Cytology, the microscopic examination of tissue samples spread onto slides, is a powerful tool for evaluation of skin lesions in small animal patients. In many cases, cytology can provide a definitive diagnosis for cutaneous masses. In others, sample evaluation can rule out some differential diagnoses and help the clinician choose the best next diagnostic test to perform.

**CYTOLOGIC SLIDE CREATION**

The first critical step in the use of cytology is obtaining and creating a good cytologic sample.

**Sample Collection**

*In vivo* collection technique can be performed with a needle alone (fenestration—this author’s preferred technique) or with a syringe attached to the needle (aspiration) (see *What You Will Need*); both of these techniques are typically called fine-needle aspiration (FNA).

1. Before obtaining the sample, lay at least 4 to 6 slides in a row.
2. Stabilize the mass in your nondominant hand.
3. For the fenestration technique:
   - Direct the needle into the mass.
   - Rapidly withdraw—yet stay within skin—and push back into the mass several times before removing the needle completely.
   - Draw several milliliters of air into the syringe, connect it to the needle, and expel the needle contents onto a slide.
   - Make sure the bevel is down and the contents are expelled near the frosted end of the slide.
4. For the aspiration technique:
   - Connect the needle and syringe, direct the needle into the mass, and draw back and release the plunger several times.
   - With pressure released, withdraw the needle from the mass, disconnect the needle, and draw several milliliters of air into the syringe.
   - Reconnect the syringe and needle; then expel the needle contents onto the slide.

**Slide Preparation**

Slide preparation is key for good cytology results. For best cellular evaluation, the samples must be smeared—in a thin layer—quickly to prevent clotting or drying. Smearing is important even if only a small dot of the sample is expelled onto the slide. If cells dry in a little round puddle of fluid, the slide is difficult to assess because the cells maintain more of a 3-dimensional appearance and their cytoplasm is not spread out.

*What You Will Need*

Sample collection and preparation for cytology are simple and require little equipment:

- Needles (usually 20- or 22-gauge)
- 6-mL syringe
- Clean glass slides
After expelling the contents onto the sample slide:
1. Use a second slide (smearing slide) to lightly touch the top of the sample, adhering a small amount of sample on its underside (Figure 1).
2. Smear the sample from the smearing slide onto a new slide, using firm pressure to keep the slides touching evenly and flatly as the smear is made (Figure 2).
3. Use the smearing slide to pick up another small bit of sample off the sample slide to smear onto another new slide.
4. Continue until only a very little bit of sample is left on the first slide, which is then smeared itself. In this manner, at least 3 to 5 good slides are generally produced from one needle sample, allowing for one slide to be stained and evaluated in-house, while other slides can be sent unstained to a clinical pathology laboratory for evaluation.

**TUMOR CYTOLOGY**

**Mast Cell Tumor**

**Description.** Feline mast cell tumors (MCTs) tend to be small, raised, hairless masses, while canine MCTs can grossly look similar to many different lesions, including lipomas and mammary tumors or infections.

**Diagnosis.** Most MCTs are easily diagnosed via cytology (Figures 3 and 4), although a small percentage of canine MCTs have granules that do not stain well with Diff Quik stain (Figure 5), and must instead have a Wright-Giemsa stain applied (Figure 6, page 30). Thus, keeping some of the cytology slides unstained is key.

**Management.** Feline MCTs typically are easily excised with narrow margins, and often completely

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**FIGURE 2.** In the left hand is a new clean slide; the slide in the right hand has been pressed against the top surface of the new slide to apply the sample to the clean slide and then smear it evenly.

**FIGURE 3.** Cytology of a feline MCT; note the numerous round cells with purple staining granules (Diff Quik stain; magnification, 20×).

**FIGURE 4.** Cytology of a canine MCT; similar to the feline MCT, this slide has a large number of round cells with varying amounts of purple staining granules (Diff Quik stain; magnification, 50×).

**FIGURE 5.** Cytology of a canine MCT showing poorly staining mast cells; scattered red blood cells and cells with segmented nuclei are seen in the background (Diff Quik stain; magnification, 20×).
removed with a lidocaine local block and skin punch biopsy. Canine MCTs are more locally invasive than the feline form; recommendations for complete surgical removal generally suggest excising the mass with 2- to 3-cm margins of visibly normal tissue included.

A more recent study reported on a modified margin technique in which tumors are excised with lateral margins equal to the widest diameter of the tumor, up to a maximum of 4 cm. Forty dogs had tumors removed with clean margins, 7 had incomplete margins, and only one tumor was suspected to have recurrence.

Histopathologic grading is critical to determine the likely systemic biologic behavior of any particular canine MCT, as well as the necessity for systemic therapy after excision.

**Squamous Cell Carcinoma**

*Description.* Squamous cell carcinoma (SCC) occurs primarily in older dogs and cats. Animals with lightly pigmented skin that spend time in the sun are predisposed to solar (actinic) induced SCC in their thinly haired areas. Multiple lesions may be present in these cases, particularly in white cats. In dogs, cutaneous SCC unrelated to sun exposure is the most commonly reported digital tumor and most often affects dark-haired dogs.

*Diagnosis.* On physical examination, cats present with facial lesions more frequently than dogs, usually at thinly haired areas, such as the ear tips, eyelids, and nasal planum. These lesions are often crusty and ulcerated. In dogs, digital SCC is usually single but may affect multiple digits in giant schnauzers and other large, black-haired breeds. SCC of the digit appears as a swollen digit with an abnormal nail; it is often diagnosed initially as a fractured nail with a nailbed infection (*Figure 7*).

