

# Do's and Don'ts of Cytology

## Do's and Don'ts for Sample Collection and Slide Preparation



## The Obvious

- Good profit center
- No additional equipment needed
- Relatively safe procedure
- Allows rapid identification of pathology in lumps, bumps, fluids and tissues from internal organs

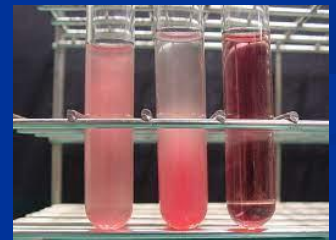


## The Not So Obvious

- Allows evaluation of tissues that would otherwise require much more invasion → Internal organs and internal masses
- Allows evaluation of material that cannot be evaluated any other way → Fluids: Effusions, urine, synovial fluid, spinal fluid

## Fluids

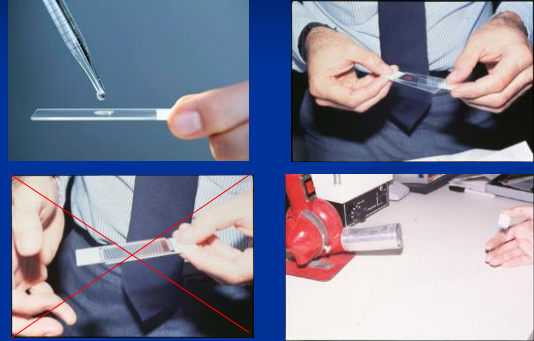
- Never send a fluid to the lab without preparing slides!
  - Cells degenerate!
  - Exception: Spinal fluid mixed 1/2 and 1/2 with hetastarch (Ship on cold pack)



## Effusions

- Total Protein with refractometer
- Cell counts: hemocytometer or your automated analyzer (Dr. Dennis DeNicola)
- Making a smear
  - Do a direct smear for cellularity
  - Spin down fluid and make a smear of the pellet

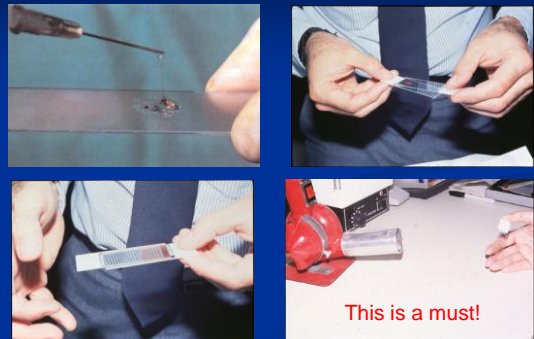
## Slide Preparation



## Synovial fluid

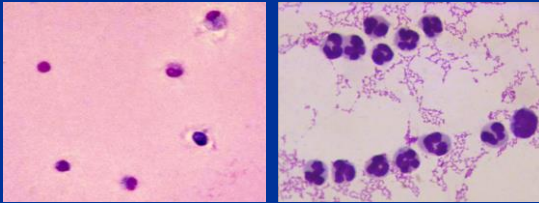
- Only way to distinguish inflammatory vs. non-inflammatory joint disease!

## Slide Preparation



## Synovial fluid

- Only way to distinguish inflammatory vs. non-inflammatory joint disease!



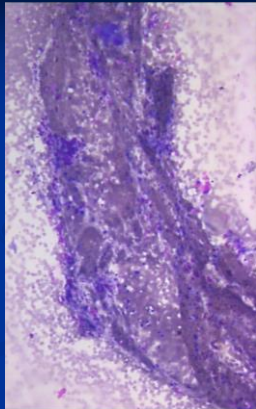
Tap more than one joint!!

## Respiratory Samples

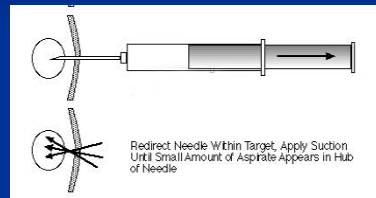
- Endoscopy and bronchial brush
- Tracheal washings
  - Don't just smear the fluid
  - Pick out particles of mucus
    - Capillary pick up
    - Filter fluid on coffee filter
    - Pick out mucus plugs with forceps
    - Smear on slide

## Sample Collection from Tissues

- Use the woodpecker technique with or without an attached syringe
  - If you use a syringe, have the plunger extended
  - Without a syringe, have the syringe ready with plunger extended before collection
- Must collect the sample and prepare the slide without delay!
  - Clots are your enemy!!

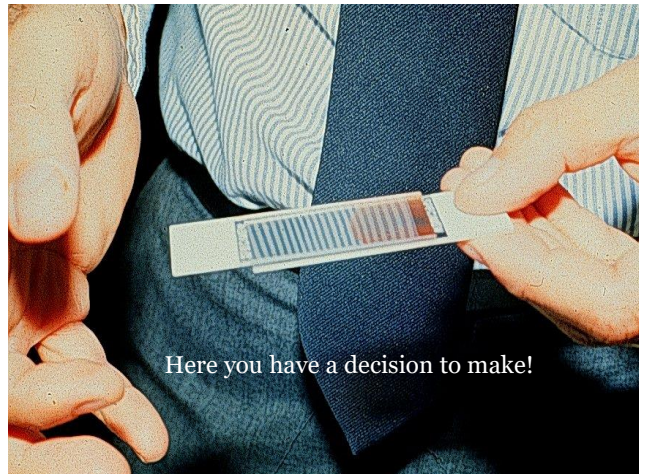


## Woodpecker Technique



Redirect Needle Within Target. Apply Suction Until Small Amount of Aspirate Appears in Hub of Needle



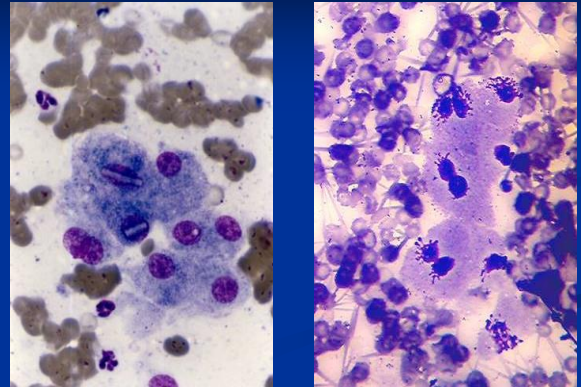




## Dry the Slides!!!



## Delayed Drying Artifact!



## Cytology / Hematology Stains

- Three-step staining set
  - Diff Quik®
  - Numerous other brands
- Aqua fixative – 2 mins.
- Red stain – 1 min.
- Blue stain – 1 min.



## Organs / Masses: Common Lesions

- Ultrasound guided sample collection
- Greatly increases utility of cytology in practice
  - Lung
  - Liver
  - Spleen
  - Pancreas
  - GI tract
  - Prostate
  - Bladder

## Interpretation (Pondering the Material)

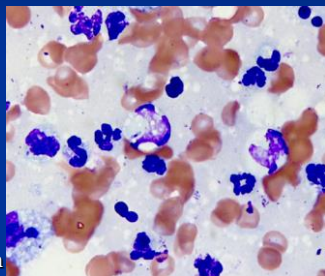


## 5 Categories of Tissue Lesions

- **Inflammatory lesion** - neutrophils
- Cystic lesion – amorphous material
- Hemorrhagic lesion – phagocytized RBCs
- **Neoplastic lesion** – monomorphic cell population
- Mixed cell population – both inflammatory and noninflammatory cells

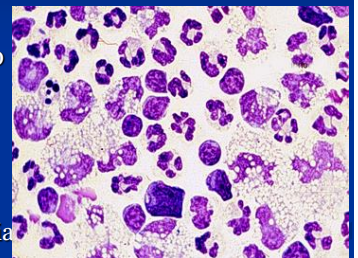
## Inflammatory Lesions

- Neutrophils above those expected from blood contamination
- Three types of inflammation
  - Purulent inflammation
  - Pyogranulomatous inflammation
  - Eosinophilic inflammation



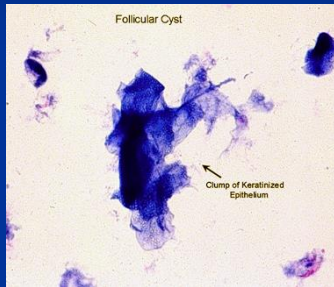
## Pyogranulomatous Inflammation

- Greater than 15% to 40% macrophages
  - Fungal infections
  - Foreign bodies
  - **Panniculitis**
  - Intracellular bacteria
    - *Mycobacteria* spp.
    - ***Bartonella* spp.**



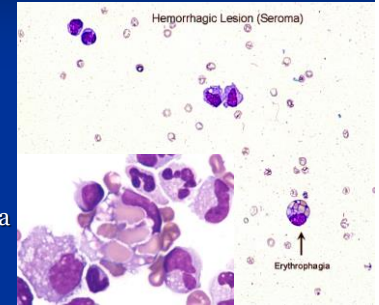
## Cyst Formation

- Follicular cyst (EIC)
- Apocrine cyst
- Sebaceous cyst



## Hemorrhagic Lesion

- Hematoma
- Seroma
- Neoplasia
  - Hemangioma
  - Hemangiosarcoma

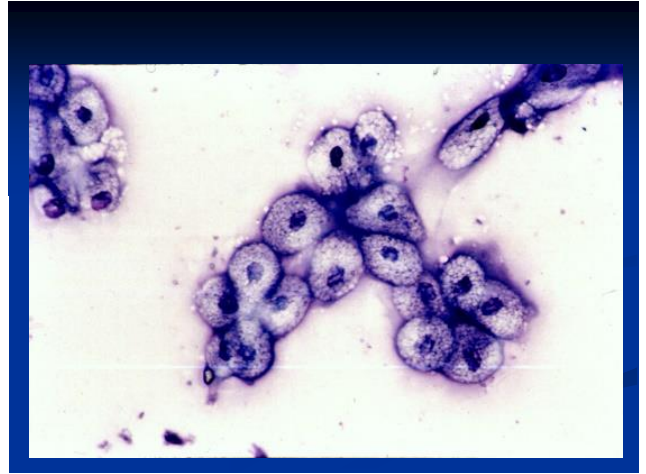
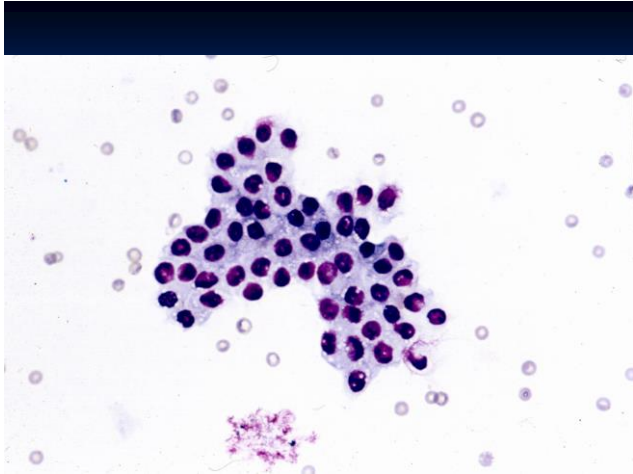


## Neoplasia

- Monomorphic population of cells
- Benign vs malignant

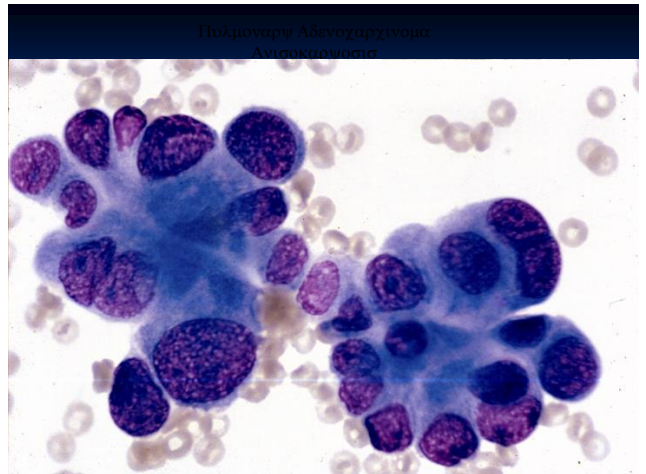
## Benign Neoplasia / Hyperplasia

- Uniformity in nuclear and cytoplasmic size
- Uniformity in N:C ratio
- Consistent size, shape, and number of nucleoli

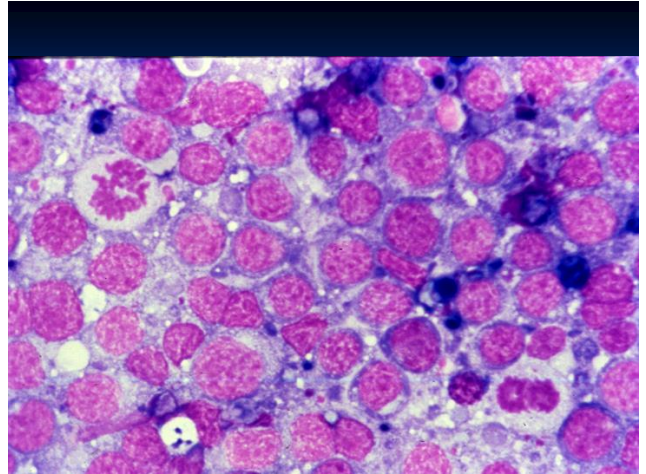
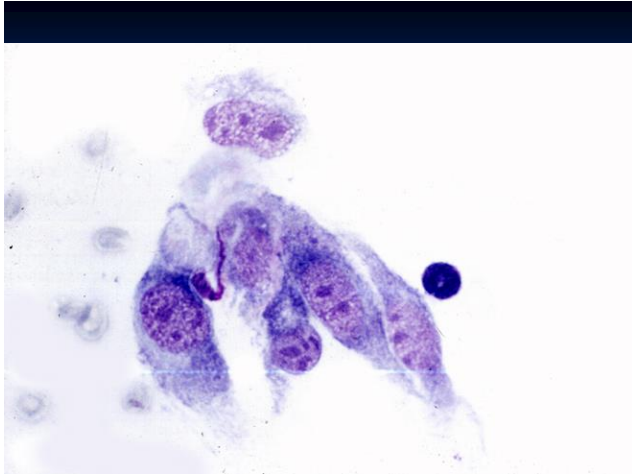


## Characteristics of Malignancy

- Anisokaryosis
- High or variable N:C ratio
- Variable nucleoli
- Coarse, clumped chromatin
- Increased Mitotic activity
- Pleomorphism
- Nuclear molding
- Multinucleation







## Special Considerations

- The cell population should contain 3 or more of the nuclear criteria for malignancy
- Presence or absence of inflammation
- Predicting biological behavior
  - Location of lesion
  - Specific tumor types
  - To be discussed later

## Special Considerations

- The cell population should contain 3 or more of the nuclear criteria for malignancy
- Presence or absence of inflammation
- Predicting biological behavior
  - Location of lesion
  - Specific tumor types
  - To be discussed later

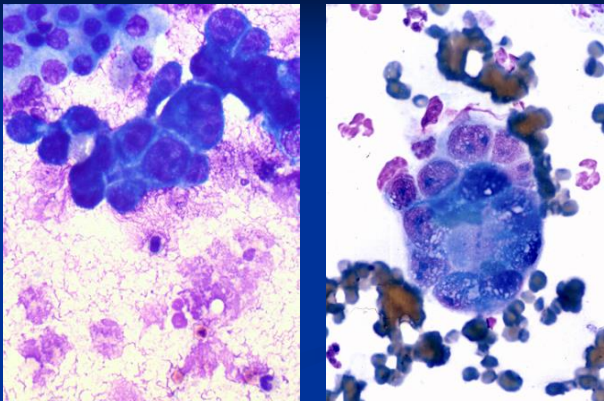
## Categories of Neoplasia

- Epithelial
- Mesenchymal
- Round cell
- Neuroendocrine

## Epithelial Tumors

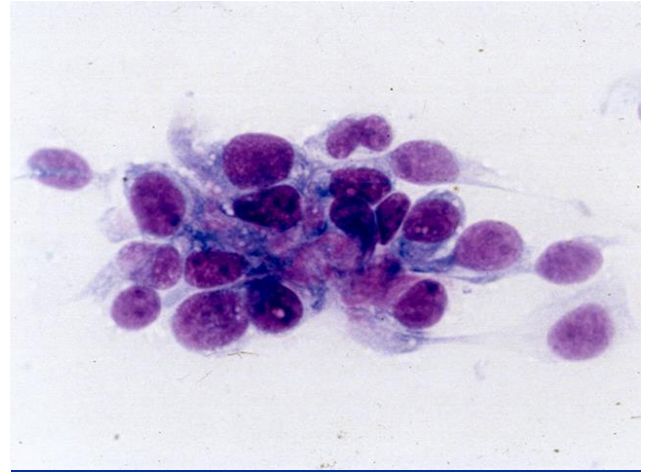
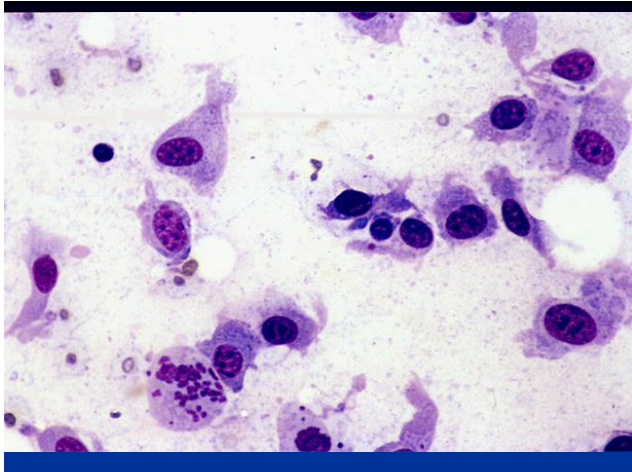
- Usually exfoliate easily
- Cells tend to occur in clumps or clusters
- Distinct cytoplasmic borders
- Cytoplasmic membranes adherent to each other displaying tight cell junctions

