INTRODUCTION TO CYTOLOGY

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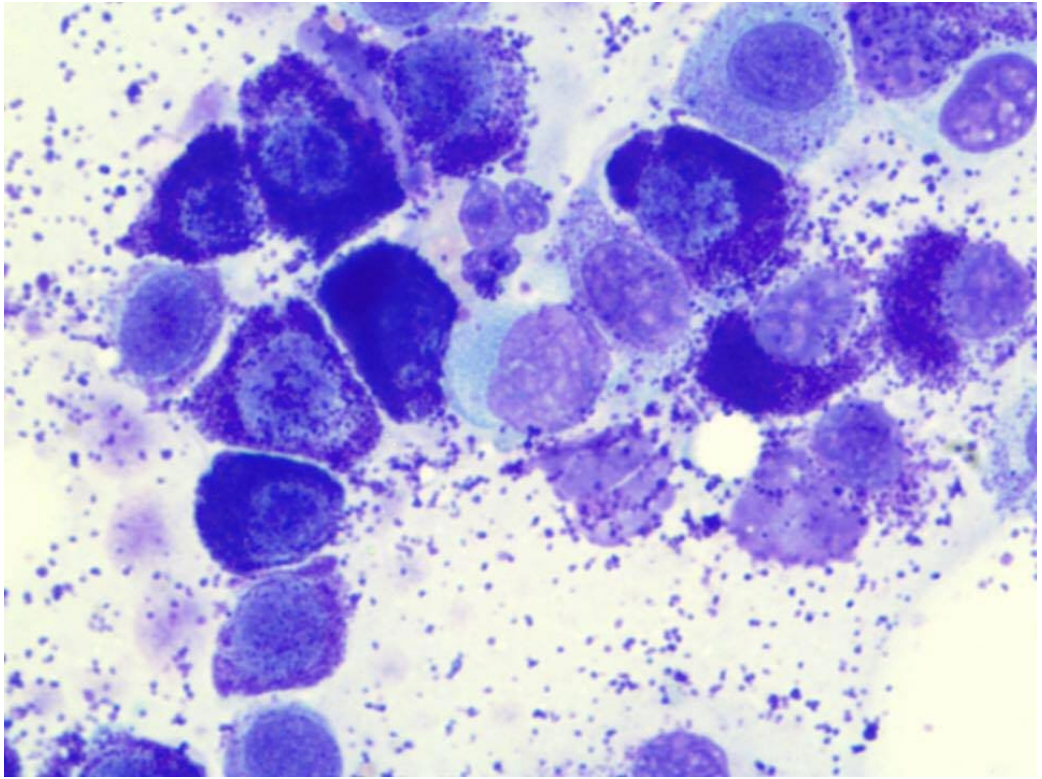
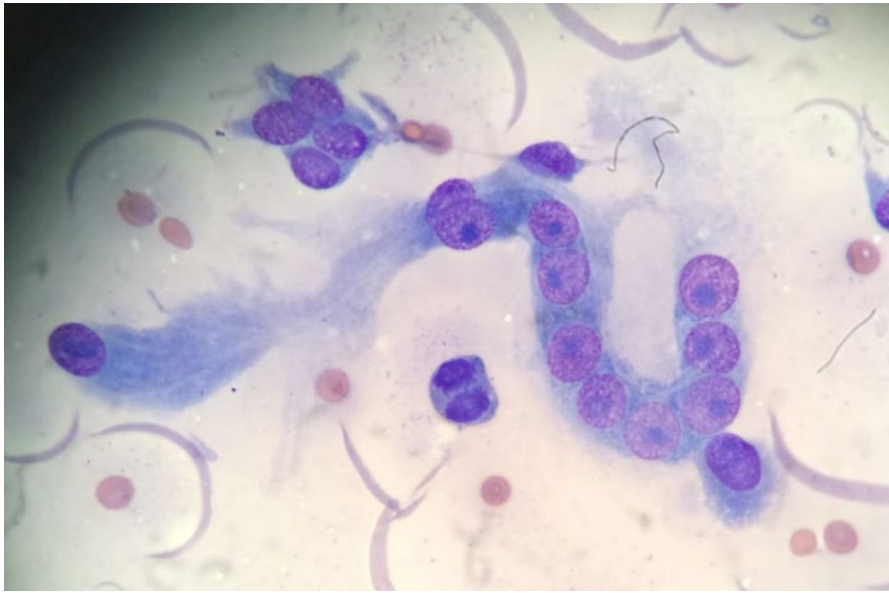


Mast cell tumor

Outline

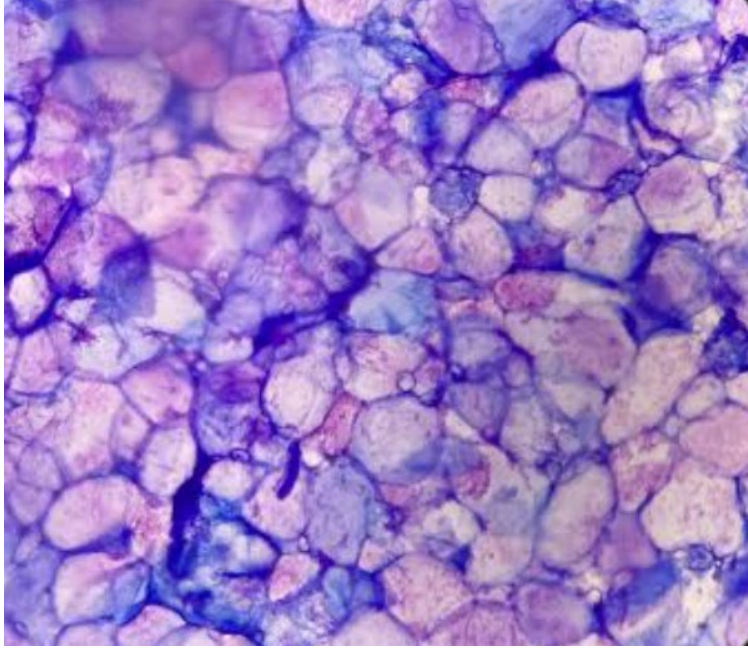
- **Indications for Cytology**
- Sample collection and handling
  - Collection method & slide preparation
- Sample evaluation
  - General approach
- Sample interpretation
  - Basic cytologic principles



Cool mesenchymal cell-sarcoma

Why Cytology??