Cytology can be diagnostic for SCC of the digit (*Figure 8*). Direct the needle deep into the midpoint of the digit; the bone is often involved and lytic; therefore, a good sample may be obtained from the bone itself. Occasionally, dogs will have so much pain that sedation is needed for FNA.

*Management.* SCC of the skin is a locally invasive tumor but rarely metastasizes; thus, the
treatment of choice is wide surgical excision. Depending on the area affected, such surgery may entail nasal planeectomy or amputation of 1 to 2 digits. For cats with erosive lesions around their eyes, cryotherapy can be effective (Figures 9 and 10). Depending on the lesion size, liquid nitrogen may be applied with a cotton swab, cryoprobe, or cryospray device, using 2 to 3 freeze–thaw cycles. Protect the eye with ocular lubrication and use extreme care to avoid the cornea.4

Basal Cell Tumor

Description. With the advent of immunohistochemical staining, tumors can be reclassified and renamed as they are investigated. This has occurred with basal cell tumors, one of the most common tumors in dogs and cats. The typical mass—benign, well-circumscribed, and previously called basal cell tumor—is now recognized as a trichoblastoma (Figure 11).5,6

The term basal cell tumor was also used as an umbrella term for a large heterogeneous group that included tumors from epidermal, trichofollicular, and adnexal tissues demonstrating basal cell characteristics. This is particularly relevant in cats, in which benign and malignant apocrine ductular sweat gland tumors and basal cell carcinomas are now recognized as having been lumped into the basal cell tumor category.

These tumors have malignant potential and, although reports are limited, feline basal cell carcinomas are often highly metastatic. Additionally, while there are no reports of canine malignant basal cell tumors in the literature, veterinary oncologists, including this author, have seen rare cases of metastasis of basal cell tumors in dogs.

Basal cell tumors arise in middle-aged to older dogs and cats, with some dog breeds, such as poodles, being over-represented.

Diagnosis. Histopathology now differentiates among trichoblastoma, basal cell carcinoma, sweat gland tumors, and other epithelial tumors, but cytologists may still use the term basal cell tumor as a broad cytologic diagnosis covering all of these tumor types.

The cytologic diagnosis of a tumor of basal cell origin is based on small, round to cuboidal cohesive cells arranged in tight clumps or ribbons (Figure 12, page 34). Features of malignancy may be recognized and support a diagnosis of carcinoma, but histopathology is needed to determine the tumor’s malignant potential because the main criterion of malignancy is invasiveness.7,8 Thus, once cytology confirms a tumor of basal cell origin, excision, followed by histopathology, is recommended whether or not cytologic features of malignancy are seen.
Management. Most tumors diagnosed as basal cell tumors via cytology are easily cured with narrow surgical excision. If histopathology reveals a basal cell carcinoma, complete staging, including locoregional lymph node aspiration, chest radiography, and abdominal ultrasonography, is recommended. The malignant variant of this disease is rare enough that studies evaluating chemotherapy are not available. If histology confirms a mass as malignant, chemotherapy may be of benefit.

Histiocytoma

Description. Histiocytomas affect primarily young dogs, but they may arise at any age. These tumors have not been identified in cats.

Diagnosis. Grossly, histiocytomas have a distinctive appearance: they are raised, rounded, and alopecic and have small indentations all over (“pock marked”), which create an appearance similar to the surface of a strawberry. The tumors are also described as “button-like” because of their shape (Figure 13).

These benign tumors will regress on their own, often over several months’ time. As they do so, the surface may become ulcerated and intermittently bleed. Experienced practitioners may diagnose these tumors on the basis of gross appearance alone, although cytologic confirmation is suggested.

On cytology, histiocytomas are in the round cell tumor category. They may be confused with other round cell tumors, such as plasma cell tumors or poorly granulated MCTs. Despite their benign behavior, histiocytomas often exhibit cytologic features of malignancy, such as numerous mitotic figures and marked anisocytosis (Figure 14). Caution needs to be used in interpreting these features, and
submission to a cytologist before alarming an owner about malignancy is recommended.

Histiocytomas can be routinely diagnosed using a combination of cytologic and gross appearance. As they begin to regress, cytology will reveal lymphocytes scattered among the tumor cells.

**Management.** Excision is generally not needed. It may be recommended if the patient is going to be under anesthesia for a different procedure, such as an ovariohysterectomy, or if the tumor is not regressing over time or begins to grow.

**IN SUMMARY**

Cytology provides a quick and minimally invasive way to evaluate cutaneous tumors in dogs and cats. It may provide a definitive diagnosis and can, thus, help the clinician to determine whether further staging tests, such as lymph node aspiration or chest radiography, are indicated. Even more important, a cytologic diagnosis can help determine the width of the surgical margins needed for complete excision, based on the biologic behavior of the tumor type identified. This preoperative information helps clients avoid the excess expense of follow-up treatment for a dirty margin, and patients the excess morbidity of a wide surgical excision that could have been more conservative.

Thus, cytology on cutaneous masses assists the clinician in patient assessment, may decrease discomfort for the pet, and may actually make the treatment less expensive for the client, with better outcomes for all.

FNA = fine-needle aspiration; MCT = mast cell tumor; SCC = squamous cell carcinoma

**References**