliative dermatitis. Disease is often chronic but overall prognosis is poor. In cats, epitheliotrophic forms are typically T-cell and non-epitheliotrophic forms are B-cell. Lesions often appear as crusty plaques and may be pruritic.

**Primary skin tumors can have a more bizarre appearance compared with lymphoma arising in lymph tissues and can also have a waxing and waning clinical course.** Cells can vary from relatively small to large. Nuclei may be round, indented or even cerebriform. Nucleoli may be indistinct or prominent. Consider lymphoma if you have an odd looking round cell tumor of the skin that doesn’t fit any of the other common round cell types. The presence of significant numbers of lymphoglandular bodies (cytoplasmic fragments) should increase your suspicion of lymphoma whenever observed in non-lymphoid tissues or organs, including peripheral blood!

**ADDITIONAL TUMORS & LESIONS...**

**Melanoma**

Melanoma is a tumor of melanocytes and in dogs and is uncommon in cats. In the dog, this is a common tumor of the skin and oral cavity. Malignancy is based on location, histology and breed; malignant tumors carry a guarded prognosis. Tumors of haired skin are usually benign (85%). Locations associated with malignant behavior include mucocutaneous junctions (90% malignant), oral cavity (90% malignant) and nail bed (50% malignant). Finding a high mitotic index on histology is predictive of malignant behavior. Doberman pinchers and miniature schnauzers are more likely to have benign tumors (75% benign); miniature poodles are more likely to have malignant tumors (85% malignant). In the cat, histologic assessment does not appear to have the same predictive value as in dogs. In general, ocular tumors behave worse than oral tumors, and skin tumors can be either benign or malignant. In gray horses, melanomas are common. They form primarily on the tail base and perineum, usually starting at around 4 years of age.

**Cytologic appearance of melanomas can be highly variable- they are known as the ‘great pretender’ or the ‘great imitator’.** Cells can be epithelioid, spindloid, or discrete/round. The most common forms seen are epithelioid (looks epithelial) or discrete/round (cells are individualized and round). **Poorly or non-pigmented forms can easily be mistaken for other tumor types thus this tumor.** Pigmentation varies both from tumor to tumor and within tumors. Well differentiated cells have abundant, fine melanin granules which stain green-black to brown-black to golden brown to blue. Heavily pigmented cells may have their nuclear features obscured – cells look like solid aggregates of pigment +/- a pale spot where the nucleus sits. Poorly differentiated tumors may have only a few cells with a very fine dusting of melanin. Even in well differentiated, well pigmented tumors, cells from the deeper margins tend to be less well granulated. Features of malignancy will vary. In locations where tumors are more likely to be malignant, malignancy should be presumed, even if cells are uniform, well differentiated and lack overt criteria of malignancy. In locations where tumors are typically benign, finding criteria of malignancy on cytology increases the likelihood of malignancy.
There can be difficulties in diagnosing melanomas. Melanophages can be mistaken for melanocytes, especially in lymph nodes. A melanophage has an oval, well-behaved nucleus with coarsely clumped chromatin, and melanin tends to be aggregated in coarse clumps contained within phagolysosomes. Melanocytes tend to have fine granules evenly dispersed in the cytoplasm. Poorly pigmented melanomas can easily be mistaken for other tumor types, although most will, upon close inspection, have at least a few cells with a slight dusting of melanin.

Mammary tumors
Canine mammary tumors can be composed of a variety of elements including mesenchymal and epithelial cells, myoepithelial cells and matrix and they may all be in the same tumor! Tumor type depends a lot on what is present. Because cytology specimens may not sample all of the elements that are present, accurately predicting exact tumor type is difficult. It is also difficult to determine benign vs. malignant. Using cellular criteria of malignancy, about 50% of canine tumors would be classified as malignant. But the problem is that about half of those will not exhibit a malignant clinical course of behavior (invasiveness and metastasis). So how do we predict which will be bad actors? Currently, the best predictor of malignant behavior is assessment of the tumor for evidence of tissue invasion; using this criterion in conjunction with tumor type, about 25% of tumors are classified as malignant and this correlates fairly well with clinical course of behavior. So, are there any rules we can use for cytology? In general, the more criteria of malignancy that are seen, the more likely it is be malignant, but remember that some benign tumors can have some pretty ugly looking cells in them. The reverse can also be stated; the more uniform the cells are, the more likely it is to be benign, but histopathology should still be done. So is cytology even useful for canine mammary tumors? It is useful for confirming a mammary tumor vs. some other diagnosis and it can be used to screen for metastatic disease, but I would advise histopathology over cytology if you know you are dealing with a mammary tumor.

