Microscopy 101

Getting the Most from Your Scope

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Learning Objectives

- Why perform cytology
- What is required
- Collection techniques
- Slide preparation and optimizing staining
- Slide examination including normal skin and artifacts
- Examination of aspirates of solid tissues and masses

Why Perform Cutaneous Cytology?

- Most common diagnostic test performed in veterinary dermatology practice
- Provides the basis for empirical selection of antimicrobial drugs
- Adds diagnostic etiologic information based on cell type or structures present → medical vs. surgical management
- Best to correlate with results of culture and biopsy

Why Perform Cutaneous Cytology?

- May be superior to histopathology for some microorganisms
- Safe for patient
- Cost effective
- Easily self taught
- Good business practice builder while improving the quality of medicine offered

What Cytology Can't Do

- Determine tissue architecture
  - Precise diagnosis of neoplasm type not always possible (i.e. spindle cell)
  - Assess invasiveness
  - Assess distribution
- If poorly cellular
  - May not be representative
  - Negative results do not rule out disease

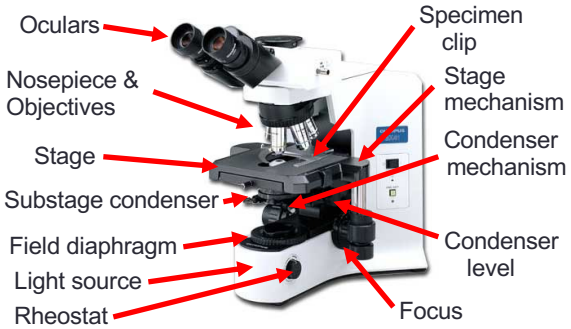
What Cytology Can't Do

- Grade mast cell tumors
- Predict malignant/benign behavior of some neoplasms: mammary, melanoma, endocrine, hepatocellular
- Distinguish infiltrative from encapsulated lipoma
- Diagnose small cell variety lymphoma (sometimes)
- Distinguish granulation tissue from sarcoma (sometimes)

What Do You Need?

- Binocular microscope with strong light source and high quality lenses
  - Ideal: 4x,10x, 40x and 100x (oil immersion)
- Slides (frosted edge preferred)
- Immersion oil
  - Usually Type A or B
- Stain - *Diff-Quik*®
  - Quick and easy Romanowsky-type stain

Anatomy of the Light Microscope



Microscope Maintenance

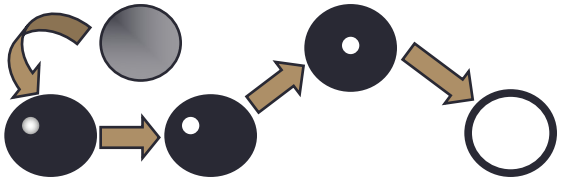
- Annually – professional cleaning, service and lubrication
- Daily – dust cover
- As needed – ocular and lens cleaning
  - LENS paper ONLY
  - Alcohol based cleaners
  - Xylene (CAUTION with plastic lenses and some lens sealants)

Kohler Illumination

- AKA "critical focusing"
- Centers and focuses light onto the specimen for maximum resolution
- Best to perform when switching from unstained to stained specimens

Steps to Kohler Illumination

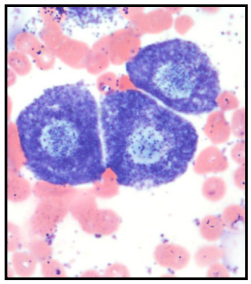
1. With slide on the stage, focus at 10x
2. Open substage condenser diaphragm fully
3. Close field diaphragm until only a dot of light is visible – FOCUS and CENTER
4. Re-open field diaphragm maximally



Collection Techniques

Impression Smear

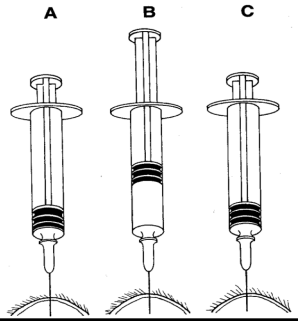
- Identify inflammatory and neoplastic cells, as well as organisms that will aid in diagnosis
- Touch slide directly to lesion if moist or exudative



