Maximizing the use of your reference lab's clinical pathologist: *Help your pathologist help you!*

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Cytology: Advantages

- No special equipment is needed (just a microscope and stain solutions).
- Fast results:
  - Slides can be stained within minutes and examined immediately
- Less invasive and less costly than tissue biopsy.
- Individual cells can be examined more closely. (They are examined in an intact state, rather than sectioned.)

Cytology: Limitations

- Cannot assess tissue architecture/cell arrangement as well. Structure is largely disrupted by the collection procedure.
- Results are highly dependent on sample quality. This depends on technique, and the nature of the lesion.
- Limited number (and variable quality) of slides makes special stains & immunostaining difficult.

Cytologic Samples

- The goal of the cytology sample collection technique is to obtain a thin layer/monolayer of intact, representative cells to facilitate staining and cytologic examination.
- The type of sample collected depends on the nature/location of the lesion and the preferences of the collector.

Submission Guidelines

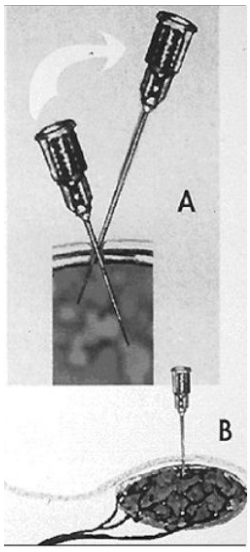
- Label slides with a pencil or permanent marker (AKA sharpie)
  - Frosted slides make this easier
- Include reasonably descriptive indication of anatomic location
- Include the relevant clinical history
- 5 slides per site is usually sufficient
- Maximum of 10-15 for bone marrow
- Please do not send more than that

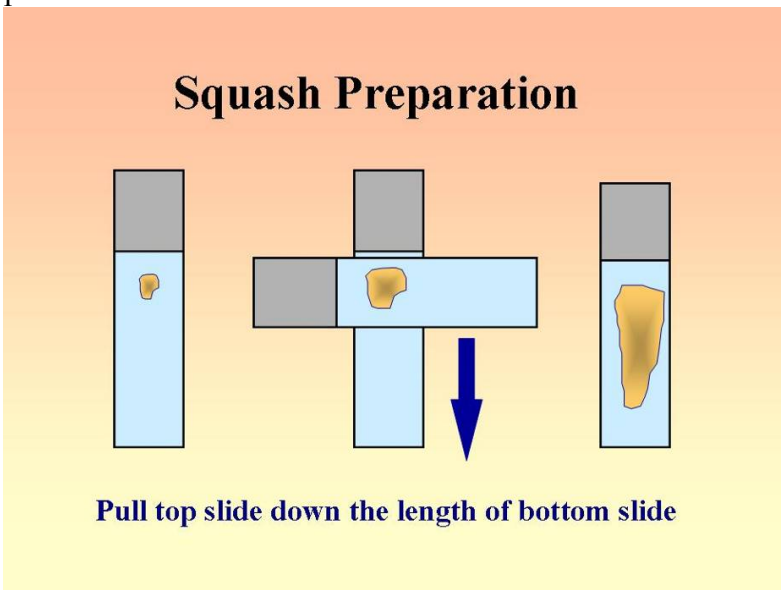
Solid Tissue Cytology-Collection Techniques

- "Tattoo" or "Pokey-pokey" technique
  - Needle with no attached syringe is packed with cells by inserting several times into lesion
  - Offers advantage of producing less hemorrhage and cell disruption
- Aspiration technique
  - Cells are aspirated using vacuum force (typically by using a syringe).
  - For solid tissue FNA, cells should remain *in the needle*. Make sure to release suction before pulling the needle out of the lesion.

- Maximizing Diagnostic Yield
  - Smear cells immediately. (If the slide is allowed to dry before smear is attempted, the cells cannot be spread, and this typically results in numerous small, thick droplets.)
  - Don't take the term "squash prep" too literally. Use only the weight of the top slide for downward pressure.

- Thickness
  - Slides that are too thick can be difficult or impossible to interpret.
  - The cells in thick areas are usually understained.
  - Morphology of individual cells cannot be effectively examined if they do not spread out on the slide.

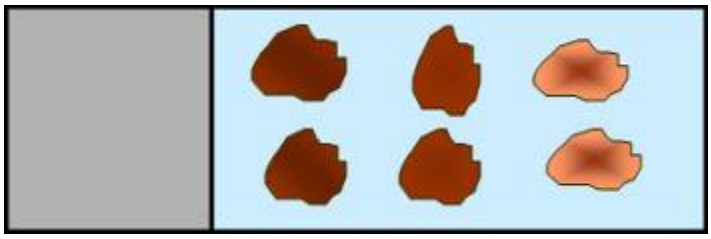




"Seaweed" Method
- Place sample on slide
- Put 2 slides together & pull apart
- Advantage
  - Can preserve cell integrity
- Disadvantage
  - Preparations are often thick
    - Difficult for optimal stain penetration
    - Cells piled up on top of each other
- Starfish method
  - Tends not to damage fragile cells,
  - Allows a thick layer of tissue fluid to remain around the cells, which may interfere with staining.
  - The tip of a needle is placed in the aspirate and moved peripherally, pulling a trail of the sample with it.