Feline mammary tumors are a little easier. Intact, cycling cats can get lobular mammary hypertrophy or fibroepithelial hyperplasia (often diffuse); both should resolve with spaying. Lobular hyperplasia should be composed of uniform glandular epithelial cells. Fibroepithelial hyperplasia looks like uniform spindle cells with lots of matrix. Actual tumors are typically epithelial and about 80% are carcinomas. On cytology, carcinomas should look epithelial with criteria of malignancy. If the cells look uniform and benign, histopathology is still warranted to rule out a carcinoma.

Mucocele (AKA Sialocele)
The aspirated fluid from a mucocele is clear or bloody and often thick and stringy. A key cytologic feature is the background appearance which is pink to basophilic and often regionally distributed in somewhat thick clumps or puddles of blue material. Inflammatory cells are often entrapped within the puddles of mucus. NOTE: this appearance is often best appreciated on low power. The predominant cell type consists of individualized, large, highly vacuolated macrophages. Sometimes they can have an almost spindled or stelate appearance because of the tenacious background fluid. Non-degenerate neutrophils are also common, especially if the lesion has been traumatized. Evidence of hemorrhage may be seen.
Dermatophytes

*Trichophyton* and *Microsporum* species are the only species of significant veterinary medical importance. These organisms are distributed worldwide and all domestic species are susceptible. Usually, the organisms only grow on keratinized structures (skin, hair, claws).

On cytology, the arthrospores have a ‘boxy’ look; they are round to cuboidal and elongated, 2-4 μm in diameter, with a thin, clear cell wall and deeply basophilic interior. You may see them adhered to keratin flakes or hair, or you may see them invading hairs. Inflammation may or may not be prominent, when present it is usually suppurative to chronic suppurative and phagocytized spores may be seen. Few thin, septate hyphae may also be seen.

Superficial lesions typically consist of flaking and crusting with hair loss, but cats can have clinically silent infections and be a source of infection for other animals. In immunocompetent dogs and shorthair cats, this disease is usually self limiting and often occurs in young animals. Longhair cats are more susceptible. Although infection is often limited to keratinized structures, dogs commonly develop folliculitis and furunculosis. Cats can develop granulomatous dermatitis consisting of well demarcated dermal nodules. Suppurative to pyogranulomatous inflammation is typical and spores and/or hyphae may be observed.
NCSU-VHC CYTOLOGY SERVICE INFORMATION:

NCSU-CVM-VHC
1060 William Moore Drive
Clinical Pathology Laboratory, Rm C269
Raleigh, NC 27607

Jaime Tarigo, DVM, DACVP
Jennifer Neel, DVM, DACVP
Carol Grindem, DVM, PhD, DACVP
Email: VHCcytology@ncsu.edu
Phone: (919) 793-5082; (919) 513-6550
http://cvm.ncsu.edu/vhc/csds/Cytology.html

Cytology Sample Submission Guidelines

Samples may be submitted for cytology, bloodsmear review and bone marrow analysis. Samples eligible for PARR will be submitted directly to the Clinical Immunology Laboratory upon request.

Slide Submission:

Please submit 6 slides maximum for each site unstained when possible. Staining one slide with Diff Quik to check cellularity is recommended if cell yield appears low. Please label each slide with the patient name and site.

DROP-OFF or SELF MAIL-IN: Samples may be mailed to the address above. For local hospitals, a drop-box is located outside of the Animal Scan Entrance of the Wellness Center (address above).

PRE-PAID FedEx MAIL-IN OPTIONS: FEDEX can be called for a pickup at your hospital using supplied pre-paid labels/envelopes (1-800-463-3339). Multiple samples/patients can be submitted in one envelope. FedEx Overnight Shipping $9 (NC) $12 (out of state)

Reporting Schedule:
Cytology reports are faxed and/or emailed within 1 to 2 business days.

