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Sample Collection

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Introduction

One of the most appealing features about diagnostic cytology is its capacity to provide a timely, inexpensive, and minimally invasive diagnosis. However, the acquisition of a diagnostic quality sample can be strewn with pitfalls contributing to pre-analytical error that can heavily influence the ability to obtain an accurate diagnosis. Understanding and preventing these sources of error will help avoid the frustration of a nondiagnostic sample or diagnostic error. Screening of sample quality is also discussed in Chapter 5; however, briefly, to be diagnostic, a cytology sample should be representative of the lesion, cellular enough to obtain a diagnosis, relatively free of contaminants (e.g. lubricant, gel, bacteria, blood, etc.), appropriately prepared to allow for the interpretation of cellular material, and sufficiently stained (see Chapter 2 for more detail). Four of these five criteria fall under the process of sample acquisition. As such, recognizing the importance of sample acquisition is particularly relevant for clinicians that are submitting their samples to reference laboratories. Based on tissue and lesion type, some samples will have a higher probability of yielding a diagnostic quality slide, and variations in acquisition and preparation methods are required to maximize obtaining diagnostic samples.[1] The specifics of sample collection for particular species or tissues are addressed in those chapters, and Chapter 50 describes the laboratory analysis of fluid samples in detail.

Methods of Sample Collection

There are a number of technical approaches that can be used in the collection of samples for cytologic analysis. The choice of a particular method should be driven primarily by the features of the lesion, the sampled tissue, and the clinical suspicion.[2]

Sampling Superficial or Subcutaneous Masses

Fine-needle aspiration (FNA) is a relatively simple noninvasive technique used to obtain tissue samples. Samples are generally considered to be sensitive with good agreement between cytology and histopathology results for many lesion types.[3,4] Although the procedure is well recognized, there is variability in recommended techniques.[5] Regardless, aspiration is typically performed without clipping or sterile preparation of the site; however, the area should be free of gross contamination. Necrotic or overtly infected areas should also be avoided as these generally yield samples that may not be reflective of the underlying process.

The term aspiration often refers to the application of negative pressure with a syringe attached to a needle. Fine needle is sometimes reserved for needles 20 G or smaller.[6,7] Another option is the capillary, core, or fenestration technique, which can also be referred to as "needle-off" since a syringe is not attached when the needle is introduced into the tissue. This technique was first described in human medicine in 1986 but has been a commonly recognized sampling method in veterinary medicine for many years.[8] Three to four short, rapid redirections are used to obtain the sample; the bevel of the needle cuts through the mass and directs the sample into the inner diameter of the needle. Redirecting the needle into multiple planes significantly increases tissue yield as opposed to unidirectional core sampling.[9] The needle is then attached to an air-filled syringe for expulsion of the sample. Avoiding suction can minimize hemodilution of the sample, and less material is lost to coating the inside of the syringe.

Capillary sampling can be superior in areas where blood contamination can negatively impact sample acquisition, which is more important in highly vascular areas such as the spleen, but can be relevant for hemorrhagic lesions of the skin and subcutaneous tissue as well.[8] Lymph nodes are often aspirated using the capillary technique.[3,5] Because lymphoid cells are prone to rupture, capillary sampling can

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improve the diagnostic yield. When the needle is inserted, the tip of a finger is positioned to cover the end of the needle hub. This prevents sample leakage from the end of the needle as it is withdrawn. Alternatively, a syringe can be attached to the end of the needle in the capillary method but without suction. The attached syringe provides negative pressure as the needle is withdrawn. Attaching the syringe can aid in manipulating the needle in difficult-to-reach areas or for operators with mobility or dexterity limitations. Sampling complications for superficial structures are typically minimal, but can include hemorrhage, particularly for vascular lesions or if there is a coagulopathy.