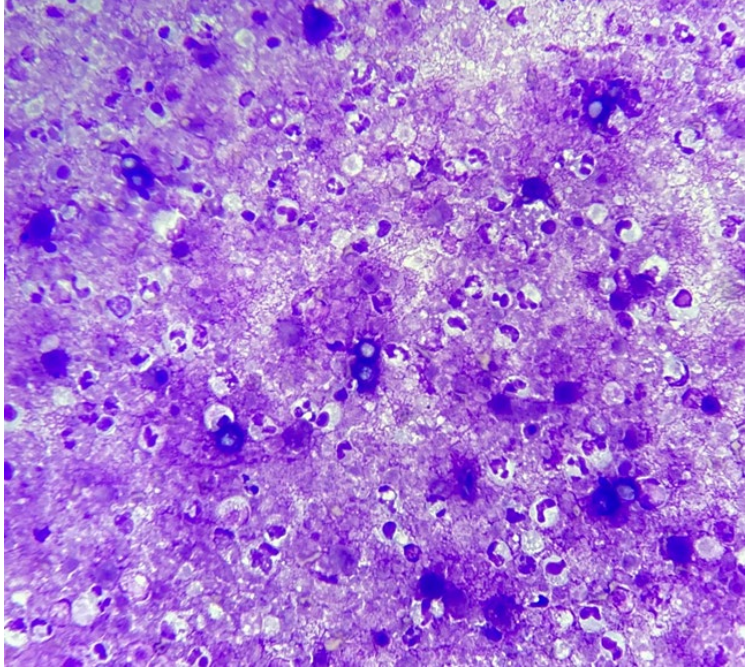
- Fast, minimally invasive, and relatively inexpensive



Keratin

Indications

- Characterize a detected abnormality
  - Mass/infiltrative
  - Organomegaly
  - Ulcerative/exudative lesion
  - Effusion
- Staging of cancer
- Diagnostic work-up for FUO, hypercalcemia, monoclonal gammopathy
- Bone marrow aspiration for investigation of hematological abnormalities



Blastomycosis with neutrophilic inflammation

Limitations

- Sometimes difficult to achieve a final diagnosis on a cytology sample
  - Histopathology (or additional diagnostics) may eventually be needed
  - May be non-representative or of low diagnostic quality
- Check one slide before sending to external laboratory or waking up the patient
- Interpretation requires experience
- ‘Quick’ stains are suboptimal for reliable cell morphology evaluation

Expectations

- Highly dependent upon the quality and cellularity of the sample submitted
- Quickest option for fluids, best option for bone marrow (BM) and round cell tumors
- Great for detecting inflammation and good at detect infection
- Detect neoplasia in *most* cases
- Differentiate between benign and malignant lesions and identify major tumor type in *many* cases
- The more complicated the lesion, the less clear the cytologic diagnosis

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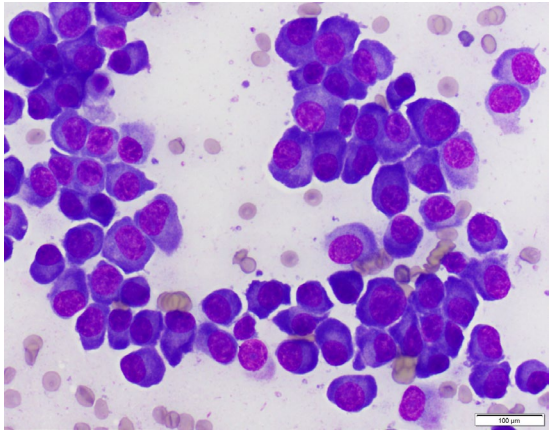


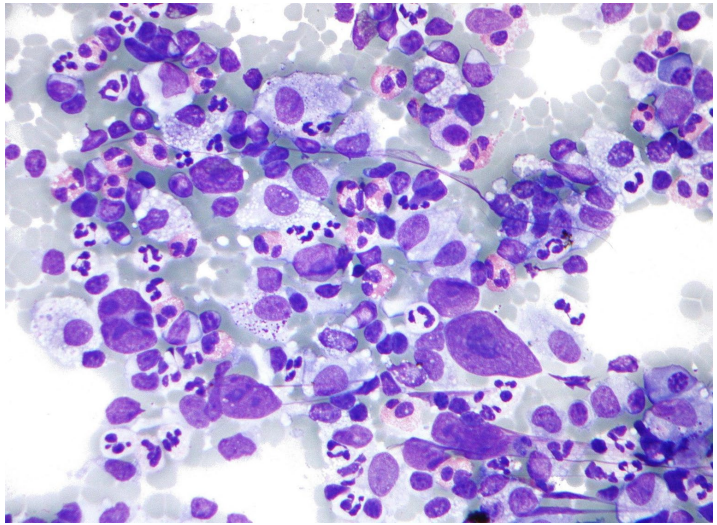
Plasma cell tumor

Sample Collection & Handling

- **DIFFERENT LESIONS EXFOLIATE DIFFERENTLY…**
  - In general:
    - Mesenchymal-poorly exfoliative
    - Epithelial & round cells (e.g., lymph node)-exfoliate well
- **…AND SPECIMEN MANAGEMENT AFFECTS ACCURACY OF INTERPRETATION…**
  - E.g., neoplastic cells (especially lymphocytes) are very fragile
- …**THEREFORE, APPROPRIATE TECHNIQUE AND PREPARATION ARE KEY!**

Sample Collection & Handling

- ROUTINE SKIN PREP!
- Collection Method
  - Fine needle aspiration
    - Preparation
      - Blood smear technique
      - Squash preparation
  - Impression smear
  - Roll preparation



https://en.wikipedia.org/wiki/Hodgkin's_lymphoma#mediaviewer/File:Hodgkin_lymphoma_cytology_large.jpg

