INSIGHTS IN CLINICAL PATHOLOGY

Obtaining a Sample for Cytology Using Fine Needle Biopsy

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Cytology is a valuable diagnostic tool. It is cost-effective, minimally invasive, and relatively easy to perform in-house, and slides can be forwarded to a veterinary pathologist for review.[1-4] Top reasons to perform cytology include investigation of cutaneous and subcutaneous lesions (e.g., masses, enlarged peripheral lymph nodes) and other easily accessible areas of interest, such as ultrasound-guided percutaneous sampling of visceral organs/structures.[5-9] This article details steps and tips for obtaining a cytology sample from cutaneous and subcutaneous masses and lymph nodes using a fine needle biopsy (FNB) procedure.

STEP 1: LESION AND SAMPLING PREPARATION

Supplies

First and foremost, it is important to gather supplies before attempting to sample a lesion for cytology. Being organized and prepared can help prevent sampling issues such as clotting or drying before it is fully processed.[10] Prior preparation of a well-stocked cytology kit or station for easy access during busy clinic days is beneficial. Supplies often used for cytology include:

1. **Microscope glass slides:** These should be fresh, clean, and not previously used. Slides with frosted edges are preferred for proper labeling.

Abstract

Cytology is a cost-effective, minimally invasive, relatively easy, and valuable diagnostic tool that veterinarians can use in general practice. It is often performed to investigate cutaneous and subcutaneous lesions (e.g., masses, lymph nodes) and frequently involves a needle biopsy procedure. This article reviews methods and techniques to obtain a fine needle biopsy (FNB) sample for cytology. FNB samples can be collected with a needle alone or a needle attached to a syringe with applied negative pressure (suction/aspiration). Ultimately, the sample is gathered in the needle and slides can be prepared by expelling the sample from the needle using a syringe or by using a wooden applicator to create a "roll" preparation. Minimal time from collection to preparation and gentle handling of the sample are crucial to generating representative slides for cytology. Attempting several methods/techniques to obtain and prepare a sample for cytology can maximize the likelihood of obtaining a diagnostic sample.

Take-Home Points

- Fine needle biopsy (FNB) for cytology is a relatively easy and minimally invasive tool to help diagnose cutaneous and subcutaneous lesions such as masses and lymph nodes.
- There are several different ways to collect and prepare an FNB sample for cytologic evaluation.
- If a lesion appears poorly exfoliative with a needle-only technique, try an aspiration/suction technique by attaching a syringe and applying negative pressure, but be sure to release the pressure before removing the needle from the lesion.
- When making cytology slides, it is crucial that the sample be gently and thinly dispersed across 1 or more slides to help minimize sample lysing and clumping, which can preclude interpretation.
- It is recommended to use different sample collection and slide preparation methods to maximize the chances of generating a diagnostic slide.
- Keep a few slides unstained in case you submit the sample for cytologic examination by a pathologist.
- If submitting slides to a pathologist, send any previously evaluated and stained slides, as they may contain more or different cytologic findings, especially if they were collected with different needles/methods, and always provide a relevant history and lesion description.
- Never store or mail cytology slides with formalin, as the fumes can severely affect staining, precluding interpretation.

2. **Sterile needles:** Typically 1 to 1.5 inch, 22 to 25 gauge; a larger-gauge needle (18 to 20 gauge) can be used for poorly exfoliative lesions but will likely cause more blood contamination.[5,10] It is imperative to review and use best practices for handling needles during the entirety of this procedure.[11]
3. **Syringes:** 6 to 12 mL
4. **Pencil:** To label the slides. Pens or markers should be avoided as they wash off during staining.
5. **Wooden applicator (optional):** Used in the "roll" slide preparation method

Lesion Preparation

Preparation of the lesion of interest can be similar to venipuncture and can include cleaning it with alcohol and clipping the hair in that region for easier access. If there is concern the lesion is infected and samples may be collected for culture, a more sterile preparation procedure is recommended.

STEP 2: SAMPLE COLLECTION

Before inserting the needle, palpate the lesion of interest, typically with the nondominant hand. During this step, try to isolate the lesion using the thumb and fingers. Keeping these digits to the sides of the lesion, use the dominant hand to insert the needle into the lesion. Depending on the nature of the lesion, 2 different collection methods can be applied to try to obtain a cellular sample (**FIGURE 1**):

1. **Nonaspiration:** Fenestration (also known as needle-only or woodpecker) technique
2. **Aspiration:** Suction technique (with or without fenestration)

FIGURE 1. (A) Nonaspiration (needle-only) and **(B)** aspiration (suction) sample collection techniques.