Fine Needle Aspiration

- Indications: nodules and plaques
- Identify inflammatory and neoplastic cells as well as infectious organisms
- Avoid zones of inflammation and/or necrosis for best specimen
- Technique: aspiration vs. needle “core biopsy”
  - Aspiration: 6 ml syringe with 20-22 gauge needle
  - Insert needle into lesion

- Aspirate about 1 ml vacuum
- Move needle within lesion → repeat aspiration
- Release vacuum, extract syringe from lesion

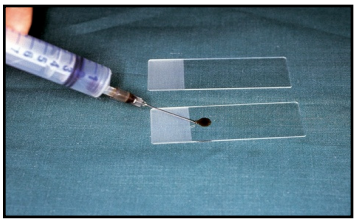


(A) insert
(B) aspirate
(C) release
(repeat)

Cowell and Tyler’s Diagnostic Cytology and Hematology of the Dog and Cat, 4th edition

Fine Needle Aspiration

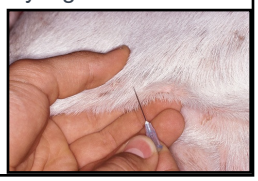
- Remove needle and add ~2 cc of air into the syringe
- Replace needle and push contents onto a clean glass side



Fine Needle Aspiration

Needle core technique

- Insert needle into lesion
- Rotate needle on axis and redirect while within the lesion
- Repeat insertion
- Add ~2 cc of air to a 3 or 6 cc syringe
- Replace needle and push contents onto a clean glass slide



Slide Preparation

- Collected materials are allowed to dry on slide
- Oily (adipocytes), waxy or dry samples may be heat fixed
- Diff-Quik®
  - ✓Minimum of five 1 second dips in each stain
  - ✓Rinse with water
  - ✓Air dry or use hair drier
  - ✓Gently blot with bibulous paper

Slide Preparation

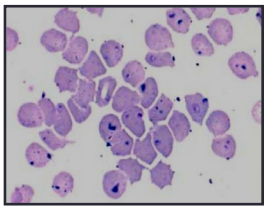
- Staining duration depends on specimen thickness
- Diff-Quik®
  - Fast, high contrast stain
  - Lacks cytoplasmic and nuclear detail
  - May miss cytoplasmic granules
- New methylene blue
  - Wet or unfixed slide may be stained
  - Little advantage over Diff-Quik®

Getting the Most from DiffQuik®

- Storage
- "Dirty" vs. "Clean" specimens
- Replace stain at least once weekly
- Adapting staining to specimen thickness
- Avoid formalin exposure
- Stain soon after specimen has dried thoroughly

Top Causes of Poor Staining

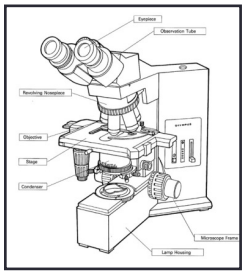
- Stain precipitate
- Stain contaminants
- Inappropriate specimen drying
- Too little fixative time
  Too much stain time
- Exposure to formalin (fumes or liquid)
- Prolonged delay before staining



Slide Examination

- Stained specimens
  - Cytology and hematology
  - Stain provides contrast
  - Settings for optimal light

ESTABLISH KOHLER ILLUMINATION

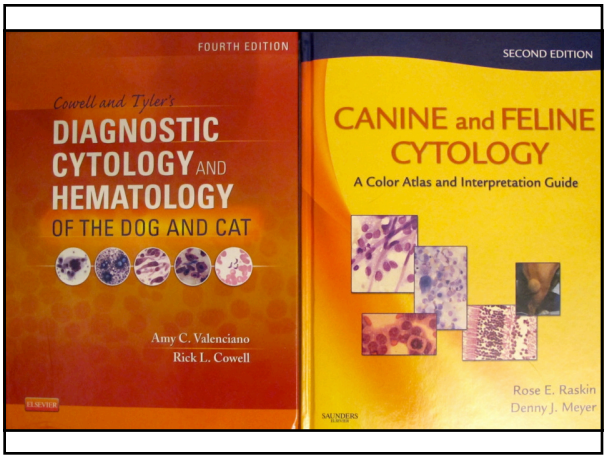


Why is the 40x Blurry?

