

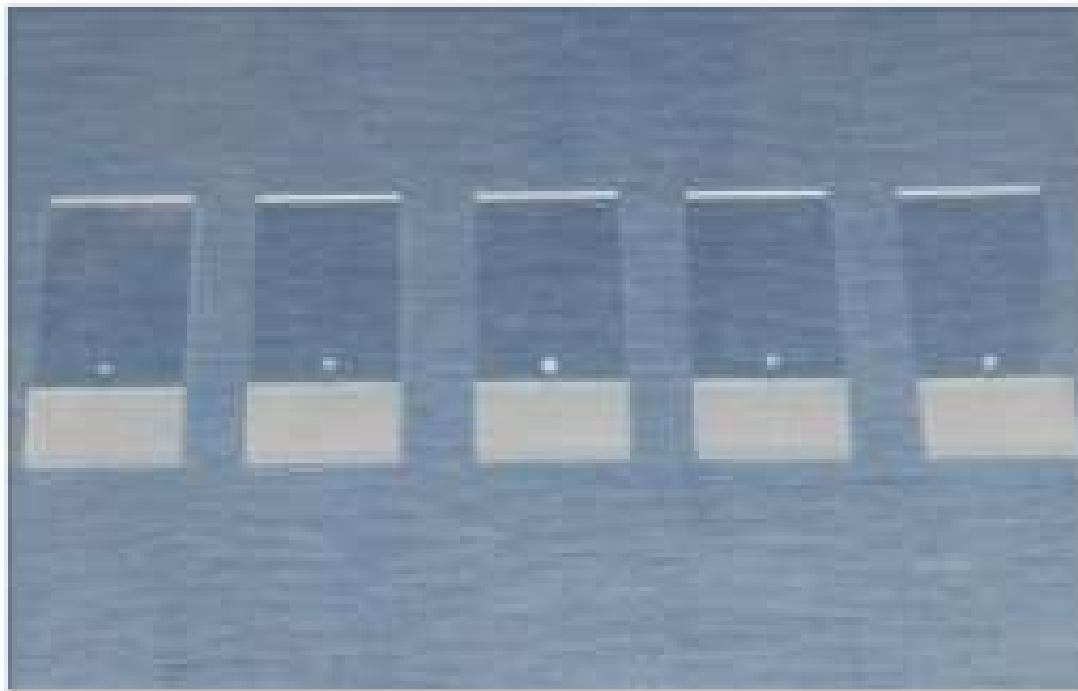


Figure 1.13 Slides on which a small amount of material, from one aspirate, has been expelled.