## Pulmonary Masses



## Mesenchymal Tumors

- May not exfoliate well?
- Cells more individually arranged
- Polygonal to wispy, spindle-shaped cytoplasm



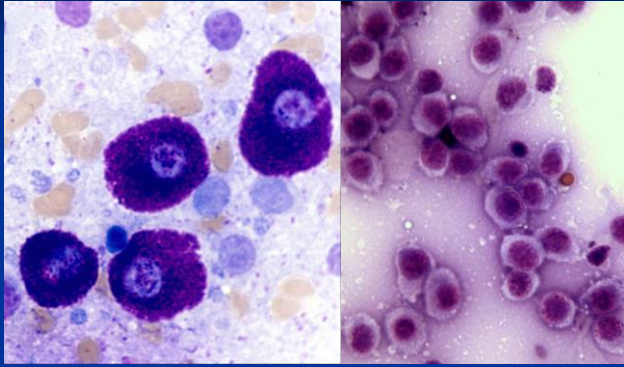
## Round Cell Tumors

- Usually exfoliate well
- Individually arranged, polygonal to round cells
- Distinct cytoplasmic borders
- Most malignant ones metastasize via lymphatics

## Round Cell Tumors

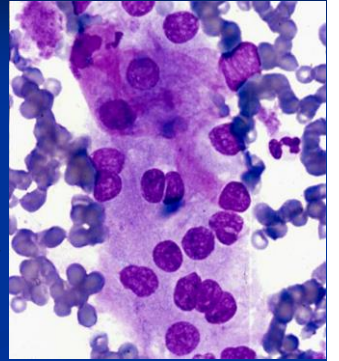
- Histiocytic tumors
- Lymphoma
- Mast cell tumor
- TVT
- Plasmacytoma
- Melanoma

## Round Cells



## Neuroendocrine Tumors

- Tumors of the endocrine and chemoreceptor glands
  - Thyroid, parathyroid, endocrine pancreas, adrenal, carotid body and aortic body
- Appears cytologically as free nuclei in background of cytoplasm



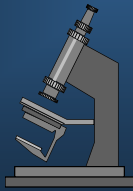
## Any Questions





# Cytology Evaluation of Lymph Nodes

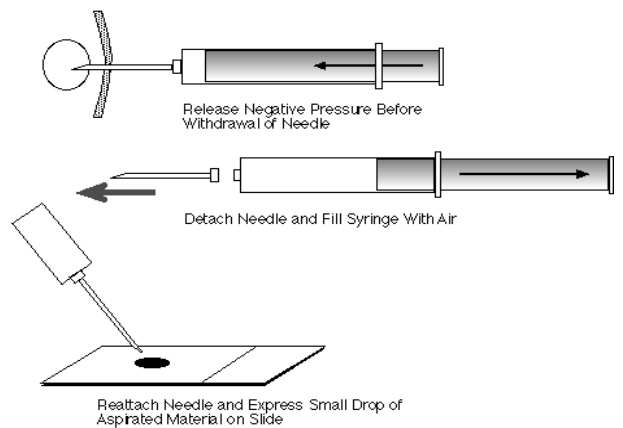
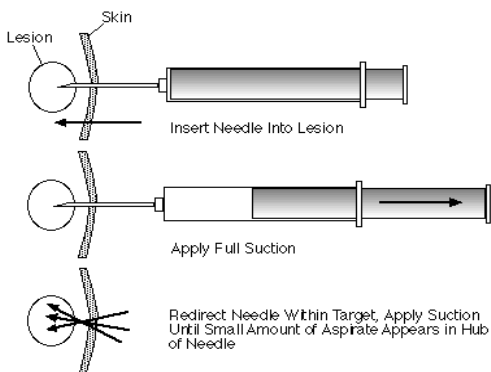
## The Cytological Evaluation of Lymph Nodes

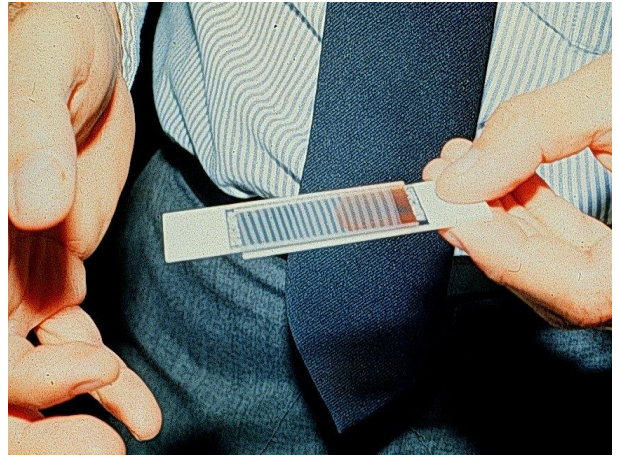


## Aspiration Technique

- 22 gauge needle or butterfly catheter, and a 6 cc or 12 cc syringe
- Insert toward periphery of the node
- + / - negative pressure

### Aspiration procedure





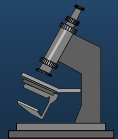
## Cytologic Stains

- Three-step staining set
- Diff Quik®



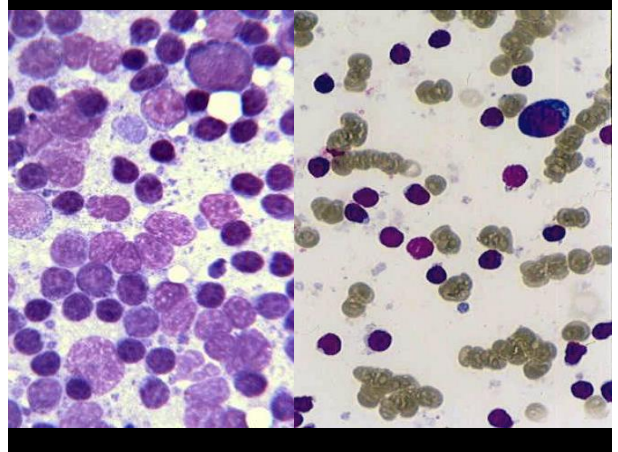
## Cytologic Interpretation

- Normal
- Reactive lymphoid hyperplasia
- Inflammatory
- Lymphoma
- Metastatic disease
  - Could be normal size



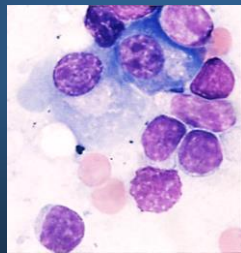
## Normal Lymph Node

- 75% - 95% small, well-differentiated lymphocytes
  - Dark dense chromatin
  - Nucleus 1 - 1.5 times size of erythrocyte
- Low numbers of intermediate lymphocytes and rare lymphoblasts
- Rare macrophages and plasma cells



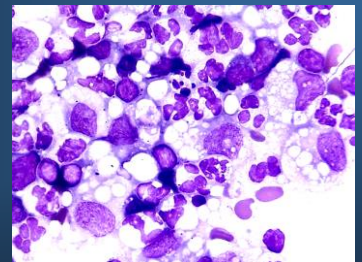
## Plasma cells

- Abundant basophilic cytoplasm
- Perinuclear clear zone (Golgi region)
- Eccentric nuclei with condensed chromatin



## Other cells

- Neutrophils
- Macrophages
- Mast cells
- Eosinophils

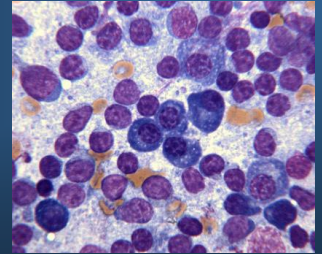


## Reactive Lymphoid Hyperplasia

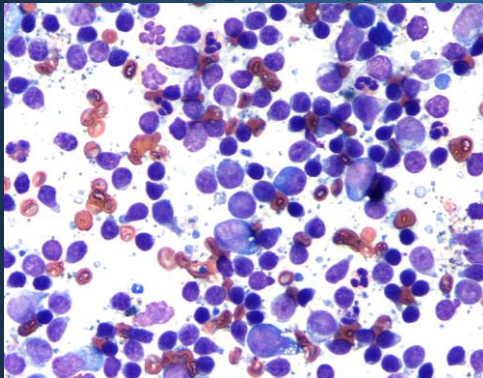
- Reactivity is usually due to antigenic stimulation of the draining areas
  - infection, inflammation, neoplasia, etc.
- If several lymph nodes are reactive, systemic disease should be considered
  - systemic infection
    - Protozoal, fungal, rickettsial, bacterial, viral, etc.
  - autoimmune disease
    - SLE, polyarthritis, polymyositis, etc.

## Reactive Lymphoid Hyperplasia

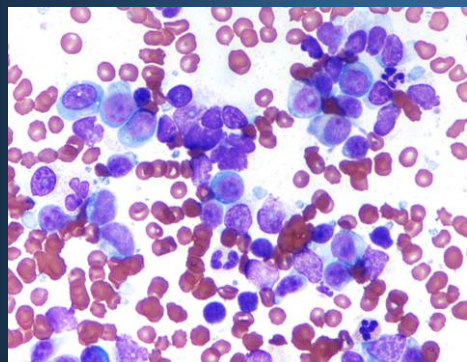
- Small lymphocytes predominate
- Increased numbers of intermediate lymphocytes and lymphoblasts
- Increased numbers of plasma cells (dog)
- Increased blast cells in the cat
- +/- low numbers of neutrophils, macrophages or mast cells



## Mild Feline Lymphoid Hyperplasia

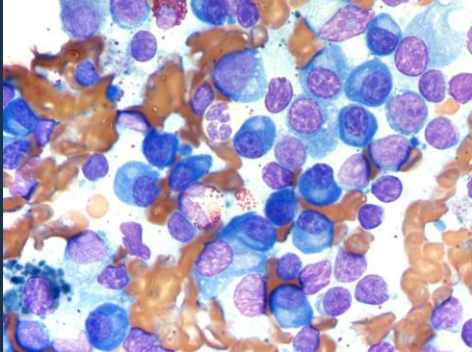


## Marked Feline Lymphoid Hyperplasia





## Marked Canine Lymphoid Hyperplasia

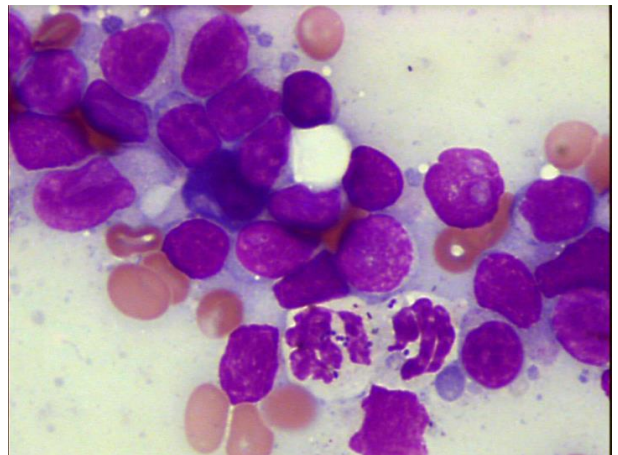
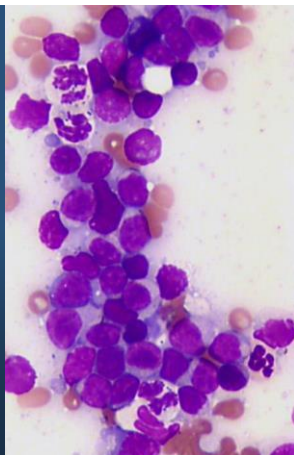


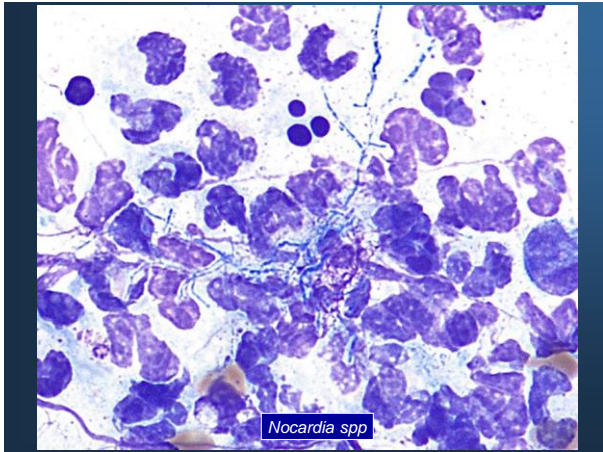
## Lymphadenitis

- Inflammation within the lymph node
- Various types
  - Purulent lymphadenitis
  - Pyogranulomatous lymphadenitis
  - Eosinophilic lymphadenitis

## Purulent Lymphadenitis

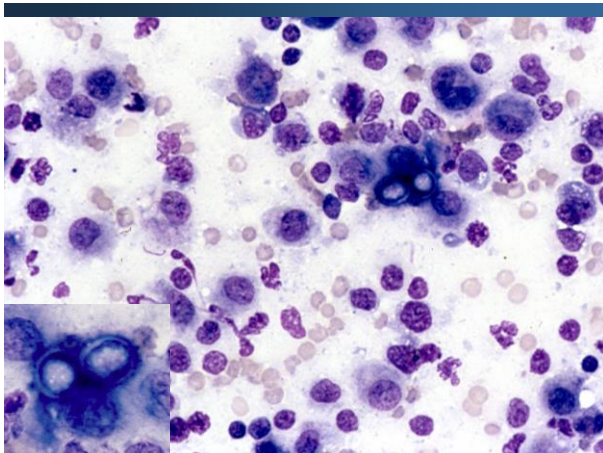
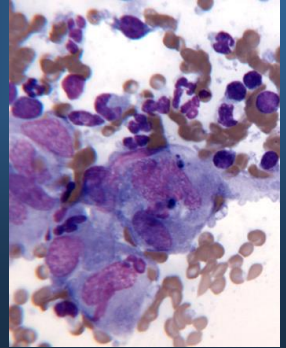
- Neutrophilic inflammation
  - Usually bacterial infection within the node or in the surrounding tissues
  - Submandibular lymph nodes





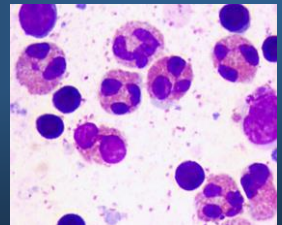
## Granulomatous or Pyogranulomatous Lymphadenitis

- Many macrophages ± neutrophils
  - Fungal - pythiosis, blastomycosis (etc.) Bacterial – *Bartonella* spp. mycobacteria, *Nocardia*, *Actinomyces*
  - Protozoal - cytauxzoonosis, toxoplasmosis, leishmaniasis
  - Protothecosis
  - Idiopathic granulomatous lymphadenitis (canine)
  - Chronic inflammation
  - Neoplasia



## Eosinophilic Lymphadenitis

- Increased eosinophils with some neutrophils and macrophages
  - Allergic dermatitis
  - Hypersensitivity reactions
  - Eosinophilic granulomas
  - Parasitic infection
  - Eosinophilic gastroenteritis / Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia
  - Hypereosinophilic syndrome / granulomas (Cats, Rottweilers, Siberian Huskies)
  - Mast cell tumors (Canine)
  - Rarely, lymphoma or carcinoma



## Lymphoma

- Population of neoplastic lymphocytes that originates in peripheral lymph nodes or tissues
- Easy to diagnose cytologically in the canine lymph node
- Difficult to diagnose cytologically in the feline lymph node
- **Lymphoid Leukemia** – by definition, originates in the bone marrow

## Lymphoma in Dogs

- Most common hematopoietic neoplasm
- Most dogs have multicentric form
- Non-painful, marked generalized lymphadenopathy