Sample Collection Using Advanced Imaging

Indications

Sampling structures that are not superficially accessible or cannot be palpated and stabilized manually can be challenging. Additionally, some lesions will have regions of necrosis or cavitation, which, if sampled, will not reflect the underlying disease process (i.e. "geographic misdiagnosis"). Percutaneous image-guided sampling with FNA, fine-needle biopsy, or tissue-core biopsy can guide precise needle placement and facilitate procurement of samples from deeper lesions in an efficient, effective, and relatively safe manner. Additionally, with image guidance, samples can be obtained from regions of viable tissue suspected to represent the true underlying disease process.[10,11] Image guidance allows the operator to avoid other important structures and minimize risk of laceration of major blood vessels or inadvertent puncture of adjacent organs.[12,13] Image-guided sampling can be more efficient and safe than other methods and result in more positive diagnostic samples, with follow-up imaging assessment for post-biopsy complications possible.[13–15]

Imaging Modalities

Ultrasound (US), fluoroscopy, computed tomography (CT), and magnetic resonance imaging (MRI) have all been described as means to directly visualize and guide sampling of lesions in veterinary patients.[10,13,15–23] The choice of modality depends upon availability of equipment, location of lesion, and preference and experience of the sampler.[10] Each modality has unique advantages and limitations for use during image-guided sampling.[10,16,23] For example, while fluoroscopy provides real-time guidance and can be utilized to sample lesions deep within the lung or bone, staff are exposed to radiation; the internal structure of the lesion cannot be visualized; and it is more challenging to accurately differentiate lesions from adjacent vital structures.[10] Cross-sectional imaging modalities (US, CT, and MRI) have mostly replaced fluoroscopy for percutaneous biopsy because of greater contrast resolution, better visualization of needle placement, and greater overall ability to procure diagnostic samples. CT provides improved tissue evaluation and provides unobstructed visualization of needle pathway and target but generally does not allow real-time guidance, resulting in blind needle passage and in longer procedure times with possible geographic misses.[1,15,16] Compared with CT, MRI has superior soft tissue resolution, lacks beam-hardening artifacts from nearby bone, and involves no radiation exposure, but image-guided sampling with MRI necessitates specialized non-ferromagnetic equipment.[10,11] CT and MRI are generally less available, are more costly, and often require deep sedation or general anesthesia; therefore they are often reserved for lesions that are difficult to biopsy with US guidance.[10,16,23] US-guided sampling is readily accessible, portable, cost effective, and rapid; affords continuous, real-time monitoring of needle insertion; and involves no radiation exposure.[12,13,15] For US-guided fine-needle biopsy, the patient often needs no or minimal sedation. The major limitation to US-guided sampling is inability to identify an acoustic window, such as when overlying gas or bone prevents identification of the lesion.[10]

Materials Required

In general, materials required for image-guided sampling, beyond the imaging equipment, are simple and similar to those needed for non-image-guided sample collection. In our practice, a small gauge (e.g. 22 G) hypodermic (1.5 in.) or spinal (3.5 in.) needle coupled to a 6 cc syringe with either aspiration (FNA) or non-aspiration techniques are employed based on radiologist preference. In some instances, an extension set is attached to the needle and syringe for gentle suction, particularly in the case of sample collection from fluid-filled or more technically challenging structures. Additional materials may be required depending upon the modality used. For example, a localizer method (localizing needles or grid) can be placed on the patient during CT-guided sampling.[16] A needle guidance system can be utilized while procuring samples in order to target very deep, small lesions.[16–18,24,25] A discussion of technique for image guidance with each modality is beyond the scope of this chapter, and the reader is referred to other sources.[10,11,16–18,20–23,26,27]

Complications

A complete discussion of the potential complications per modality is beyond the scope of this chapter. Potential complications depend upon operator experience, sampling needle size, and the location and nature of lesions sampled. Potential complications include pain, fever, hemorrhage, intraparenchymal hematoma, hematuria, peritonitis,

pneumothorax, epistaxis, hemoptysis, seizures, spread of infection, tumor seeding, and death.[12,16,17,21,28–34] Complications occurring during image-guided sampling will depend upon the location of the lesion but are reported to be low for fine-needle and tissue-core biopsy with higher risk for bleeding during tissue-core biopsy in the presence of thrombocytopenia.[13,14,35–39] Bleeding at the site is the most commonly reported complication of sampling abdominal structures, but is often minor and self-limiting.[12,40] The risk of hemorrhage is reported to be 0–16.9% in the veterinary literature, with higher risk associated with renal biopsy.[12,13,40–42] A review of FNA utilizing image guidance (fluoroscopy, scintigraphy, CT, and ultrasonography) in over 11 000 human patients demonstrated low risk for any type of complication with a mortality rate of 0.008%, major complication rate of 0.05%, and other complications at a rate of 0.49%.[28] Pneumothorax has been the most commonly reported complication of sampling thoracic structures, especially when aerated lung is penetrated. Typically only a small volume of air is identified and no intervention is necessary.[21,32] Imaging can be repeated after the sampling procedure to assess for any immediate or delayed complications from the procedure.