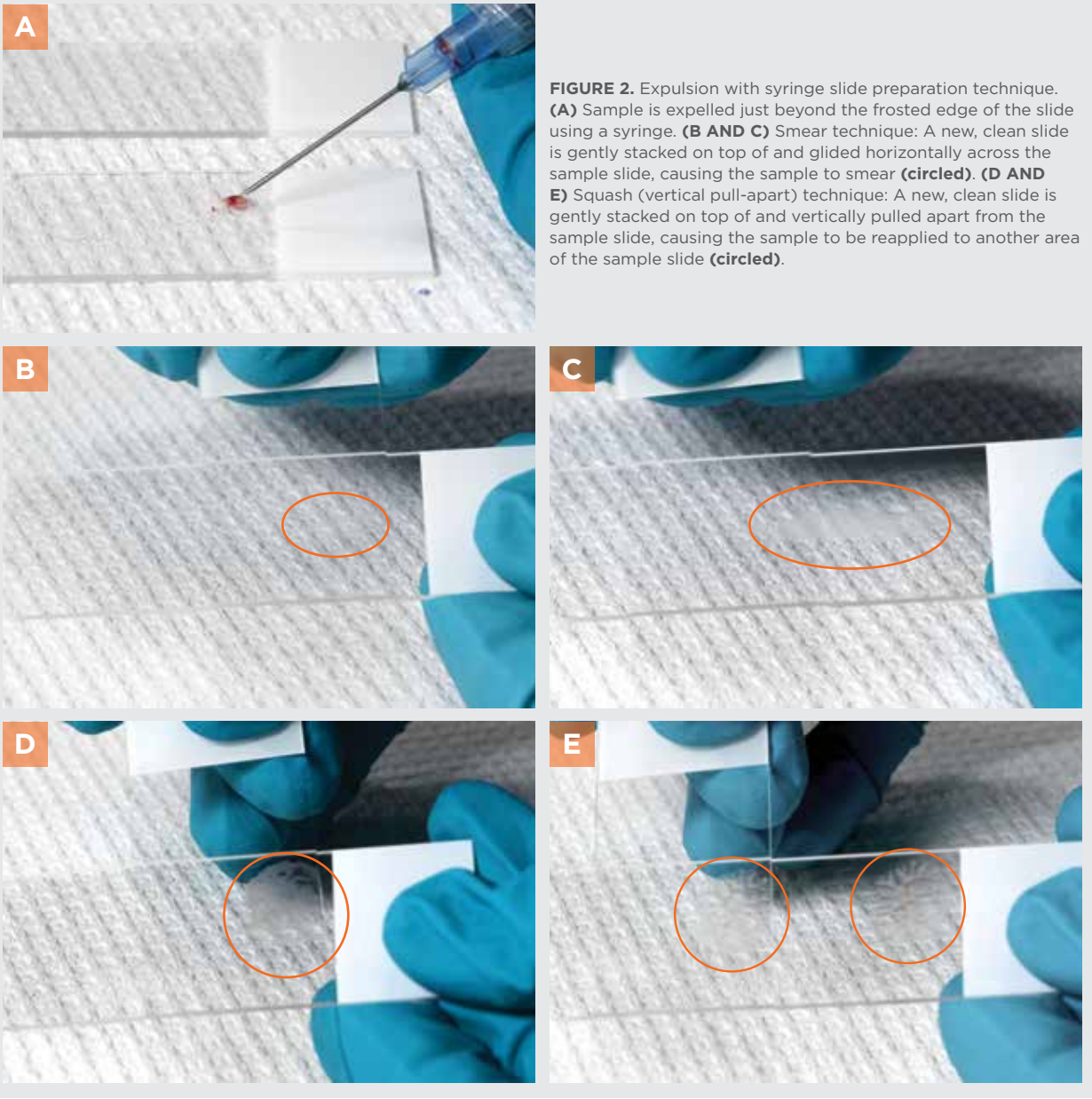

FIGURE 2. Expulsion with syringe slide preparation technique. **(A)** Sample is expelled just beyond the frosted edge of the slide using a syringe. **(B AND C)** Smear technique: A new, clean slide is gently stacked on top of and glided horizontally across the sample slide, causing the sample to smear **(circled)**. **(D AND E)** Squash (vertical pull-apart) technique: A new, clean slide is gently stacked on top of and vertically pulled apart from the sample slide, causing the sample to be reapplied to another area of the sample slide **(circled)**.

