CYTOLOGY IN PRACTICE

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Introduction

The primary goal of a cytologic examination is to gain valuable information about a lesion, thus it has to be considered as a SCREENING tool: while in many cases it can give definitive diagnoses, in others it will help narrow our differentials, or justify further, potentially more invasive/expensive procedures.

Indications

Cells may be collected by a number of methods including aspiration, imprinting, washing, squashing and scraping. Fine needle aspiration is most commonly performed on skin masses and enlarged lymph nodes. Fluid analysis can be performed on joint fluids, body cavity fluids/effusions, respiratory tract washings (TTW, BAL), or cerebrospinal fluids. With the advance of ultrasound guidance, organs (e.g. spleen, liver, kidney, mesenteric lymph nodes) and lesions deep within the body (e.g. mediastinal mass) can also be sampled. Naturally, different sampling techniques and different sources of samples may have different limitations or difficulties in getting nice samples.

Cytology vs. histopathology

Cytologic sampling/examination is relatively simple, quick, inexpensive, minimally invasive, and has quicker turn around time then biopsies when sent off to a pathologist. However, with good basic training and a quality microscope, many straight forward lesions can be diagnosed in house, right next to the patient. Furthermore, when cell detail is critical (e.g. round cell tumours), it can even out perform routine histopathologic examination. Biopsy procedures are more time consuming, more expensive

(sterile procedure, anaesthesia) with a slower turn around time; however it will provide valuable information about tissue architecture, which can be essential for diagnosis. Also grading, invasion etc. can only be evaluated via the latter procedure. Unusual lesions (e.g. poorly differentiated neoplasia) commonly need immunohistochemical examination, which is currently much more readily available for tissues than cells on a smear. Lastly, the representativeness of the sample is important. While entire masses excised (excisional biopsy) will obviously be the most representative samples, in my experience, properly acquired aspirates can be actually more representative then tru-cut biopsies.

Common mistakes during sampling

For a long time, FNAs were performed via aspiration, as the name implies. However, creating vacuum with our syringe within the tissue will hurt our samples more time then help! First always attempt to only use a small gauge needle (22-24G) without an attached syringe, and gently advance it (with one hand) into the well stabilized (with the other hand) lesion, then pull out partially (still within the mass), redirect, and advance more deeply into the mass again, multiple times to increase representativeness of the sample. While it is somewhat counterintuitive, but very small amount of material is needed to prepare nice smears – literally just the content within the length of the needle is enough. We have to avoid “contaminating” our sample with blood, as it really makes the evaluation much more difficult. Blood will represent the “haystack”, while the cells of interest will be the “needles”. Furthermore, blood doesn’t only have erythrocytes, but neutrophils will come with it, again making judgement more difficult.

Actual aspiration should only be attempted, if couple of attempts with the needle only failed (e.g. poorly exfoliating neoplasia). As mentioned above, very small amount of material is needed, thus if fluid has already appeared in the hub of the needle, it is likely too much (unless it is an abscess or cyst type lesion)!

Common problem is caused by large necrotic areas in the middle of lesions (e.g. malignant neoplasia). Just simply going for the middle of the lesion (without redirecting the needle) will provide highly diagnostic slides of necrosis, but not necessarily for the underlying pathology. Therefore, if we suspect a mass with a “soft middle”, make sure to redirect the needle into the more outer “walls” of the lesion as well.

Acquiring highly diagnostic material from the lesion however is only the first challenge. Then comes the preparation of the smears, which is another common source of problems. Many people will try to spread the cells throughout the entire glass slide via forceful spraying action, which unfortunately only creates droplets of fluid on the slide, which will be very thick after drying, and nothing in between. The primary goal during slide preparation is the spreading of the material to create thin areas where cells will flatten out (cell should appear as fried eggs vs. a boiled egg cut in half) without much lysis of them. The best practice is the attach the syringe (e.g. 5 mL) to our needle containing the valuable sample, and very gently squirting it out to one end of the slide. Then another slide is placed at 90°, creating a square between the two slides. After the material has started spreading within the “square”, the top slide needs to be glided along the bottom slide. The amount of pressure applied is something that needs fine tuning, which can be greatly achieved by examining our own prepared slide. Too little pressure will create too thick preparations, while too much pressure will lysed many cells (especially the fragile ones, such as neoplastic cells). Only intact cells can be reliably assessed, thus our goal is to have many intact cells, with adequate spreading as mentioned above.

Imprints can also provide excellent preparations, if the following tips are followed. Try to create a fresh cut surface (e.g. cutting the excised tissue in half – will also aid the penetration of formalin), which HAS to be blotted dry with a paper towel. Without this critical step, we are only “imprinting” the tissue juices and blood on the surface, but not the cells. On the other hand, with the dried, granular appearing surface of the mass will exfoliate really well even with gentle pressure, which is important to avoid to thick specimens (with too much pressure). Multiple imprints can be done on one glass slide, dependent on the size of the cut surface of course.

Even though it sounds very convenient to imprint an excised tissue right above the open formalin jar (so we can just drop it in when we are done), unfortunately even just the fumes of formalin will completely destroy cytologic preparations! Never have open formalin jars anywhere close to blood smears or cytologic preparations! Even closed jars, packed together with smears for mailing will have detrimental effects, thus at minimum double pack each, separately.

As most lab samples commonly kept in the refrigerator until processing or shipping, it is not uncommon for the glass slides (blood smears, cytologies) to end up in the fridge as well. However, those with glasses will know well what happens to cold glass in a warm room! Water condensation on

the glass will completely destroy the cells! Just air drying smears (but also avoid hot air of a hairdryer) will preserve the cells very well, so there is no need for fixation or refrigeration.

Lastly, but not the least, good communication with the pathologist (both ways) is essential to increase the efficiency of our cytologic diagnostics. This process starts with properly labelled and packaged slides, and providing adequate information about the case. It is a common myth to try to keep the pathologist “blinded” to avoid bias. Most well trained/certified clinical pathologists will only report what is present on the slides, however in our comment, we can give much better information if we know more about the lesion. In other works, letting me know of the dog’s hypercalcemia will not make me call a lymph node aspirate a lymphoma, unless I am certain based on the cytologic picture. However, if I can’t make such a conclusion on the submitted slide, I may still comment, that while this slide was not diagnostic of lymphoma, but further tests (e.g. excision of the node) could be considered in face of such a biochemical finding to make sure that an important diagnosis is not missed! If e.g. the differential diagnoses are provided, it is easy to comment, whether the cytologic picture is consistent or inconsistent with each of the listed differentials.

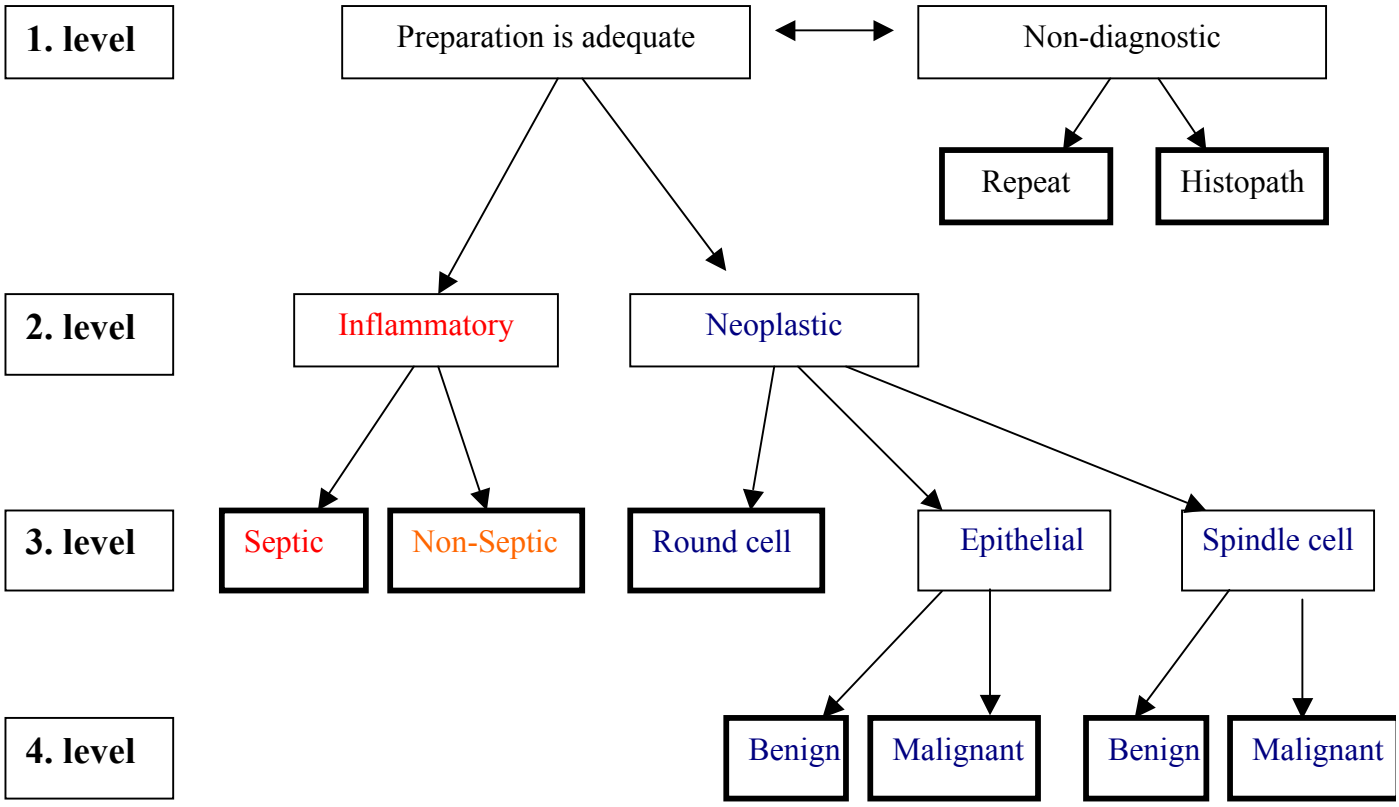
Even such “simple” information, whether the lesion is from the surface of the skin, or being dermal (moves with the skin) or subcutaneous (skin moves above it), makes a significant difference in the interpretation. Another simple information commonly hidden such as signalment can make a big difference. With a large mass in the abdomen, neoplasia of a retained testicle can have similar cytologic picture as an ovarian mass, thus just knowing the sex of the animal will help greatly in the evaluation of the sample.