Sample Preparation and Staining

Once an aspiration sample is obtained, it is transferred to a slide. It is helpful to lay out several clean glass sides before the aspiration procedure. Some slides may retain glass or dust debris, which can significantly interfere with creation of a monolayer of cells on the slide surface. If the slides are not pre-cleaned, wiping with lens paper or a Kimwipe® can significantly improve outcomes. Care should be taken to minimize handling of slides to avoid contamination with keratinocytes. A 10–12 mL syringe can be used with 6–8 mL of air in the syringe to transfer fenestration and capillary samples to glass slides. The syringe with the air is attached to the needle with the sample, and the air should be gently expelled from the syringe to allow a single drop of material to be placed on each slide. Control is important at this step; if excessive amounts of material are placed on the slide, an excessively thick preparation may result. Thick preparations are very difficult to evaluate as they do not stain well, individual cells may not be visible, and excessive pressure may be needed to create the slide increasing the risk of cell rupture (Figure 1.1).[5]

Several methods have been described for preparing slides, but no significant case–control studies have been performed to validate various techniques. Individual outcomes, operator preference, and prior instruction will generally dictate the method. When preparing a slide, any technique creating multiple small droplets should be avoided. Droplets will dry quickly, resulting in excessive thick smears. Many veterinary oncologists use squash preparation when preparing slides. The goal of the slide preparation is to create a monolayer of intact cells that will stain readily and for optimal cytomorphology. To create a squash preparation, two slides are placed at right angles to each other centered over the sample drop on the slide. As the slide is applied, the sample will begin to slowly spread under the slide. When the sample begins to spread, the top slide is gently drawn to the bottom of the other slide to smear the material in a uniform layer on the glass surfaces. It is recommended that the two slides be held in the air while this is done and not placed on a hard surface to avoid excessive downward pressure. This method is not as well suited to liquid specimens, and there is a risk for thick smears if too little pressure is applied or for nondiagnostic ruptured cells if excessive pressure is applied.[5]

The blood smear technique is more appropriate for serous or fluid samples. This method is similar to blood smear preparation. To begin, a clean slide is placed on a stable, hard surface; a drop of the sample is deposited at one end of the slide; and a second slide is directed a third of the way into the sample at a 45° angle. The angled or spreader slide is then quickly drawn down to the bottom of the first slide using gentle pressure to create the feathered edge. A modified method is to combine the squash prep and blood smear techniques and use a 90° rotated flat slide to smear the fluid sample. This is sometimes referred to as a line technique.

Once a slide is created, it must be allowed to dry for one to two minutes at a minimum. Direct heat application especially with an open flame is not recommended because of uneven distribution of heat, cell deformation, and risk for injury. When the slides are dried, a representative slide should be stained and evaluated for adequate cellularity. Diff-Quik is a modified Romanowsky stain readily available in most practice settings. It is generally considered to be a reliable stain for screening smears and most diagnostic applications. Some mast cell granules may not stain with Diff-Quik (Chapter 13), and the lipid contained in lipoma cells is dissolved by the alcohol fixative in step 1 of the staining process.[43,44] Dipping rather than passive immersion is recommended to enhance stain uptake and reduce the time for stain to set.[45] Additional detail on staining is presented in Chapter 2. It is essential that a slide be evaluated for adequate cellularity and quality prior to lab submission. In a UK study, lack of cellularity was the most common reason for a sample to be rejected as nondiagnostic.[1] Screening can also help confirm successful sampling of the target tissue. For example, aspiration of salivary tissue can inadvertently occur during attempts to aspirate