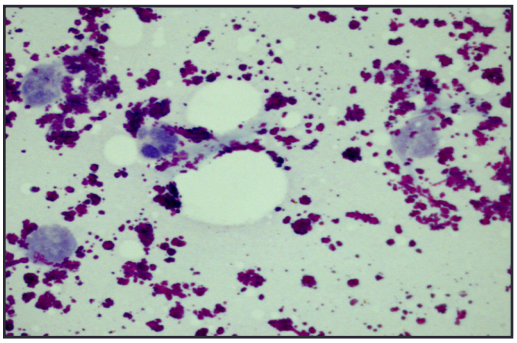
- Designed for use with cover slipped specimens
- HOW TO WORK AROUND:
  1. Stain and dry specimen as usual
  2. On top of stained specimen, place 1-2 drops immersion oil
  3. On top of oil, gently place cover slip (avoid bubbles!)
  4. 40x resolution should be improved
  5. Oil can be wiped off *gently* with Kleenex tissue

Slide Examination

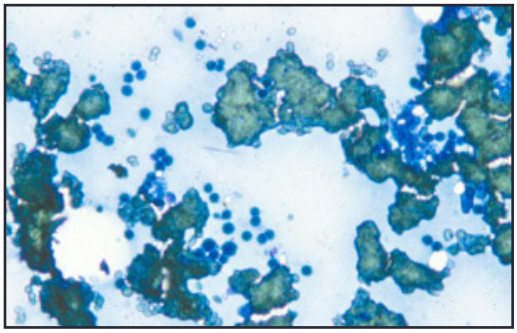
- Practice learning what is normal
- Learn to recognize artifacts (stain precipitate, hair, cotton fibers, pollen) that occur with cutaneous sampling
- Consider evaluating one sample in house and sending the other to a clinical pathologist
- PRACTICE!



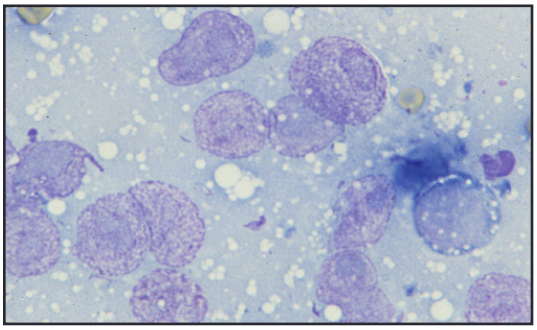
Artifacts – Aqueous Lubricant



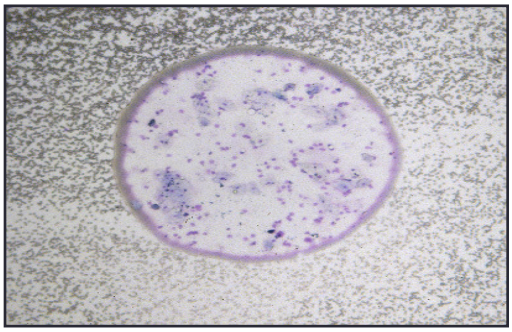
Artifacts – Inadequate Spreading



Artifacts – Cell Rupture



Artifacts – Contaminants

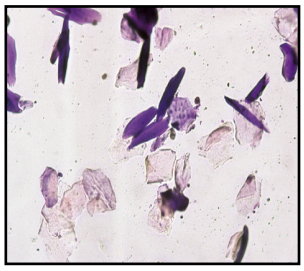


Normal vs. Abnormal

- Normal skin
  - < 1 organism (yeast, cocci, rod)/OIF
  - No inflammatory cells
- Ears
  - Malassezia
    - ✓Cats: > 1 Malassezia/OIF → significant
    - ✓Dogs: > 3 Malassezia/OIF → significant
  - Cocci
    - ✓>5 cocci/OIF → significant
    - ✓>1 rod/OIF → significant