If one is looking for metastasis, knowing the primary tumor type will greatly enhance the search for the metastatic cells.

Lastly, a common problem originates from the fact, that when a lesion is right in front of our eyes, it is easy to describe it too superficially, but we have to keep in mind that the pathologist will not have the same luxury. Favourite example: abdominal mass – yes, it is very obvious when somebody is standing in front of a radiograph with a giant mass in the abdomen, but by the time it comes to us, the question arises: are we talking about an intraabdominal mass, or a skin mass on the abdomen ☺.

SYSTEMATIC APPROACH TO CYTOLOGY SPECIMENS

1. Evaluate the quality of the preparation
    a. Adequate amount of cells
    b. Adequate spread (thin areas)
    c. Adequate staining
2. Scan the entire slide on low magnification (4-10X) to find the best areas to spend you time on!
3. Assess the cellular arrangement on low power – clusters of cells, sheets, individual cells, etc…
4. Try to decide if it is inflammatory or neoplastic (or normal, if the tissue sample is such, that this is an option)
    a. Inflammatory
        i. Identify the cell types involved (neutrophils, macrophages, eosinophils, lymphocytes, etc…)
        ii. Decide if **septic** or **not**
    b. Neoplastic – try to decide which of the 3 main categories
        i. Round cell tumor – histiocytoma, MCT, lymphoma, plasma cell tumor …
            - Biologic behavior is more specific to the type then cytologic features
        ii. Epithelial tumor – look for features of malignancy, key word: pleomorphism!
            1. **Benign** (e.g. adenoma)
            2. **Malignant** (e.g. carcinoma)
        iii. Mesenchymal/spindle cell tumor – features of malignancy…
            1. **Benign** (e.g. fibroma)
            2. **Malignant** (e.g. sarcoma)



Limitations and things to keep in mind during interpretation

- A perfect cytologic diagnosis may not represent the actual lesion (e.g. squamous cell carcinoma commonly has necrotic, inflammatory and possibly septic areas, especially within the oral cavity).
- Spindle cell tumours are a cytologic minefield. Fibroblasts within granulation tissue may appear more atypical then cells of a well-differentiated fibrosarcoma. Interpretation of many lesions with spindle cells needs considerable experience. This is the type of lesion where most commonly histopathologic examination is required (and suggested in the cytology conclusion) for a reliable diagnosis. Nonetheless, these cause the most frustrations in clinicians: "why didn't I just biopsy it?" It is important to keep in mind, that cytology is a screening tool! Before aspirating the lesion, we didn't know anything about it (could have been an abscess, MCT, lipoma, etc), thus getting this far is actually very helpful within the context of our diagnostic investigation, even if the final diagnosis is not reached via cytology.
- Concurrent presence of tissue and inflammatory cells: inflammatory lesions can easily contain epithelial (e.g. nasal epithelium) or spindle cells (fibroblasts), while malignant tumours (both carcinomas and sarcomas) can have necrotic and inflammatory areas within the mass. The dilemma is further intensified by the fact, that epithelial cells will commonly become dysplastic, when the tissue is inflamed, giving cytologic features of malignancy to the cells. There is a grey zone between dysplasia and neoplasia when cells are examined via light microscopy. Squamous, respiratory and transitional epithelia, and mesothelium are common to appear "malignant" for the untrained eye due to the dysplastic/reactive features. Spindle cells are similarly challenging; fibroblast walling of an abscess will also look quite "atypical", and easily misclassified as malignant without experience. Thus, whenever tissue cells and inflammation are both present, it is wise to seek a second opinion, and in fact these cases may also be reported out by the pathologists with the recommendations for histopathologic examination.
- Mammary masses – only aspirate to confirm whether it is a mammary mass, and not a mast cell tumour, abscess, etc… Unfortunately mammary tumours can have several different areas (normal, dysplastic, benign, malignant) within the same mass, and cytologic pleomorphism does not correspond well with the true biologic behaviour. Only stromal invasion and evidence of metastasis appears to correlate with survival, both of which needs to be evaluated via histopathologic examination.

- Cytologic evaluation for malignancy relies on cellular pleomorphism – the more atypical and variable the cells are, the more likely to be malignant. However, certain highly malignant tumours may appear very uniform (e.g. thyroid carcinomas), thus location, and in depth knowledge of certain tumours can be vital in order not to miss malignancy.
- Certain malignant cell types (e.g. transitional cell tumours) easily metastasize, thus aspiration may seed the neoplastic cells into surrounding tissues (e.g. aspirating prostate through the abdominal wall).
- Bacteria – stain precipitation can mimic bacteria, especially cocci. With well kept stain, bacteria should have a dark blue to almost dark green colour, while stain precipitates will always be shades of purple and tend to be variable in size. It is a subtle but well visible difference. Try to compare these colours critically the next time when you are certain that you are looking at a septic smear. Other issue to remember, that cytology is not very sensitive to pick up low numbers of bacteria, thus not seeing intracellular organisms on a slide does not rule out sepsis. Furthermore, if antibiotics have been already used, the odds of seeing bacteria decrease significantly!

Conclusion

Cytology is not just fun, but can be extremely useful in our diagnostic work. Many of the straight forward lesions can be easily diagnosed with good basic training and a quality microscope. Cytology is based on pattern recognition, and contrasting the seen image with pictures of colour atlases are a very valid way of evaluating slides, even on a professional level! The references listed are great resources to have next to our microscopes (previous editions are great as well).

Furthermore, knowing about the limitations of cytology in general, and of our own training level will greatly help in deciding, when to seek second opinion, or choose a different type of diagnostic process (e.g. histopathologic examination). Most well trained pathologist will give detailed descriptions of the slides (not just a conclusion), thus making notes of your own opinion and contrasting that with the report is a great way to continually train yourself, and in fact this doesn't even cost anything to you ☺!

Further reading

Raskin, R; Meyer D. Canine and Feline Cytology: A Color Atlas and Interpretation Guide. Saunders, 2009.

Cowell, RL; Tyler, RD; *at al*. Diagnostic Cytology and Hematology of the Dog and Cat. Mosby, 2008.

Baker, R; Lumsden; JH. Color Atlas of Cytology of the Dog and Cat. Mosby, 1999.

Cytology in Practice

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Advantages of Cytology

- Quick, easy, inexpensive
- Non-invasive
- Minimal risk to patient
- **Screening tool**: determining what diagnostic procedures should be performed next
- Can be useful in establishing a diagnosis or identifying a disease process
  - Certain diagnoses are easy to make!

Limitations of Cytology

- Greatly relies on sample quality
  - Skill of collector
  - Smear quality
  - Type of tissue (exfoliation), site of collection
- Limited by the ability/experience of the person examining the smears
  - Quality of the microscope
- Lack of information about tissue architecture
- Diagnostic challenges of cytology (later)

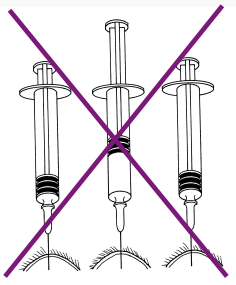
Histopath

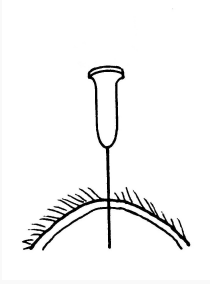
- - More expensive procedure
  - Sterile
- - Slower turn around time
  - 48 hr minimum
- - Poor detail for round cell tumours
- + Tissue architecture
- + Tumour grading
- + Immunohistochemistry more available

Samples for cytology

- **Aspiration or imprints**
  - Superficial masses
  - Lymph node (enlargement, metastasis?)
  - Organs, deep masses (US guidance)
- **Fluids**
  - Body cavities (peritoneum, pleural space, pericardium)
  - Joints
  - Respiratory tract (TTW, BAL)
  - Cerebrospinal fluid

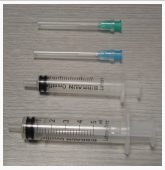
Fine Needle "Aspiration"



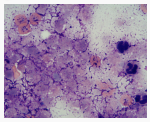


Modified from Cowell et al: Diagnostic Cytology and Hematology of the Dog and Cat, Mosby, 1999

Fine Needle Biopsy

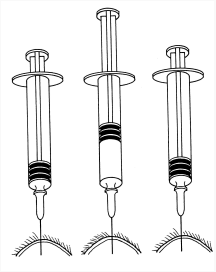


- For solid or fluid filled masses
- Visual or ultrasound guidance
- Similar to FNA but **NO negative pressure** is applied to syringe (no aspiration)
- Use **small gauge** needle (22 -24 gauge)
- Insert into mass several times
- Masses with **necrotic centre** – sample the 'wall' not just the centre
- Then use air filled syringe to expel cells onto slide



Fine Needle Aspiration

- Only if previous method unrewarding
- With needle in centre of mass, apply negative pressure to the syringe by withdrawing plunger
- Redirect needle 2-3 times to ensure representative sample
- Release plunger (neg. pressure) before removing needle from mass