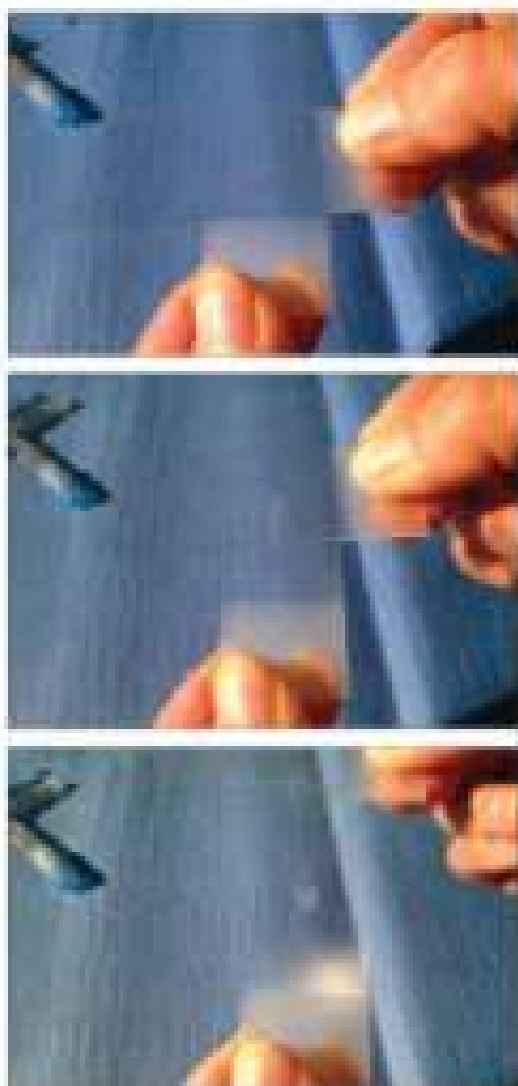


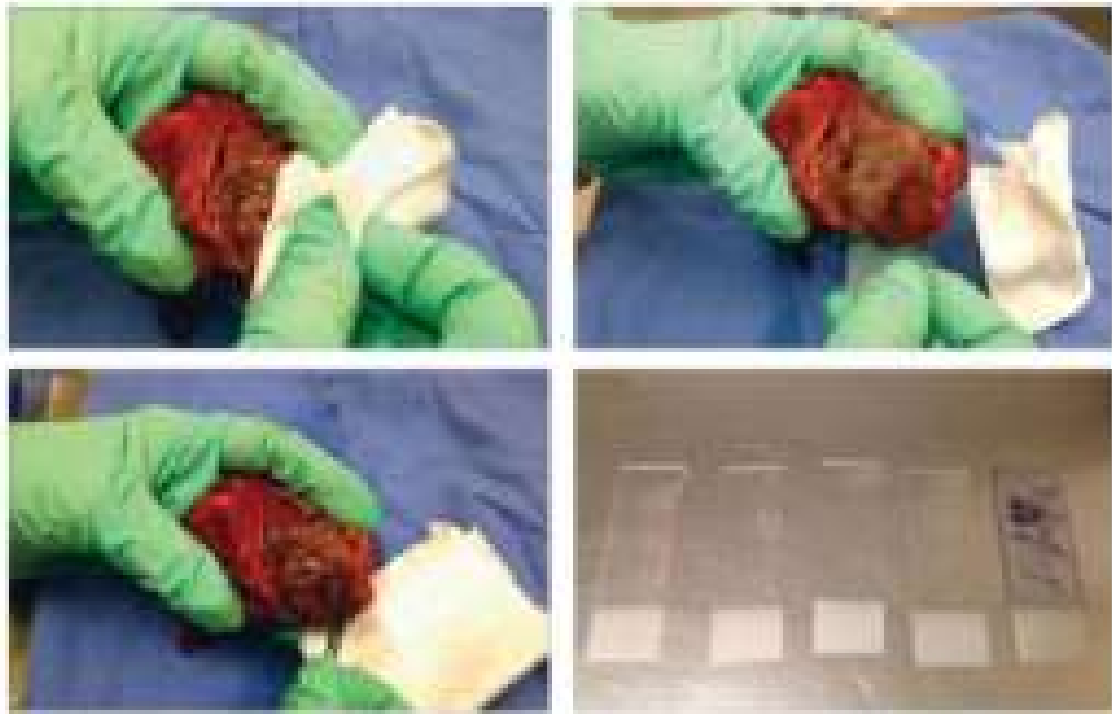


Figure 1.27 The slides, unstained and stained, that result from the squash prep technique. Note the thick and thin areas.

IMPRESSION SMEARS

Cytologic assessment of tissues that have been biopsied can be a worthwhile diagnostic tool for several reasons. The turnaround time for results is much quicker for cytology versus histopathology, and such results may be critical to guide decisions in time-sensitive situations. Additionally, some diagnoses can be made more readily with cytology compared with histopathology, including the diagnosis of round cell tumors and mycobacterial infections. Preparing slides for possible cytologic evaluation is a quick and easy procedure to do after a tissue sample has been obtained. Even if the slides are not submitted immediately to save cost when a biopsy is going to be submitted, it is worthwhile saving the slides for later submission if the histopathology suggests that cytology could be of additional advantage.

To make an impression smear, the tissue of interest is blotted with gauze to remove surface blood ([Figure 1.28](#)). The cut surface of the tissue can then be pressed, quite firmly, to a slide ([Figure 1.29](#)). Multiple impressions are made along the length of the slide, and multiple slides are created in this way. For very firm masses, a scalpel blade can be used to scrape at the cut surface in an effort to increase exfoliation ([Figure 1.30](#)). Any material on the blade can be smeared onto a slide, and the surface of the tissue itself can again be pressed against slides. When stained, the impression smears will look similar to squash prep samples, with thick and thin areas ([Figure 1.31](#)).



Figures 1.28-1.31 Preparing a surgically excised mass to obtain an impression smear. (1.28) First, gauze is used to dry blood from the surface. (1.29) A slide is pressed firmly against the surface of the mass. (1.30) A scalpel blade is used to scrape cells from the mass; the material on the blade will then be smeared on a slide. (1.31) Impression smear slides, both unstained and stained, showing thick and thin regions.

It is important to remember that the presence of formalin near cytology slides will fix the slides and ruin them for evaluation. Be sure to keep the slides away from the formalin jar, even when it is closed. Histopathology and cytology samples should not be submitted in the same package. Also, if the biopsy samples are very small, care must be taken not to destroy the sample for histologic evaluation. Care and gentle pressure are needed when making impressions of small pieces of tissue, and if multiple biopsies of the same lesion are obtained, only use one or two for impressions.