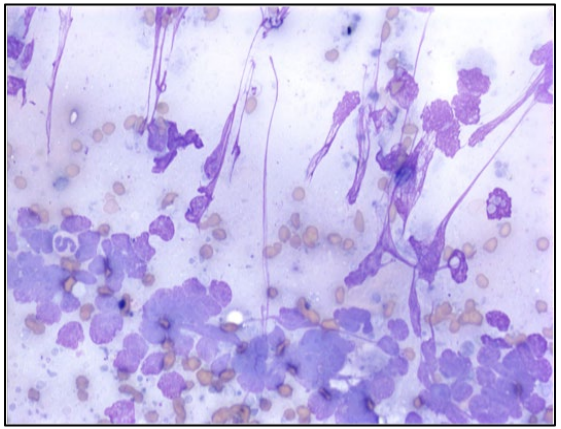
Mast cells, eosinophils, neutrophils

Sample Collection & Handling

- Collection Method
  - **Fine needle aspiration**
    - Most common!
      - Solid tissue
      - Fluid aspiration
    - Supplies
      - 6 or 12 mL syringe
      - 20 or 22 gauge needle (bigger is not better)
      - NEW slides (wipe!)
- +/- Ultrasound guidance
- Needle vs. needle + syringe
- Aspiration vs. capillary
  - Vascular lesion?
  - Release negative pressure!
- If you have doubts about quality, you're probably right!

Sample Collection & Handling

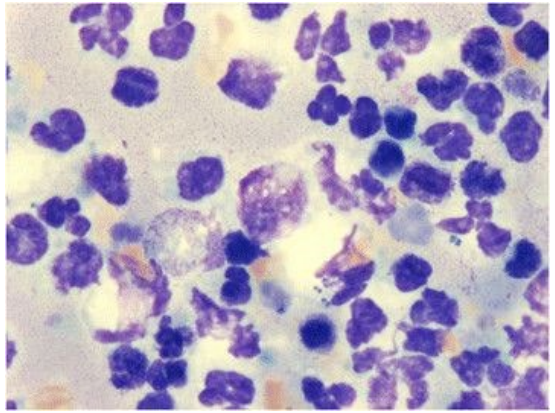
- Collection Method
  - Fine needle aspiration
    - **Preparation**
      - Blood smear technique
        - » Ideal for more vascular lesions
      - "Squash" preparation
        - » Ideal for little blood/fluid, semisolid, mucoid/thick specimens
        - » Don't actually squash…a firm, gentle, continuous pull!



Broken cells

Sample Collection & Handling

- Collection Method
  - Fine needle aspiration
  - **Impression smear/Touch Imprint**
    - Ideal for ulcerated lesions & evaluation of cut surface of biopsy
      - If ulcerated, MUST aggressively debride!
    - BLOT tissue until DRY
    - Touch several areas to slide – **should be sticky!**
    - Fibrous tissue samples can be “roughened” with a scalpel



https://link.springer.com/article/10.1186/s12917-014-0201-z

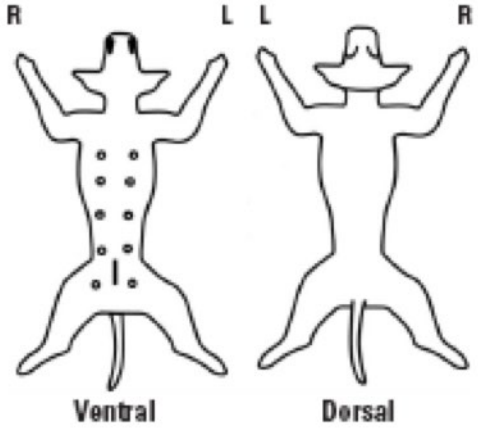
Neutrophilic inflammation with intracellular cocci

Sample Collection & Handling

- Collection Method
  - Fine needle aspiration
  - Impression smear
  - **Roll preparation**
    - Used for very small tissue samples, bone marrow cores
    - GENTLY roll the sample along the slide using a needle

Sample Collection & Handling

- Submitting Slides
  - Properly label the slide!!!
    - Patient ID
    - Clearly identify sample/tissue of origin
      - Specify multiple samples (i.e., which lymph nodes)
  - FILL OUT THE HISTORY FORM
    - Provide relevant clinical information!!!



Sample Collection & Handling

- Submitting Slides
  - A word on staining…
    - **Always stain the "worst" slide and assess adequacy** (send this too!)
    - **Always send unstained slides**
  - **KEEP AWAY FROM FORMALIN FIXED SAMPLES!**

- Romanowsky-type stains
  - "Diff-Quik"
    - **Mast cell granules**
  - Wright Giemsa
- New methylene blue stain
  - Non-permanent aqueous stain
  - Useful for ID'ing mast cells, fungal organisms, reticulocytes, Heinz bodies

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  - **General approach**
- Sample interpretation
  - Basic cytologic principles

Sample Evaluation

- Know your limits!
- Get to know your pathologist
- When in doubt, get a second opinion!
- Record keeping – provide a description