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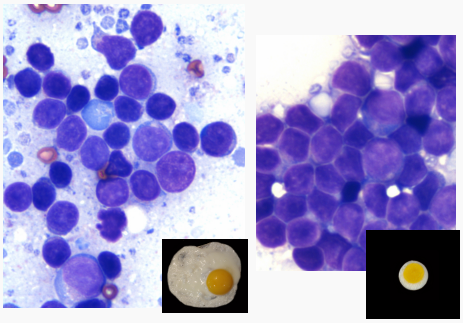
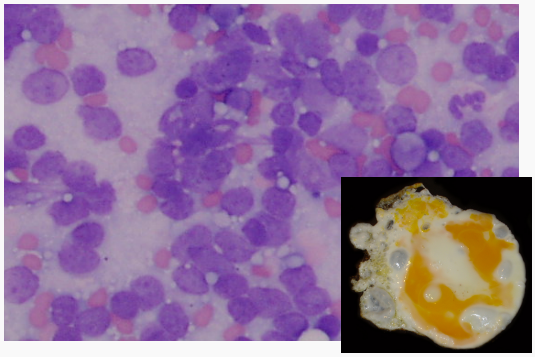
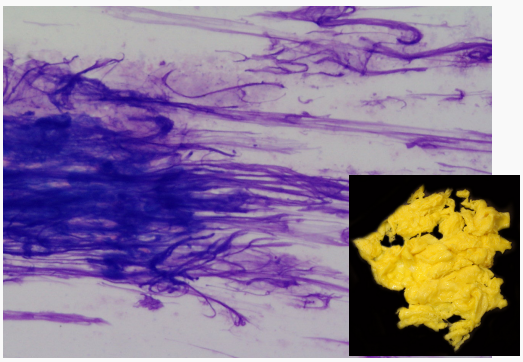
Quality of the Smear



Quality of the Smear

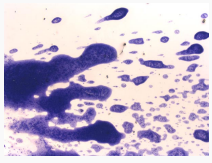


Quality of the Smear



Smear Preparation

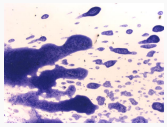
- (Remove needle from syringe)
- Draw air into syringe
- Replace needle
- Expel aspirated fluid onto slide from needle lumen with air-filled syringe
  - Be gentle!
- **Smearing, not spraying!**



Smear preparation

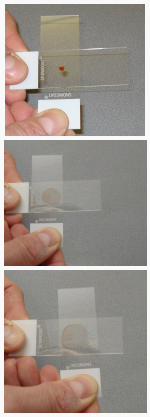
Goals:

- Thin areas with good cell spread
- Minimize cell damage
- Minimize blood content



Making Smears

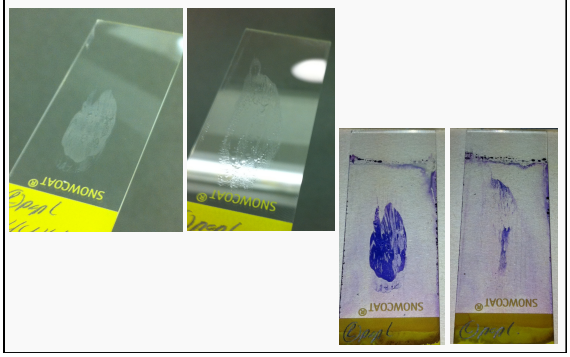
- Put another slide over top – this will spread sample
- Pull slides apart – use gentle pressure
  - If pressure is excessive – rupture cells (especially true of neoplastic cells and lymphocytes)
  - If not enough pressure – too thick



What Should it Look Like?

Blood also contains neutrophils!

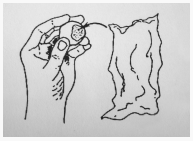
What Should it Look Like?



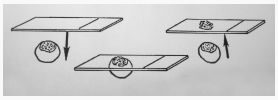
Touch Impression (Imprints)

- Good for evaluation of excised tissue or superficial lesions
- Imprints are made before the excised tissue is placed in 10% buffered formalin and submitted for histopathology

Imprints



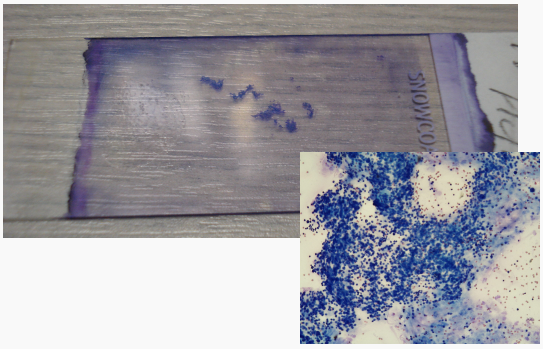
- Use fresh cut surface of the tissue
- Blot until **dry** with paper towel
- Imprint directly onto glass slide
  - Tissue should be rolled against the slide
  - 4-5 imprints per slide
  - Air dry slides and stain



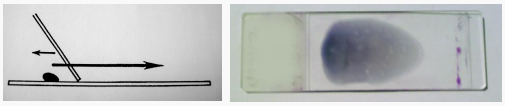
Collection of the Fluid

- Clot prevention – **EDTA!**
- Bacteriology – **Sterile pot!**
- Slide preparations – **Fresh!**
  - Direct smear
  - Line preparation
  - Squash preparation
  - Concentration techniques

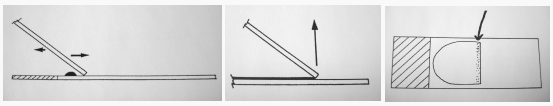
Sample Quality – Clots!

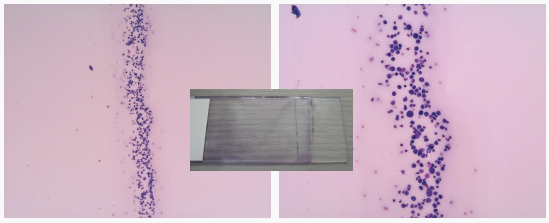


Fluids - Direct Smear



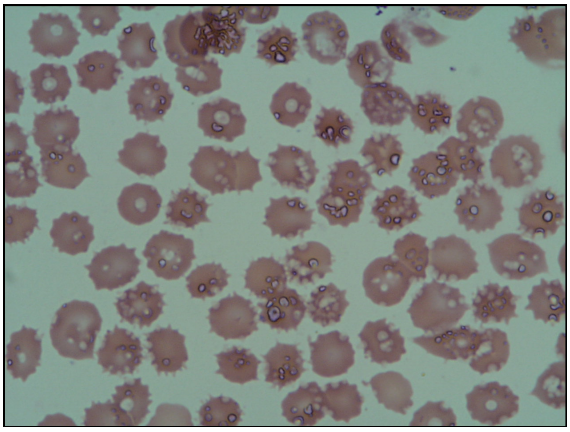
Fluids - Line Preparation





Staining

- **Automated**
- **Manual**
  - Diff-Quik type stains



Diff-Quik "Secrets"

- 1st jar: alcohol – longer the better!
- 2nd jar: red stain – can not overstain!
- 3rd jar: blue stain – number of dips!
  - Can always go back and dip more
  - Blot "mirrory" film from top
- Replace frequently (about 2 weeks)
  - MUST clean jars properly

- + Great penetration, nuclear detail
- - Mast cell granules, basophils
- Use separate set for ear canal, skin

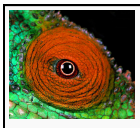
Gram Stain

- Designed for bacterial culture – alive
- Dead bacteria is Gram –
  - Antibiotics
- Nuclear material stains Gram –
- Time of de-staining (false gram + or -)
  - Thick or thin preparation

Objectives: Magnifications

- 4x
  - Great overview of cytology slides and feathered edge of blood smears
- 10x
  - Essential for haematology overview, good in cytology
- 20x
  - Good in cytology and haematology
- 40x
  - "High dry", needs coverslip!
  - Will only give sharp image with the extra layer of glass!!!
  - Drags into oil on slide – need to be careful
- 50x oil
  - Great objective, but expensive
  - Great for leukocyte differential, fantastic in cytology
- 100x oil
  - Essential if 50x oil is not available
  - Highest magnification – best to confirm organisms and fine structures

Eye Pieces: Diameter

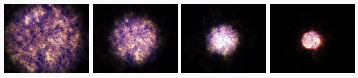


20 mm 22 mm 25 mm 26.5 mm

- Wide angle preferred (22-25 mm is ideal)
- Number of objects (e.g. platelets) per field changes with diameter!
  - Need to modify estimation schemes

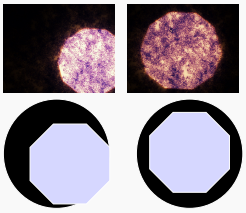
Condenser: How to Set Height

- Köhler illumination
  - Helps to find proper height of condenser
    - 1. Close field diaphragm
    - 2. Elevate the condenser close to top
    - 3. View in eyepiece will change:



How to Centre the Light

- After Köhler illumination is set, start opening the field diaphragm
- Use the two knobs to centre light



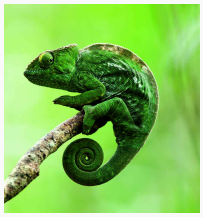
- Open field diaphragm completely

Basic Care and Cleaning

- Remove excess oil from oil objectives with lens paper!
  - Don't use other type of tissues
- If dry objective becomes oily, clean it right away!
  - Will need Q-tip and cleaning solution
- Don't use xylene or similar solvents!

