Getting the most out of your FNA cytology samples

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Obtaining a diagnostic quality sample

Supplies needed for fine needle aspiration include clean glass slides, a 6-12 cc syringe, and a 22 gauge needle. To obtain a cytologic sample, the tip of the needle is inserted into the tissue of interest and redirected several times. Aspiration may be performed with or without the use of suction by retracting the plunger of the attached syringe. Suction may increase cellular yield but may also increase the amount of blood contamination and cause cytolysis (cellular rupture).

To prepare slides for cytologic evaluation, the syringe is detached from the needle, filled with air by fully retracting the plunger, and then reattached to the needle. Pushing quickly on the plunger to expel air will allow cellular material within the hub of the needle to be expelled onto the surface of a glass slide. A second slide is used to gently spread the cellular material across the sample slide without exerting downward pressure. Multiple slides can be prepared from the same sample by "blotting" a small amount of cellular material from the original slide and gently spreading across a clean slide; this process is then repeated until minimal cellular material remains on the original slide.

Screening samples in-clinic

When multiple slides are prepared, screening one or more slides in-clinic may confirm sample quality and provide a preliminary interpretation. If submission to a reference laboratory is planned, sending at least one unstained slide is preferred. Diff-Quik or other rapid commercial Romanowsky stains can be used in-clinic staining. Multi-use stain jars should be regularly maintained. An adequately stained sample will have pink-red coloration of erythrocytes and deep purple staining of cell nuclei. Staining technique and contact time may need to be adjusted based on sample thickness and cellularity.

When dry, the sample is first scanned at low-power (10x objective) to characterize the sample cellularity. A highly cellular sample will have many nucleated cells identifiable at low-power and is more likely to be diagnostically rewarding. The presence of intact nucleated cellularity should be confirmed at mid- or high-power (40-50x or 100x objective). If many ruptured cells are present, resampling without the use of suction followed by gentle spreading of cellular material may be indicated. The degree of blood contamination can also be evaluated during initial slide screening.

Inflammatory cells include neutrophils, macrophages, lymphocytes, plasma cells, and eosinophils. The presence of these cells should be correlated with the degree of peripheral blood contamination to differentiate true inflammation. Neutrophils should be evaluated for karyolysis ("degenerate neutrophils"), and if present, thorough high-power evaluation for bacterial organisms is indicated. Fungal disease typically results in a pyogranulomatous inflammatory response, which can be identified by the presence of multinucleated giant cell macrophages or epithelioid sheets of macrophages. Eosinophilic inflammatory responses are often associated with parasitic disease, hypersensitivity/allergic reactions, or paraneoplastic processes.

Tissue cells include epithelial, mesenchymal, and round cell populations. Epithelial tissue typically exfoliates in cohesive clusters or sheets with distinct intercellular junctions. Mesenchymal cells appear individualized to loosely aggregated with indistinct cellular margins. These cells are often tapered or spindloid in shape and may exfoliate poorly with fine needle aspiration resulting in a low cellularity

sample. Round cell populations also tend to be individualized to aggregated but are round in shape with discrete cellular margins. Round cell tumors tend to be highly cellular samples, with examples including mast cell tumors, lymphoma, plasma cell tumors, and histiocytomas. Tissue cell populations may be evaluated for cytologic criteria of malignancy, including anisocytosis, anisokaryosis, multinucleation, and bizarre mitotic figures, among others. If the clinical and cytologic impressions support neoplastic disease, submission of the slides to a reference laboratory is recommended for confirmation.

Best practices for sample submission

Label slides clearly with site of lesion and patient's name or ID number. Placing samples ~1 inch from one end of the slide and spreading across the long edge of the slide is preferred, resulting in samples being centrally located on the slide. Many reference laboratories use automated slide stainers which do not stain the far edges of the slide. Heat fixation is not necessary for samples other than ear swabs and may induce artifact in FNA cytology samples. Protect slides from moisture and formalin when shipping. If unstained cytology slides and formalin-fixed biopsy samples are being submitted concurrently, separate packaging is recommended. Package slides securely to prevent breakage during transit; durable plastic slide holders are preferred to cardboard slide booklets. Submission paperwork should be completed with as much detail as possible, including the patient signalment, specific location and description of the lesion or tissue sampled, and pertinent clinical history.

Suggested reading

Barger AM & MacNeill AL. 2016. Small Animal Cytologic Diagnosis. Boca Raton, FL: CRC press.

Raskin R & Meyer D. 2015. Canine and Feline Cytology. Philadelphia: Elsevier-Saunders.