# Preparation of Cytology Samples: Tricks of the Trade



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#### **KEYWORDS**

• Digital image • Cytology • Sample preparation • Romanowsky stain

#### **KEY POINTS**

- Cytology has many advantages; it is relatively quick, minimally invasive, and provides clinically useful results.
- To get ideal results, cytology does require some investment in time to acquire the skills to collect and evaluate the sample.
- Beyond reading and learning about how to perform these techniques, the next step is to
  practice and experiment with those techniques until the skills can be reliably used in the
  clinical setting.

#### TECHNIQUES FOR CYTOLOGIC PREPARATION

The quality of every cytologic evaluation is directly impacted by the quality of sample selection, collection, and preparation. Adherence to basic principles can significantly improve the usefulness of this simple and minimally invasive diagnostic test. Application of the principles covered in this article will help to improve the quality of cytologic preparations.

#### **SAMPLE COLLECTION**

#### Equipment

Minimal equipment is needed to collect and prepare cytologic samples. Additional information on staining and microscopic evaluation are discussed in following sections.

#### Needles

A 21- to 27-gauge needle is used for most settings. Generally, studies have found that needle size did not significantly affect cytologic adequacy, though admittedly, only needles ranging between 21 and 27 gauge were used. Larger gauge needles may be helpful in poorly exfoliative sites, such as bone and suspected mesenchymal

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tumors. Needle length depends on the depth of the lesion being evaluated; a 1- to 1.5-inch needle usually is sufficient.

#### Syringe

A 6-mL syringe will generally suffice for those techniques that require aspiration. Too large a syringe will apply too much pressor or be ungainly in the hand, whereas small syringes may not apply sufficient aspiration. A section of extension tubing between the needle and syringe can make collection easier.

#### Slides

Clean glass slides are imperative. Greasy or dirty slides prevent even distribution of the sample and introduce contaminants. Although these contaminations can usually be recognized as such by an experienced cytologists, they can also occasionally cause diagnostic confusion.

#### Location Selection

When deciding which area(s) to sample, start by considering the likely architecture of the lesion. There is typically an interface of normal host reactive or inflammatory cells surrounding many lesions. The center of fast-growing lesions often are composed of necrotic debris. Surfaces, especially ulcerated ones, often contain opportunistic bacteria, acellular crusts, and inflammation. Therefore, there are distinct advantages to aspirating multiple locations within a lesion.

Typically, sampling off the center of mass lesions are most rewarding diagnostically. Ulcerated lesions may need to be sampled deep to the ulcerated surface.<sup>3</sup> If multiple lesions are present, collection from both the older and newer lesions can demonstration the chronologic course of the pathology.

#### Site and Patient Preparation

One of the main advantages of cytology is the minimally invasive nature of sample collection. Minimally invasive does not mean that it is stress free to the patient. The patient should be restrained adequately; this may mean simple physical restraint for a compliant patient that is not undergoing a highly invasive technique. Painful procedures or anxious patients may benefit from analgesia and/or chemical restraint. If a sample is being collected from a lesion on the surface of the skin using the imprint/ scraping technique, the surface should be sampled without clipping hair or cleaning the lesion. If a sample is being collected using fenestration or aspiration techniques, the area of interest can be cleaned (if necessary); however, hair does not need to be clipped.

#### **Collection Techniques**

#### Nonaspiration/fenestration collection

Nonaspiration collection has also been called fenestration or capillary collection. The lesion of interest is stabilized manually and the needle is advanced into the lesion and then withdrawn partly out of the lesion, redirected, and advanced again several times. This action will pack cells into the needle while causing a minimal amount of trauma. Multiple areas of the mass can thus be sampled at essentially the same time. One study in human thyroid tissue found that redirecting fewer than 4 times increased the nondiagnostic rate of subsequent samples.<sup>4</sup>

After redirecting the needle multiple times, the needle is removed from the lesion and connected to an air-filled syringe. The sample in the needle is then gently expelled onto a glass slide. A gentle hand is helpful when placing the aspirated material on the

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