Examination of Smears

- Gross examination
  - Texture (greasy vs. dry)
  - Large cellular clumps
- Microscopic examination
  - Low magnification
  - High magnification



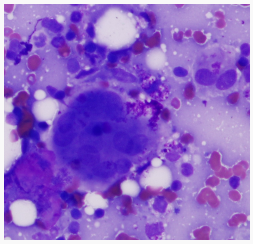
Low Magnification

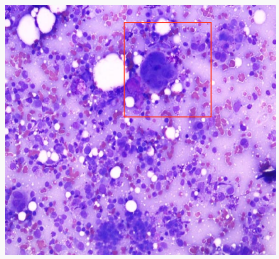
- Cellularity
  - Arrangement – clusters, bundles
  - Shapes
  - Variability
  - Degree of cellularity
- Background – mucus, blood
- Large organisms – larvae, fungi

High Magnification

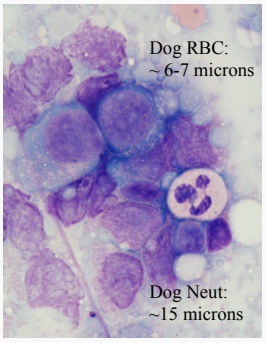
- Evaluate cells populations
  - Inflammatory, epithelial, mesenchymal, round cells
- Evaluate cellular detail
  - Nucleus: chromatin, shapes, number
  - Nucleoli: number, shapes, variability
  - Cytoplasm: colour, amount, inclusions
- Organisms
  - Intracellular or extracellular

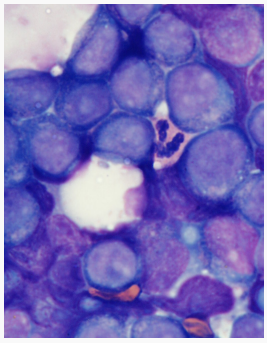
Essential Microscopic Skills



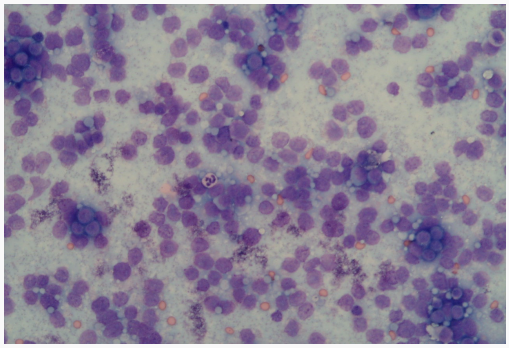


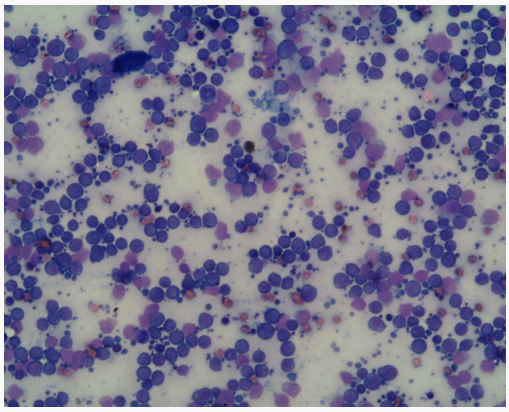
Size Judgement





Finding the Area to Spend Our Time on!

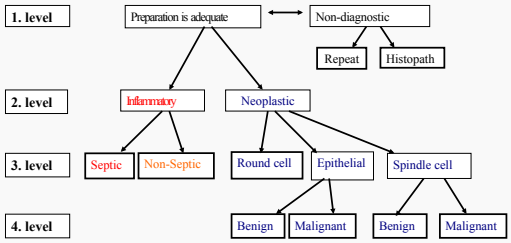




Essential Microscopic Skills

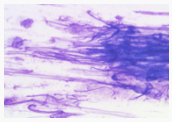
- Your time is expensive – don' t waist it!
- Try to recognize more and more things at lower power
- Use high power to confirm suspicion
- Never lurk around on high power!

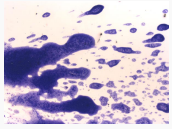
Systematic Approach

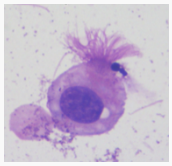


1. Sample Quality

- Enough cells to examine?
- Preservation of cells
- Adequate spreading
- Is it representative of the lesion
- Do we expect normal cells?
  - Tracheal wash – ciliated cells
  - BAL – alveolar macrophages
  - Joint fluid – mononuclear cells







2. Inflammation vs. Neoplasia

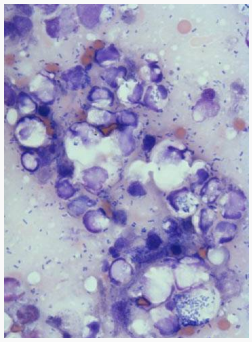
- Sample dominated by inflammatory cells
  - Neutrophils, eosinophils, lymphocytes, macrophages
- Sample dominated by tissue cells → neoplastic
- If both are present – need experience!
  - Inflammation with secondary dysplasia
  - Neoplasia with secondary inflammation

3. Inflammatory: Septic vs. Non-Septic

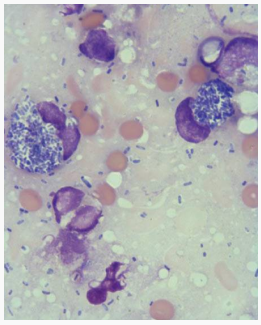
- Septic
  - Contains bacteria/organisms
  - Degenerate neutrophils
  - Bacteria must be intracellular within neutrophils to be significant
  - If extracellular may be contaminants
- Non - septic
  - No bacteria or organisms seen
  - Neutrophils non-degenerate
  - Lack of identifiable bacteria and the presence of non-degenerate neutrophils

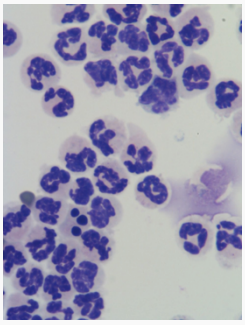
Degenerative Changes in Neutrophils

- Nuclear change
- Nucleus swells, loses lobulation and becomes paler (chromatin becomes less condensed)
- Secondary to release of bacterial toxins
- If degenerate neutrophils are present consider septic cause for the inflammation even if bacteria are not seen

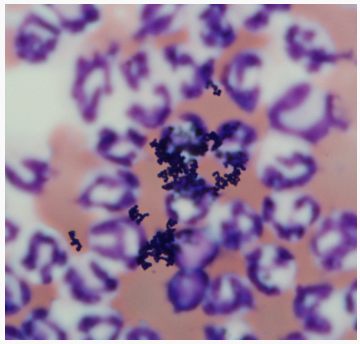


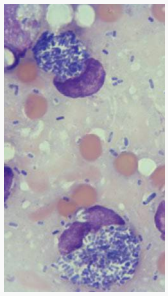
Degenerate vs. Non-Degenerate Neutrophils





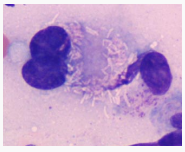
Stain precipitate vs. bacteria

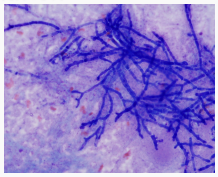




Increased Numbers of Macrophages

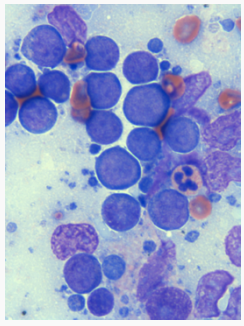
- Granulomatous inflammation
  - Mycobacterium sp
- If neutrophils also – pyogranulomatous inflammation
  - Fungal infections
- Either can occur with foreign body reactions



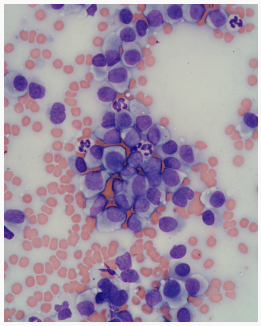
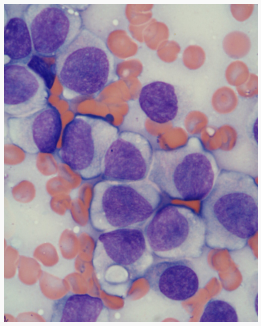


Round Cells

- Individual cells
- Small to medium size
- Round to oval cells
- Round to oval nuclei
- Well defined cell borders
- Good cell yield
- Advantage over histopathology

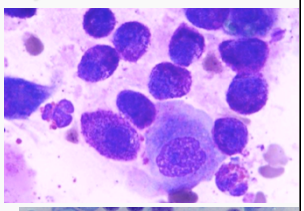
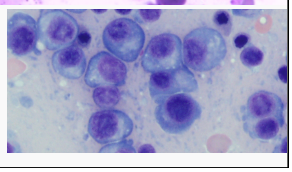


Cell Organization – Beware!

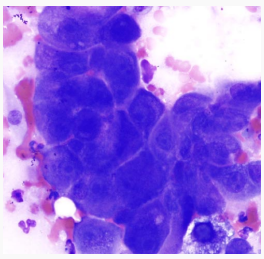
Round (Discrete) Cell Tumours

- Lymphoma
- Histiocytoma
- Mast cell tumor
- Plasmacytoma
- Transmissible venereal tumour (TVT)
- (Melanoma)

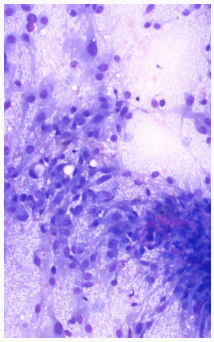
Epithelial Cells

- Often found in sheets/ rafts/clusters
- Large cell size
- Cell-to-cell junctions
- Oval to angular in shape
- Nuclei round, centrally located
- Cytoplasm often abundant
- Good cell yield
- Sebaceous, mammary, liver



Mesenchymal Cells

- Individual cells or clumps
- Small to medium size cells
- Spindle to fusiform to stellate
- Indistinct cell borders
- Elongated nucleus
- Poor exfoliation
- Matrix production - collagen, osteoid/bone



4. Benign vs. Malignant

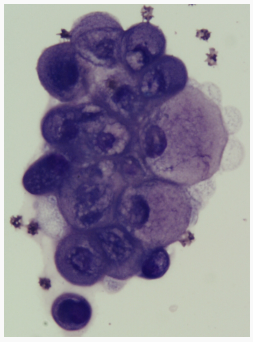
- Epithelial
  - Benign: adenoma
  - Malignant: carcinoma
- Mesenchymal
  - Benign: fibroma
  - Malignant: fibrosarcoma
- (Round cell tumors
  - Biologic behaviour dependent on type)

How to Assess Malignancy

- **Criteria of Malignancy**
- Cytoplasmic and nuclear features associated with malignant behaviour
  - Uniformity vs. pleomorphism (carcinoma, sarcoma)
  - Monotony (lymphoma)
- Nuclear criteria are most reliable
- Need at least 3 nuclear criteria of malignancy before calling a tumour malignant

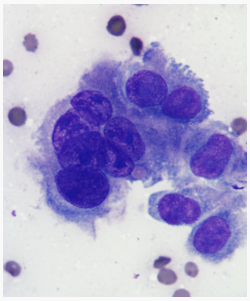
Cellular Criteria of Malignancy

- Anisocytosis - variation in cell size
- Macrocytosis - large cells
- Cell crowding