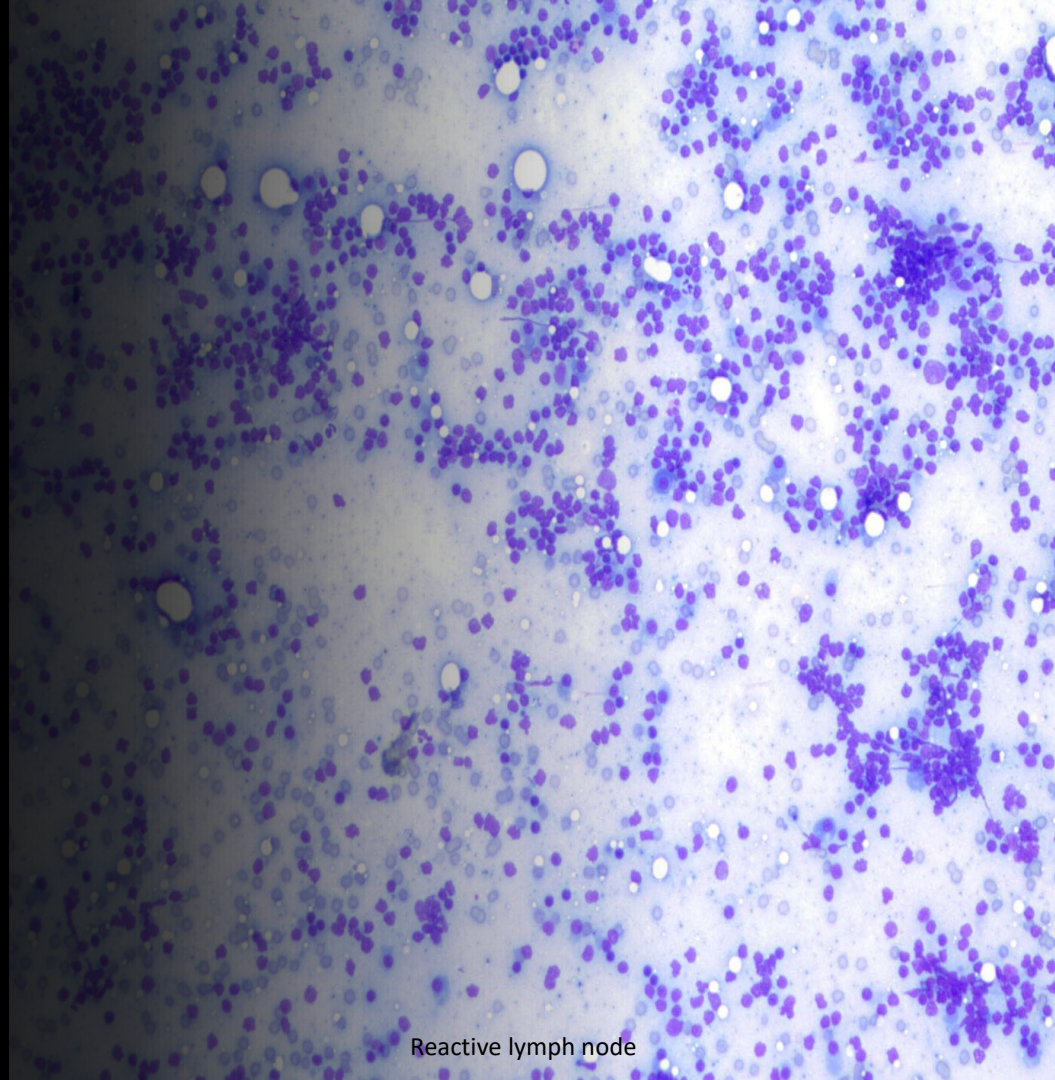
Sample Evaluation

- GENERAL APPROACH: JUST LIKE A PHYSICAL EXAM!
  - Systematic: same order, every time!
  - Just like TPR: **Before even looking, always ask yourself "what is normal for this tissue?"**
  - Start from big picture and zoom in
    - **Resist the urge to go to 100x!**

Sample Evaluation

- 4-10x
  - General impression: similar to observing

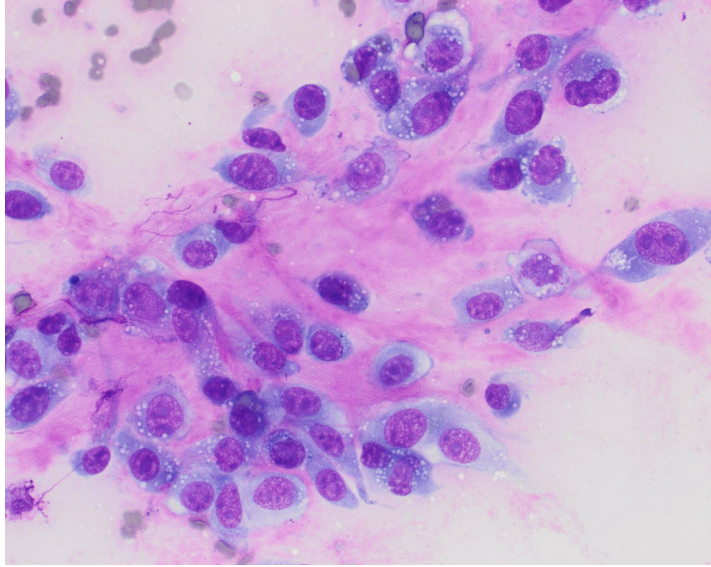
    your patient from afar as you are taking a history from an owner
    - Cellularity?
    - Single population or mixed?
    - How are cells distributed?
    - Background?
    - Blood contamination?
    - Large structures (infectious agents, big cells)?
    - What areas do I want to look at more closely?



Reactive lymph node

Sample Evaluation

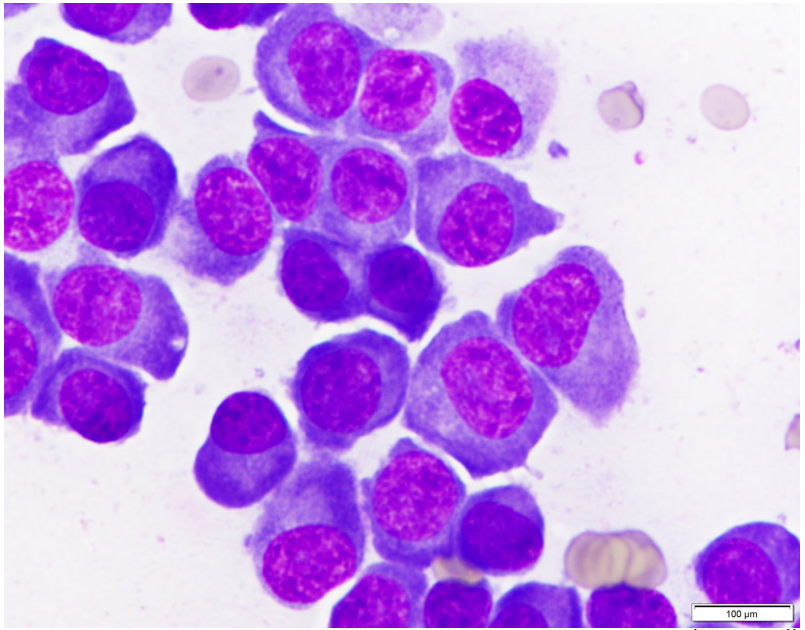
- 40-50x
  - Similar to hands on physical exam
    - Focus on diagnostic areas – look at several
    - What are the cell populations that are present?
    - Are these the cells you were expecting?
    - Are they increased or decreased in number?
    - Do they look normal or abnormal?
    - Any infectious agents (e.g., bacteria)?