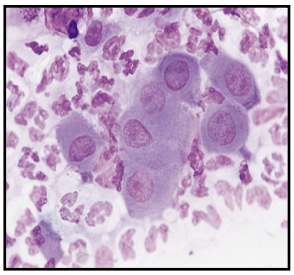
Cytology of Normal Skin

- Epidermis
  - Corneocytes
    - Anucleated
    - Flat
    - Often folded



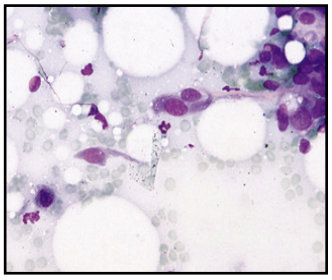
Cytology of Normal Skin

- Epidermis: basal and spinous keratinocytes
  - Nucleated
  - Round nuclei
  - Basophilic cytoplasm



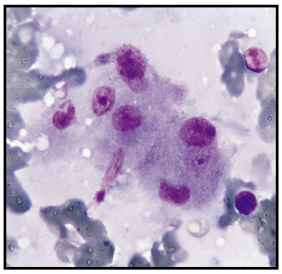
Cytology of Normal Skin

- Dermis
- Fibroblasts
  - Spindle shaped
  - Oval nuclei



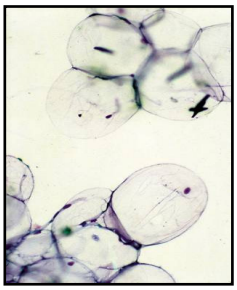
Cytology of Normal Skin

- Dermis: sebacytes
  - Sebaceous glands
  - Round nucleus
  - Abundant cytoplasm with small clear vacuoles



Cytology of Normal Skin

- Subcutis: adipocytes
  - Small displaced nucleus
  - Abundant clear cytoplasm (lipid)



HOW TO EXAMINE ASPIRATES OF SOLID TISSUES AND MASSES

Logical Approach to the Specimen

- Scan entire slide at low mag (4x and10x)
  - Evaluate staining quality, cell density, arrangement of cells, hemodilution
  - Are cells intact or lysed?
  - Do cells “match” the site sampled?
  - Find an area to examine at higher magnification
- Examine areas at higher magnification (40x and100x)
  - Identify normal cells
  - Identify inflammatory cells, infectious agents, neoplastic cells

Common Questions to Answer

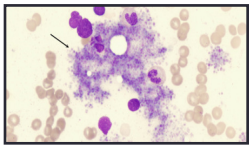
- Is there hemodilution or hemorrhage?
- Is there inflammation?
  - What kind of inflammation?
  - What is causing the inflammation?
  - Is there an infectious agent?
- Is there a neoplastic population?
  - What type of neoplasia (round, epithelial, spindle cell)?
  - Is it benign or malignant?

Hemodilution or Hemorrhage?

Procedural blood contamination

Look for:

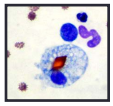
- Leukocytes in proportion to blood
- Platelet clumps

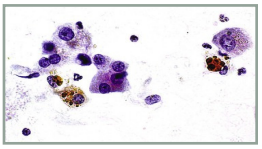


Hemorrhage

Look for:

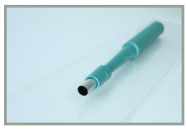
- Macrophages consuming RBCs
- Hemosiderin
- Hematoidin





Combinations – TRICKY!

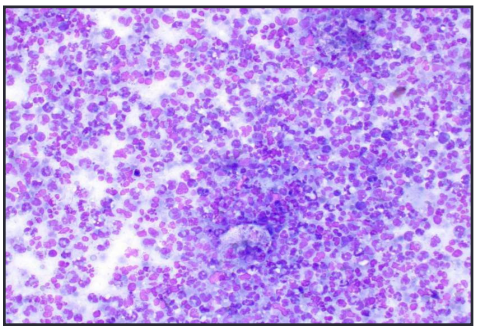
- Inflammation/infection + atypical cells = primary inflammatory lesion with dysplastic change OR
- Primary neoplastic lesion with superimposed inflammation, infection, hemorrhage and/or necrosis
- GET A BIOPSY!