## Lymphoma in Dogs

- Typically middle-aged dogs
- Higher incidence in Golden retrievers, boxers, Scottish terriers, German shepherds, Basset hounds, Bernese Mountain dogs, and others



## Lymphoma in Dogs: Tendencies

- May involve B or T lymphocytes
- Breed differences
  - Shih Tzus, boxers, and Siberian Huskies more likely T-cell origin
  - Cocker Spaniel, Doberman pinscher, Basset Hound, German shephard, Rottweilers more likely B-cell origin

## Lymphoma in Dogs

- Some use the terms high grade (large / blast cells) and low grade lymphoma (small lymphocytes)
- Intermediate grade
- There are classification systems with specific criteria

## Lymphoma Classification (Dogs)

- Diffuse large B-cell lymphoma (52%)
- Peripheral T cell lymphoma, not otherwise specified (15%)
- Nodal marginal zone lymphoma (8%)
  - Specific type of B-cell lymphoma that develops in the marginal area of lymph nodes of dogs
  - Indolent lymphoma - low mitotic rate and slow clinical progression (some cases can be aggressive)
- Other T or B cell lymphomas (13%)
- T zone lymphoma (4%)
- T-cell lymphoblastic lymphoma (3%)

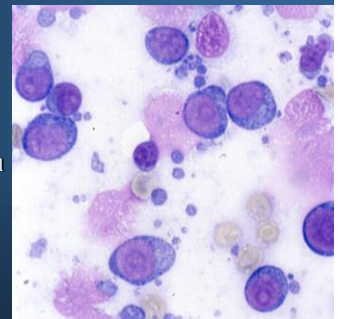
## Canine Lymphoma

- Lymphoma often occurs in peripheral lymphoid tissue (multicentric)
- Often is a large cell variant
- **Lymphoblasts predominate**, typically representing 50% - 90% of the cell population
- Increased mitotic figures
- Large number of lymphglandular bodies



## Canine Diffuse Large B-cell Lymphoma

- Lymphoblasts usually predominate
  - Nuclei are 2-5 times the size of a RBC
  - Chromatin pattern is diffuse
  - Cytoplasm is abundant and deeply basophilic
  - Nucleoli can be identified
- Lymphoglandular bodies are common
- Increased numbers of mitotic figures may be seen





## Immunophenotyping

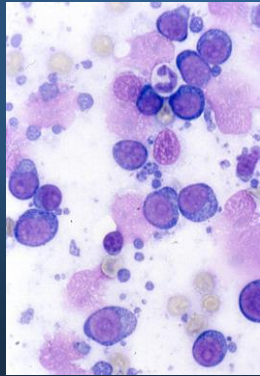
- Most accurate way to determine cell type involved (B-cell vs. T-cell)
  - Prognostic and therapeutic information
  - Surface protein markers
    - Cluster differentiation (CD) CD3, CD4, CD8 = T-cell
    - CD21, CD79a = B-cell
  - **Technically, does not identify population as neoplastic**
- Must first diagnose lymphoma cytologically
- **Morphological characteristics** of the neoplastic lymphocytes may also indicate cell type
  - Cytological typing of canine lymphomas

## PCR Analysis of Lymphoid Tissue

- PCR for **Antigen Receptor Rearrangements (PARR)** (DNA analysis)
- Used to help **identify a population as neoplastic**
- Tests for clonality in antibody receptor and T-cell antigen receptor
- Not for typing as B or T cells because some Bs have T cell antigen rearrangements etc.
- Good for canine and feline small cell lymphoma, (new primer development >80% in feline)
- Clinical immunology laboratory at CSU
  - 970-491-1170

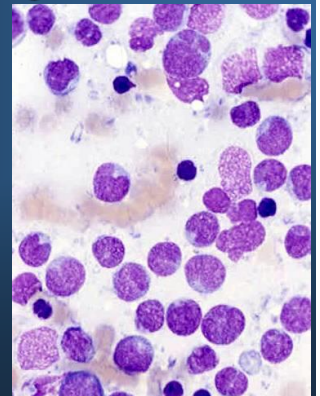
## Cytological Typing: B-cell Lymphoma

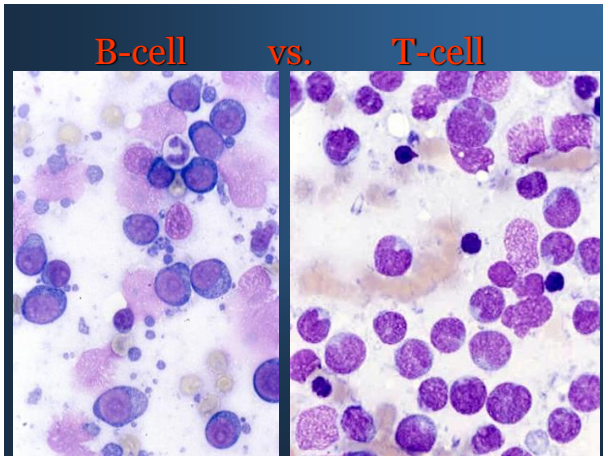
- Plasmacytoid appearance with eccentrically located nuclei and a perinuclear clear zone
- Single, prominent, centrally located nucleoli
- Also called Immunoblastic Lymphoma



## Cytological Typing: T-cell Lymphoma

- Nuclei are sometimes cleaved or convoluted
- Nucleoli are often indistinct or absent
- Often associated with hypercalcemia
- Also called Lymphoblastic Lymphoma

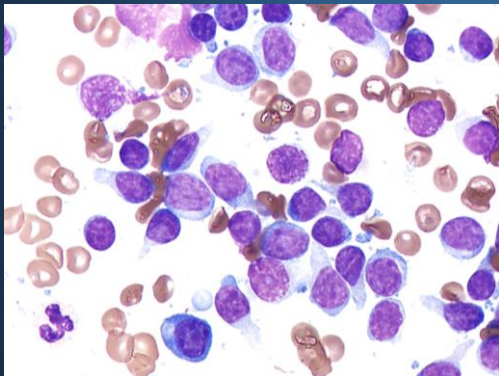




## T Zone Lymphoma (4%)

- Expansion of small to intermediate lymphocytes (30-90% of cells in the LN); may resemble lymphoid hyperplasia
- Round nucleus with coarse chromatin and rare small, faint nucleoli
- Moderately expanded pale blue cytoplasm with a **wide-base pseudopod**
- CD45-, variable T-cell antigen expression

## Also Called Hand-Mirror Lymphoma



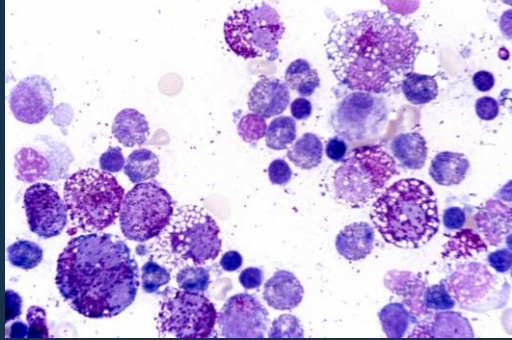
## Metastatic Disease

- Homogeneous population of cells not normally seen in a lymph node
- **Size of node not a factor**
- **Confirmatory only if metastasis is found**
- Early infiltration not detected cytologically
- Lymphoid population if present may appear reactive

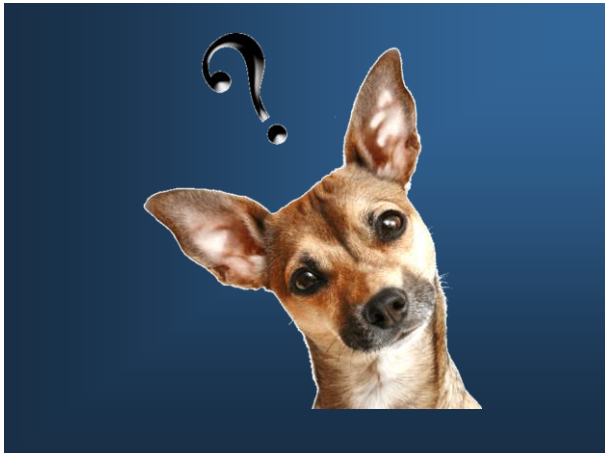
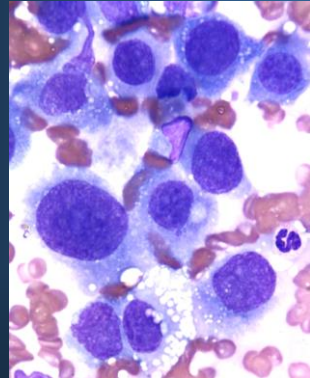




Metastatic Mast Cell Tumor (mod. differentiation)



Metastatic Histiocytic Sarcoma





## Cytology Case Challenges: The Splenic Mass

### Cytology Case Challenge: The Splenic Mass



### Sadie

- 7 year old, female, spayed golden retriever
- Presentation (Emergency)
  - Episodic weakness (twice) over 2 week period
  - Acute collapse day of presentation, but recovered



### Physical Exam Findings

- Pale mucous membranes, tachycardia (120 BPM)
- CRT prolonged @ 4 sec.
- Abdominal distension
  - Effusion and/or mass was difficult to determine on abdominal palpation
- PCV 19%
- TPP 5.4
- Plan: CBC

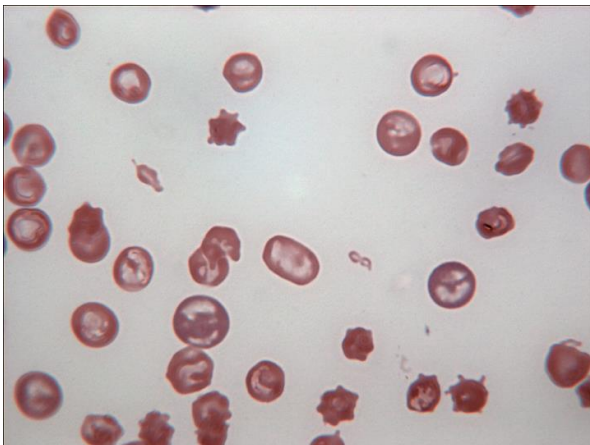
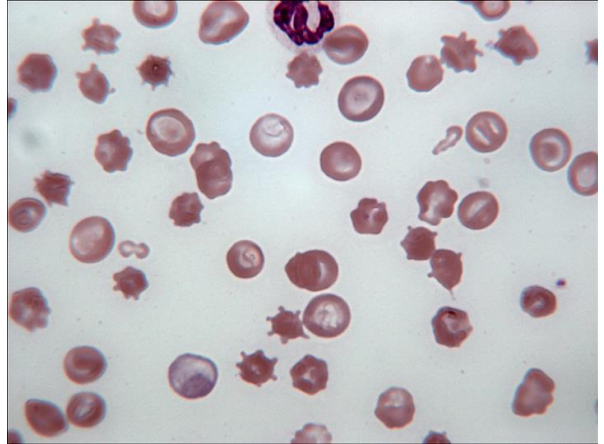
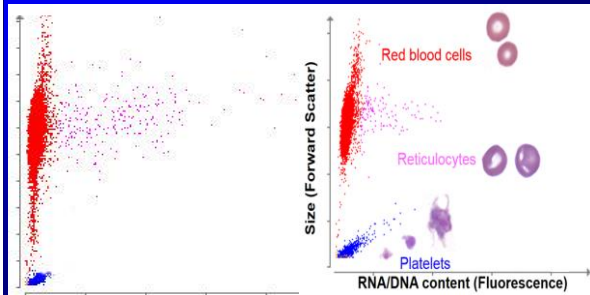
### CBC Results

WBC	19.1	(6.0 – 17.0) x 10 <sup>3</sup>	RBC	2.51	(5.4 – 7.8) x 10 <sup>6</sup>
Neuts	15.2	(3.0 – 11.5) x 10 <sup>3</sup>	HGB	6.8	(13.0 – 19.0) g/dL
Bands	0.900	(0.0 - 0.3) x 10 <sup>3</sup>	HCT	18.2	(37.0 – 54.0) %
Lym.	0.700	(1.0 – 4.8) x 10 <sup>3</sup>	MCV	76.2	(66 – 75) fL
Mon.	2.3	(0.15 – 1.35) x 10 <sup>3</sup>	MCHC	36.3	(34.0 – 36.0) g/dL
Eos.	0.0	(0.1 – 1.25) x 10 <sup>3</sup>	Plts	25.0	(150 – 430) x 10 <sup>3</sup>

Reticulocyte count (6%) = 150,600 /  $\mu$ l (>80,000 = regenerative)

- Blood film evaluation

## RBC and Platelet Dot Plot

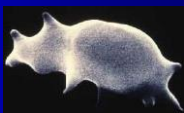


## Findings from Blood Film Evaluation

- Regenerative anemia (polychromasia)
- Poikilocytosis
  - Acanthocytes
  - Schistocytes
- Thrombocytopenia

## Schistocytes

- Hallmark of fragmentation hemolysis
- Fragmentation of cells passing through tortuous or abnormal vessels
  - DIC
  - Neoplasia (HSA, Thyroid ACA)
  - 50% of dogs with Splenic HSA
  - Vasculitis
  - Thromboembolism (Cushing=s, HWD)
  - Caval Syndrome
  - Glomerulonephritis
- Increased fragility of erythrocytes
  - severe iron deficiency anemia



## Abdominal Ultrasound

- Free abdominal fluid
- Large mass in cranial abdomen (14 cm)
  - Cavitated with mixed echogenicity
  - Appeared to be associated with the spleen

## CBC findings that support Dx of HSA

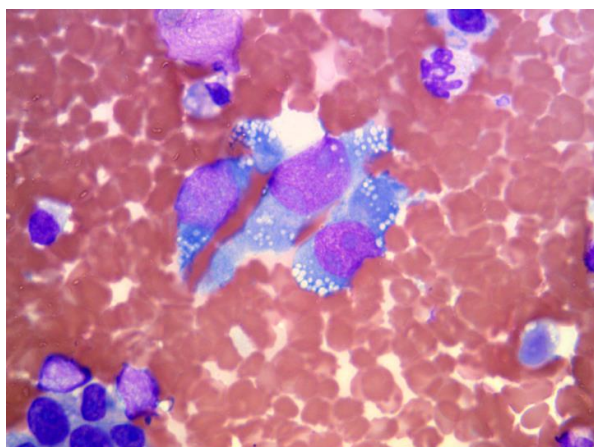
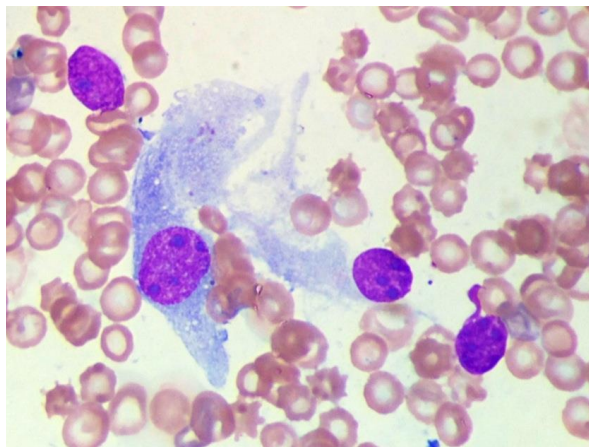
- Anemia – (80%) of dogs
  - Hemolysis and/or hemorrhage
- Thrombocytopenia – (75%) of dogs
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels
- Schistocyte formation – (50%) of dogs
  - Hallmark of red cell fragmentation
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels

## Hemostasis Profile

- PT and APTT – normal
- FDPs - negative
- D-dimers – (ref. range < 250 ng/ml)
  - Not useful in this case due to hemoabdomen
  - Can result in d-dimer levels > 1,000 ng/ml in dogs without evidence of TE disease

## Fine-needle Aspiration of Splenic Mass

- Potential for definitive, presurgical diagnosis
- Potential for complications
  - Seeding the abdomen with tumor cells
  - Hemorrhage
  - Dog is already bleeding likely due to rupture of neoplastic vessels, not DIC



## Plan for Sadie

- Owners elected surgery and chemo if possible
- Sadie was transfused (PCV 26%)
- Surgery was performed and a 14 cm x 16 cm mass was identified in the spleen
- Multiple, red-purple, raised nodules were present in all lobes of the liver
- The spleen and biopsies taken from the hepatic masses were submitted for histopathology
- Final Dx: hemangiosarcoma



## Treatment

- Chemotherapy was initiated approximately 1 week post-op (once histopath confirmed a diagnosis) (PCV 35%)
- 21 day cycle of VAC
  - Vincristine 0.75 mg/m<sup>2</sup> BSA (IV) (Day 8 & 15)
  - Doxorubicin 30 mg/m<sup>2</sup> BSA (IV) (Day 1)
  - Cyclophosphamide 200 - 300 mg/m<sup>2</sup> BSA (PO) (Day 10)
- Sadie received 4 cycles of therapy