Supplies
Please contact (919) 793-5082 for slide holders and prepaid labels for overnight FedEx shipping envelopes.

Pricing
Cytology $30.00
Bloodsmear Review $14.00
Bone Marrow $40.00 (submit slides only with CBC report, no EDTA)
STAT $13 additional (STAT samples will be read and reported within the same day received- please call 919-513-6550 if a STAT is needed).
NCSU FLOW CYTOMETRY/PARR ASSAY – GUIDE TO SAMPLE SUBMISSION

SAMPLE SUBMISSION

What type of sample to submit: Submit a sample that represents the disease process. See below. The samples are similar for flow cytometry and PARR. For flow cytometry, we need to know the species, and we need a sample that can be made into a single cell suspension. If there are enough cells, we can run both assays from the same sample. No formalin fixed samples for flow! Formalin fixed samples OK for PARR (at an additional cost – see below).

<table>
<thead>
<tr>
<th>Presenting complaint</th>
<th>Best sample to submit</th>
<th>Form of sample</th>
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<tbody>
<tr>
<td>Lymphadenopathy</td>
<td>Lymph node aspirate</td>
<td>Tube w/ saline</td>
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<tr>
<td>Lymphocytosis</td>
<td>Blood</td>
<td>EDTA</td>
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<tr>
<td>Suspect bone marrow disorder</td>
<td>Bone marrow aspirate</td>
<td>EDTA</td>
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<tr>
<td>Mass</td>
<td>Aspirate</td>
<td>Tube w/ saline</td>
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<tr>
<td>Effusion w/ cells</td>
<td>Fluid</td>
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<tr>
<td>CSF w/ cells</td>
<td>CSF</td>
<td>EDTA</td>
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</tbody>
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How to send a sample: Refrigerate sample immediately after collection. Send sample with a cold pack (be sure sample will not freeze) for overnight delivery. Do not send for Saturday/Sunday or holiday receipt. Send by FED EX, UPS, or DHL. Do not send US postal, as the delivery will be to the main mail office on campus and can take a day or two to reach us. Samples collected on Fridays may be refrigerated over the weekend and sent out the following Monday morning.

NOTE: For blood, we absolutely need CBC results from as close to time of sample submission as possible.

Formalin fixed samples for PARR: 3 or 4 20-25 micron sections in a 1.5ml eppendorf tube or equivalent-(NOT ON A SLIDE). Formalin fixation can degrade DNA, therefore, PARR may not provide pertinent information in approximately 10% of these cases.

Typical turnaround time for flow cytometry results is 24-48 hours after receipt of the sample. The typical turnaround time for the PARR assay is 2-3 days after receipt of the sample.

Note about low cellularity samples: Some samples (e.g. CSF) may not have enough cells to run flow or PARR. In some cases, we can determine this prior to starting the assays. However, often we do not know until we have completed the assay and begun to analyze the results. In these cases, as it costs us same amount to run as a sample with enough cells, you will be charged for the non-diagnostic sample.

Prices:
Flow cytometry - $90/sample; additional samples at the same time from the same case - $55/additional sample

PARR assay - $105/sample (LN/mass FNA, BM, effusions, blood), additional samples from the same case at the same time, $55/additional sample
Formalin-fixed samples - $130 (as additional processing is required) Call the Clinical Immunology Lab (919-513-6363) prior to sending a sample. If no one answers the phone, leave a message. This way we can begin to track the sample if it does not arrive in a timely manner. Additionally, there are times when we are short-staffed or the University is closed and we cannot receive samples. If this is the case, there will be a message to that effect on the phone.
## Cytology Submission Form

### Contact & Billing Information

<table>
<thead>
<tr>
<th>Veterinarian</th>
<th>Clinic</th>
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<tbody>
<tr>
<td>_________________</td>
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<tr>
<th>Address</th>
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<table>
<thead>
<tr>
<th>City</th>
<th>State</th>
<th>Zip</th>
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<tr>
<th>Phone</th>
<th>Fax</th>
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<tr>
<th>Email</th>
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### Patient Information