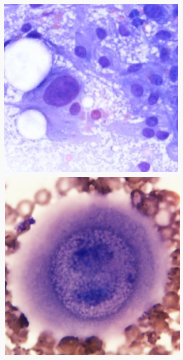
Nuclear Criteria of Malignancy

- Anisokaryosis - variation in nuclear size
- Multinucleation –
  - Nuclei vary in size
  - Odd numbers of nuclei



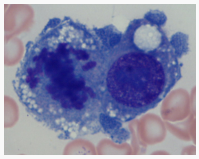
Nuclear Criteria of Malignancy

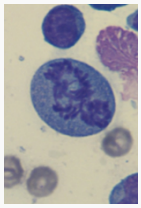
- Macrokaryosis - giant nuclei
- High nuclear to cytoplasmic (N:C) ratio (in large cells)

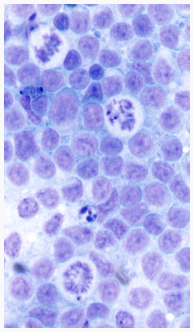


Nuclear Criteria of Malignancy

- Increased mitotic figures
- Abnormal mitotic figures

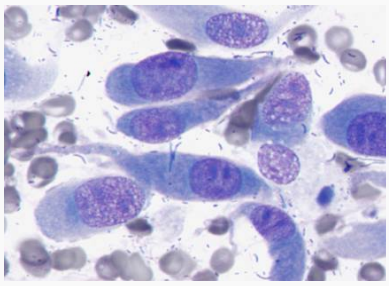






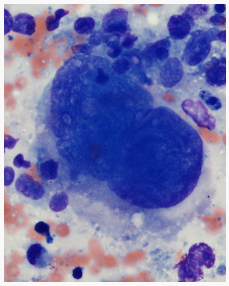
Nuclear Criteria of Malignancy

- Coarse chromatin



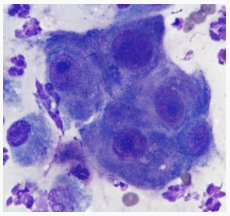
Nuclear Criteria of Malignancy

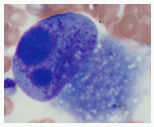
- Nuclear moulding - deformation of nucleus by other nuclei (1 nucleus appears to be growing around another

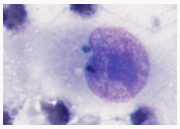


Nuclear Criteria of Malignancy

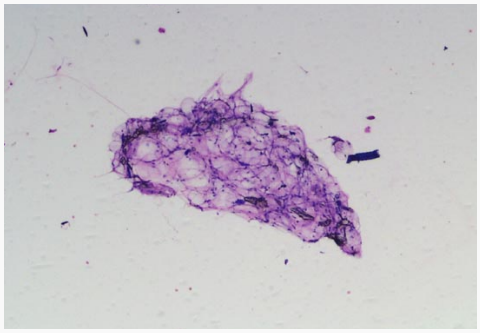
- Macronucleoli - large, prominent nucleoli (RBC)
- Varying nucleolar shape (rectangular, triangular etc)
- Variation in nucleolar size





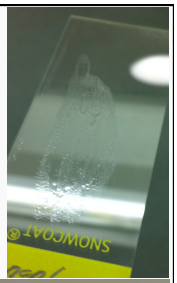


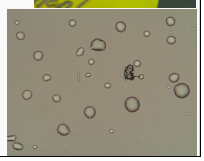
The Important Ones That Doesn't Fit



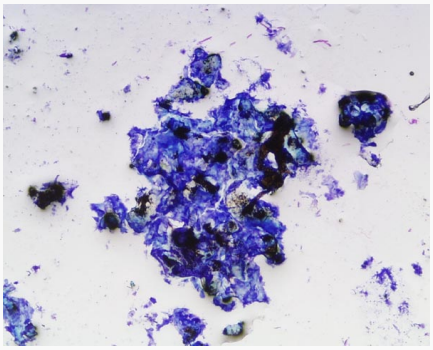
Lipoma

- Most common subcutaneous mass
- Wet appearance before staining
- Subcutaneous fat looks the same





Dermal Mass With "No Cells"



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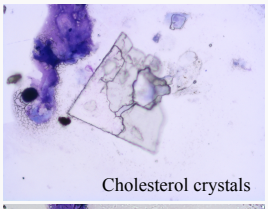
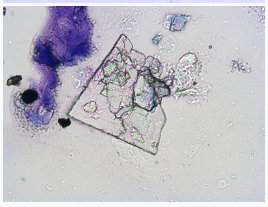
Follicular/ Epidermal Inclusion Cyst

- Common dermal mass
- White chalk like material when dries
- Excision is curative
- Hair follicule tumors

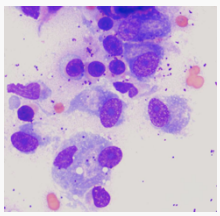


Cholesterol crystals



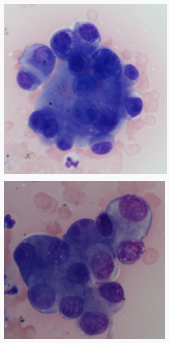
"Unrewarding" Results

- Spindle cells
  - Granulation tissue can have cytologic features of malignancy
  - Well-differentiated sarcoma can look very benign
  - *"Why didn't I just took a biopsy???"*



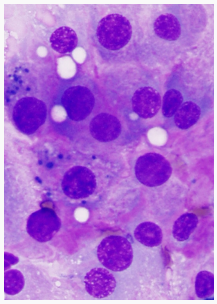
"Unrewarding" Results

- Mammary tumours – mine field
  - Only to differentiate abscess, MCT, lipoma vs. mammary tumour
  - Stromal invasion and metastasis !



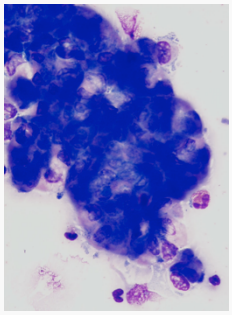
"Unrewarding" Results

- Malignant tumours with uniform cells
  - Thyroid carcinoma - 95%



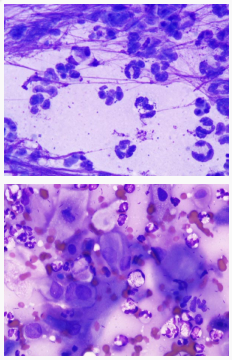
"Unrewarding" Results

- Dysplasia caused by inflammation
- Malignant neoplasia with necrosis, inflammation



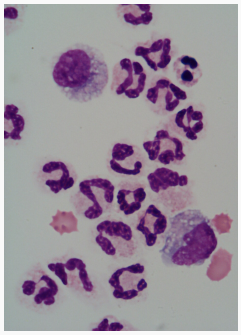
"Unrewarding" Results

- Cytologic diagnosis vs. clinical diagnosis
  - Representative sample



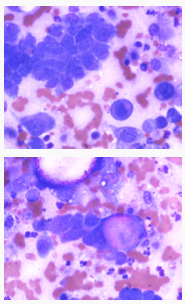
"Unrewarding" Results

- Lack of bacteria does not rule out sepsis
  - Antibiotics
  - Joints



Other Considerations

- Transitional cell carcinoma
  - But how to get the sample?
  - FNA – potential for seeding*
  - Cells mixed with urine
    - Cytopathologist nightmare
    - *Fresh preparation is important!*



*Nyland et at: Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate; Vet Radiol Ultrasound. 2002 Jan-Feb;43(1):50-3

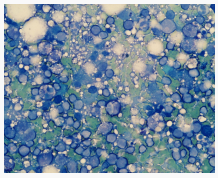
*Vignoli M et al: Needle tract implantation after fine needle aspiration biopsy (FNAB) of transitional cell carcinoma of the urinary bladder and adenocarcinoma of the lung; Schweiz Arch Tierheilkd. 2007 Jul;149(7):314-8

Submitting Samples to the Lab

- Send multiple unstained smears
- LABEL slides/tubes
- Use pencil – will not wash off in fixative/stain
- Signalment (breed, sex, age)
- History, important clinical findings (↑Ca^{2+})
- Describe location of the lesion ("abdominal mass", dermal vs. subcutaneous)
- Duration and rate of growth, previous lesions and diagnosis
- Current therapy (antibiotics, cytotoxic drugs)

Common Problems

- Formalin fumes
- Refrigerating glass slides
- Breakage during shipping
- Lack of freshly made smears (urine!)
- (Flies)



Blind or not blind ?