## Prognosis

- Long-term prognosis extremely poor
- Death from exsanguination from rupture of metastatic site
- Surgery alone rarely curative with MST of 1 to 3 months
- Multi-drug chemotherapy MST 6 to 9 months

## Sadie

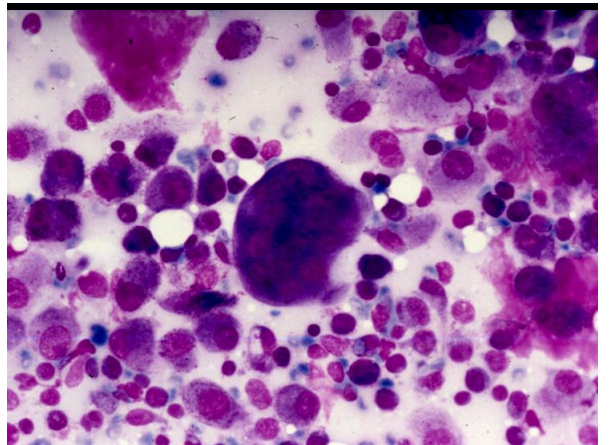
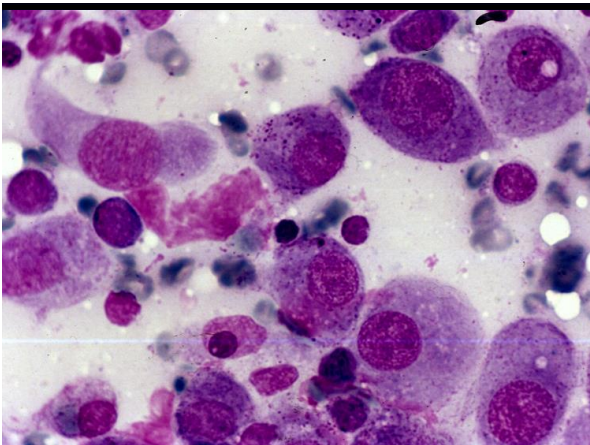
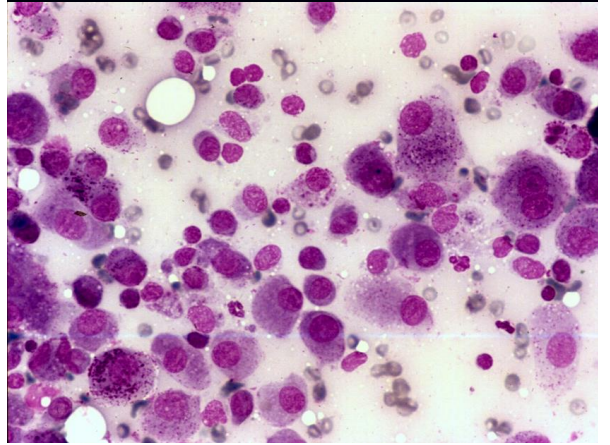
- Sadie was found dead in her bed 9 months after splenic surgery
- Likely the result of ruptured metastatic lesion



## Patient: Toby

- 6 year old, intact, male German shepherd
- Acute onset of lameness
  - Right front leg



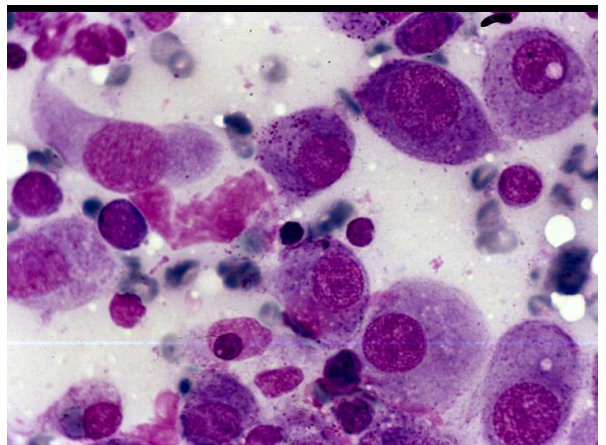
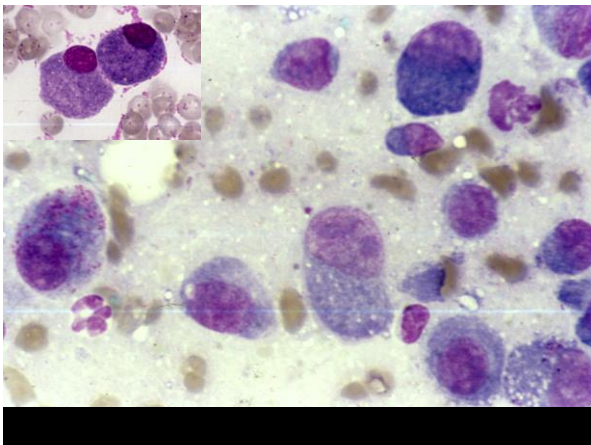
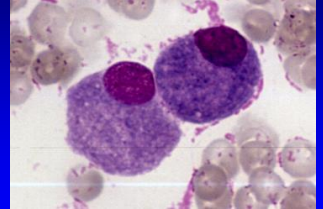


## Osteosarcoma

- Most common malignant bone tumor of the limbs
- Appendicular forms typically in giant or large breed dogs
- Uncommon in cats
- Site predilection for metaphyseal regions
  - Away from elbow, toward the knee
    - Proximal humerus
    - Distal radius and ulnar
    - Proximal tibia
    - Distal femur

## Cytologic Features of Osteosarcomas

- May look like round cell tumor
- Large, oval to spindle-shaped cells
  - Individually arranged
- Discrete cytoplasmic borders
- Eccentric nuclei
- Multinucleated giant cells
- Dense, amorphous pink material
  - Osteoid



## Staining for Alkaline Phosphatase

Vir Pathol 42:161-165 (2005)

### Use of Alkaline Phosphatase Staining to Differentiate Canine Osteosarcoma from Other Vimentin-positive Tumors

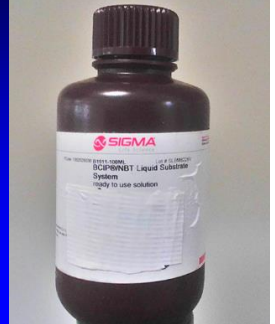
A. BARGER, R. GRACA, K. BAILEY, J. MESSICK, L.-P. DE LORIMIER, T. FAN, AND W. HOFFMANN

Departments of Veterinary Pathology (AB, RG, KB, JM, WH) and Veterinary Clinical Medicine (LPD, TF), College of Veterinary Medicine, University of Illinois, Urbana, IL.

- Sensitivity near 100% (rare to get false negative)
- Specificity 89%
  - One of four chondrosarcomas were positive
  - Malignant histiocytic sarcomas may be positive, seen clinically, not tested in original study
  - Rottweilers and other predisposed breeds

## Staining for Alkaline Phosphatase

- BCIP / NBT Liquid Substrate System
  - Sigma: P Code 1002028038
  - B1911-100ml (~ \$80 USD)
  - Lasts 2 or more years (refrigerated)

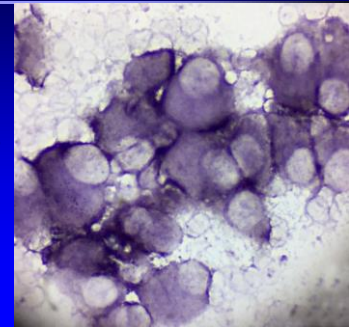


## Staining for Alkaline Phosphatase

- Place air-dried slide on flat surface (cardboard slide holder)
- Apply a few drops of substrate
- Incubate at room temp. for 10 min. (1 hour if previously stained)
- Rinse with tap water and air dry

## Staining for Alkaline Phosphatase

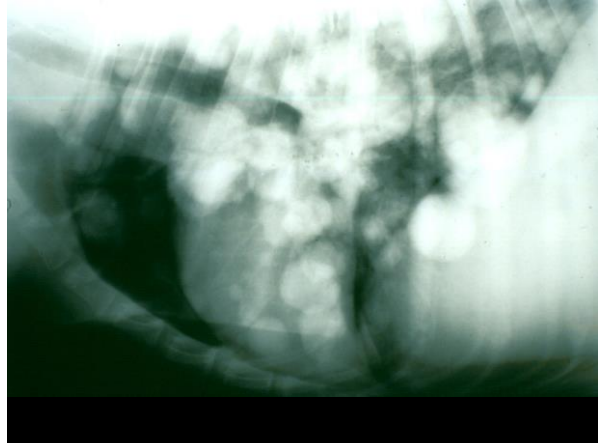
- Place air-dried slide on flat surface (cardboard slide holder)
- Apply a few drops of substrate
- Incubate at room temp. for 10 min.
- Rinse with tap water and air dry





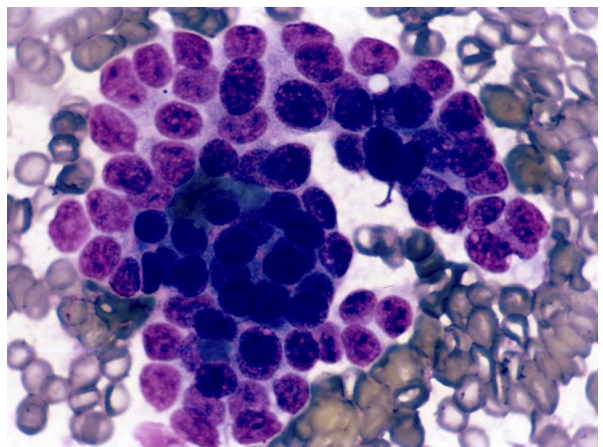
## Biological Behavior of Osteosarcomas

- Location of lesion and signalment of patient
- Appendicular skeleton
  - Rapid hematogenous spread
  - 90% have mets by time of Dx
    - Pulmonary
  - Median survival 2 – 4 months
- Axial skeleton less aggressive
  - Mandibular OS: 1 year survival 71%
  - Small dogs (<15 kg) 60% affects axial skeleton
- Feline OS
  - Femur
  - Less aggressive, reduced incidence of metastasis
    - Median survival time 4 – 5 years



## Patient: Tangy

- 10 year old, M/C DLH
- Swelling on left cheek
- Physical exam
  - Lobulated, pigmented mass caudal to the left commissure

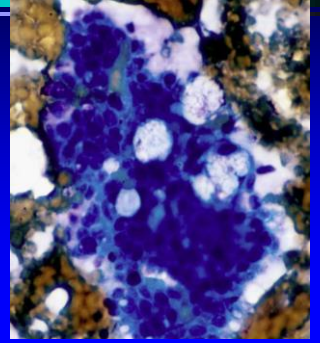



## Basal Cell Tumor

- Benign epithelial tumor
- Very common cutaneous tumor in dog and cat
- Rare in other species
- Site predilection for skin on head and neck
  - Also on legs in cats

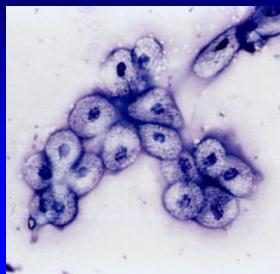
## Cytologic Features of Basal Cell Tumors

- Tightly adherent clumps of deeply basophilic epithelium
- High N:C ratio
- Mild to moderate anisokaryosis
- Occasional nucleoli
- Variable amounts of melanin pigment
  - Especially in the cat
- Sebaceous differentiation



## Sebaceous Gland Adenomas

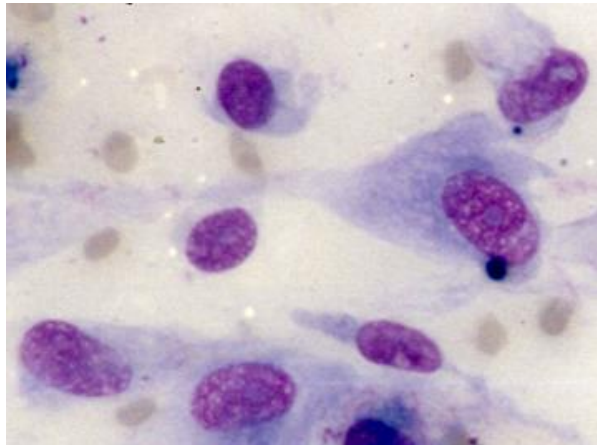
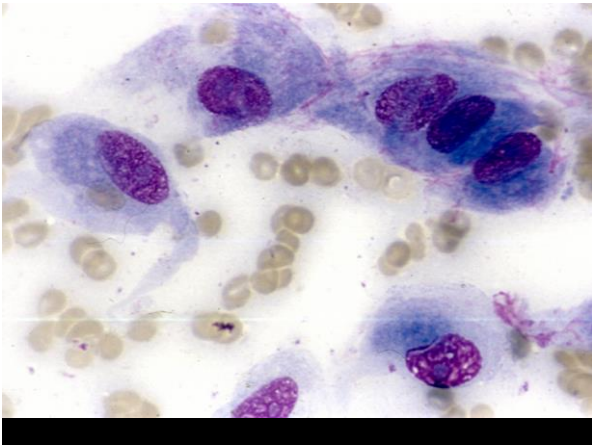
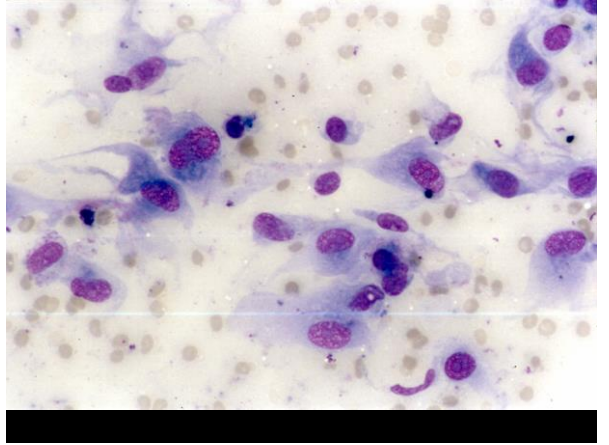
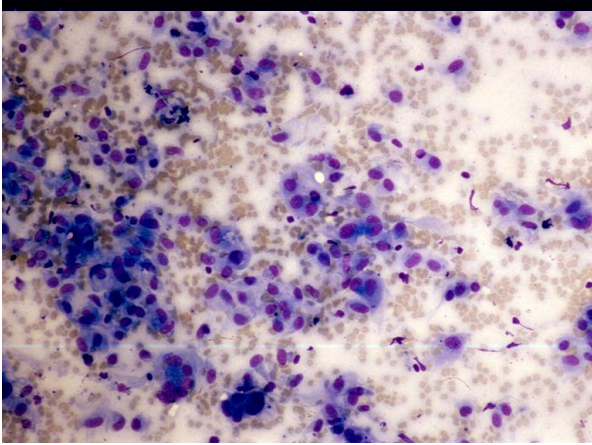
- Common cutaneous tumor of dogs
- Uncommon in other species
- Sebaceous gland ACA rare
- Site predilection for skin of head, neck and eyelids

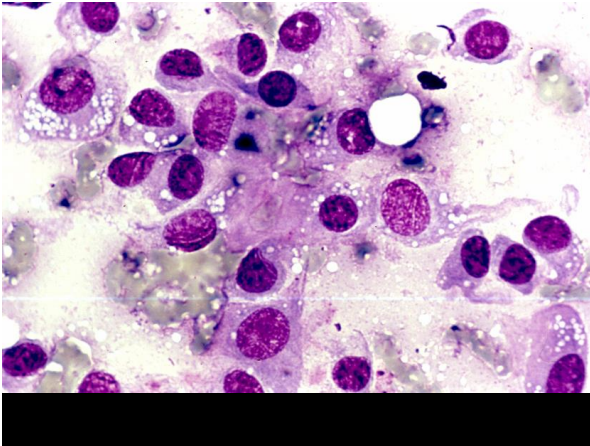


## Patient: Bandit

- 9 year old, male, Husky
- Left rear leg lameness
- Physical exam findings
  - Firm, large, swelling on caudal aspect of left thigh and perineal area
  - Attached to underlying tissue
  - No bone involvement







## Hemangiopericytoma

- Perivascular wall tumors
- Frequently reported mesenchymal neoplasm in the dog
- Site predilection for the extremities, especially lateral surface
- Originates from vascular pericytes