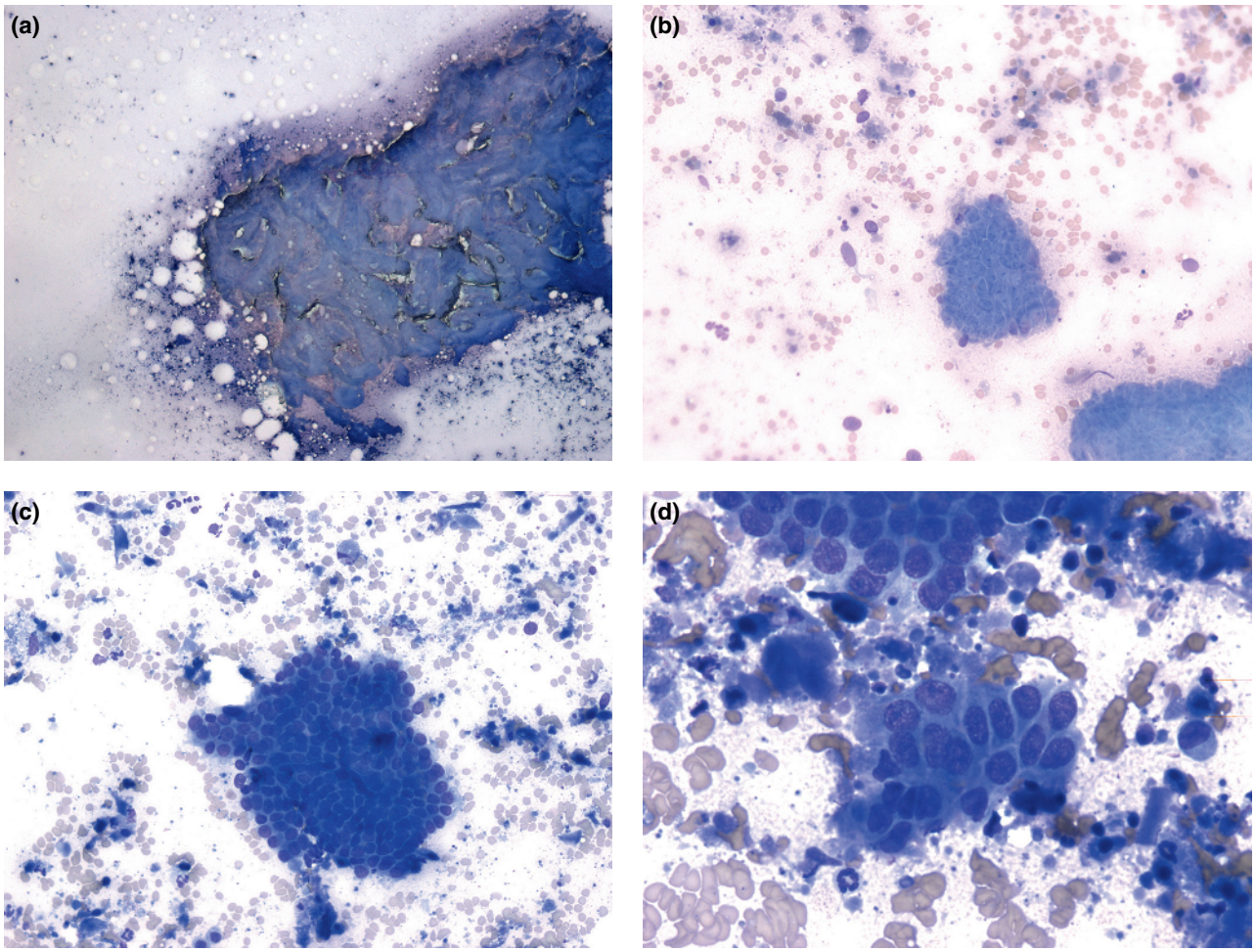

Figure 1.1 Cytology smears from a subcutaneous mass on the ventral abdominal midline from an adult dog with a history of removal of a colonic adenocarcinoma. Multiple smears were submitted and there was regional variation in the thickness of the smears. (a) A region of the smear demonstrating clusters of cells that are too thick to stain (Wright Giemsa, 200×). (b) A region of the smear that was understained, but thin enough that repeat staining might improve cytologic detail (Wright Giemsa, 200×). (c and d) Thinner, better stained areas with good cytologic detail (Wright Giemsa, 200× and 500×, respectively). A cytologic diagnosis of metastatic carcinoma due to seeding of the laparotomy incision site was made.