Tissue Imprint/Scrape

- AKA "impression smear." These are typically collected from a tissue biopsy sample
- Gently scraping the cut surface with a scalpel blade & spreading the cellular material on a slide can increase diagnostic yield
- ***Before the sample is placed in formalin***, blot (to remove excess blood) & then touch to a slide. Cells from the surface (the interior/cut surface should be used) adhere to the slide.
- Touch preparations or scrapings from a biopsy or excised tissue
- Impression smears of ulcerated lesions of limited value – often only see inflammation



Diff-Quik: Friend or Foe?

- Advantages
  - Rapid
  - Inexpensive
  - Good for viewing Distemper inclusions
- Disadvantages
  - Lack of consistency
  - Prone to contamination
  - Weakening/Dilution from overuse
  - No metachromatic reaction-Leaving slide in longer will not help

Getting Good Stain Results

- Most cytologists prefer to do their own stain on all samples for consistency.
- Submit unstained (air-dried) slides if possible.
- Use fresh or filtered stain to avoid contaminating microorganisms.
- Stain thicker samples for longer periods. "Light" slides can often be re-stained to darken the staining.

Lubricant/ultrasound gel

- All things in moderation
- Excessive amounts
  - Obscure visualization
  - Affect differential staining
- Try to remove excess prior to aspirating

Cystic/Fluid Filled Lesions

- Fluid is generally of low cellularity
  - Typically macrophages and lymphocytes
  - Tissue/neoplastic cells may or may not exfoliate

    - Do not perform fluid analysis
        - No reference intervals, meaningless numbers
- Improve diagnostic yield
    - Remove all fluid, place in red/white top/EDTA tube
    - Aspirate remnant "tissue" component

Sample storage

- Do not refrigerate unstained slides until they are dry
    - Moisture/condensate can lead to cell rupture
- Place fluids in refrigerator to prolong sample viability
    - Place a portion in EDTA for cytologic analysis
    - Place a portion in red/white top tube for possible culture
        - Use non additive containing tubes for fluids

Why no additive?

- Crystalline material can obscure visualization of cells particularly in low volume, low cellularity samples.

Urine cytology

- Advantages
    - Cell detail not well appreciated on wet mount preps
    - Useful to identify
    - Neoplastic cells (TCC)
    - Infectious agents (Fungi)
- Challenges
    - Cells deteriorate rapidly
    - Labile epithelium
    - Epithelial atypia, primary or secondary?
- Suspect neoplasia
    - Empty bladder to remove necrotic debris, deteriorated cells
    - Catheterize/traumatic catheterization
        - Submit fresh smears/ sediment preparations
        - Please, no sedi-stain

Formalin is BAD!!!

- For cytology, that is
    - Fumes will penetrate most any packaging
    - Results in partial fixation that interferes with staining
- Double-bag biopsy samples
- Do not even STORE near cytology samples

In house Cytology

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Solid Tissue Cytology-Collection Techniques

- "Tattoo" or "Pokey-pokey" technique
  - Needle with no attached syringe is packed with cells by inserting several times into lesion
  - Remove needle, attach pre-evacuated to syringe and express material on to slide
  - Offers advantage of producing less hemorrhage and cell disruption
- Slide preparation
  - Smear cells immediately. (If the slide is allowed to dry before smear is attempted, the cells cannot be spread, and this typically results in numerous small, thick droplets.)
  - Don't take the term "squash prep" too literally. Use only the weight of the top slide for downward pressure.
- **ALWAYS SPREAD THE CELLS!!!**
- Do not use the technique for blood smears

Getting Good Stain Results

- Use fresh or filtered stain to avoid contaminating microorganisms.
- Change stains often to avoid dilution
  - At least once/week
- Stain thicker samples for longer periods. "Light" slides can often be re-stained to darken the staining.