- **Trained cytopathologist will look at slide objectively**
- **History is critical to formulate comment**
  - Findings are consistent/inconsistent with suspected differential
  - Looking for metastasis – good to know what to look for

Summary

- Microscopic skills
- **Pattern recognition** – experience!
- Use resources (see next slide)
- Some lesions are easy to recognize - lipomas, mast cell tumours, melanoma, follicular cysts
- If in doubt - submit to the lab or biopsy
  - Continued training (paid by the client ☺)

Real Life ☺

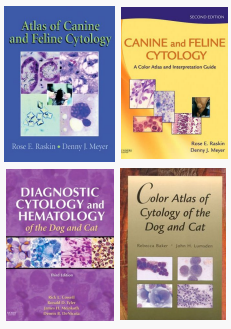
Table 2. Specific diagnoses achieved within category 1

Specific diagnosis	Frequency
Lipoma	105
Mast cell tumour	49
Epidermal inclusion cyst	17
Lymphoid neoplasia	16
Histiocytoma	8
Sebaceous hyperplasia/adenoma	6
Perianal adenoma	5
Anal sac adenocarcinoma	4
Haemangiopericytoma	1
Hepatocellular carcinoma	1
Leishmaniasis	1
Melanoma	1
Multiple myeloma	1
Plasmacytoma	1
Thyroid adenoma (in a horse)	1
Transitional cell carcinoma	1
Total	218

*Skeldon, N and Dewhurst, E: The perceived and actual diagnosticutility of veterinary cytologicalsamplesThe perceived and actual diagnosticutility of veterinary cytologicalsamples; JSAP (2009) 50, 180–185

References

- Atlas of Canine and Feline Cytology – Raskin
- NEW: Canine and Feline Cytology: A Color Atlas and Interpretation Guide – Raskin
- Diagnostic Cytology and Hematology of the Dog and Cat – Cowell
- Color Atlas of Cytology of the Dog and Cat - Baker



SYSTEMATIC APPROACH TO LYMPH NODE ASPIRATES

1. Evaluate the quality of the preparation
    a. Adequate amount of intact cells
        Immature lymphocytes are very fragile, thus many lysed cells are common
    b. Adequate spread
        Thin areas – good aspirates tend to be very cellular, and quite thick!
    c. Adequate staining (thick areas tend to under-stain)
        NEVER INTERPRET UNDER-STAINED AREAS – they always look like lymphoma!
2. Scan the entire slide on low magnification (4X) to find the best areas to spend you time on!
3. Assess the cellular arrangement on low power – look for "foreign" cells (clusters, etc.)
4. Try to decide if it is a uniform population (small matures mostly, or medium to large immature) or a variable population
5. Lymph nodes are "easy", as there is only really 5 big categories in which you have to fit it into based on the proportion (%) of cells types present.

a. Normal

i. Dominated by small, mature lymphocytes (>90%; size of nucleus: **1-1.5 RBC** in diameter, clumped (blocks of) chromatin, small amount of cytoplasm)
ii. Low numbers of medium (size of nucleus: **2-2.5 RBC** in diameter) to large (size of nucleus: **>3 RBC** in diameter), immature lymphocytes (finely granular chromatin, occasionally prominent nucleoli, increased amount of light to deeply basophilic (blue) cytoplasm (<5-10%).
iii. Occasional macrophages, rare neutrophils, eosinophils, mast cells, etc…

b. Hyperplastic or reactive

i. Could be similar populations as normal, but the node is enlarged
    1. If node is enlarged, and the proportions look similar to normal node, by definition it is a hyperplastic node
ii. Increased % of medium to large lymphocyte, but less than 50%!
iii. Possibly increased % of plasma cells (small eccentric nucleus, clumped chromatin, abundant deeply basophilic cytoplasm with prominent perinuclear halo (Golgi zone).
iv. Possibly increased numbers of mitotic figures
v. ALWAYS try to look for reason of hyperplasia/reactivity! (e.g. metastatic tumor, organisms, etc…)

c. Lymphadenitis

i. Increased percentages of inflammatory cells
    1. neutrophils (>5%): 'neutrophilic lymphadenitis'
    2. eosinophils (>3%): 'eosinophilic lymphadenitis'
    3. macrophages: 'histiocytic'/'macrophagic' lymphadenitis
    4. combination of the above, also mast cell % will increase

d. Lymphoma

i. More than 50% immature (medium to large) lymphocytes – *monotony*!
    1. It is not so much the *appearance*, but the *numbers* that count!
ii. Possibly increased numbers of mitotic figures
iii. Only low numbers of small, mature lymphocytes
iv. Plasma cells may or may not be present

e. Metastatic neoplasia

i. Presence of "foreign" cells (even if they don't have ample features of malignancy)
ii. Need to examine all slides
iii. Not finding metastatic cells does not rule it out!

Lymph Node Cytology

Balázs Szladovits - DVM, MRCVS, FHEA, Diplomate ACVP

Pathology and Pathogen Biology

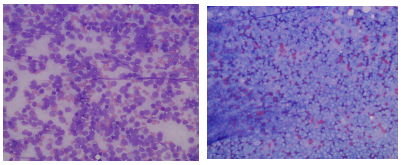
RVC Royal Veterinary College
University of London

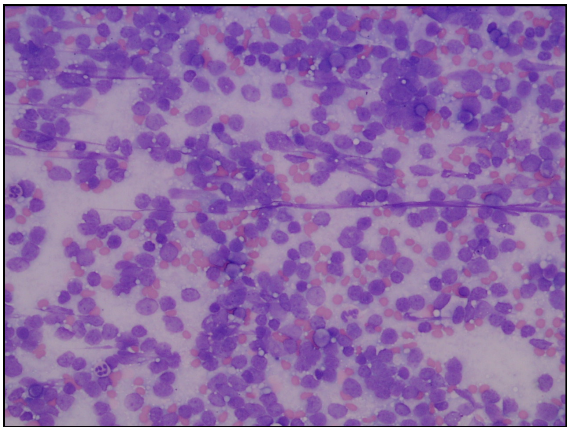
Indications for lymph node sampling

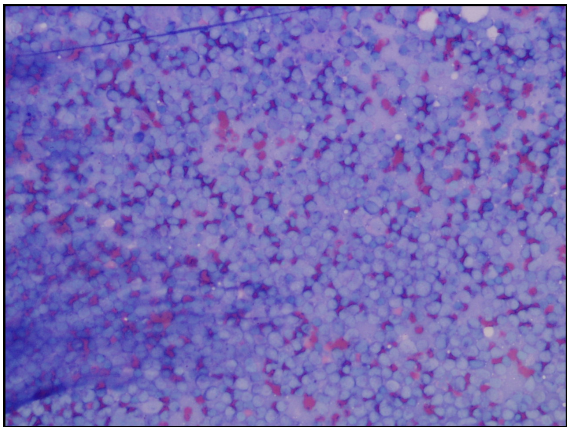
- Lymphadenomegaly
  - Single or multiple nodes
- Evaluation of metastatic disease
  - Based on drainage
    - Submandibular – head (incl. rostral oral cavity)
    - Prescapular – head caudal (pharynx, pinna), thoracic limb, part of thoracic wall
    - Axillary – thoracic wall, deep structures of thoracic limb and neck, thoracic and cranial abdominal mammary glands
    - Superficial inguinal – caudal abdominal and inguinal mammary glands, ventral half of abdominal wall, penis, prepuce, scrotal skin, tail, ventral pelvis, medial part of thigh and stifle
    - Popliteal – distal to stifle
- Classification of lymphoma (?)

Lymph node sampling considerations

- Submandibular vs. popliteal or prescapular
- Aspirating very large nodes
  - Necrotic, hemorrhagic center
- Smearing technique!







Aspirate vs. biopsy

- Invasiveness, cost, turn around time
- Cell detail vs. architecture
  - Lymphoma
  - Metastasis
- Immunocytochemistry

Systematic approach to lymph node aspirates

1. **Evaluate the quality of the preparation:**

- Adequate amount of intact cells
  - Immature lymphocytes are very fragile, thus many lysed cells are common
- Adequate spread
  - Thin areas – good aspirates tend to be very cellular, and quite thick!
- Adequate staining (thick areas tend to under-stain)
  - ***NEVER INTERPRET UNDER-STAINED AREAS*** – they always look like lymphoma!

Systematic approach to lymph node aspirates

2. **Scan the entire slide on low magnification (4X) to find the best areas to spend you time on!**
3. **Assess the cellular arrangement on low power – look for "foreign" cells (clusters, etc.)**
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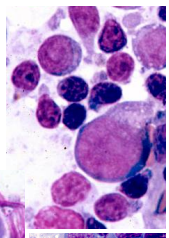
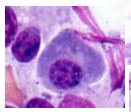
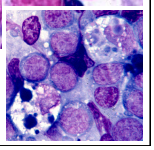
5. **Lymph nodes are "easy", as there is only really 5 big categories in which you have to fit it into based on the proportion (%) of cells types present.**

Lymph node classifications

- **Normal**
- **Hyperplastic/reactive lymph node**
- **Lymphadenitis**
- **Lymphoid neoplasia**
- **Non-lymphoid neoplasia**

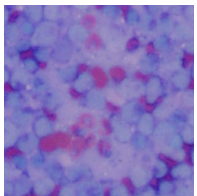
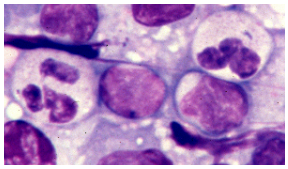
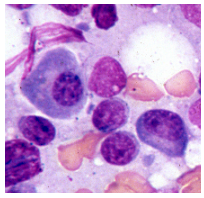
Systematic approach to lymph node aspirates

- Only few cell types to recognize
  - Small lymphocyte
    - 1-1.5x RBC
  - Medium lymphocyte
    - 2-2.5x RBC
  - Large lymphocyte
    - >3x RBC
  - Plasma cell
  - Macrophage
  - Few others
    - Inflammatory cells (neuts, eos)
    - Mast cells
    - Foreign cells

Size judgement

- Adequate spread
- RBCs
- Neutrophils

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- Occasional macrophages, rare neutrophils, eosinophils, mast cells, etc…

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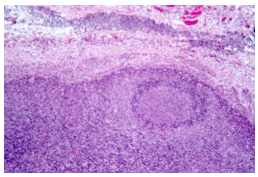
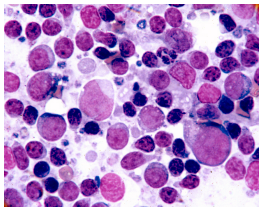
- Could be similar populations as normal, but the node is enlarged
  - If node is enlarged, and the proportions look similar to normal node, by definition it is a hyperplastic node
- Increased % of medium to large lymphocyte, but less than 50%!
- Possibly increased % of plasma cells (small eccentric nucleus, clumped chromatin, abundant deeply basophilic cytoplasm with prominent perinuclear halo (Golgi zone).
- Possibly increased numbers of mitotic figures
- ALWAYS try to look for reason of hyperplasia/reactivity! (e.g. metastatic tumor, organisms, etc…)

Hyperplastic lymph node

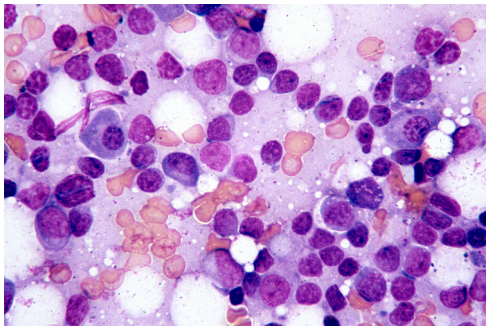


Lymph node section (H & E stain)