## Characteristic Features of Hemangiopericytomas

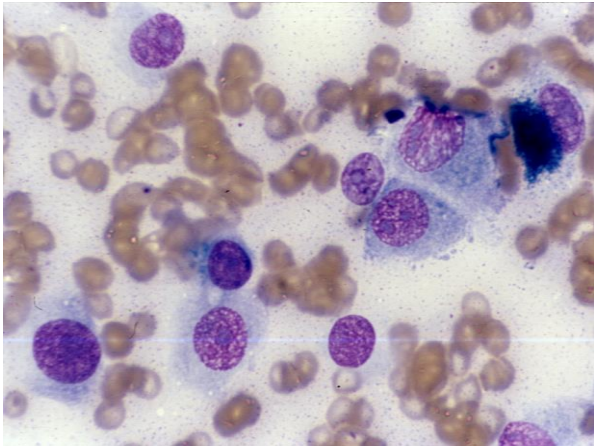
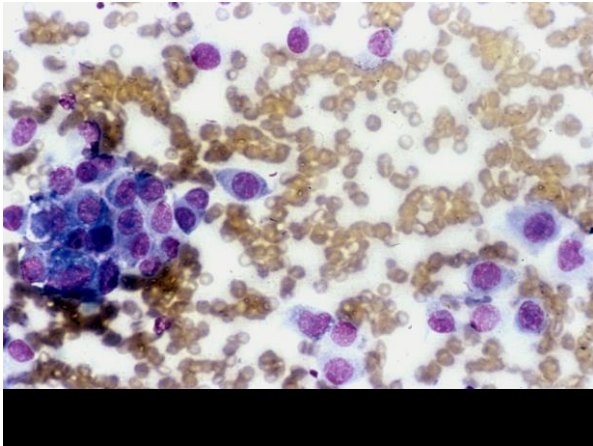
- Extremely cellular aspirates
- Anaplastic mesenchymal cells with very wispy cytoplasm
- Small, punctate cytoplasmic vacuoles
- 1 or 2 prominent nucleoli
- Cells branching off of capillaries
- Locally very invasive
  - Best chance for cure
    - First surgical resection
- Metastasis is rare


## Patient: Sam

- 9 year old, F/S, mix breed
- Swelling on gum
- Physical exam
  - Mass surrounding lower right incisors

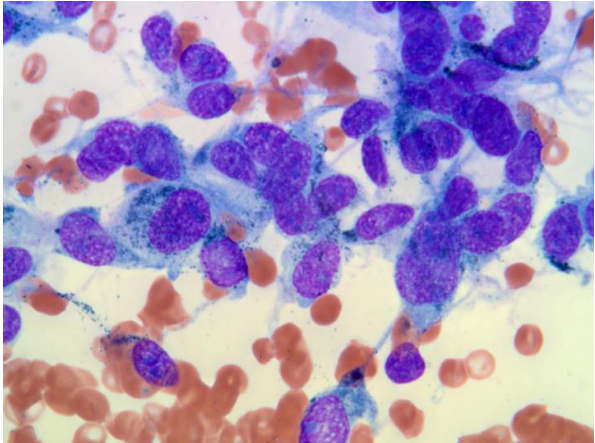
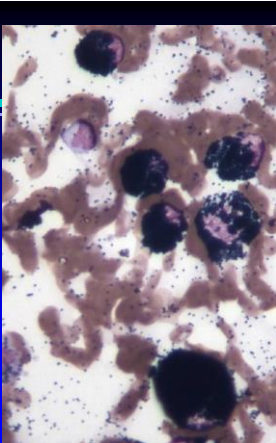






 **Melanoma**

- Round cell tumor of neuroectodermal origin
- Found cutaneously anywhere on the body
- Canine melanomas
  - Site predilection for oral cavity and digits

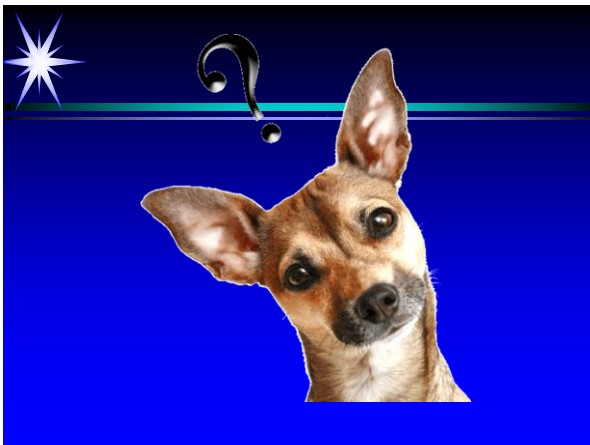


## Cytologic Appearance of Melanomas

- Epithelial-appearing and spindle-shaped forms
- Variable degrees of differentiation (pigmentation)
  - Dark green to black, small granules
- Approximately 1/3 of oral melanomas lack pigment
  - Amelanotic
  - Pale cytoplasm with nuclear criteria for malignancy
  - Suspected with oral tumor of undefined tissue of origin

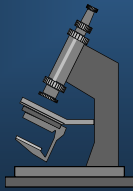
## Biological Behavior of Melanomas

- Most well-differentiated cutaneous melanomas are benign
- Most oral (and digital) melanomas are malignant
  - Rapid metastasis to regional nodes
- Feline melanomas
  - Uncommon



# The Diagnosis of Feline Lymphoma: A Diagnostic Dilemma

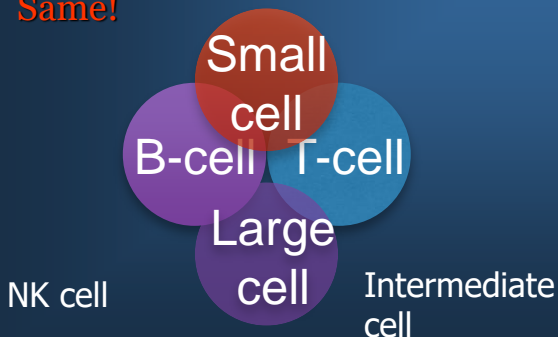
## The Diagnosis of Feline Lymphoma: A Diagnostic Dilemma



## What is Lymphoma?

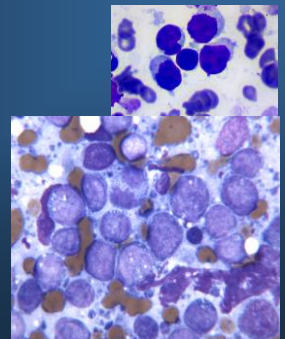
- “Clonal” proliferation of neoplastic lymphocytes that originates in peripheral lymph nodes or tissues
- Often easy to diagnose cytologically in the canine
- Difficult to diagnose cytologically in the feline

## All Lymphomas are not the Same!



## Lymphoma: Variable biological behavior

- Different lymphocyte phenotypes (B, T, NK)
- Clonal proliferation at any stage of maturation



## Morphologic classification of lymphoma and prognosis

- Prognostic – gastric vs. intestinal lymphoma in cats
- Treatment changes with subtype
- Morphology, immunophenotype (IPT), genetic features and anatomic location

## Morphologic classification of lymphomas and prognosis: What do we know?

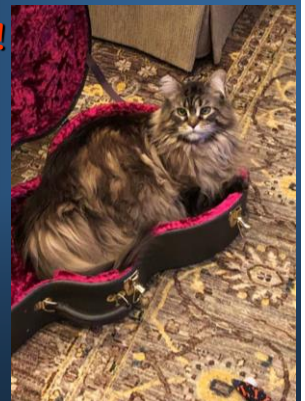
- What is all this fuss about??
- Isn't it “**B for Bad and T for Terrible**” when it comes to TREATMENT?
- Traditional thinking: Prognosis depends on the treatment. **Wrong!**
- Current evidence:
  - PROGNOSIS depends on lymphoma type (100s) (WHO) – **considering all factors previously discussed**
- Need of SPECIFIC THERAPIES “Targeted”

## The Cytological Diagnosis of Feline Lymphoma

A diagnostic dilemma!

## Cats are so Weird!

- Most lymphomas in cats do not involve peripheral lymphadenopathy
  - Internal organs
- Small cell lymphoma in internal organs
- Bizarre Distinctive peripheral lymph node hyperplasia (DPLH)
- Hodgkin's-like lymphoma

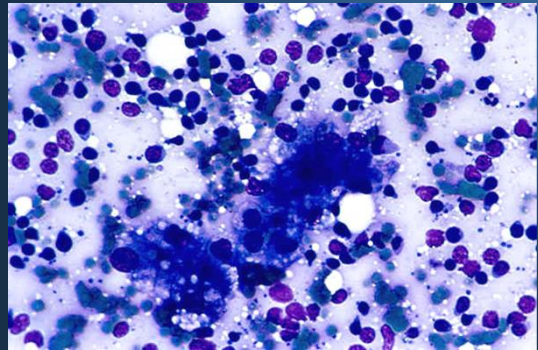




## Feline Hepatic Lymphoma

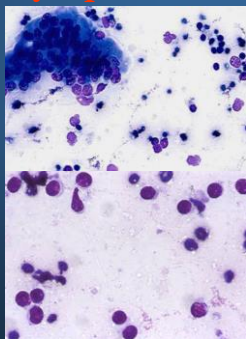
- May be composed of small, well-differentiated lymphocytes
- Similar to those seen in cats with Lymphocytic / **Plasmacytic** cholangiohepatitis
- Distinguishing features
  - Signalment – age, severity of hepatomegaly
  - Cytological appearance – numbers and monomorphic appearance

## Lymphoma or Lymphocytic / Plasmacytic Cholangiohepatitis?

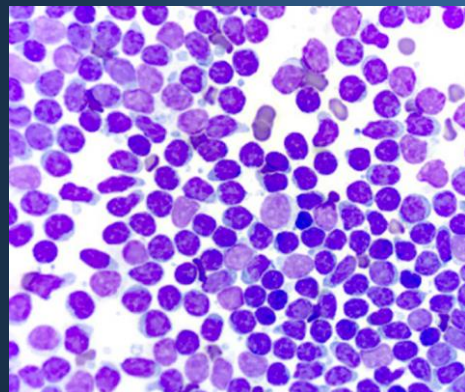


## Feline Intestinal Lymphoma

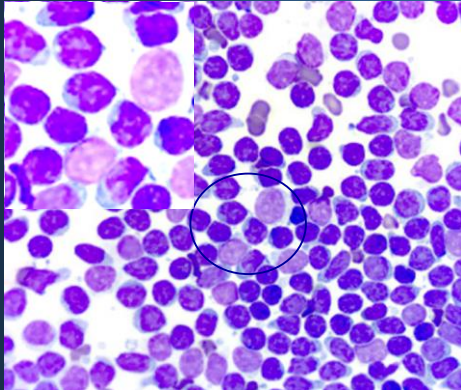
- Small cell, well-differentiated lymphomas are frequently observed
- GI Lymphoma is often seen in older cats
- Cellularity of the preparation, age of the cat and lack of lymphoid heterogeneity and plasma cell population aids in making the distinction
- Biopsy may be necessary
- **Aspiration of mesenteric lymph**



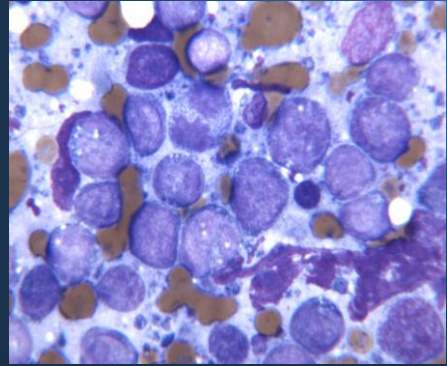
## Mesenteric Lymph Node



## Mesenteric Lymph Node

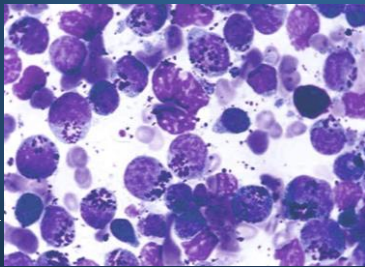


## Gastric Lymphoma in a Cat

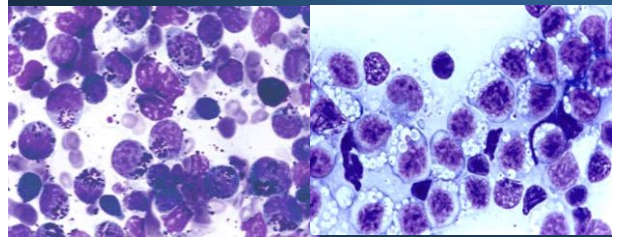


## Feline Large Granular Lymphoma

- Often involves the gastrointestinal tract
- Cytological diagnosis possible due to large population of granular lymphocytes



## Staining Properties of LGLs



Wrights-Giemsa

Diff-Quik®

## Peripheral Lymphadenopathy in Cats

- Difficult to diagnose lymphoma from a lymph node aspirate
  - Multicentric form, as seen in dogs, is unusual in cats
- DPLH
- Hodgkin's-like Lymphoma

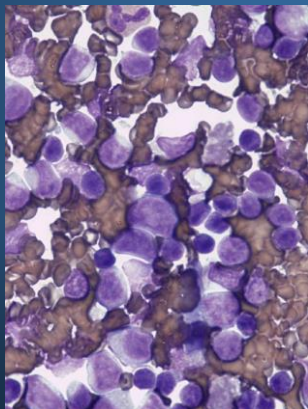
## Distinctive Peripheral Lymph Node Hyperplasia (DPLH)



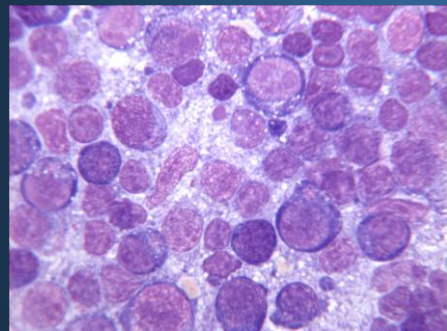
- Distinctive peripheral lymph node hyperplasia
- Generally young cats (2 to 4 years old)
- Mimics multicentric lymphoma clinically, cytologically and histologically
- However, peripheral lymphadenopathy regresses in 1 to 17 weeks
- May be associated with underlying infection

## DPLH

- See high numbers of lymphoblasts
- Lower numbers of small lymphocytes, intermediate lymphocytes and plasma cells
- Requires histological diagnosis



## DPLH in a Young Cat



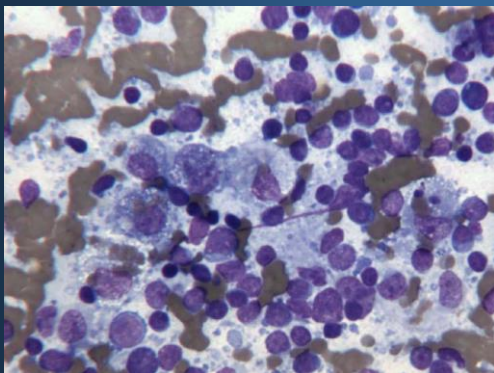
## Feline Hodgkin's-like Lymphoma

- Resembles the condition in humans
- Most often seen in older cats
- Most animals present with:
  - A mass in the ventral cervical region
  - Submandibular LN enlargement and/or cervical / prescapular LN enlargement
- Difficult to diagnose cytologically, as only neoplastic cells comprise only 1-5% of cells in LN
- Reed-Sternberg Cells

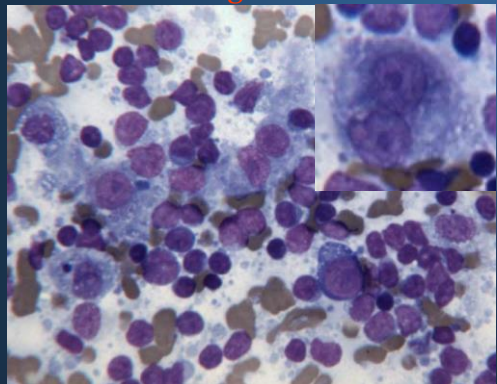
## Feline Hodgkin's disease

- Remaining cells are non-neoplastic lymphocytes, macrophages and granulocytes (neutrophils)
- Diagnosis is confirmed by histopathology – several histological types exist
- Prognosis – good as disease is generally less aggressive than non-Hodgkin's lymphoma
- Many cats survive months to years

### Feline Hodgkin's-like Lymphoma



### Reed-Sternberg Cells in Fe - HLL





## Advanced Diagnostics Currently Available

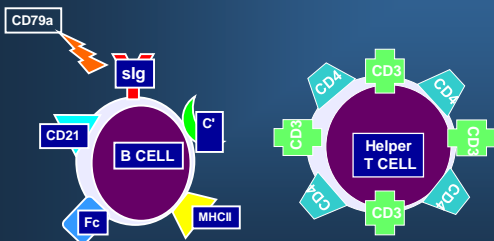
- If it comes down to “PARR” ticular tests
- Don’t choke on a hairball
- You just go with the “Flow”



## Immunophenotyping (Flow Cytometry)

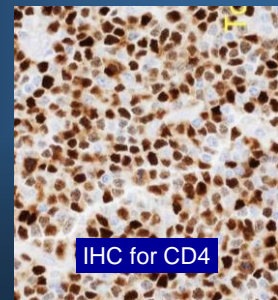
- Most accurate way to determine cell type involved (B-cell vs. T-cell)
  - Prognostic and therapeutic information
  - Surface protein markers
    - Cluster differentiation (CD) CD3, CD4, CD8 = T-cell
    - CD 20, CD21, CD79a = B-cell
    - **Technically, does not identify population as neoplastic**
- Prevalence of specific subtypes may indicate lymphoma
- Can be done on blood or lymphocytes in solution
  - Flow cytometry
  - Histopath - IHC

## Lymphocyte Surface Antigens



## Immunohistochemistry (IHC)

- Immunohistochemistry for T and B markers can be performed on histologic sections



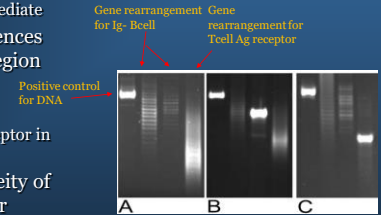
www.gotpath.com

## PCR Analysis of Lymphoid Tissue

- PCR for **Antigen Receptor Rearrangements (PARR)** (DNA analysis)
- Used to **help identify a population as neoplastic**
- Tests for **clonality** in antibody receptor and T-cell antigen receptor
- Good for canine, sensitivity 80% in feline ☹
- Clinical immunology laboratory at CSU
  - 970-491-1170

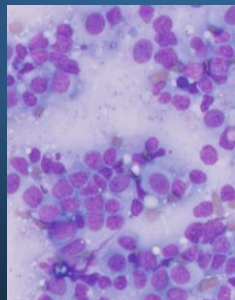
## PARR: Clonality Assay - When do I use it?