the mandibular lymph nodes. All slides, including those pre-stained, should be submitted for evaluation. Smear quality and content can vary and impact the diagnosis, so submission of multiple smears optimizes the diagnostic process (Figures 1.1 and 1.2). Cytology slides should never be submitted in the same shipping container as a formalin sample. Formalin is known to destroy the cells on the slide and render them nondiagnostic (Figure 1.3).

Diagnostic Yield

Maximizing diagnostic yield can require use of multiple collection methods. The recovery rate (or the rate of collecting diagnostic quality samples) is also dependent upon the experience of the sampler and the nature of the lesion.[8,46–49] Interestingly, reporting the diagnostic recovery rate is not emphasized in veterinary medicine, although it is reported in some studies.[3,37,50–53] Recovery rate should be noted by clinicians in evaluating the appropriateness of cytology as a diagnostic tool in individual cases. Absence of nucleated cells, poor cell preservation, and cell disruption are cited as causes of nondiagnostic samples.[54,55] Many studies of the diagnostic value of cytology focus on the agreement between cytologic and histologic diagnoses. Often, correlations are performed using only diagnostic cytology samples, which can minimize the impact of difficulties obtaining diagnostic specimens. In studies that choose to report the number of nondiagnostic samples as part of their findings, the diagnostic accuracy of cytology