Sarcoma with matrix

Sample Evaluation

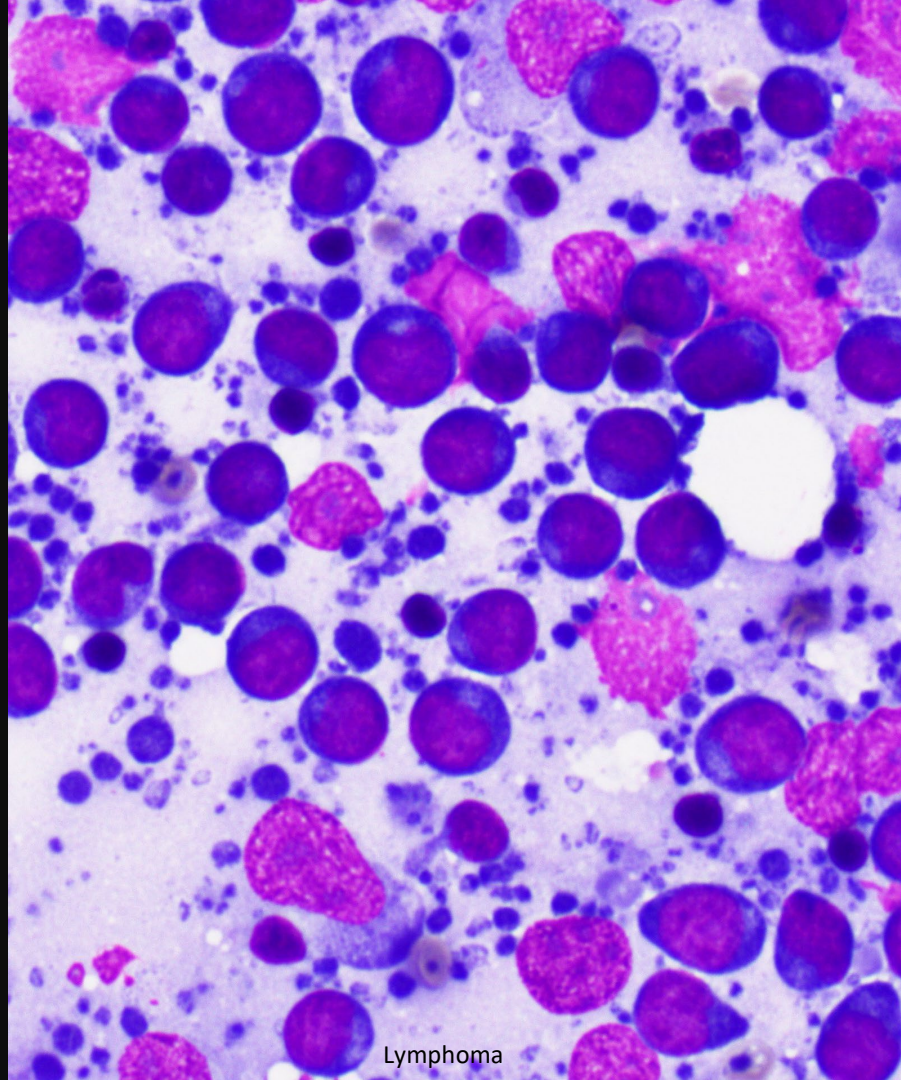
- 100x
  - Zoning in on the abnormalities found on PE
    - What are the features of the normal/abnormal cells?
      - Shape? Size? Cytoplasm? Nuclear features? Etc…
    - Look for small organisms



Plasma cells

Sample Evaluation

- **Checklist:**
  - Sample adequacy and cellularity/number of cells
    - E.g., “mild, moderate, or highly cellular”
  - Describe cell populations present with relative frequency, type of cell
    - E.g., “large numbers of small lymphocytes”
  - Do the cells from the same population look alike?
  - Association with other cells
  - Cell margins? Shape?
  - Cytoplasm: Amount? Color? Inclusions?
  - Nucleus: Number? Shape? Chromatin? Nucleoli?
  - Are there abnormal features?
    - E.g., “mitotic figures”
  - Describe background
    - E.g., “proteinaceous”
  - Are there any etiologic agents or other materials?