Lymph node aspirate (Wright stain)

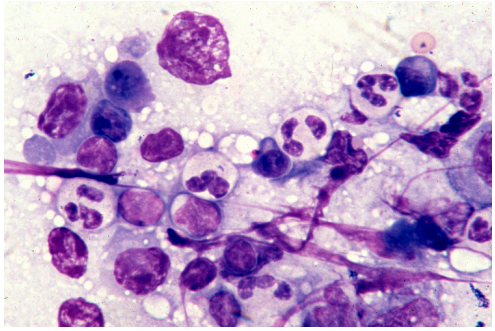
Reactive lymph node



Lymphadenitis

- Increased percentages of inflammatory cells
  - Neutrophils (>5%): 'neutrophilic lymphadenitis'
  - Eosinophils (>3%): 'eosinophilic lymphadenitis'
  - Macrophages: 'histiocytic'/'macrophagic' lymphadenitis
  - Combination of the above, also mast cell % will increase

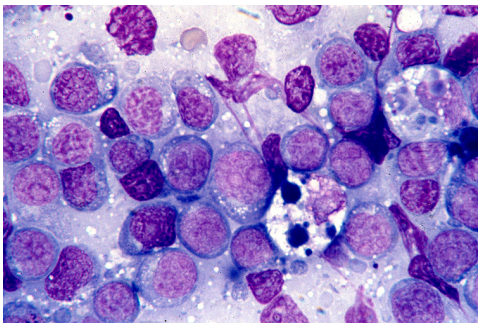
Lymphadenitis



Lymphoid neoplasia

- More than **50%** immature (medium to large) lymphocytes – ***monotony***!
  - It is not so much the ***appearance***, but the ***numbers*** that count!
- Possibly increased numbers of mitotic figures
- Only low numbers of small, mature lymphocytes (rare really large)
- Plasma cells may or may not be present

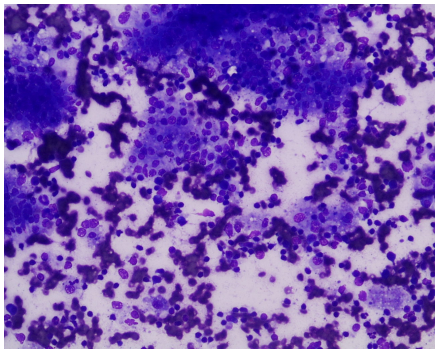
Lymphoid neoplasia



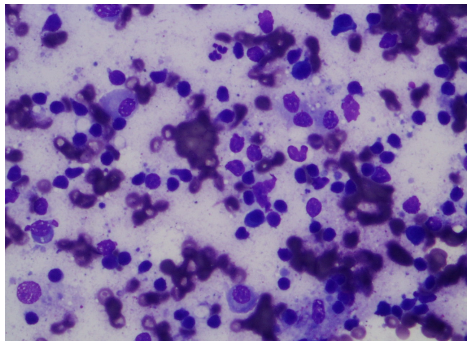
Metastatic neoplasia

- Presence of "foreign" cells (even if they don't have ample features of malignancy)
- Need to examine all slides!
- Not finding metastatic cells does not rule it out!

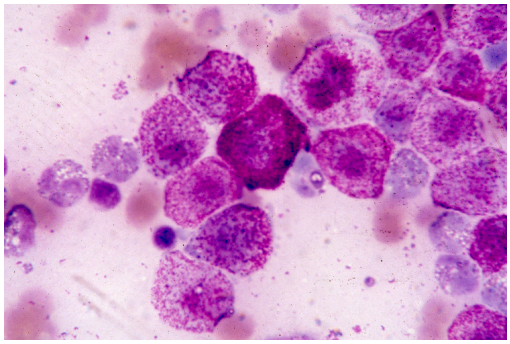
Metastatic neoplasia



Metastatic neoplasia

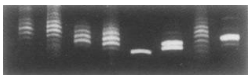


Metastatic neoplasia

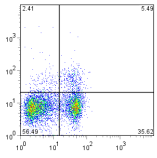


New diagnostic techniques

- PCR for T or B cell receptor re-arrangement
  - Clonality



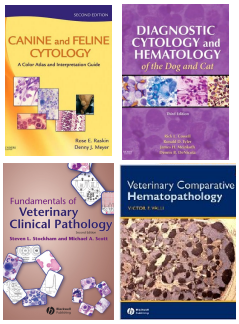
- Flowcytometry
  - Immunophenotyping



- Immunocytochemistry

References

- NEW: Canine and Feline Cytology: A Color Atlas and Interpretation Guide – Raskin
- Diagnostic Cytology and Hematology of the Dog and Cat – Cowell
- Fundamentals of Veterinary Clinical Pathology (2nd ed) – Stockham and Scott
- Veterinary Comparative Hematopathology - Valli

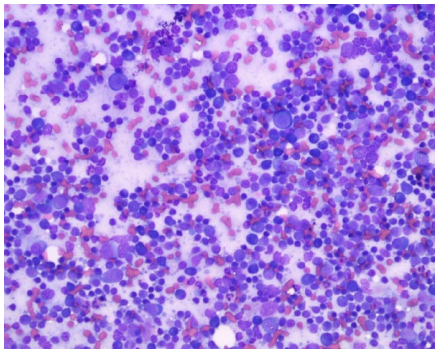


Lymph nodes – Case 1

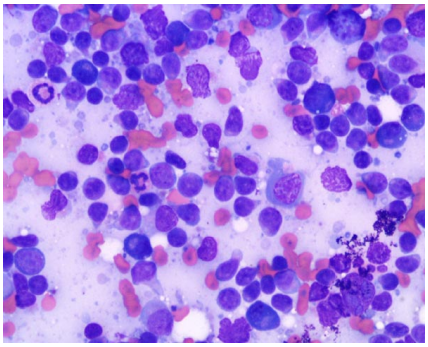
Case history

- 5 year old male neutered Cocker Spaniel with intermittent fever (39.8-41.0 °C) over the last 2 weeks presenting to you with slight generalised peripheral lymphadenomegaly. You perform a fine needle aspirate of the popliteal lymph node.

Lymph nodes – Case 1



Lymph nodes – Case 1



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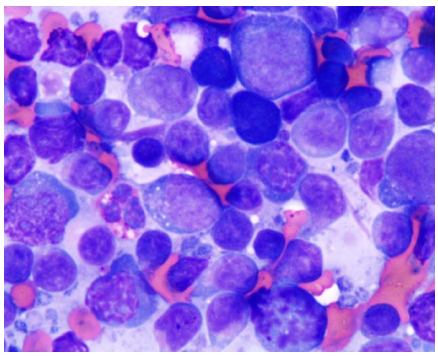
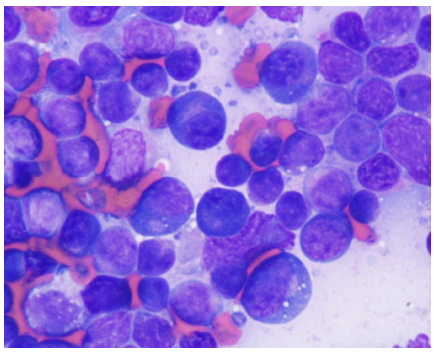


Lymph nodes – Case 1

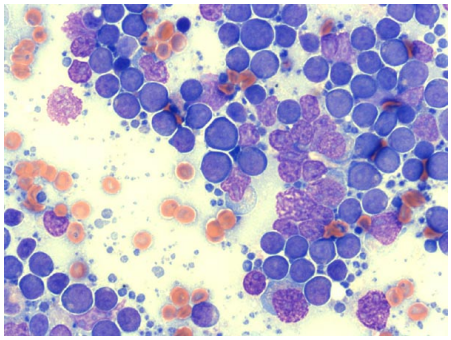


Lymph nodes – Case 2

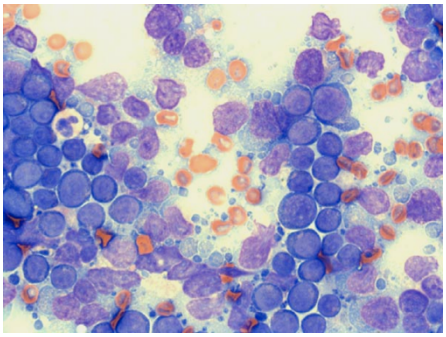
Case history

- 3 year old male intact Boxer presenting to you with PU/PD of 3 week's duration. On physical examination, you notice severely enlarged prescapular and popliteal lymph nodes. You perform FNA's of the lymph nodes.