- Cytology can't accurately diagnose (Reactive vs Lymphoma)
  - Small cell and intermediate
- Amplifies DNA sequences coding for variable region of:
  - T-cell receptor
  - Immunoglobulin receptor in B-cells
- **Reactive** - heterogeneity of B- and T-cell receptor
- **Neoplastic** - same configuration – clonality



## What do I submit for PARR?

- Cytology slides (even stained)
- Needs enough lymphoid tissue
- 70-90% sensitivity
- Potential for **false-negatives**
  - In NK lymphomas, with aberrant lymphoid receptors
- Potential for **false-positives**
  - Ehrlichia canis, Borrelia, Leishmania, histiocytomas, **feline IBD (pseudocloneality)**, hepatitis from drug hypersensitivity...



## Results of histopathology, immunohistochemistry, and molecular clonality testing of small intestinal biopsy specimens from clinically healthy, client-owned cats

Sina Marsilio, et.al. J Vet Intern Med. 2019;33:551-558

- 20 – clinically healthy, client owned cat (≥ 3 years of age)
- Gastric and duodenal biopsies (histopath., immuno. & clonality)
- Cats followed for development of chronic enteropathy (CE) (Median=709 days) (Range =219-869 days)
- 12 diagnosed as SCL; 1 diagnosed as emerging SCL
- 6 diagnosed with lymphocytic enteritis (1 with pseudocloneality)
- 3 cats eventually developed Chronic Enteritis - 2 were euthanized
- 17 cats remained healthy



ACVIM consensus statement guidelines on diagnosing and distinguishing low-grade neoplastic from inflammatory lymphocytic chronic enteropathies in cats. Sina Marsilio et al. *J Vet Intern Med.* 2023;37:794–816

- Panel: (2 internists, 1 radiologist, 1 anatomic pathologist, 1 clonality expert, 1 oncologist)
- Evaluate current peer-reviewed publications and compile a consensus statement
- **Most recommendations given by the panel were supported by a moderate or low level of evidence in the literature**
- Several under-studied areas were identified
- Bottom-line: no single diagnostic criterion or biomarker reliably differentiates inflammation from lymphoid neoplasia in the intestinal tract of cats
- **Diagnosis established by integrating all available clinical and diagnostic data**
- Histopathology remains the main-stay for differentiation?

## SDMA: Potential Marker in Low Numbers of Cases

- Symmetric dimethylarginine
- Sensitive indicator for renal insufficiency
- Can be elevated in dogs and cats without concurrent elevations in BUN +/- Creatinine
- SDMA levels near 100 in some dogs and cats with lymphoma
  - All had normal BUN, low numbers had mildly elevated CR

Received: 13 January 2022 | Revised: 15 June 2022 | Accepted: 15 June 2022  
DOI: 10.1111/avo.12845

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

### The association between symmetric dimethylarginine concentrations and various neoplasms in dogs and cats

Michael J. Coyne | Corie Drake | Donald J. McCrann | David Kincaid

**1803 dogs and cats with neoplasia**

SDMA concentrations were significantly higher in dogs and cats with lymphoma ( $p < .0001$ ) compared with non-tumor controls.

Cancer type	N	OR (95% CI)	p
Canine lymphoma	307	10.00 (5.98-16.72)	$p < .001$
Feline lymphoma	224	3.04 (1.95-4.73)	$p < .001$
Feline visceral mast cell tumour	55	1.63 (0.67-3.92)	$p = .275$
Canine hemangiosarcoma	230	1.11 (0.66-1.87)	$p = .691$
Canine mammary carcinoma	387	0.49 (0.28-0.84)	$p = .009$
Canine mammary adenocarcinoma	388	0.41 (0.231-0.71)	$p = .001$
Canine lipoma	212	0.39 (0.18-0.85)	$p = .013$

RESEARCH ARTICLE PLOS ONE | <https://doi.org/10.1371/journal.pone.0250839> May 14, 2021

## Validation of protein arginine methyltransferase 5 (PRMT5) as a candidate therapeutic target in the spontaneous canine model of non-Hodgkin lymphoma

Shelby L. Sloan<sup>1,2\*</sup>, Kyle A. Renaldo<sup>3\*</sup>, Mackenzie Long<sup>1,2</sup>, Ji-Hyun Chung<sup>2</sup>, Lindsay E. Courtney<sup>2</sup>, Konstantin Shilo<sup>4</sup>, Youssef Youssef<sup>2</sup>, Sarah Schlottner<sup>2</sup>, Fiona Brown<sup>2</sup>, Brett G. Klamer<sup>2</sup>, Xiaoli Zhang<sup>2</sup>, Ayse S. Yilmaz<sup>2</sup>, Hatice G. Ozer<sup>2</sup>, Victor E. Vali<sup>5†</sup>, Kris Vaddi<sup>7</sup>, Peggy Scherie<sup>7</sup>, Lapo Alinari<sup>2</sup>, William C. Kisseberth<sup>2,2†\*</sup>, Robert A. Balocchi<sup>2†\*</sup>

- 42.4% of lymphomas positive for PRMT5
- PRMT5 inhibition → Cell death





# Feline Cytology Case Challenges

## Feline Cytology Case Challenges

- Challenge?
- How do I get down from here?



## "Herc" The Cat with Diarrhea

- Starts dressing in strange attire
- Sleeping in unusual places

## "Herc" The Cat with Diarrhea

- Starts dressing in strange attire
- Sleeping in unusual places

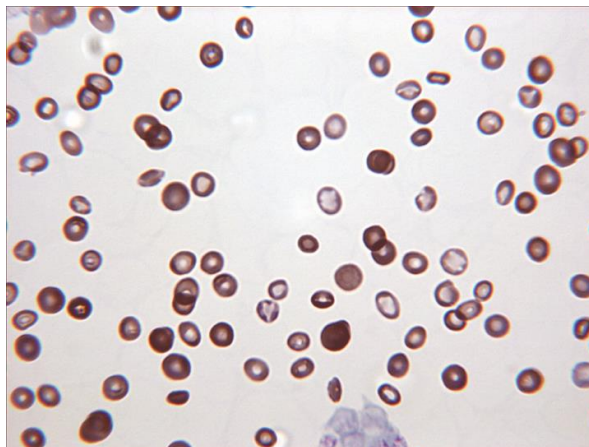


## Differentials

- DDx?
  - Lymphoma
  - Other neoplasms (Carcinoma if TD were Siamese)
  - Lymphocytic / plasmacytic enteritis
  - Infections / parasitic
  - Dietary etc. etc.
- Plan?
  - MDB, CBC, Chem. Profile
  - FeLV/FIV test
  - Imaging of abdomen

## CBC Values

- PCV 18% (30 - 45)
- RBC 7.5 (5-10.0)
- Hgb 4.9 (9.8-15.4)
- MCH 9.0 (13-17)
- MCHC 24 (30-36)
- MCV 20.1 (39-55)
- RDW 24.1 (17-22)
- WBC 6.83 (5.5-19.0)
- Bands 1.3 (<.3)
- Neuts 5.4 (2.5-12.5)
- Lymphs .08 (1.5-7.0)
- Monos .03 (<.9)
- Eos 0 (<.8)
- Platelets (Adq) (300-800)



## Interpretation

- Microcytic, hypochromic anemia
- Iron deficiency
- What causes a microcytic, hypochromic, iron deficiency anemia in cats?

## Interpretation

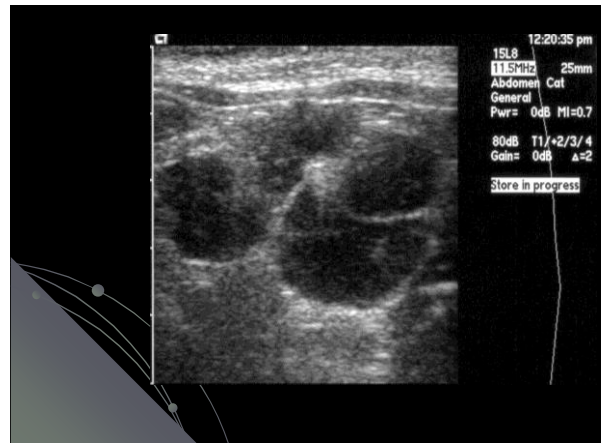
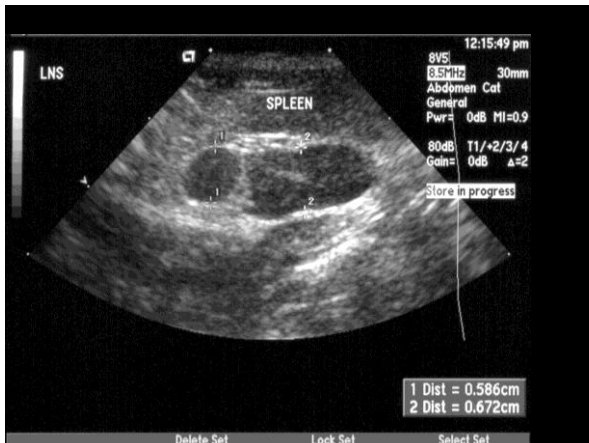
- Microcytic, hypochromic anemia
- Iron deficiency
- Reasons?
  - Chronic blood loss

## Biochemical Profile

BUN (19-34)	45 H	ALT (25-98)	98
Creat (0.9-2.2)	1.6	AST (7-38)	38
Phos (3.0-6.1)	3.9	ALP (0-45)	32
Ca (8.7-11.7)	10.2	Bili (0-1.0)	0.1
TCO <sub>2</sub> (13-21)	17	Alb (2.8-3.9)	2.3 L

## "Herc"

- T<sub>4</sub>: 2.1 µg/dl (1-3 µg/dl)
- FeLV/FIV: negative



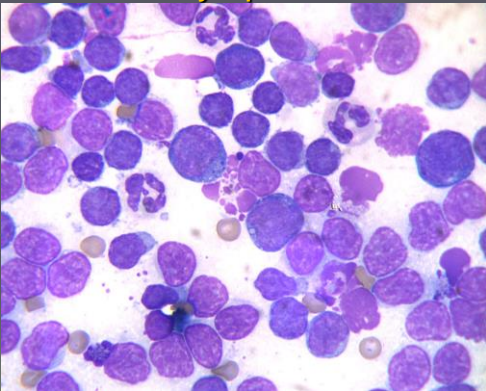
## Problems

- Iron deficiency anemia due to chronic blood loss
- Diarrhea
- Enlarged mesenteric lymph nodes
- Thickened intestinal wall
- DDX
  - IBD
  - Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia
  - Lymphoma
  - Other neoplasms

## “Herc”

- What do we do with them big lymph nodes?
  - Take them out?
  - Biopsy them?
  - FNA?
  - Histopathology
  - Immunophenotyping
  - PARR analysis
  - All of the above? Good luck with that!

## FNA Lymph Node



## Herc Results

- Cytological Diagnosis
  - Reactive lymphadenopathy
- So what do we do now?

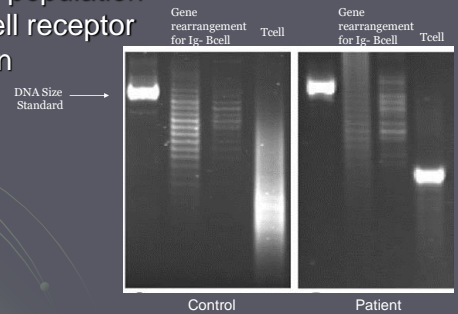


## Herc Results

- So what do we do now?
  - PARR Analysis
  - You already have the sample collected
  - Relatively inexpensive
  - 80% sensitivity; Higher specificity
  - False negatives in very low numbers of lymphomas (NK cells)
  - False positives in cats with IBD and pseudoclonality

## Herc PARR Results

- Clonal population of T-cell receptor antigen

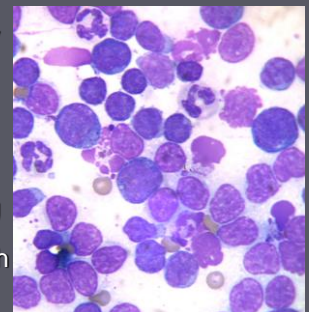


## Plan for Herc?

- COP chemo
- Sucralfate/famotidine
- Iron supplementation
- SQ fluids
- Are we experiencing a blast transformation or high / intermediate grade ITL?

## Chemo for Herc

- COP chemotherapy
  - Cyclophosphamide 300 mg/m<sup>2</sup>, PO, q3 wks
  - Vincristine 0.5 mg/m<sup>2</sup> IV q1 wk
  - Prednisolone 10 mg PO q24h X 1 wk; then 10 mg PO q48h



## Herc Follow-up

- Follow-up:
  - Started Tx on 10/14/23
  - GI signs resolved; no other abnormalities
  - Abdominal US on 12/29/23 WNL

## "Small Cell" GI Lymphoma

- Conservative Tx
  - Leukeran 20 mg/m<sup>2</sup>, PO, q/2 weeks
  - Prednisone 5-10 mg PO q48h
  - Vincristine 0.5 mg/m<sup>2</sup>, IV, q/2 weeks

## Tabby Thompson

- 9 year old, F/S DSH
- Presented with complaint of listlessness, anorexia and rapid respiration
- Physical exam findings
  - Muffled heart and lung sounds
  - Cranial thorax not easily compressed

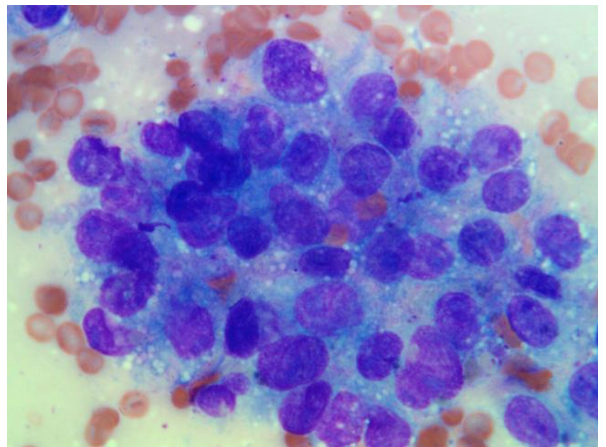
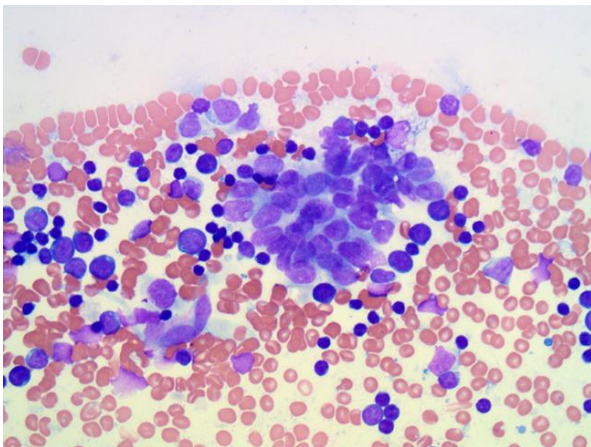
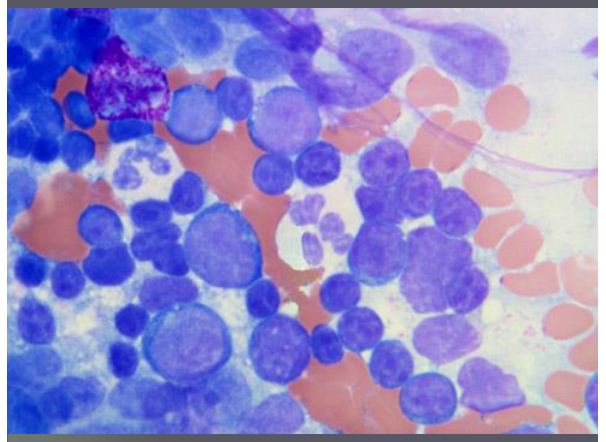
## Tabby Thompson

