ZRL Cytopathology Sample Collection Best Practices

Cytopathology Air-fixed Slides – Sample Collection & Handling – Best Practices

- **Use new, clean glass slides with frosted edge.**
- **Ensure slide labeled clearly with a pencil on the frosted edge with the patient name and site sampled with a pencil.**

» **Ink labeling can wash off during staining.**

» **Do not** label the slide box only.

- **Collect your sample using appropriate method for the lesion type.**

» **Fine needle aspiration (FNA):** FNA is the most common method used for sampling mass lesions and organs. We recommend use of a 22 ga needle that is directed rapidly in and out of multiple planes of the mass lesion in a "woodpecker-like" motion to place cells within the hub of your needle.

» Alternatively, or in addition to this method, you can attach your aspiration needle to a syringe, then, while directing the needle in and out of multiple tissue planes within the mass, apply ***gentle*** suction by pulling back on the syringe plunger. This latter technique may be useful for some less exfoliative lesions, like soft tissue sarcomas.

» Once sampling is complete, remove the needle from the syringe (if using) and pull back on the plunger of a clean, 3 mL syringe to fill it approximately 2/3 with air, then attach your sampling needle. Direct the opening of the needle, bevel down, toward the slide, gently and rapidly expel the air contents of the needle with the syringe (you will have to remove and reattach the syringe for each expulsion to avoid aspirating the sample from the needle into the syringe).

» Immediately spread the expressed sample by placing another new, clean glass slide on top of the sample. Let the weight of the new glass slide disperse the sample. Do not add extra pressure. Then in a swift motion, pull the spreader slide along the entire length of the bottom slide until it has naturally run off the sample side.

- **Do not** pull the spreader slide up prematurely as this will rupture cells.
- **Do not** add extra pressure (this will rupture cells) - let the glass do the work for you.

» **Swabs:** This method may be used for sampling exudates (for example, nasal, vaginal, preputial, fistulous tracts) and ear canals. If the area to be sampled is not moist, gently moisten the swab with sterile saline prior to sampling. This helps to minimize cell rupture. Following sampling, roll the swab onto a clean slide in a slow, continuous and gentle fashion. Additional downward force should **not** be applied as this can cause cellular rupture. Other movements (fanning of the swab) may also lead to cellular rupture and are not recommended.

- **Scraping:** This method may be used for lesions that are crusted or ulcerated. The back or blunt end of a scalpel blade is used to gently scrape across the top of a lesion or tissue until a small amount of material is collected. The sample is then gently spread across the slide.
- **Imprints:** Touch imprints can be made from ulcerated or crusted lesions, or from surgical biopsies. Dried crusts should be removed prior to making imprints, as cells within crusts are often not representative of the lesion, are poorly preserved, and do not stain well. Gently blot the tissue to be imprinted with a paper towel to remove surface blood or fluid. Gently press the tissue several times onto multiple areas of a clean glass slide. Please note that imprints from ulcerated lesions often demonstrate surface inflammation without evidence of infectious agent involvement or a neoplastic cell population, even when these are present. Fine-needle aspiration or biopsy is recommended to more fully assess such lesions for identification of the primary lesion.

- **As mentioned under FNA, prepare your sample so that there is a single layer of well-preserved and intact cells for cytopathological evaluation. Avoid spraying material onto a slide without spreading the sample, as this often produces thick cellular areas that hinder microscopic evaluation. The following preparation recommendations apply to samples taken as fine-needle aspirates.**

- "**Squash**" or compression preparation: This technique is most commonly used to spread FNA samples expelled onto glass slides and can be used for semi-solid, mucus-like, or pelleted material. A common error is the use of excessive amounts of material, so that samples are too thick for cytopathological evaluation. Sample to be spread should be placed near the frosted/labeled end of the slide. A second slide is aligned perpendicular to the first and rests gently on the first slide. The second slide is gently and smoothly moved across the first slide, keeping both slides in the same horizontal plane, which spreads the material evenly and creates two smears that can then be used for cytopathological evaluation.

  Note: The weight of the slides together is generally adequate for sample spreading. Further downward force is NOT recommended, as this typically results in large numbers of ruptured cells in the sample.

  Note: Do not lift the top spreader slide up vertically while spreading the sample as this will also rupture cells.