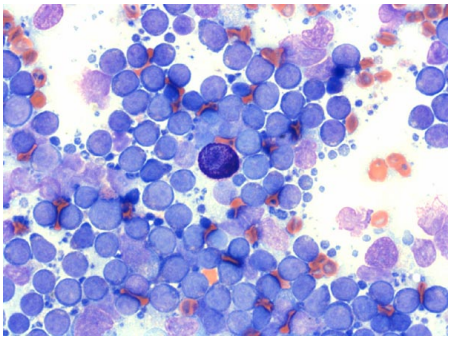
Lymph nodes – Case 2



Lymph nodes – Case 2



Lymph nodes – Case 2



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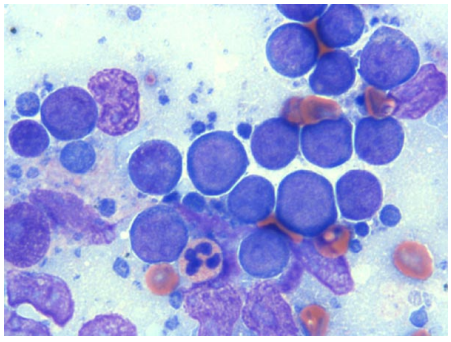
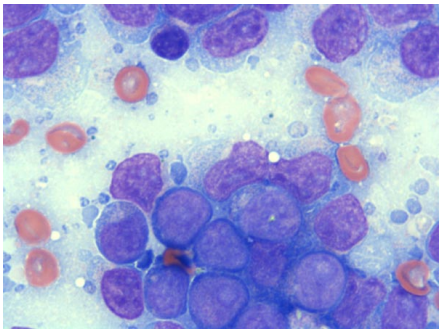


Lymph nodes – Case 2

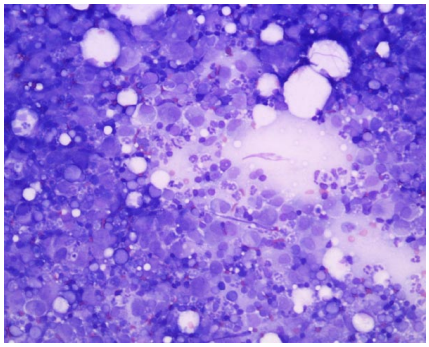


Lymph nodes – Case 3

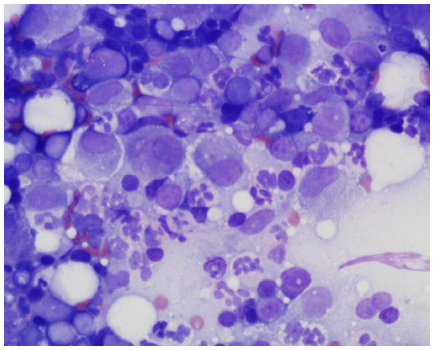
Case history

- 12 year old male intact Flat Coated Retriever presenting to you with sneezing, mucopurulent discharge from both nostrils and epistaxis from the left nostril over the last 2 weeks. Radiographs of the nose show a destructive rhinitis on the left side, suggestive of neoplasia. You perform rhinoscopy and at the same time, take a FNA of the mandibular lymph nodes, as they are enlarged.

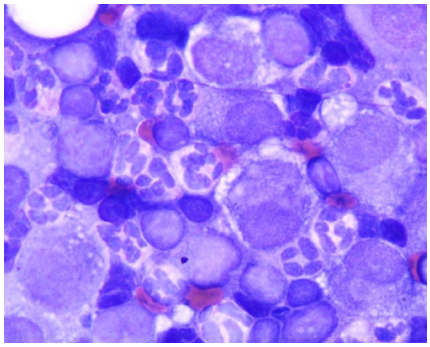
Lymph nodes – Case 3



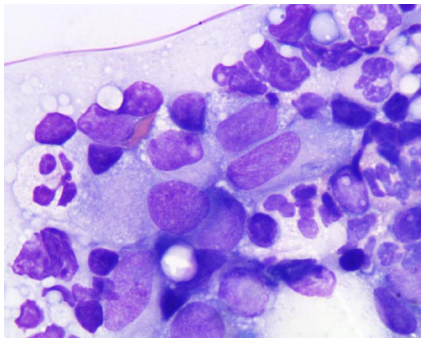
Lymph nodes – Case 3



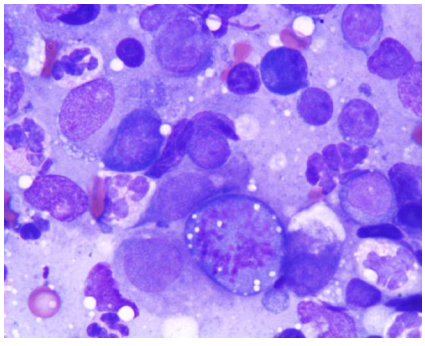
Lymph nodes – Case 3



Lymph nodes – Case 3



Lymph nodes – Case 3

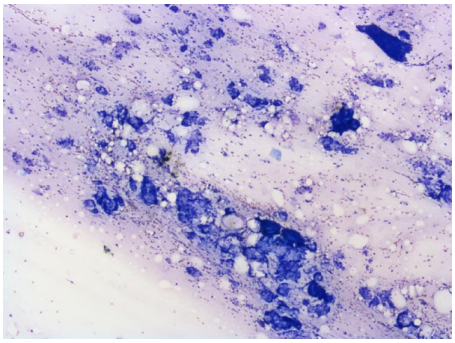


Lymph nodes – Case 4

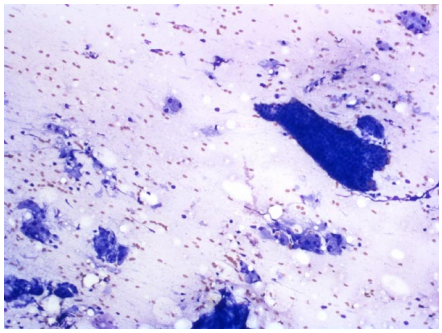
Case history

- 10 year old female neutered West Highland White Terrier presenting to you with lethargy and inappetence. You examine the dog and find that all peripheral lymph nodes are moderately enlarged. You take an FNA of the submandibular lymph nodes.

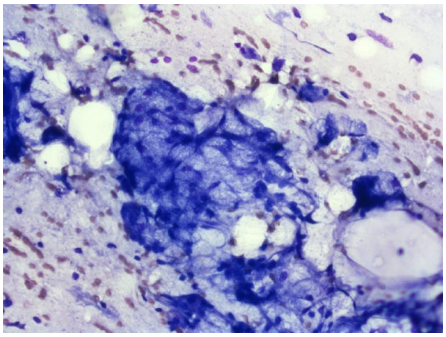
Lymph nodes – Case 4



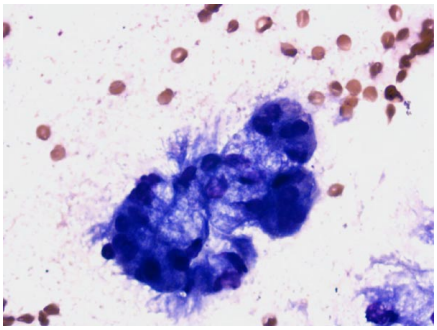
Lymph nodes – Case 4



Lymph nodes – Case 4



Lymph nodes – Case 4

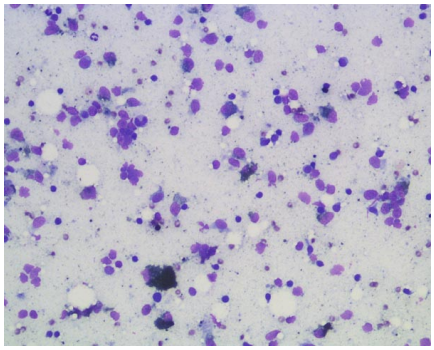


Lymph nodes – Case 5

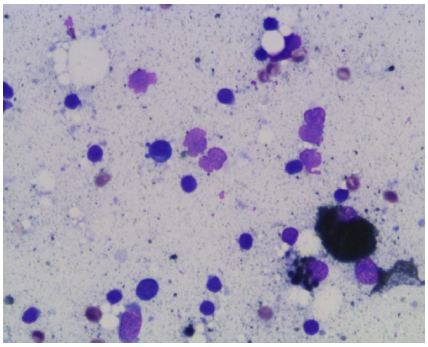
Case history

- 3 year old male intact Boxer presenting to you with an ulcerated oral mass on the left side of the lip. On physical examination, the submandibular lymph nodes are enlarged, and you perform an FNA of the lymph nodes.

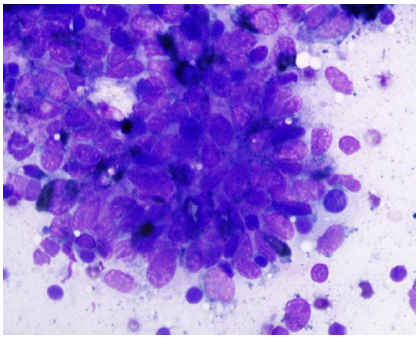
Lymph nodes – Case 5



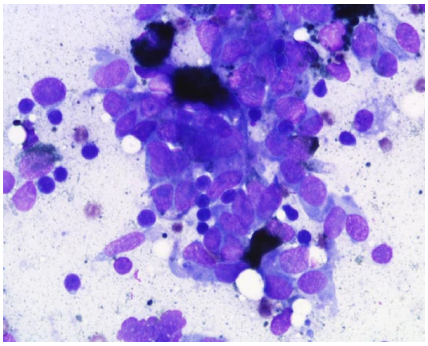
Lymph nodes – Case 5



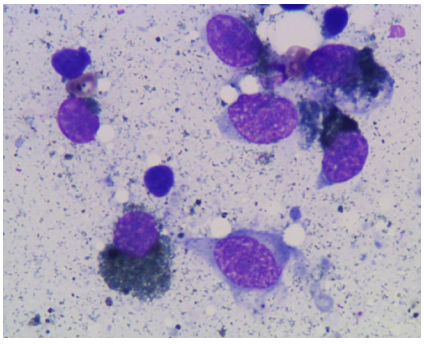
Lymph nodes – Case 5



Lymph nodes – Case 5



Lymph nodes – Case 5



Lymph nodes – Case 5

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Survival of Dogs Following Surgical Excision of Histologically Well-differentiated Melanocytic Neoplasms of the Mucous Membranes of the Lips and Oral Cavity

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Abstract. Postsurgical follow-up information was obtained on 64 dogs with 69 histologically well-differentiated melanocytic neoplasms that involved the mucous membranes of the lips and oral cavity. The patients received no adjunct therapy. Sixty one of 64 dogs (95%) were alive at the end of the study or had died of causes unrelated to the tumor, with a mean survival of 23.4 months and a median survival of 34 months after surgery. Twenty-eight dogs alive at the end of the study had a mean survival of 31.3 months after surgery. There were 2 dogs, which had recurrent tumors, that were still alive at the end of the study. All dogs that died of tumor-related causes (3) and all dogs with recurrent tumors (2) had tumors in the oral cavity.

Results of this study indicate that a favorable clinical course and prolonged survival can be expected in most dogs with histologically well-differentiated melanocytic neoplasms of the mucous membranes of the lips and oral cavity, with only local excision of the lesions and no adjunct therapy.

Key words: Canine; lips; melanocytic neoplasms; oral cavity.