Lymphoma

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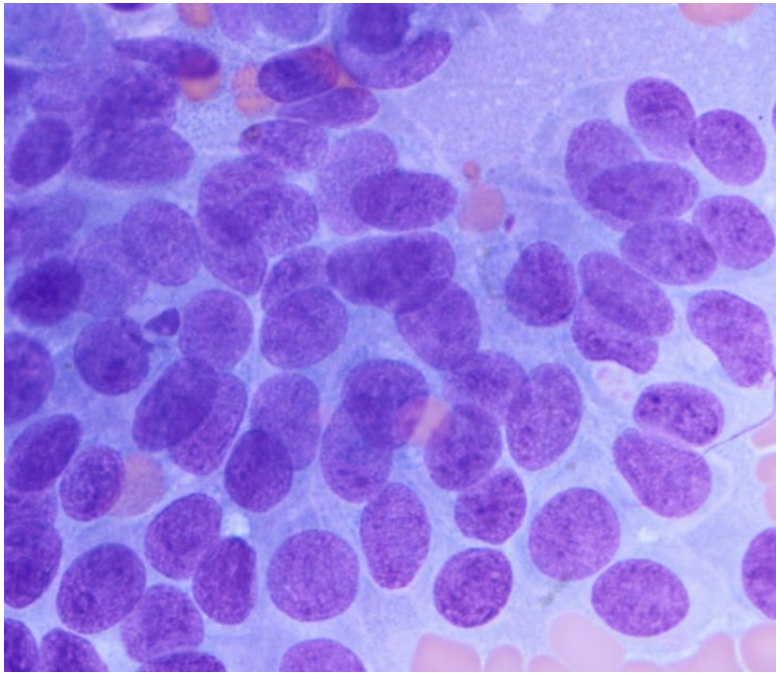
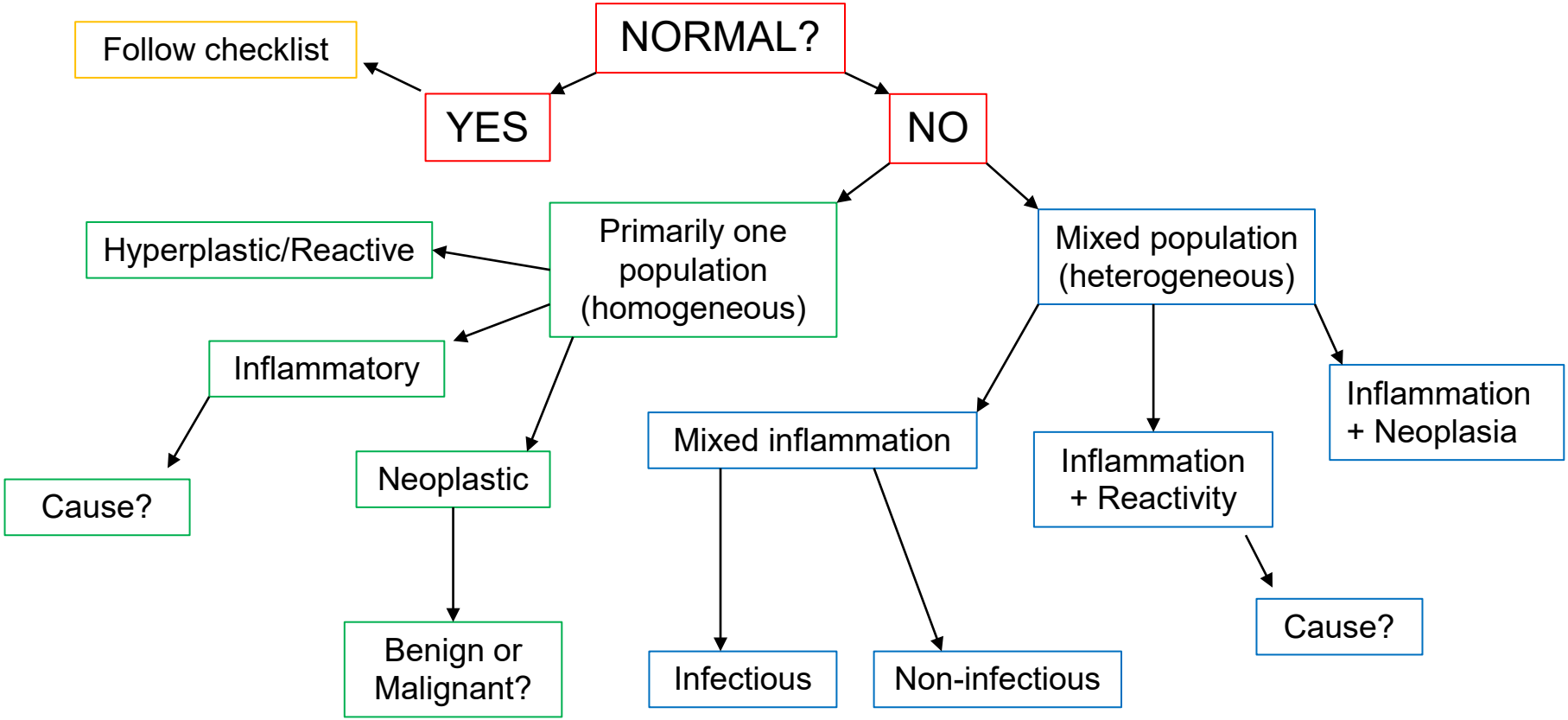


Anal gland adenocarcinoma

Sample Interpretation

- Normal or abnormal (i.e., is this what I expected)?
- Inflammatory vs. tissue (neoplasia)
- How many populations?
- Cellular features?
  - Reactive/Hyperplastic? Inflammatory? Neoplastic?
- Criteria of malignancy?
- Other processes
  - Infectious agents? Are they primary or secondary?
  - Hemorrhage?

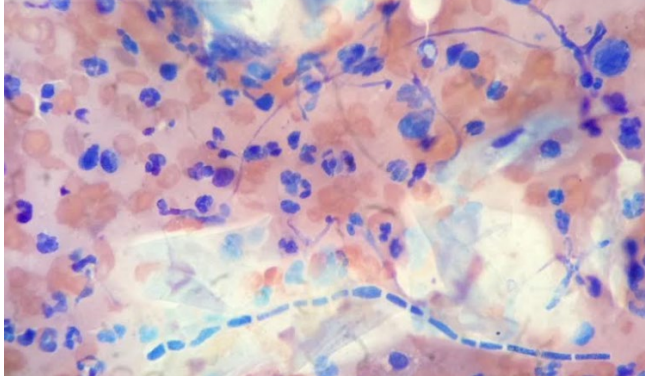

NORMAL?
Follow checklist
YES
NO
Primarily one population (homogeneous)
Mixed population (heterogeneous)
Hyperplastic/Reactive
Inflammatory
Neoplastic
Cause?
Benign or Malignant?
Mixed inflammation
Inflammation + Reactivity
Inflammation + Neoplasia
Infectious
Non-infectious
Cause?