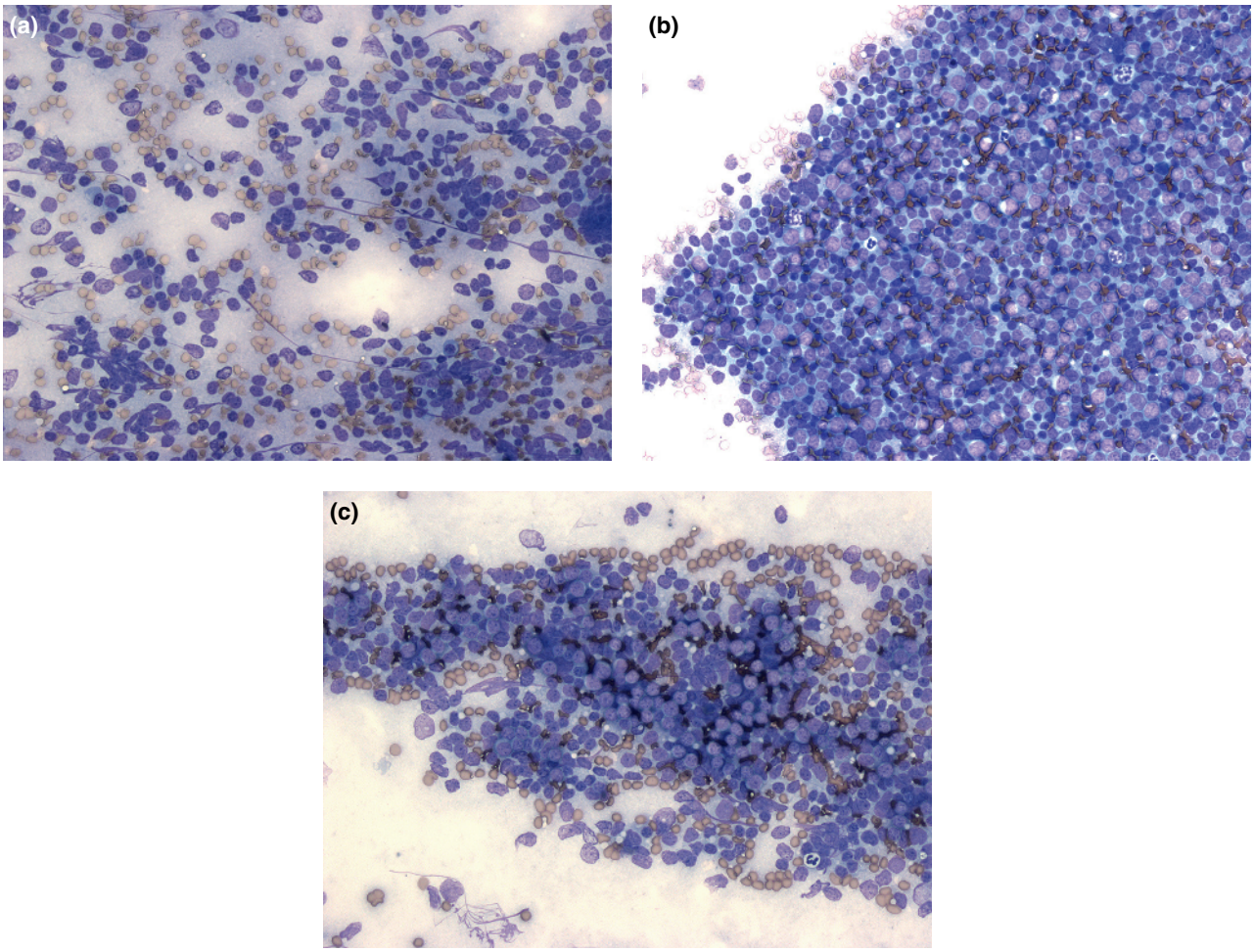

Figure 1.2 Prescapular lymph node aspirate from an adult dog. (a) Broken cells that cannot be interpreted are present in several of the smears. (b) Other areas of the smear were composed of predominantly intact well-stained cells. The population was heterogeneous and included many small cells as well as intermediate and some large cells. Occasional mitotic figures and non-degenerate neutrophils were observed. (c) Some regions contained moderate numbers of broken cells, and intact cells were predominantly a uniform population of medium lymphocytes (Wright Giemsa, 200×).

tends to be lower.[56] Studies in human medicine report that up to 32% of aspirates obtained by clinicians were considered unsatisfactory. More specifically in one study, half of these patients either had to repeat the procedure or undergo additional diagnostic testing.[46] Definitive diagnosis was obtained in 61% of human abdominal FNA cases with the first needle pass, with a further increase of 21% with the second FNA attempt using a new needle, 8% with the third FNA attempt, and 6% with the fourth FNA attempt.[57]

Implications of Nondiagnostic Samples

Nondiagnostic or low cellularity samples are frustrating for clinicians and cytopathologists; however, interpreting inadequate samples is not recommended. Sample quality can affect the diagnostic accuracy, with low quality samples risking being reported as either a false negative or a false positive. Although this seems intuitively obvious and is often alluded to in studies of veterinary cytology, the impact is rarely quantitatively assessed. One study of bone cytology in dogs revealed that cellularity significantly affected rates of cytologic-to-histopathologic correlation with high, moderate, and poor cellularity samples having concordant primary process in 88, 77, and 47% of cases, respectively.[58] This study highlights the potential harm that may result from forcing a cytologic interpretation on substandard samples. Unfortunately, in many cases, the slides are not examined prior to submission, making it difficult, if not impossible, for the clinician to know if a high-quality sample has been obtained. In a study of veterinary