SAMPLING OF INTRA-ABDOMINAL OR INTRATHORACIC FLUIDS

Fluid analysis with cytology can be extremely helpful in identifying processes leading to fluid development, including infectious and neoplastic causes. Thoracentesis and abdominocentesis are fast procedures with a very low risk of complications. For either body cavity, if a moderate to large amount of fluid is present, it is easy to obtain a sample blindly. Equipment needed includes a 1/4 inch 22-gauge needle, a 1-6 ml syringe, and red and purple top tubes. Unlike when sampling tissues, the needle is always placed on the syringe for this procedure.

For abdominocentesis, similar to the standard procedure for cytocentesis, the skin is not usually clipped or scrubbed. To avoid the liver and spleen, the sampling site should be mid-abdomen and towards the right side of the midline. In an upright patient, the needle is inserted into the ventral abdomen, as fluid will sink (Figure 1.32). If the patient is recumbent, the needle is directed into the cavity from below the midline point. The plunger on the syringe is then withdrawn and the sample obtained (Figure 1.33). Several milliliters of fluid can be obtained

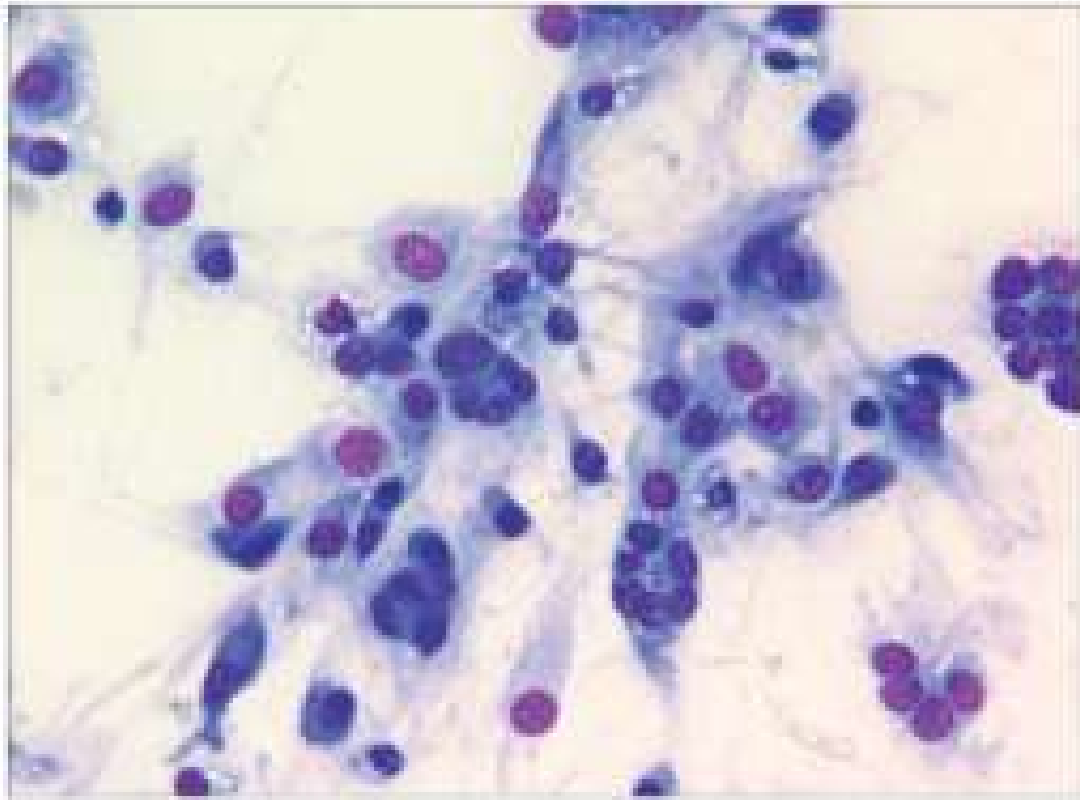


Figure 1.39 Subcutaneous mass on the carpus of a dog. The sample is highly cellular and consists of a population of neoplastic mesenchymal cells. The sample is adequately stained with basophilic cytoplasm, classic purple staining of the nucleus and basophilic nucleoli (Wright-Giemsa, 1,000 $\times$ magnification).

Romanowsky stains

In veterinary medicine, Romanowsky stains are the most commonly used and readily available stains. These stains include May-Grimwald-Giemsa, Leishman-Giemsa, Wright-Giemsa, and Diff-Quik[®] and its variants. The Romanowsky stains include a combination of reagents that include azure B/polychromed methylene blue and the eosin family of stains (Horobin, 2011). The characteristic staining with this combination results in a classic purple staining of the nucleus (Figure 1.39). If the nucleus of the cell is not purple, concern for inappropriate staining, inadequately dried sample, overly thick preparation, or exposure to aldehyde fixatives should be considered (Horobin, 2011; Kraft's, 2011).