Nonaspiration Technique

This method is frequently used first to obtain a sample. As the name implies, this method does not involve aspiration or suction. It simply uses capillary action and redirection of the needle to collect the sample. After the needle is inserted, redirect the needle to different areas of the lesion without removing the needle completely (the bevel should remain in the lesion). This can be achieved by changing the angle/direction of the needle several times. For very large lesions, sampling multiple areas of the lesion, particularly areas away from the center, is recommended as the center could be necrotic. This can be achieved by using separate needles and collection attempts for different parts of the lesion, which can be noted on the slide and in submission information for the pathologist.

Tip: Monitor the needle hub as you obtain the sample. If the hub fills up quickly, it is possible you have nicked a vessel, have a cystic or fluid-containing lesion, or have a lesion that is excellently exfoliative and the FNB procedure may need to be completed sooner so the sample can be immediately prepared on a slide. If you wait too long to prepare a slide after FNB, the sample can dry or clot within the needle and will be difficult to expel for microscopic review.[10,12]

Aspiration Technique

This method can be used for poorly exfoliative or more firm lesions and is the preferred method for flow cytometry (an ancillary diagnostic test that is used for immunophenotyping lymphoma and leukemia).[13] For this method, a syringe is attached to the needle before the needle is inserted into the lesion; be sure to break the seal of the syringe to facilitate aspiration, but rezero the syringe before inserting the needle into the lesion.

Once the needle is seated in the lesion, pull back on the plunger to generate negative pressure (around 0.5 to 2 mL). After suction is obtained, either:

1. Release the negative pressure, redirect the needle, and reapply negative pressure, or
2. Hold the negative pressure and redirect the needle as in the previously described nonaspiration technique.

While suctioning, there should be resistance on the syringe plunger. If there is no resistance, then there is likely air in the system, which could mean that the needle is no longer in the lesion or the needle is not appropriately sealed on the syringe.

As with the nonaspiration method, monitor the needle hub and syringe barrel for sample material during the procedure. Most importantly, be sure to release the negative pressure *prior* to removing the needle from the lesion to prevent the sample from being aspirated into the syringe barrel; the idea is to keep the sample within the needle shaft/hub.

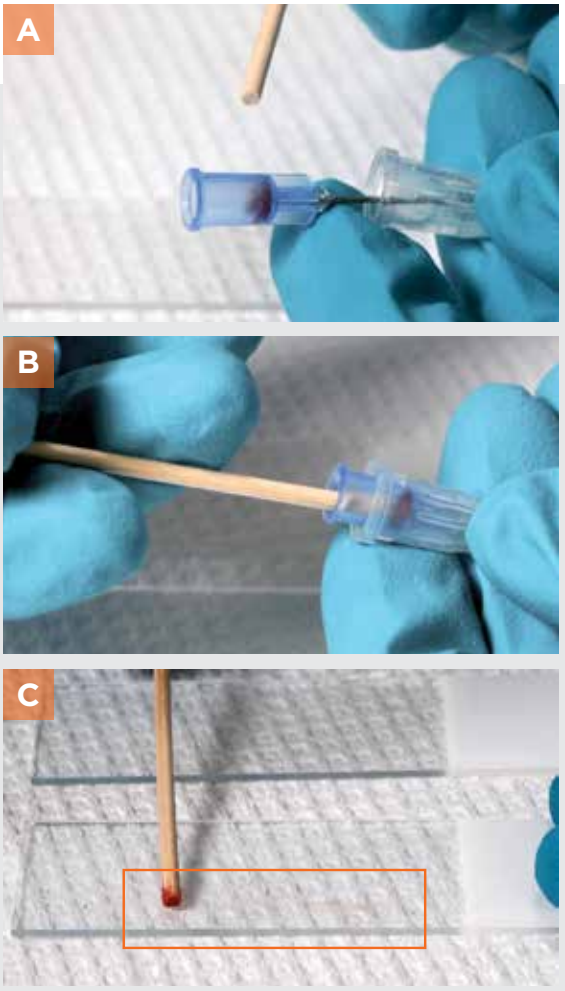

FIGURE 3. Roll preparation technique. **(A)** Sample is collected in the hub of the needle during the needle biopsy procedure. **(B)** The blunt end of a wooden applicator is gently swirled in the hub. **(C)** The wooden applicator is gently rolled across a new, clean slide in linear arrangements **(outlined)**.