- Classic criteria of malignancy
  - Anisocytosis & Anisokaryosis
  - Variable/Increased N:C ratio
  - Multinucleation
  - Increased mitotic activity
  - Large, multiple nucleoli
  - Vacuolization
- Caveats
  - Changes can be seen w/ inflammation and hyperplasia
  - Macrophages & mesothelial cells can be mitotic
  - Multinucleated giant cells in granulomas
  - Rxtv fibroblasts have prominent, multiple nucleoli
- Do not always denote malignancy

Real Criteria of Malignancy

- Nuclear molding
- Karyomegaly
- Abnormal, prominent nucleoli
  - Large size
  - Abnormal shape

- Atypical mitotic figures
- Micronuclei

Lesion Classification
- Round/Discrete cell
- Mesenchymal
- Epithelial
- Inflammatory/Infectious
- Non-neoplastic, Non-inflammatory

Round/Discrete Cell
- Cells usually individualized
- Examples
    - Histiocytoma
    - Mast cell tumor
    - Lymphoma/plasmacytoma
    - Transmissible Venereal Tumor
    - +/- Melanoma

Mesenchymal
- Connective tissue origin
    - Bone, Cartilage, Muscle, Stroma, Vessels, Fat
    - +/- Melanocytes
- Often spindle shaped
- Aggregates/bundles
- Benign & Malignant variants

Epithelial Tumors
- Round to polygonal
- Clusters
    - Distinct cell borders
- Multiple origins
    - Glands
    - Solid organs
    - Mucosa

Commonly Encountered Lesions

Round cell tumors

Histiocytoma
- Discrete round cells
- Mild to moderate anisocytosis and anisokaryosis
- Lymphocytic infiltrate-Occurs with regression
- 2° inflammation
- Can persist as long as 3 months

Plasmacytoma
- Discrete round cells
- Variable morphology
- Possible perinuclear clear/Golgi zone
- Frequent binucleate & multinucleated cells
- Morphology not related to clinical behavior (dogs)

Mast Cell Tumor
- Variable numbers of fine magenta granules
- Other elements
    - Eosinophils, Fibroblasts, Collagen, PMN, Macrophages

Lymph Node:
Reactive
- Heterogeneous
    - Small lymphocytes
    - ↑ plasma cells
    - ↑ blasts
    - Mitotic figures
    - Mott cells
        - Russell bodies = packeted Ig
        - Seen in reactive/inflammatory lesions, though not specific

Lymphoma
- Homogeneous
- Blasts: +/- nucleoli
- Small # of smalls & plasma cells
- Intermediate lymphoma
    - Difficult
    - Homogeneous
        - 10-12 micron diameter ~ size of a PMN

Sialocele
- Thick background
  - "lakes" of basophilic mucin
- Vacuolated macrophages
  - Hemosiderin/hematoidin
  - Multinucleated cells

Mesenchmal tumors

Perivascular wall tumor
- Spindle shaped cells
- Often vacuolated
- Variably sized aggregates
- Mild anisocytosis and anisokaryosis
- Multinucleated cells-crown cells

Adipose tissue/Lipoma
- Cannot differentiate b/w the two on cyto
- Slides appear greasy
- Other elements seen
  - Vessels, collagen, macrophages
- Different variants
  - Angio-,fibro-, myxolipoma
- Liposarcomas = rare
- Inflamed lipoma/chronic steatitis
  - Increased numbers of macrophages & PMNs

Epithelial tumors

Trichoblastoma (formerly basal cell tumor)
- Benign neoplasm of follicular germ cell origin
- Occur most frequently on the head and neck
- Cuboidal cells, tightly cohesive clusters, minimal pleomorphism

Sebaceous Lesions
- Adenoma, hamartoma, hyperplasia
  - Cannot differentiate based on cytology
  - All are benign
  - May see basal reserve cells
- Sebaceous carcinomas are relatively uncommon
- Epitheliomas can behave more aggressively

Perianal Masses
- Perianal/Hepatoid tumor
  - Modified sebaceous
  - Cells resemble hepatocytes

        - Prominent nucleoli
    - Basal reserve cells
    - Most are benign
        - Carcinomas are rare
            - Variably pleomorphic
    - Prepuce, tail, thigh

- Apocrine anal gland carcinoma
    - Mild atypia
    - Many free nuclei
    - Indistinct cell borders

Body Cavity Fluids

- Reactive mesothelial cells
    - Moderate to marked aniso
    - Prominent nucleoli
    - Bi/multinucleation
    - Present in clusters
    - ↑ ↑ pleomorphism in pericardial & hemorrhagic effusions

- Suppurative inflammation
    - Degenerate and nondegenerate neutrophils
        - Degenerate = Swollen, pale staining nuclei
    - Septic = intracellular bacteria
    - Absence of visible organisms does not preclude infection
    - Always culture!

Non-neoplastic, Non-inflammatory

- Cysts
    - Follicular-Can be inflamed
    - Apocrine-Usually fluid filled
    - Sebaceous-Actually uncommon in domestic animals
- Hyperplasia
- Hamartoma

Summary

- All criteria of malignancy are not created equal
- No single criterion denotes malignancy
- Evaluate cytologic findings in the context of the clinical presentation
- Utilize/consult your pathologist