<table>
<thead>
<tr>
<th>Owner Name</th>
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<tbody>
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<td>_________________</td>
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<table>
<thead>
<tr>
<th>Patient Name</th>
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<tbody>
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<td>_________________</td>
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<table>
<thead>
<tr>
<th>Species:</th>
<th>FELINE ☐</th>
<th>CANINE ☐</th>
<th>OTHER __________</th>
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</thead>
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<table>
<thead>
<tr>
<th>Age</th>
<th>Breed</th>
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<td>______</td>
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<table>
<thead>
<tr>
<th>Gender</th>
<th>MN ☐</th>
<th>FS ☐</th>
<th>M ☐</th>
<th>F ☐</th>
</tr>
</thead>
</table>

### Shipping Method

- DROP-OFF or SELF MAIL-IN*  ☐  Prepaid o/n FEDEX LABEL ☐  
  *Mail or DROP-OFF to address above (DROP BOX located at Wellness Center/Animal Scan Entrance)

### Additional Tests

- ☐ Cytology  ☐ Bloodsmear Pathology Review  ☐ Bone Marrow

### Cytology Specimen - Tissue Aspirates, Fluid- Smears Only (contact for guidelines for fluid smear preparation).

<table>
<thead>
<tr>
<th>Date</th>
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<table>
<thead>
<tr>
<th>STAT REQUIRED?</th>
<th>☐ CALL (919) 513-6550 TO NOTIFY</th>
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<table>
<thead>
<tr>
<th># Sites</th>
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<tbody>
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<th>(6 slides maximum per site)</th>
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### Source(s) (If lymph node, please specify location)

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### History

**Clinical presentation including symptoms, duration.**

__________________________________________________________________________________________

__________________________________________________________________________________________

__________________________________________________________________________________________

### Gross appearance of lesion(s) When applicable list size/growth rate/mobility/texture/color. (Please include ultrasound report for internal aspirates)

__________________________________________________________________________________________

__________________________________________________________________________________________

__________________________________________________________________________________________
NCSU FLOW CYTOMETRY/ K9/FELINE PARR ASSAY SUBMISSION FORM

Patient Information

Animal name_______________________________
Owner last name____________________________
Species/Breed_____________________________
Age______________________________________
Sex: FI_____  FS_____   MI_____   MC_____

Clinic Information

Clinic name________________________________
Clinic Address______________________________

phone_________________ Fax________________

SAMPLE TYPE      DATE COLLECTED      TEST REQUESTED

Aspirate: site_______________  ______________ Flow cytometry______  PARR ________
Blood (EDTA)_______________ ______________ Flow cytometry______  PARR ________
Bone Marrow (EDTA)_________ ______________ Flow cytometry______  PARR ________
CSF ______________________ ______________ Flow cytometry______  PARR ________
Other________________________ ______________ Flow cytometry______  PARR ________

We will send your results via (check one) ______Fax    _____email (address____________________________)

**HISTORY** (IMPORTANT!!!!)

1) Signs or symptoms leading to test request (check all that apply)

___Lymphadenopathy (include most recent cytology report if available)
___Splenomegaly       ___Hepatomegaly
___Lymphocytosis;  lymphocyte number________________(include most recent CBC/cytology report if available)
___Other peripheral blood abnormality (include most recent CBC/cytology report if available)
___Bone marrow abnormality (include most recent cytology report if available)
___Mass;  location____________________________ (include most recent cytology report if available)
___Effusion/fluid containing suspicious cells;  pleural____ peritoneal____ CSF____ other____________
___Hypercalcemia (value_____________)       ___Hyperglobulinemia (value__________________)

2) Other signs and/or additional history or concurrent conditions including any treatment.

__________________________________________________________________________________________
__________________________________________________________________________________________

3) Is this patient an ehrlichiosis suspect?  ____No  ____Suspect  ____Confirmed

Send overnight for morning delivery to: College of Veterinary Medicine  Questions?
(Please use FED EX, UPS or DHL)  ATTN: Linda English, B-324  Clinical Immunology
College of Veterinary Medicine  ATTN: Linda English, B-324
College of Veterinary Medicine  4700 Hillsborough St.
College of Veterinary Medicine  Raleigh NC 27606  Questions?
College of Veterinary Medicine  Phone: 919-513-6363
College of Veterinary Medicine  Fax:  919-513-6703
College of Veterinary Medicine  email: Linda_English@ncsu.edu

NOTIFY LAB BY PHONE OR EMAIL PRIOR TO SENDING SAMPLE