Sample Interpretation

- Abnormal→one population →
  - **Hyperplastic or Reactive Tissue**
    - Looks similar to normal tissue, just more of it than you would expect
    - Cells are often uniform or have very mild changes
    - Epithelial clusters are well-organized
    - EXAMPLES:
      - Reactive fibroplasia
      - Glandular hyperplasia (such as BPH)
      - Reactive lymphoid hyperplasia
      - Epithelial hyperplasia

Sample Interpretation

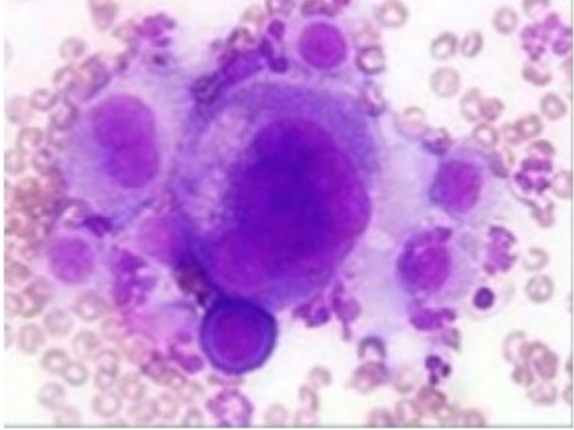
- Abnormal → one population →
  - **Inflammatory Lesion**
    - CHARACTERIZE BY THE PREDOMINANT CELL TYPE:
      - Neutrophilic/suppurative
      - Lymphocytic or lymphoplasmacytic
      - Histiocytic/macrophagic
      - Eosinophilic (>10%)
      - Mixed (no clear predominant cell type)
    - **ALWAYS LOOK FOR A CAUSE!!**



Mycotic infection with neutrophilic inflammation

Sample Interpretation

- Abnormal → one population →
  - **Neoplasia**
    - CHARACTERIZE BY CELL TYPE
      - Epithelial
      - Mesenchymal
      - Round
      - Neuroendocrine
    - BENIGN OR MALIGNANT?
      - Are there criteria of malignancy?



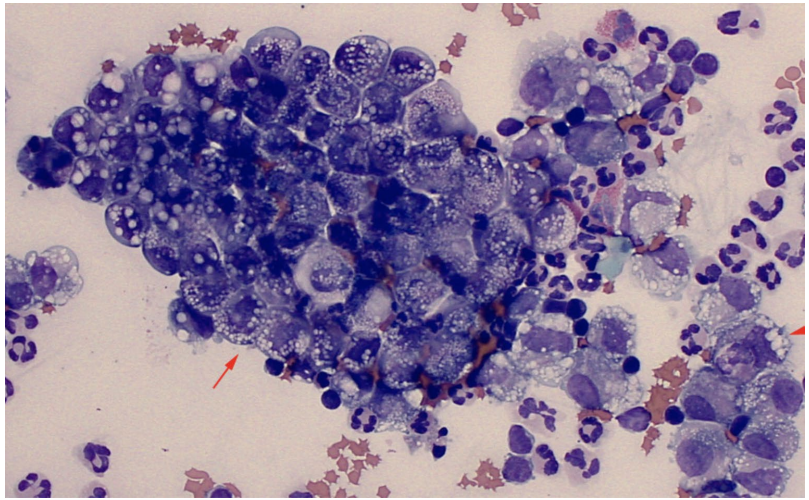
carcinoma

Neoplastic Characterization

- Epithelial
- Mesenchymal
- Round cell
- Neuroendocrine

Epithelial tumors

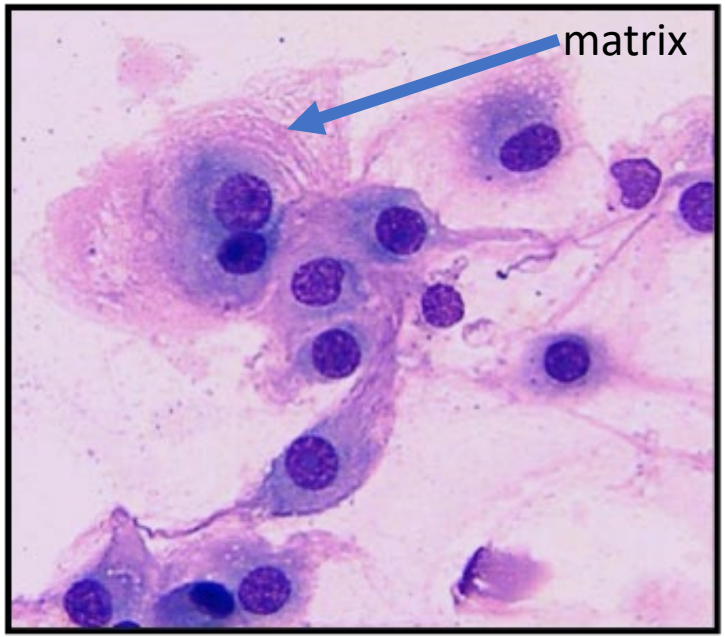
- Benign or malignant (e.g., carcinoma)
- Often exfoliate well
- Cells often present in cohesive **clusters** or **sheets**
  - **“Sticky”** or associate with one another
- **Distinct cell margins**
- Often cannot determine tissue of origin → Histopath!
  - Exception: Squamous cell carcinoma



http://www.eclinpath.com/atlas/cytology-2/feline-peritoneal-fluid/ carcinoma

Mesenchymal Tumors

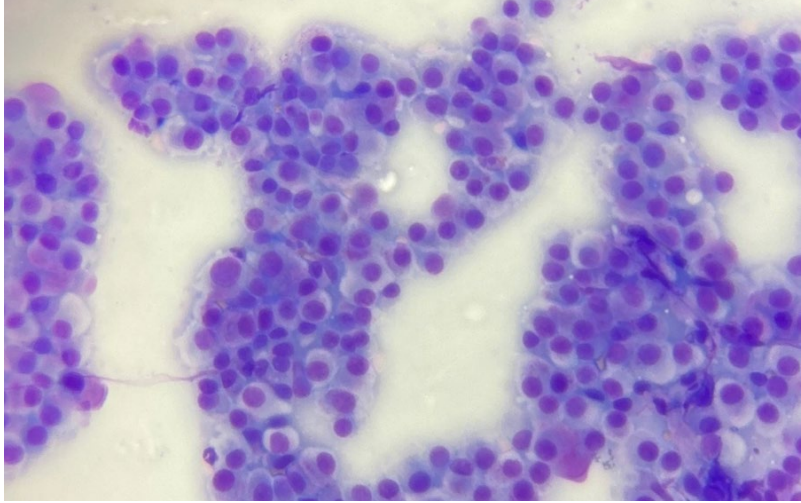
- Differentiate from reactive fibroplasia
- Often low cellularity
- Cells **individualized** or in **loose aggregates**
- May be associated with matrix material
- Variably-distinct to indistinct cell margins
- Spindloid, fusiform
- Again – often cannot tell tissue of origin