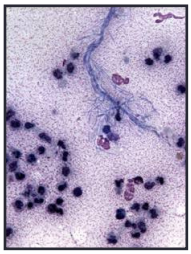
If Inflammatory. . .

Offers clue as to cause since different types of inflammation are seen with different etiologic agents

NEUTROPHILIC

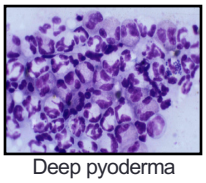


NEUTROPHILIC



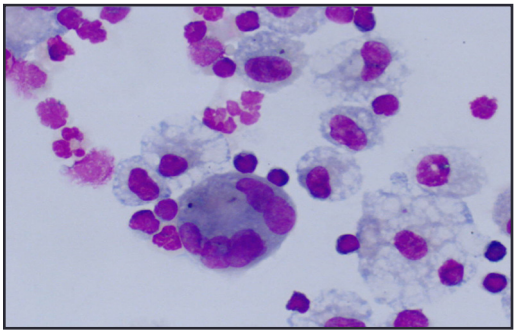
Feline abscess

- Trauma
- Foreign body
- Bacterial infection
- Immune-mediated disease
- Neoplasia



Deep pyoderma

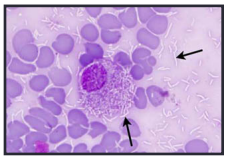
GRANULOMATOUS



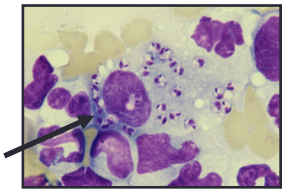
GRANULOMATOUS

Pyogranulomatous = with neutrophils

- Mycobacteria
- Fungi
- Protozoa
- Foreign body
- Hemorrhage
- Neoplasia



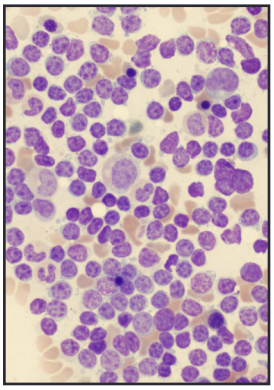
Mycobacterium



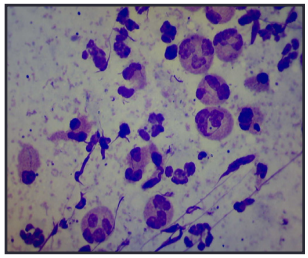
Canine Leishmania

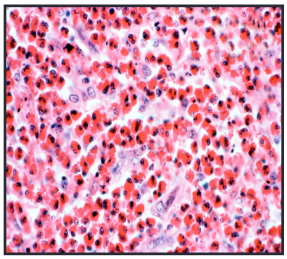
LYMPHOCYTIC

- Foreign body (vaccine granuloma)
- Immune-mediated disease
- Regressing histiocytoma
- Secondary to neoplasia (cell-mediated response)
- Lymphoma



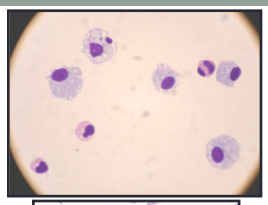
EOSINOPHILIC

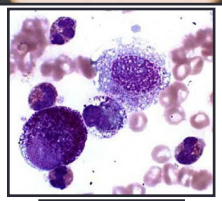




EOSINOPHILIC

- Parasitic
- Fungal
- Hypersensitivity
- Foreign body
- Eosinophilic disorders
- Neoplasia