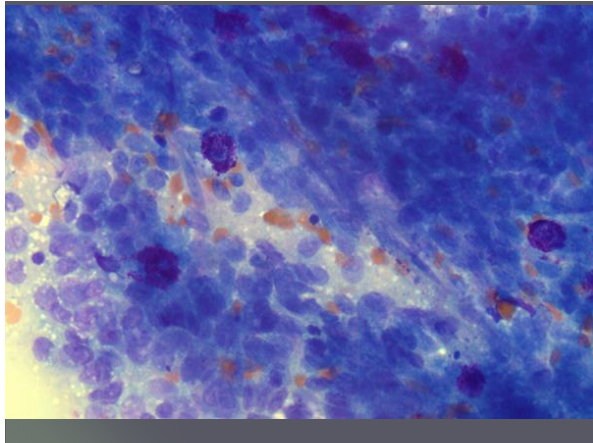
- Radiographs
  - Very large mass in cranial mediastinum



## Tabby Thompson

- DDx for **most common** cranial mediastinal mass in the cat
  - Lymphoma
  - Thymoma
- Problems
  - Drastically different therapies
  - Both contain lymphocytes





## Thymoma

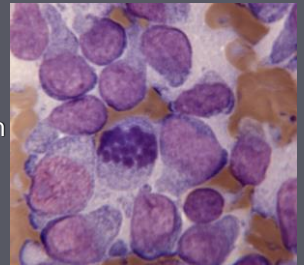
- Neoplasia of the epithelial cell population of the thymus
- Small lymphocytes predominate in most aspirates
- Low numbers of neoplastic epithelium are often seen
- Mast cells frequently observed in feline thymomas

## Thymoma

- 60% in the cat are cystic
- Benign forms well encapsulated (80%)
- Malignant forms invasive
  - Rare to metastasize
  - Cytology incapable of distinguishing forms
- Paraneoplastic syndromes
  - Myasthenia (40% dogs, rare in cats)
  - Megasophagus and or aspiration pneumonia
  - Immune-mediated anemia, polymyositis
  - Dermatitis (reported in cats)

## Thymic Lymphoma

- Lymphocyte is the neoplastic cell population
- T-cell in origin
- Large blast cells with occasionally clefted nuclei
- Small cell thymic lymphoma
  - Never seen one



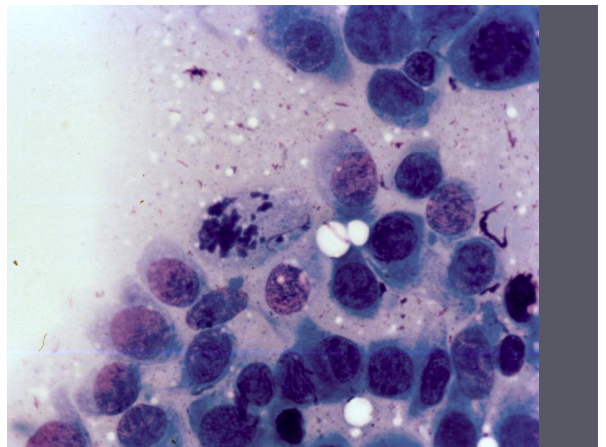
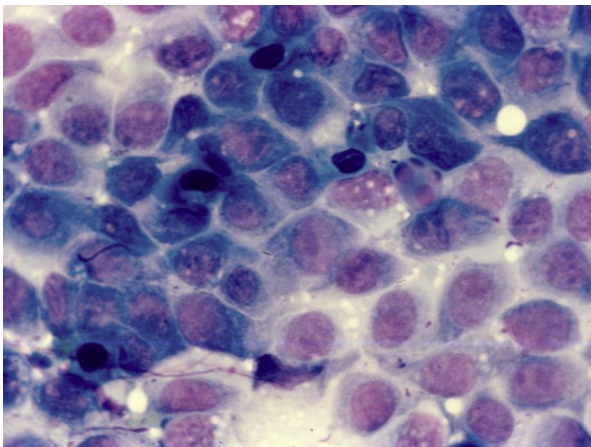


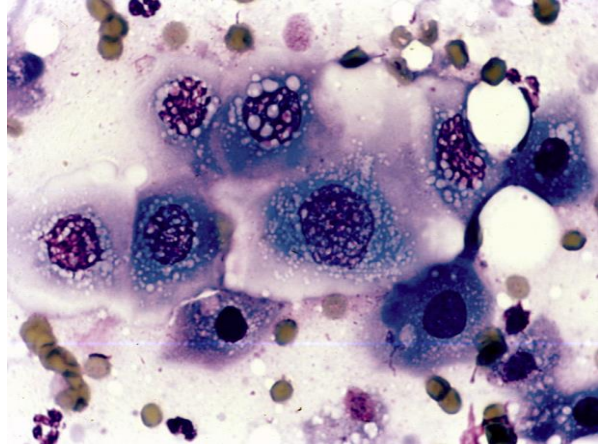
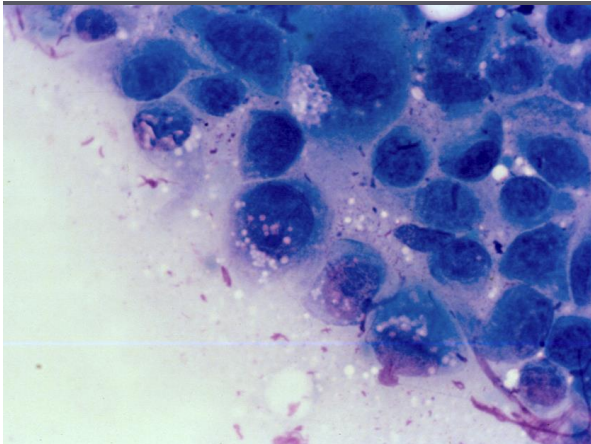
## Patient: Cricket

- 11 year old, male, DSH
- Presented for halitosis and decreased appetite
- Salivation
- PE or oral cavity
  - 1.5 cm, ulcerated mass on left side of the pharyngeal cavity
  - Possibly involving the tonsil

## Differentials

- Neoplasia
  - Squamous cell carcinoma
  - Lymphoma
  - Plasmacytoma
- Infection / inflammatory
  - Fungal (cryptococcus)
  - Sever stomatitis?
    - Eosinophilic, Lymph. / Plasma., pyogranulomatous
- Plan
- Anesthesia → FNA





## Squamous Cell Carcinoma

- Most common malignant, epithelial tumor of the oral cavity
- Strong site predilection for different areas on the head
  - Palate, lip, cheek, gingiva, tongue and tonsil
  - Ears and nose
  - Also, digits

## Cytological Appearance of SCC

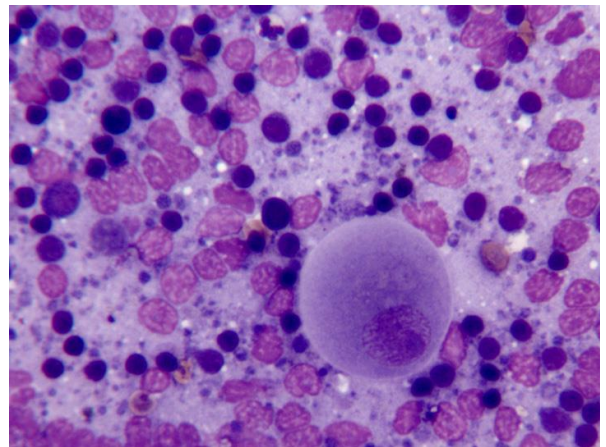
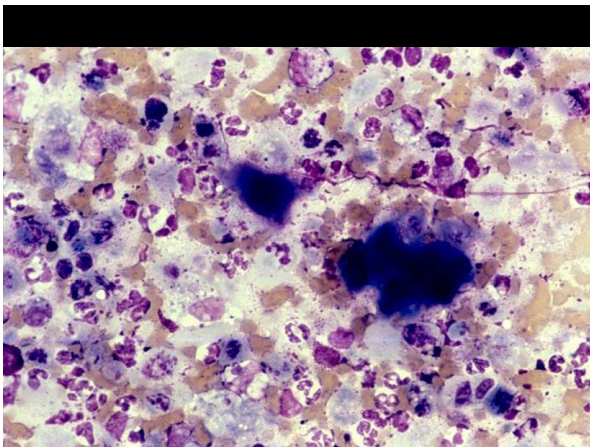
- Epithelial neoplasm with some degree of squamous differentiation
  - Angular cytoplasmic borders
  - Dysplasia with inappropriate keratinization
  - Small, perinuclear vacuoles
  - Some mature squamous cells
  - Loss of cohesion

## Biological Behavior of SCC

- Site dependent and aggressive
- Most oral and cutaneous tumors are locally aggressive and invasive
  - Especially in the cat
- Metastasis typically later in course of disease
- Tumors located at base of tongue, tonsil of digits
  - May more readily met to regional lymph nodes

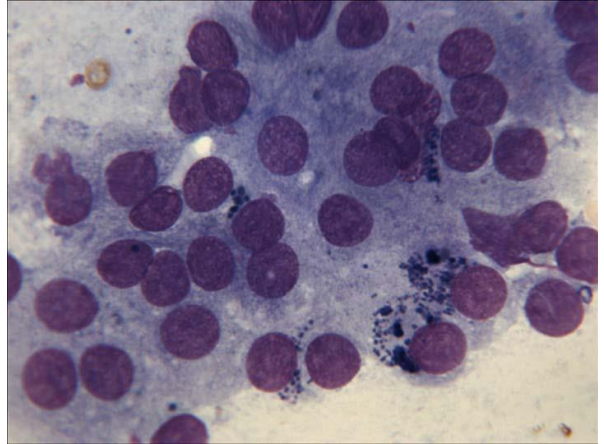
## Patient: Rascal

- 12 year old, M/C DSH
- Rapidly growing lesion on the nose



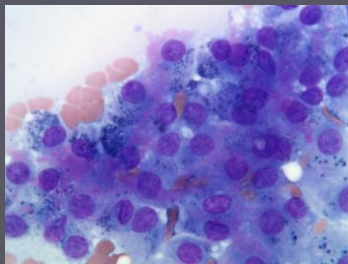
## Patient: Pounce

- Physical exam findings
  - Small, subcutaneous mass on right side of trachea, near thoracic inlet



## Thyroid Neoplasia

- Typical neuroendocrine appearance of clumps of epithelium with few distinct cell borders
- Tyrosine granules
- Colloid



## Biological Behavior (Feline)

- Adenomas most common in cats, often bilateral
- Distinction between benign and malignant done histologically
  - Even adenocarcinomas do not typically have criteria of malignancy
  - Invasion in to capsule or surrounding tissues / lymphatics



## Biological Behavior

- Most in cat are biologically active
- Hypersecretion of thyroid hormones
- Adenocarcinomas locally invasive
  - 40% to 70% have metastasis to regional lymph nodes



## Canine Thyroid Tumors

- Clinical presentation
  - Mass on ventral neck to thoracic inlet (intrathoracic)
- Breed predilection
  - Boxers, beagles, golden retrievers
- >85% malignant
- Adenomas and adenocarcinomas cytologically similar



## Biological Behavior

- Usually not biochemically secretory
- Carcinomas locally invasive and will metastasize – Blood or Lymphatics
  - Tumors < 5cm in diameter
    - Potential for metastasis low
  - Tumors > 5cm in diameter
    - 40% chance of metastasis at time of Dx
- DIC

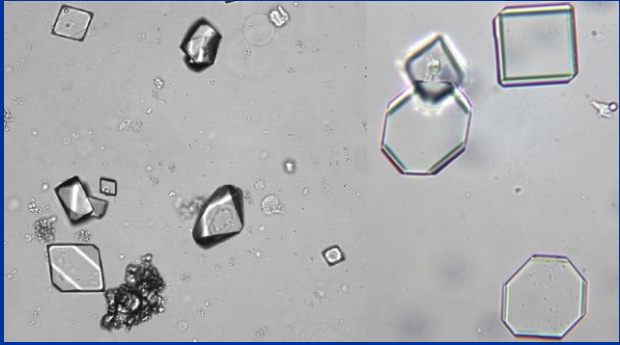
## Thank You A Ton!





# The Complete Urinalysis

## Either Urine or UR-Out: The Complete Urinalysis with Images from a Fully Automated Analyzer



## Urinalysis Procedure

- Sample collection
- Chemical analysis
- Evaluation of urine sediment
- Ideally, should be performed within 2 hours after collection
- Refrigeration allows prolonged storage prior to analysis
  - Artifacts
  - Crystals
  - Cellular degeneration

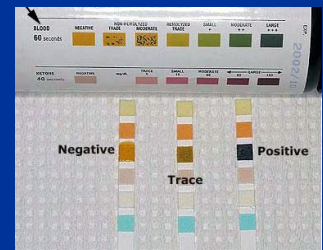


## Urine Specific Gravity: Supernatant is best!



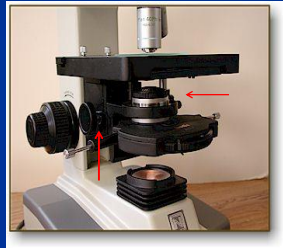
## Urine Hemoprotein

- Normally negative
- Positive results
  - Hemorrhage, hemoglobinuria
  - myoglobinuria
- False negatives
  - Patients on Captopril
- False positives
  - Iatrogenic blood contamination
  - Bleach & other disinfectants
- Positive on test pad but no red cells in sediment?



## Microscopic Evaluation of Urine Sediment

- Wet mount
  - Unstained drop of sediment with coverslip
  - Lower substage condenser
  - Look for crystals, casts and cells
  - If cells are identified . . . .
- Dry mount
  - For the active sediment: red blood cells, white blood cells and / or suspect bacteria
  - Diff Quik®



## The Active Sediment

- Best visualized on stained preparation
- Detailed evaluation – done on all urine with **active sediment**
  - Make a “**linear smear**” preparation of pellet
  - Air dry
  - Stain with Diff Quik®



(<https://www.idexxlearningcenter.com/mod/video/view.php?id=2036>)

## The Linear Smear



## IDEXX SediVue Dx™

- Urine sediment analyzer
  - IDEXX Laboratories, Inc.
- 165 µl unspun urine (4-5 drops)
- Gentle centrifugation (260 RCF)
- Provides 70 high quality digital images, equivalent to approximately 45 HPFs
- Quantitative and semiquantitative analysis using image evaluation software**
- Results in approximately 3 min.