One of the many advantages of Romanowsky stains is the ease of fixation. Air drying is all that is required. Even heat fixation is unnecessary for these types of stains. A recent study showed that even for ear swabs, heat fixation provided no benefit (Toma et al., 2006). Slide preparation, therefore, is so important because quick, even drying is essential for uniform staining. Prolongation of the drying time can result in cellular rupture and poor cell preservation (Jeundsson et al., 1999). Additionally, fixation will influence the uptake of the stain. The result of an inappropriately fixed slide is poorly stained cells with blue rather than purple nuclei (Horobin, 2011). Incomplete staining or understaining of samples is a common problem. As mentioned earlier, the nuclei of cells stained with Romanowsky stains should be purple. If the nuclei are blue, the sample may have not stained long enough, had prolonged drying time, be too thick, or been exposed to aldehyde fixatives (Figures 1.40a, b). A blue-green appearance to the erythrocytes

indicates the sample has been exposed to aldehyde fixatives or the pH of the stain is too high (Hirshbin, 2011).



Figure 1.40a,b Lymph node aspirate from a dog with lymphoma. (a) Represents a sample that is poorly stained. The nuclei are staining blue rather than purple and individual cytologic features are difficult to discern. (b) Represents a sample exposed to formalin fumes. Note the green staining of the erythrocytes and the blue staining of the nuclei (Wright–Giemsa; a, 500 $\times$ magnification; b, 1,000 $\times$ magnification).

There are several different Romanowsky stains available. Many practices will use a manual stain like Diff-Quik[®]. The manual stains are presented as three solutions: methanol, solution 1 or eosin, and solution 2 consisting of azure A and B. The slides are dipped in each solution. This is an easy stain to use; however, there are some limitations. The solutions must be changed frequently to limit sedimentation and bacterial growth. Additionally, mast cell granules, basophils, and granules from granular lymphocytes do not stain as reliably with the aqueous Romanowsky stains (Allison & Velguth, 2010). For this reason, many clinical pathology laboratories use Wright–Giemsa or May–Grünwald–Giemsa because the Giemsa component stains mast cell granules vibrantly. If performing in-house cytology, it is important to leave some of the slides unstained so one of these stains can be used.

There are several advantages to the use of Romanowsky stains for cytology, including detailed staining of the cytoplasm, background material, and microorganisms (Kraft's, 2011). A more complete list is shown in Table 1.1.

Papanicolaou stain

The Papanicolaou (Pap) stain has a long history of use in cytology. It was developed by George Papanicolaou and is most commonly associated with the Pap smear used to diagnose cervical cancer. The classic stain involves a combination of five dyes including hematoxylin, orange G, and eosin 5B, which is a combination of eosin, Bismark brown, and light green. The eosin gives a pink color to the cytoplasm of mature squamous epithelial cells, nucleoli, and cilia. Light green stains the cytoplasm of metabolically active cells blue, such as parabasal cells, squamous epithelial cells, and intermediate and columnar epithelial cells. Hematoxylin is used to stain nuclei (Pérez et al., 2003).

The original Pap stain is a little cumbersome for in-house cytology, requiring many reagents and steps. However, simplified Pap stains have been evaluated for easy cytologic use. [Sawa et al. \(2012\)](#) evaluated RADPap[®] and found the stain was easy to perform and only took 15 minutes;

Figures 1.14-1.16 A clean 'smearing' slide is used to smear the material on each of the slides shown in Figure 1.13. (1.14) First, the smearing slide is used to press the sample flat. (1.15) Next, the smearing slide is pulled across the sample slide, while maintaining even pressure and contact, to create a smear. (1.16) The smearing slide is pulled all the way off of the sample slide, and the even smear that was created can be seen.



Figure 1.17 Sample slides stained with Diff-Quik®. The slide on the right is the goal of good sample creation. The slide on the left shows what unequal pressure from the smearing slide can result in. Note the irregular shape of the smear with very thin and thick areas.

The second, and authors' preferred, technique for making smears is to expel all of the sample onto one slide (Figure 1.18). From there, the smearing slide is used to lightly touch the top of the sample drop, pick up a small amount of sample, then smear that sample evenly across a new slide (Figures 1.19-1.25). The same smearing slide is then used to pick up another small bit of sample and smear it onto a new slide. This goes on until only a very small amount of sample is left on the first slide, which is then smeared itself. In this manner, at least 3-5 good slides are generally produced from one FNA (Figure 1.26).