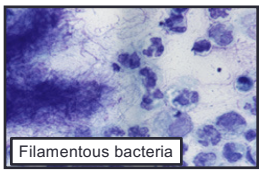




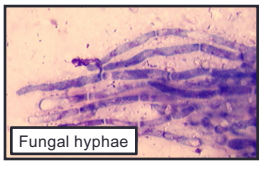
Canine MCT

INFECTIOUS AGENTS

- Bacteria
- Fungi/algae
- Viral inclusions
- Protozoa
- Parasites



Filamentous bacteria



Fungal hyphae

Neoplasia

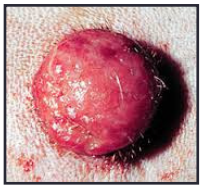
For a diagnosis, must find:

- **Atypical** cells
- Cells in an **abnormal** location
- Cells in **abnormal** proportions

Inflammatory cells + atypical cells =
USE CAUTION

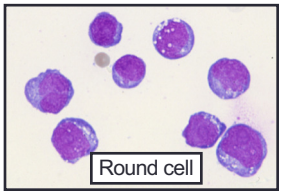
Neoplasia - Steps in Evaluation

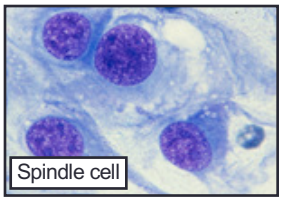
1. Determine cell type
2. Evaluate for benign vs. malignant

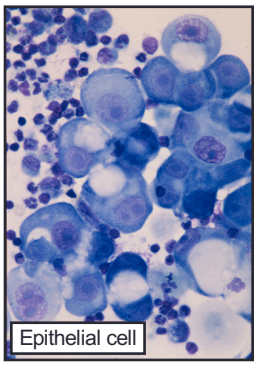


Three Tumor Types

- Round - non-adherent, round-oval
- Epithelial - display cell-cell adhesion
- Spindle (mesenchymal) cell - non-adherent, fusiform

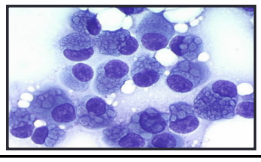


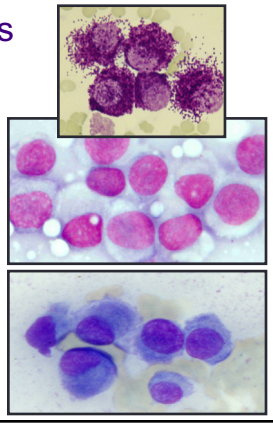




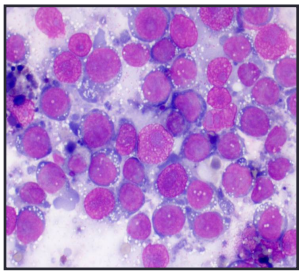
Round Cell Tumors

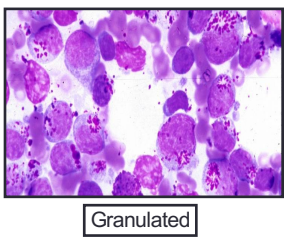
- Lymphoma
- Mast cell tumor
- Transmissible venereal tumor (TVT)
- Cutaneous histiocytoma
- Plasmacytoma





Round Cell Tumors: Lymphoma

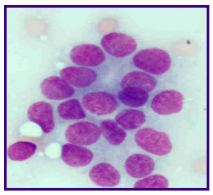


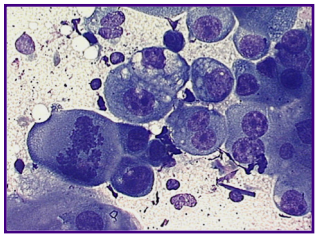


Epithelial Tumors – Generic Names

BENIGN
- Epitheliomas
- Adenomas

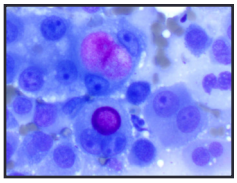
MALIGNANT
- Carcinomas
- Adenocarcinomas