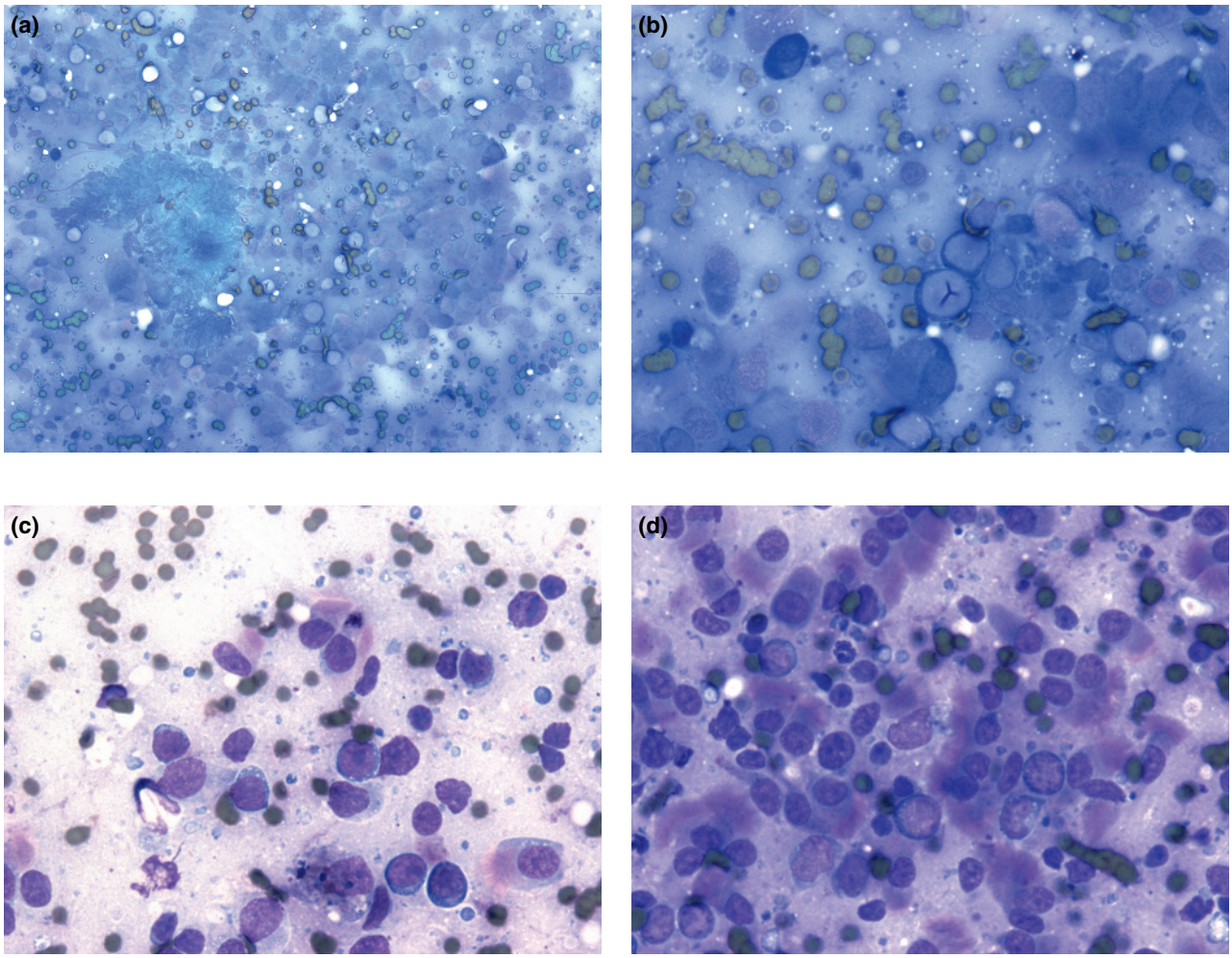

Figure 1.3 Nasal flush cytology from an adult cat. Smears were submitted in the same container with a histology sample in a container of formalin. (a and b) Smears that were unstained prior to submission and exposed to formalin fumes. Staining is inadequate and smears cannot be interpret (Wright Giemsa, 200× and 400×, respectively). (c and d) Smears pre-stained prior to submission were unaffected and revealed numerous mature ciliated columnar respiratory epithelial cells and a population of medium to large atypical lymphocytes, suggesting a potential diagnosis of lymphoma (Diff-Quik, 200× and 400×, respectively). The histologic diagnosis was intermediate cell lymphoma.

practitioners in the United Kingdom, the majority of samples submitted (51%) were not evaluated prior to submission. Of those samples submitted, 19% were classified as low cellularity. Low cellularity may not always reflect poor sampling techniques as many of these samples were classified as a "suspect lipoma" but were of insufficient quality to allow for any diagnostic interpretation.[1]

Conclusion

Aspiration is a skill that any practitioner can gain proficiency with repeated use. By avoiding the pitfalls of inadequate sampling, excessive cell damage, or thick samples in slide preparations, the diagnostic utility of cytology can be implemented to its fullest for both specialist and general practitioners.

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