STEP 3: SLIDE PREPARATION

Slide preparation should be performed *immediately* after needle sampling. As with sample collection, multiple methods can be used to ensure the sample is adequately preserved and presented for cytologic review. The following cytology slide preparation methods can be used for FNB samples:

1. Expulsion (using a syringe)
    - **b.** Smear
    - **c.** Squash (also known as pull-apart)
    - **d.** Starfish
2. Roll preparation (using a wooden applicator)

Expulsion With Syringe

The most common method of preparing an FNB sample for cytology is expulsion with a syringe (**FIGURE 2**). With this method, a clean, fresh syringe is prefilled with air (about 3 to 6 mL), then the needle containing the sample is attached to the syringe.

Tip: If the suction method was used to obtain the sample, the sampling needle must be removed from the syringe (recap it using the one-hand scoop method or forceps).[11] The syringe is then filled with air and reattached to the sampling needle for expulsion. If the syringe is filled with air without removing the sampling needle, the sample could get sucked into the barrel of the syringe, where it is extremely hard to retrieve.

Next, angle the needle bevel side down close to the proximal edge (if a frosted edge is present, aim for slightly beyond it) of a clean microscope slide. Then, quickly depress the syringe to propel the sample toward

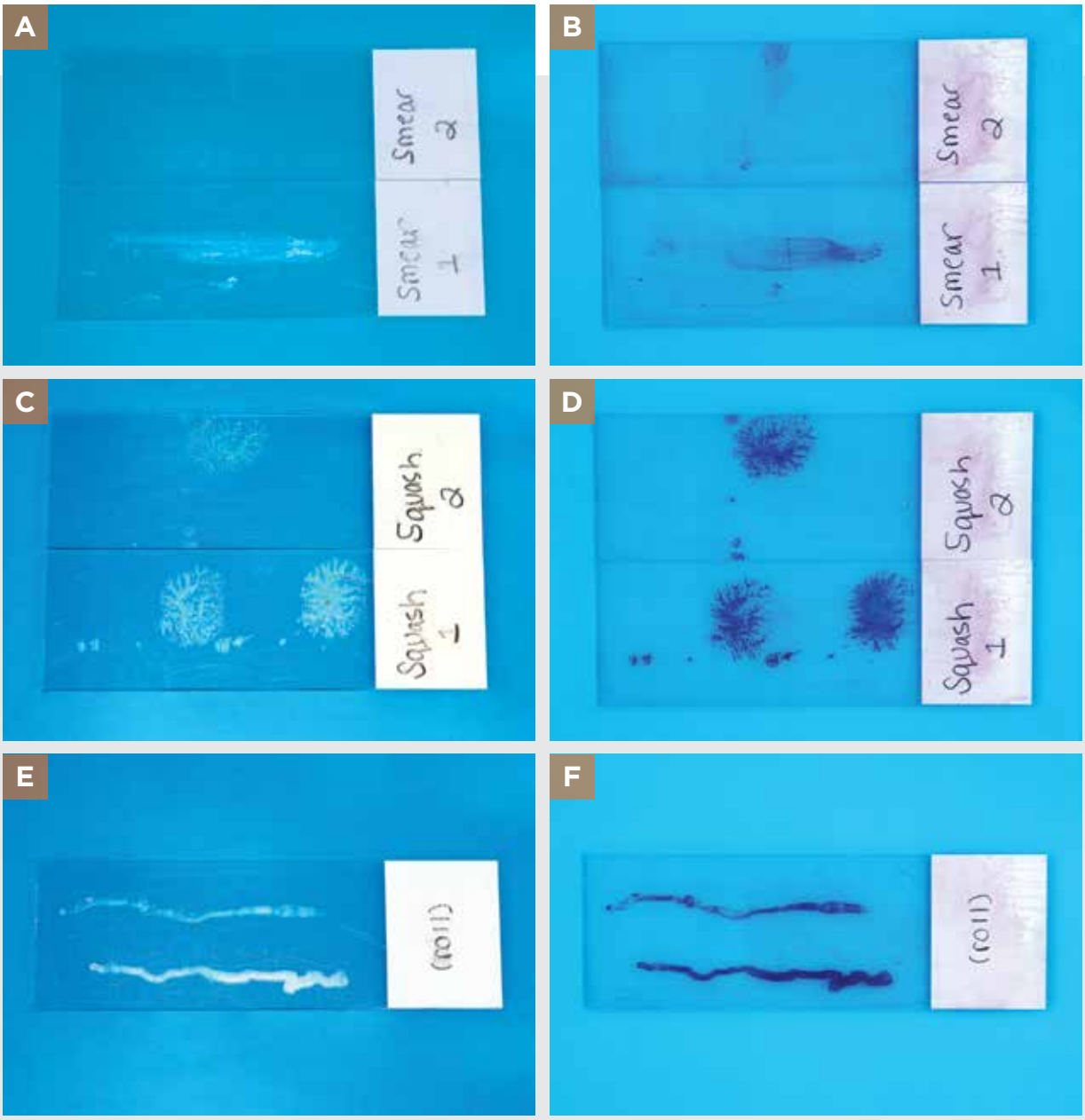

FIGURE 4. Slide processing steps. **(A, C, E)** Slides are labeled with preparation information in pencil and allowed to dry. **(B, D, F)** Labeled slides after staining.