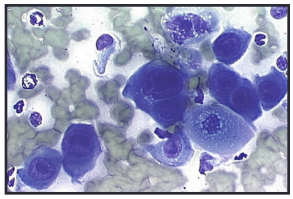


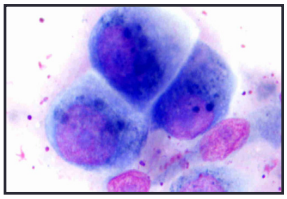


Epithelial Tumors

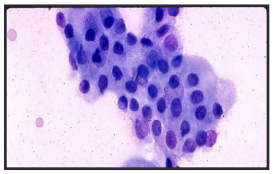
- Trichoblastoma
- Transitional cell carcinoma
- Sebaceous adenoma
- Mammary adenocarcinoma

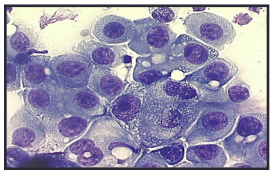


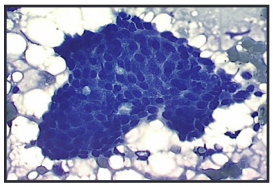


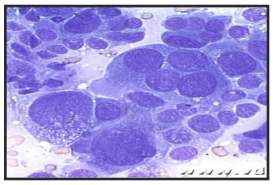


Epithelial Tumors







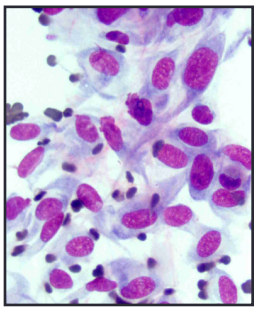


Spindle Cell Tumors

- Reactive proliferation, benign and malignant neoplasms have considerable morphologic overlap

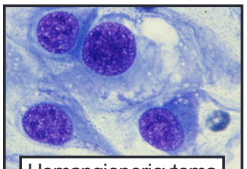
BIOPSY OFTEN NEEDED

- Benign = fibroma
- Malignant = sarcoma

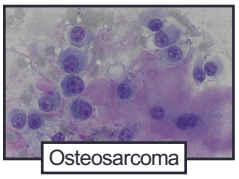


Spindle Cell Tumors

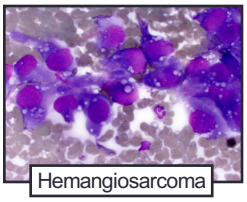
- Hemangiopericytoma
- Osteosarcoma
- Lipoma
- Chondrosarcoma
- Hemangiosarcoma



Hemangiopericytoma

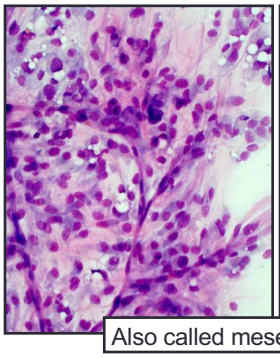


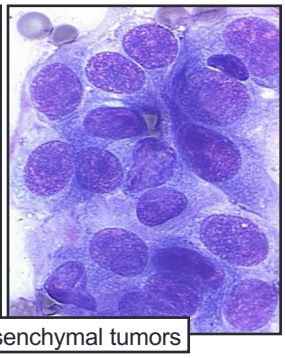
Osteosarcoma



Hemangiosarcoma

Spindle Cell Tumors

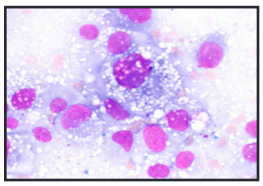




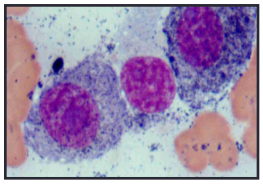
Also called mesenchymal tumors

Variable Features

Histiocytic sarcoma
Round to spindle



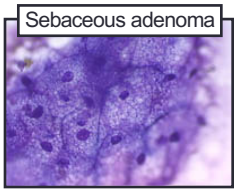
Melanoma
Round to spindle
to epithelial



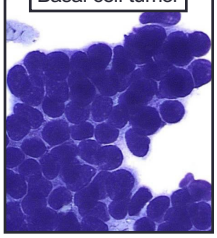
Benign Neoplasia

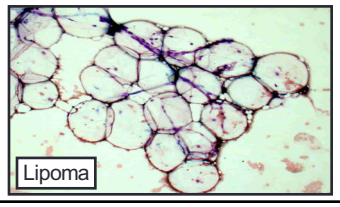
- Well-differentiated
- Uniform morphology