- "**Blood smear**" preparation: Some aspirates contain blood or fluid components and may benefit from this preparation method. Material is expressed near the frosted/labeled end of a slide. The short edge of the spreader slide is placed in front of the sample at an approximately 45-degree angle. The spreader slide is then pulled backward into the material, and once the fluid has spread along the width of the spreader slide (via capillary action), it is smoothly and quickly slid forward along the length of the bottom slide. This creates a classic "feathered" edge appearance. The speed at which the slide is moved depends on the viscosity of the sample; generally, the thinner the sample, the faster the spreader slide will need to be moved to distribute the sample contents evenly.
- "**Line smear**" preparation: This type of preparation is most commonly used for urine (or other low cellularity fluids) when concentration of cells is desired for easier examination. Material is expressed near the frosted/labeled end of a slide. The short edge of the spreader slide is placed in front of the sample at an approximately 45-degree angle. The spreader slide is then pulled backward into the material, and once the fluid component of the sample has spread along the width of the spreader slide, it is smoothly and quickly slid forward. In contrast to the blood smear technique, the spreader slide is then abruptly stopped and lifted up vertically and off of the bottom slide prior to formation of a feathered edge. This results in a high concentration of cells at the edge of the smear in a line, rather than throughout the feathered edge of the smear.
- "**Starfish**" preparation: **This technique is highly undesirable for any sample type and is not recommended.** This preparation is made when sample material is placed in the center of a glass slide and a needle is used to drag/tease the material circumferentially in multiple directions, creating a florid or starfish-like pattern. This technique results in a high degree of cellular rupture and contains many regions that are too thick to evaluate. **Do not use this technique.**
- **Allow slides to completely dry prior to placing them in a slide box. Rapid drying of wet preparations (by waving slides in the air or using a hairdryer on a low, cool setting) can reduce drying artefact and improve cellular preservation. Slides are considered "air-fixed" once they are fully dry.**
- Slides do not require any other fixation (e.g., heat, alcohol) prior to shipping.

- **Unstained smears are preferred, but it is recommended to stain at least one smear in-clinic to assess the cellularity of your sample prior to submission.**
  - » If you do pre-stain slides for in-house evaluation, please submit these slides along with the unstained smears. In some cases, pre-stained slides are the most diagnostic samples from a lesion.
- **Submit slides in rigid, plastic slide holders, taped shut to keep slides from slipping out and breaking during transit, in an appropriate shipping container. Cardboard slide holders do not adequately protect slides, and often break during shipping.**
  - » **Do not ship slides in the same package as formalin -containing specimens (biopsy jars or collection bags)**
    - Formalin may leak during shipment, bathing cytopathology slides in formalin, which renders the slides useless for cytopathology.
    - Even simple exposure to formalin fumes can render cytopathology samples useless, as it prevents the sample from being able to take up stain.

Fluid Samples – Best Practices

- **Any fluid sample intended for cytopathology, regardless of its origin (effusions, washes, fluid from masses), can be handled the same way.**
- **All samples intended for cytopathological evaluation must be submitted in an EDTA tube.**
  - » This allows for the best cell preservation with the highest likelihood of a diagnostic cytopathology sample arriving at the lab.
  - » Please note, however, that **EDTA is bacteriostatic!** Therefore samples submitted in EDTA tubes are **not** appropriate for culture.
- **If a bacterial culture of the fluid may be needed, submit at least 1 mL of fluid in a sterile, no additive tube (no-additive RTT or WTT).**
  - » **Please Note:** Any liquid submitted in a sterile, no-additive tube will not be suitable for cytopathological evaluation by the time it arrives at the laboratory. The cells will degrade and an inconclusive or non-diagnostic cytopathology report is likely.
- **Store all tubes containing fluid samples for cytopathology in the refrigerator until packaged for shipment/courier pick up.**
  - » When shipped, fluid cytopathology samples should be packed with a cold pack (wrapped in paper towels/ packaging paper), but never frozen or placed directly in contact with an ice pack.
- **When submitting fluid samples for cytopathology, odds of obtaining a diagnostic sample are greatly increased when slides are prepared at the time of sample collection and submitted along with the tubes of fluid.**
  - » If possible, submit two direct smears along with fluid samples.
  - » Prepare using the "blood smear" or "line smear" technique (described above).
  - » If a smear is prepared from a concentrated or sediment specimen, **be sure to label the slide as a concentrated or sediment preparation**.
    - If there are any aggregates of flocculent matter within the fluid, individual "squash" preparations of this material can be made.
  - » For in-clinic made smears from fluid samples, follow packing and shipping instructions as outlined above for routine air-fixed cytopathology slides.
    - **Do not** store in-clinic made smears from fluid samples with the fluid sample in the refrigerator. Storage of blood smear and cytopathology specimens in the refrigerator may destroy the sample due to humidity artefact.
    - Be sure to allow slides to completely dry prior to placing them in a slide box. Rapid drying of the slides (by waving slides in the air or using a hairdryer on a low, cool setting) can reduce drying artefact and improve cellular preservation.

      This is especially important with cytopathology smears made from fluid samples.

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