the slide. Most of the sample should remain in the proximal region of the sample slide (**FIGURE 2A**). If the sample is very bloody and/or the hub of the sampling needle contains a lot of material, it can be more gently expulsed and distributed across several slides.

The expelled sample must be dispersed to ensure that it is thin enough to evaluate individual cell detail and mitigate cellular crowding. This is a crucial step and must be done straightaway to prevent sample drying before dispersion. As mentioned above, the smear, squash (pull-apart), and starfish techniques are all options to disperse the sample.

Smear

This is the most common and often most effective technique. A new fresh, clean microscope slide is oriented perpendicular to and gently stacked on top of the slide with the sample on it (**FIGURE 2B AND 2C**). Then, the top slide is gently glided across the length of the sample slide.

Tip: Do not apply too much downward pressure as this can rupture cells and could preclude cytologic interpretation.

Squash

In the squash or pull-apart technique, a new fresh, clean microscope slide is gently stacked on top of the sample on the sample slide; then the slides are pulled apart vertically (**FIGURE 2D AND 2E**). The sample on the pull-apart slide can then be gently applied to an empty portion of the sample slide or another fresh, clean slide to continue to disperse the sample. With this method, mirror-image preparations will be generated on the sample and squash slides.

Starfish

The starfish method uses the needle to disperse the expelled sample across the slide in different directions.[10,12] This method seems to be infrequently used for cytologic preparations in the author's opinion, and it may not disperse the sample as effectively as other methods described.

Roll

In the author's experience, some oncologists find a "roll" preparation method useful, particularly for lymph nodes, as it can mitigate cell lysing that can occur with some of the previously described techniques. With this method, after sample collection, the sampling needle is recapped (one-hand scoop method or forceps),[11] and the blunt end of a wooden applicator is gently swirled within the hub of the needle. Subsequently, the swirled end of the wooden applicator is gently rolled across a fresh, clean microscope slide to form lines (**FIGURE 3**). A few lines can be made from the wooden applicator sample on 1 or multiple slides depending on how much sample is present and how thick the preparations are.

STEP 4: SLIDE PROCESSING

After slides are prepared, they should be labeled with appropriate patient and lesion information with a pencil (**FIGURE 4**). Slides must be allowed to dry before staining and shipping and should be stored at room temperature. Some samples (e.g., thick, bloody, oily) may require longer drying times; a small, low-speed fan can sometimes be used to facilitate drying. Heat fixation is not generally recommended or needed.[14]

Once dry, slides can be stained with an in-house rapid stain such as Diff-Quik. After staining, they are assessed for diagnostic yield—cellularity and presence of intact cells—and the cells can be characterized. A few slides should be left unstained in case they are to be sent to a veterinary pathologist for review.[4] This is because reference/diagnostic laboratories typically have a stain that can highlight certain cells (e.g., mast cells) better than rapid stains.[6,8,10,12] Assessing the sample for cellularity and cell preservation before submission to a pathologist can help avoid a nondiagnostic interpretation.

When submitting slides to a pathologist, be sure to include the in-house stained slides as well, as they may contain more or different cytologic findings, especially if they were collected with different needles/methods. Additionally, whenever sending a sample for pathologist review, always include a relevant history and lesion description; this helps with generating differential diagnoses for the cytologic findings in the context of the patient. Never store or mail cytology slides with formalin, as the fumes can severely affect staining, which precludes interpretation.[4,6,10]

SUMMARY

Cytology is a relatively easy and useful diagnostic tool that can be used in everyday practice. FNB samples can be collected using aspiration and nonaspiration techniques. Slides can be prepared by needle expulsion with a syringe or using a wooden applicator in a "roll" preparation. It is recommended to use different methods/techniques to maximize the chances of obtaining a diagnostic sample. Remember that minimal time from collection to preparation and gentle handling of the sample are crucial in generating representative slides for cytologic evaluation. TVP

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