## 5.0 Neural Network Imaging

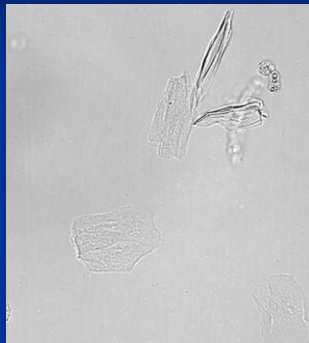
Element type	Parameter	Image tag	Reported results					
Blood cells	WBC	WBC	None detected	<1/HPF	Quantitative numerical result/HPF			>50/HPF
	RBC	RBC						
Bacteria <sup>1</sup>	Rods	N/A <sup>1</sup>	None detected	Suspect presence	Present			
	Cocci							
Epithelial cells	Squamous	sqEPI	None detected	<1/HPF	1-2/HPF	3-5/HPF	6-10/HPF	>10/HPF
	Nonsquamous	nsEPI						
Casts	Hyaline	HYA	None detected	Suspect presence	>1/LPF			
	Nonhyaline (e.g., granular, waxy)	nhCST						
	Unclassified (all other crystals)	CRY						
Crystals	Calcium oxalate dihydrate	CaOxDi	None detected	<1/HPF	1-5/HPF	6-20/HPF	21-50/HPF	>50/HPF
	Struvites	STR						
	Ammonium biurate	AmmBi						
	Bilirubin	BILU						

## Epithelial Cells

- Squamous cells
- Non-squamous cells
  - Transitional epithelium
  - Caudate epithelium
  - Renal tubular epithelium

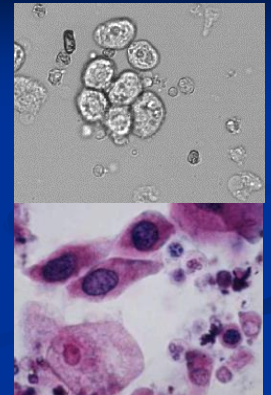
## Squamous Cells

- Large, flat cells with angular sides and small nuclei
- Distal urethra, vagina or prepuce
- Lower urinary tract contamination



## Non-Squamous

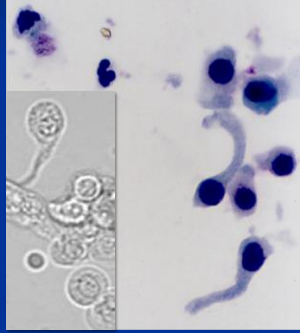
- Transitional epithelium
  - Round to pear-shaped
  - Variable size and shape
  - Higher N:C ratio
  - Ureter, urinary bladder and proximal 2/3 of urethra & ureter



## Non-Squamous

### • Caudate epithelium

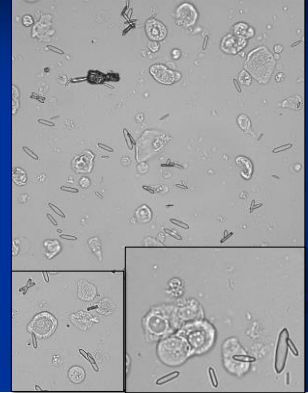
- Spindle or tadpole-shaped cells
- Renal pelvis
- Not normally seen in sediment
  - Pyelonephritis
  - Calculi



## Non-Squamous

### • Renal tubular epithelium

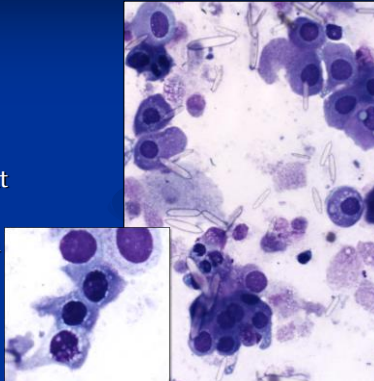
- Small round to rectangular cells
- Vacuoles in the cat
- Originate from renal parenchyma
- Indicate renal damage / inflammation



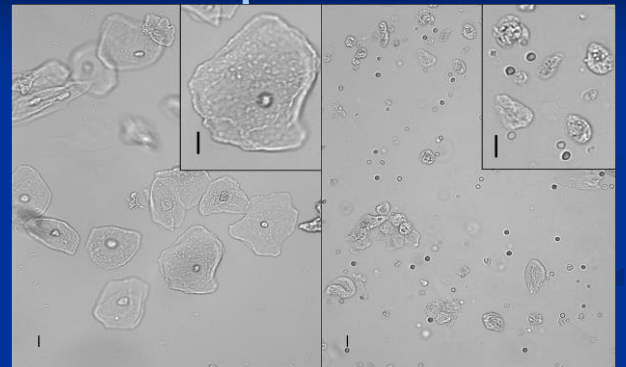
## Non-Squamous

### • Renal tubular epithelium

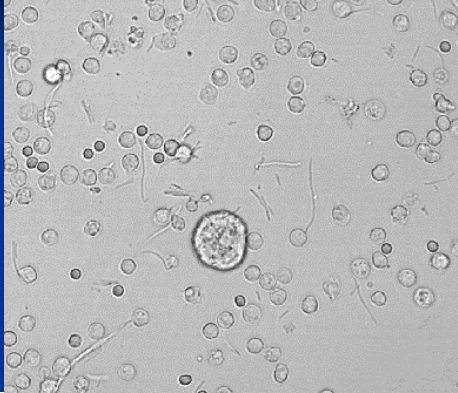
- Small round to rectangular cells
- Vacuoles in the cat
- Originate from renal parenchyma
- Indicate renal damage / inflammation



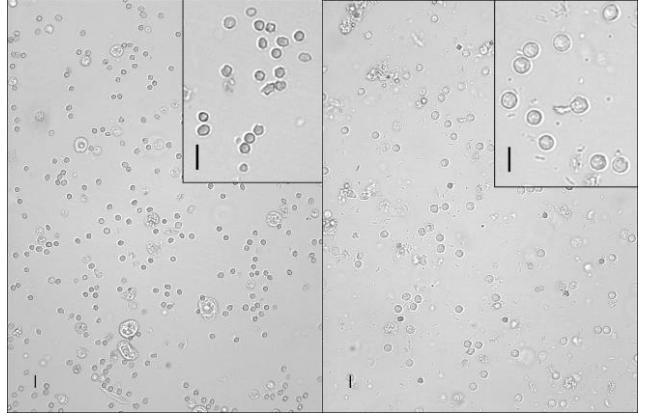
## Squamous and Non-squamous



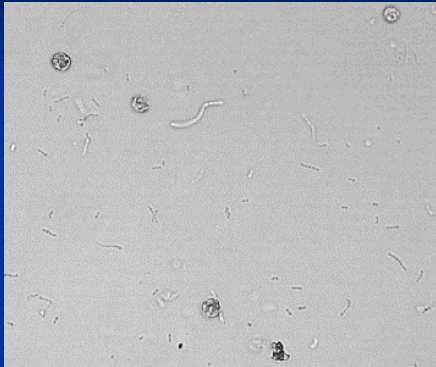
**Red Blood Cells, White Blood cells and Bacteria**



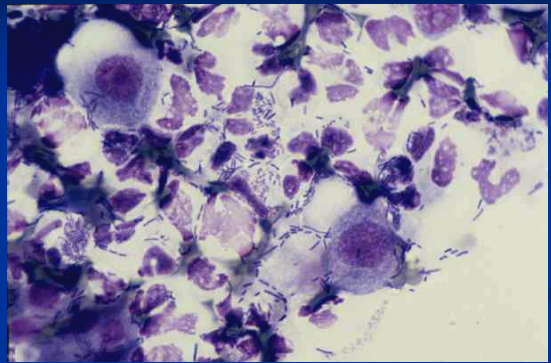
**RBCs and WBCs**



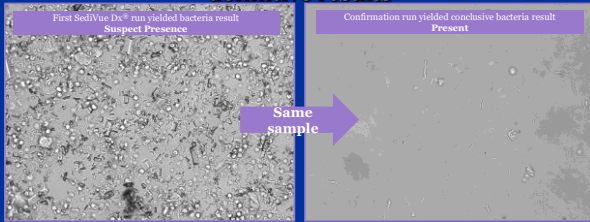
**WBC and Cocci**



**Air-Dried Diff Quik**



**The SediVue® Bacteria Confirmation Kit removes cells and crystals that can hide bacteria, revealing definitive results**



*"The reagent helps to clear out the debris, so that we can see bacteria. They're so small that they're hard to see in crowded samples."*

**Removing common "clutter" prevents SediVue Dx® from "suspecting" bacteria is present when truly negative**

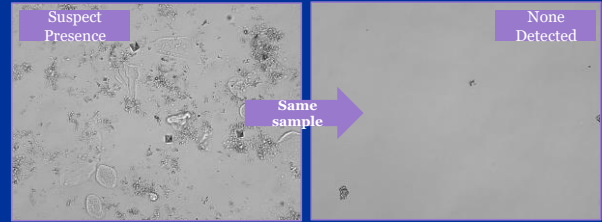


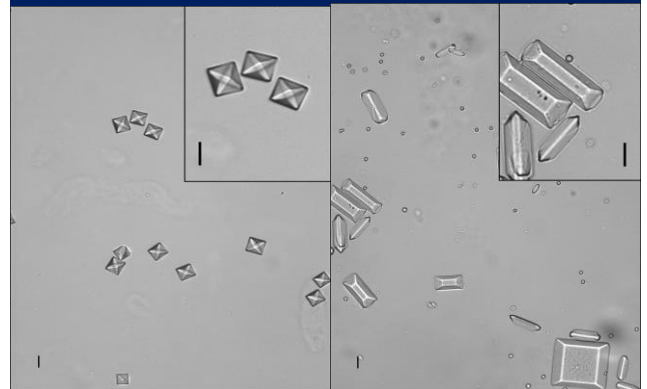
Image from initial sample run: crystalline debris clutters the sample making the result inconclusive

Image from second run is decluttered and the result is clearly negative for bacteria

**Crystalluria**

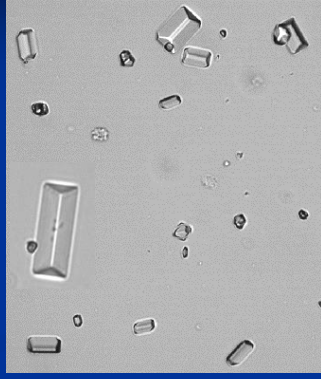
- SediVue will report
  - CaOxDi
  - Struvite (magnesium, ammonium phosphate: MAP)
  - Ammonium biurate
  - Bilirubin
  - Unclassified crystals

**CaOxDi & Struvite**

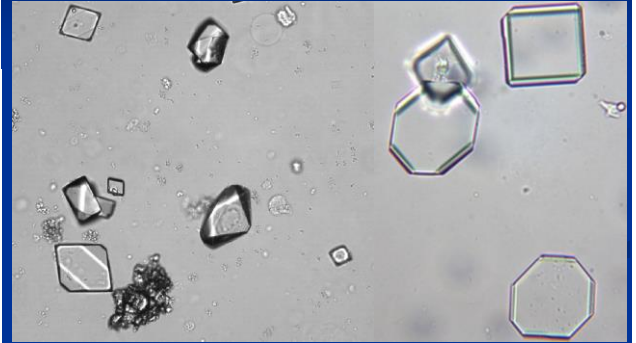


## Crystalluria

- Magnesium ammonium phosphate (MAP, Struvite)
  - Variably shaped, 3 dimensional rectangular prisms
  - Coffin lids
  - Alkaline urine
  - Secondary to UTI (dogs)
  - Sterile cystitis (cats)
  - Refrigerated samples

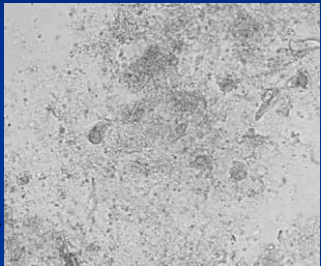


## Atypical Struvites



## Crystalluria

- Amorphous phosphate
  - Colorless, amorphous debris
  - Small spheroids
  - Seen in alkaline urine
  - Commonly found in clinically normal animals



## Crystalluria

- Calcium oxalate crystals
  - Two forms
    - Dihydrate form
      - Envelope-shaped
      - Can be normal
      - Ingestion of oxalate containing plants
      - Refrigerated samples or prolonged storage at room temperature
    - Monohydrate form
      - Elongated, flat crystal with pointed ends
      - Seen in acute cases of ethylene glycol poisoning

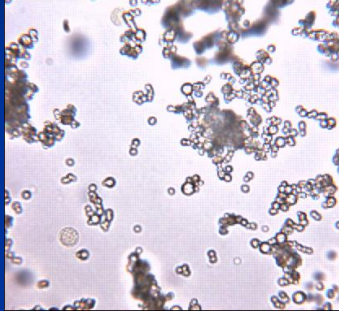




## Crystalluria

### Urates

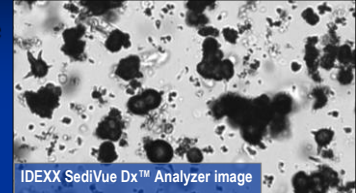
- Amorphous ground material
  - Small spheroids
- Flat prisms of various geometrical shapes
- Seen in acidic urine
- Predisposed breeds
  - Dalmatian
  - English bulldogs
  - Predisposition to urate urolithiasis



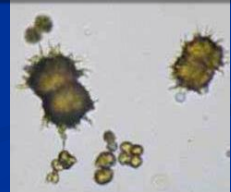
## Crystalluria

### Ammonium biurate

- Golden to brown
- Spherical with irregular protrusions
- Smooth aggregates of spheroids (cats)
- Severe hepatic disease
  - Portovascular malformations



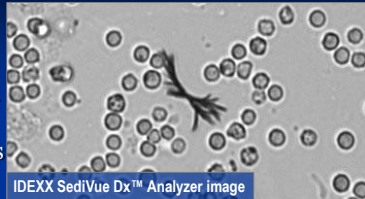
IDEXX SediVue Dx™ Analyzer image



## Crystalluria

### Bilirubin

- Orange to reddish-brown granules or needle-like crystals
- Disorder in bilirubin metabolism
  - Liver disease (hepatic or post-hepatic)
  - Extravascular hemolysis



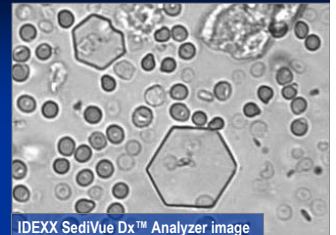
IDEXX SediVue Dx™ Analyzer image



## Crystalluria

### Cystine

- Colorless, flat hexagons
- Unequal sides
- Always abnormal
- Inherited defect in tubular transport of cystine
- Breed predisposition
  - Males
  - Dachshunds
  - English bulldogs
  - Siamese cats
  - American Bully



IDEXX SediVue Dx™ Analyzer image



## Casts

- Recorded as number per LPF
- Best visualized on unstained preparation
- Typically an early indicator of renal tubular disease
  - Hyaline casts

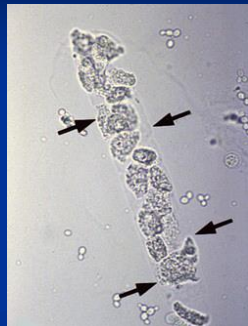
## Casts

- Hyaline casts
  - Tamm-Horsfall mucoprotein precipitates
  - Colorless tubular structures
  - Low numbers are insignificant
    - Exercise
    - Hyperthermia



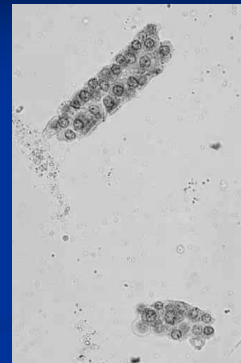
## Casts

- Epithelial (cellular) casts
  - Round to polygonal epithelial cells in tubular arrangement
  - Nephritis / pyelonephritis
  - Undergo degeneration to produce granular casts



## Casts

- Epithelial (cellular) casts
  - Round to polygonal epithelial cells in tubular arrangement
  - Nephritis / pyelonephritis
  - Undergo degeneration to produce granular casts



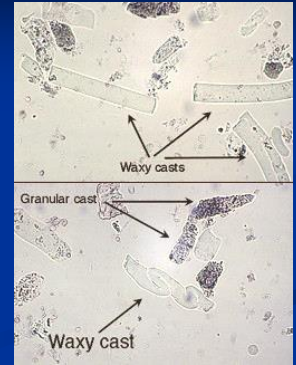
## Casts

- Granular casts
  - Seen when epithelial casts begin to degenerate
  - Coarse granular casts
    - Fine granular casts
    - Waxy casts



## Casts

- Granular casts
  - Seen when epithelial casts begin to degenerate
  - Coarse granular casts
    - Fine granular casts
    - Waxy casts

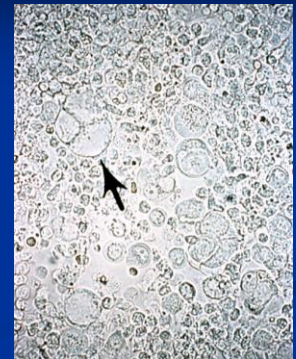


## Bladder Neoplasia

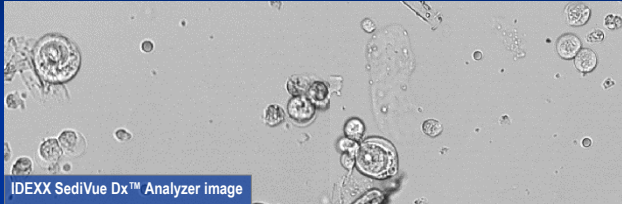
- Difficult to diagnose from urine sediment
  - Atypical transitional epithelium
  - Lack of inflammation
- Imaging required to visualize mass
  - Tissue sampling to confirm
    - FNA?
    - Traumatic catheterization
    - Cystoscopic biopsy

## Transitional Cell Carcinoma

- 90% of bladder tumors
- Middle-age to older dogs
  - Uncommonly cats
- Atypical transitional cells
  - Absence of inflammation

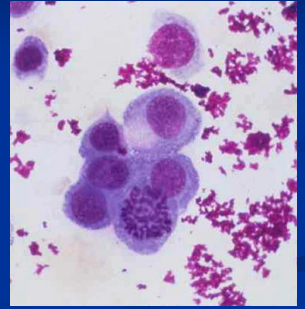


## IDEXX SediVue™



## Transitional Cell Carcinoma

- Ultrasound-guided FNA and T. C.
- Visualization of mass
- More representative of lesion
- Seeding of tumor cells



## Transitional Cell Carcinoma