Sebaceous adenoma



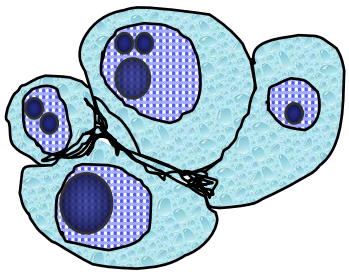
Basal cell tumor





Lipoma

Malignant Neoplasia
"Criteria of Malignancy"



Cytoplasmic Criteria of Malignancy

- Anisocytosis
- Macrocytosis
- Variable basophilia
- Atypical vacuolization
- Absence of granules in granulated cells
- Variable to high nucleus/cytoplasm ratio

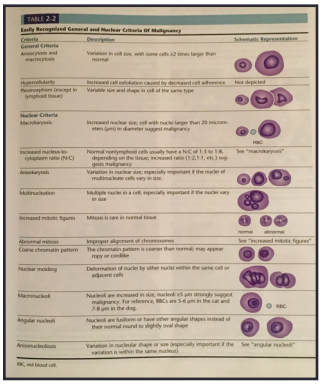


TABLE 2-2
Easily Recognized General and Nuclear Criteria Of Malignancy

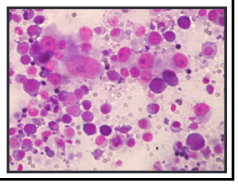
Criteria	Description	Schematic Representation
General Criteria		
Anisocytosis and macrocytosis	Variation in cell size, with some cells ≥2 times larger than normal	
Hypercellularity	Increased cell exfoliation caused by decreased cell adherence	Not depicted
Pleomorphism (except in lymphoid tissue)	Variable size and shape in cell of the same type	
Nuclear Criteria		
Macrokaryosis	Increased nuclear size; cell with nuclei larger than 20 micrometers (μm) in diameter suggest malignancy	RBC
Increased nucleus-to-cytoplasm ratio (N:C)	Normal nonlymphoid cells usually have a N:C of 1:3 to 1:8, depending on the tissue; increased ratio (1:2,1:1, etc.) suggests malignancy	See "macrokaryosis"
Anisokaryosis	Variation in nuclear size; especially important if the nuclei of multinucleate cells vary in size.	
Multinucleation	Multiple nuclei in a cell; especially important if the nuclei vary in size	
Increased mitotic figures	Mitosis is rare in normal tissue	normal abnormal
Abnormal mitosis	Improper alignment of chromosomes	See "increased mitotic figures".
Coarse chromatin pattern	The chromatin pattern is coarser than normal; may appear ropy or cordlike	
Nuclear molding	Deformation of nuclei by other nuclei within the same cell or adjacent cells	
Macronucleoli	Nucleoli are increased in size; nucleoli ≥5 μm strongly suggest malignancy. For reference, RBCs are 5-6 μm in the cat and 7-8 μm in the dog.	RBC
Angular nucleoli	Nucleoli are fusiform or have other angular shapes instead of their normal round to slightly oval shape	
Anisonucleolosis	Variation in nuclear shape or size (especially important if the variation is within the same nucleus)	See "angular nucleoli"

RBC, red blood cell.

Valenciano A, Cowell R. Diagnostic Cytology and Hematology of the Dog and Cat, 4th Ed, p. 41.

Nuclear Criteria of Malignancy

- Anisokaryosis
- Macrokaryosis with large nucleoli
- Abnormal nuclear or nucleolar shapes
- Multiple nuclei, multiple nucleoli
- High mitotic index
- Atypical mitoses



Criteria of Malignancy