sarcoma

Round Cell Tumors

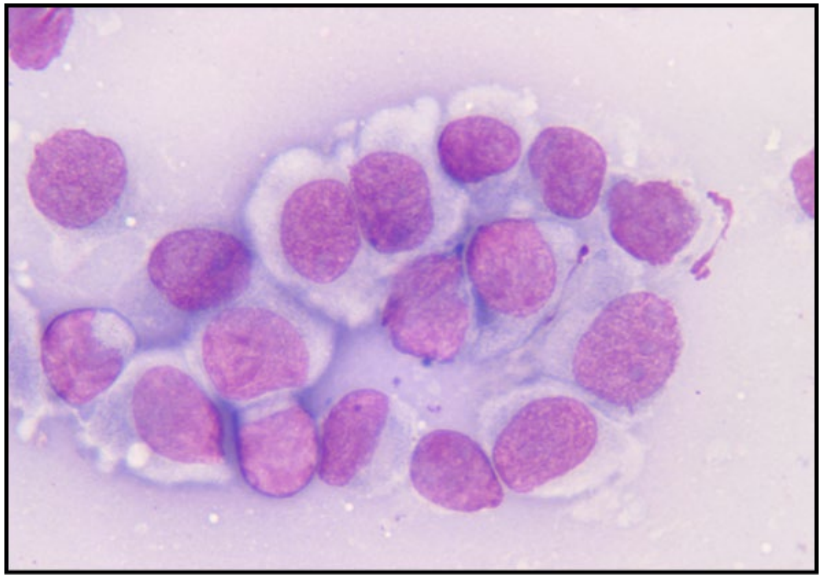
- Usually highly cellular
- Cells are **individualized** or **discrete**
  - Are red cells squeezing between?
- Round/rounded cell margins
- Other cell types present?
  - E.g., eosinophils in mast cell tumors



Poorly granulated mast cell tumor

Round Cell Tumors-T-LYMPH

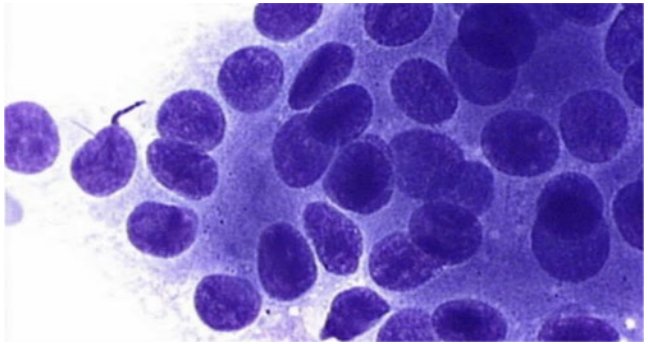
- **T**ransmissible venereal tumor (TVT)
- **Ly**mphoma
- **M**ast cell tumor
- **P**lasma cell tumor
- **H**istiocytoma
- **Melanoma*



Histiocytoma

Neuroendocrine Tumors

- Often appear as **free nuclei** on **lakes of cytoplasm**
- Specific types:
  - **Anal sac adenocarcinoma (not actually neuroendocrine)**
  - Thyroid, insulinoma, other…

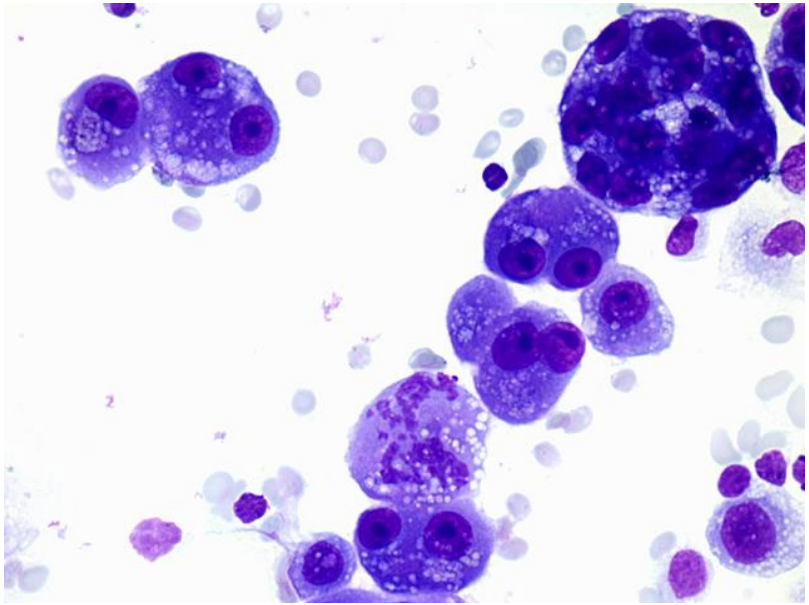


http://the-vet-life.tumblr.com/post/35267419138/alright-so-i-bombed-my-last-viva-i-got-asked

Anal gland adenocarcinoma

Criteria of Malignancy

- Anisocytosis (variation in cell size)
- Anisokaryosis (variation in nuclear size)
- Increased nuclear:cytoplasmic ratio (N:C)
- Multinucleation
- Multiple and prominent nucleoli
- Abnormal mitotic figures



http://pathology.jhu.edu/cytopath_tut/Considerations/ShowImage.cfm?ModuleID=1&CaseInfoID=3&ImageID=84&ConsiderID=7

carcinoma