REVIEW

Fine needle aspiration of abdominal organs: a review of current recommendations for achieving a diagnostic sample

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With the improved accessibility to ultrasound, fine needle aspiration of abdominal organs is now performed frequently in many veterinary practices. Despite this, there are many 'unknowns' as to the best method to produce a high-quality sample. This report begins by highlighting recent literature on the risks and benefits of abdominal fine needle aspiration. It follows with recommendations about the equipment and method best suited to the procedure, including needle and syringe size and aspiration *versus* non-aspiration techniques. Various smear preparations and laboratory submission requirements are also discussed. The final aspect of the review more specifically discusses fine needle aspiration of specific abdominal organs: the liver, kidney, spleen, pancreas, urinary bladder, prostate and abdominal lymph nodes.

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INTRODUCTION

'Fine needle aspiration' (FNA) is a method used for the aspiration of tissue cells via the introduction of a small-gauge needle into a lesion. The samples obtained from FNA are then used to prepare a specimen that can be analysed by various methods, such as microscopy. If successful, these results can then be used to obtain a definitive diagnosis, to guide additional testing or to rule in or out specific diseases. There is rarely a need for general anaesthesia during the procedure, and the small gauge of the needle seldom leads to clinical complications.

With the advent of advanced imaging modalities such as ultrasound, fluoroscopy and CT, sampling and the diagnosis of deep-seated lesions within the abdomen are now possible without the requirement for surgical exploratory laparotomy. Ultrasound is readily accessible in many general practice clinics and can be used to obtain abdominal FNA samples. Ultrasound-guided sampling has several advantages over blind aspiration, including the direct and constant visibility of the needle and target organ, enhanced accessibility of deep-seated lesions and increased accuracy and safety when compared with blind aspiration (Grant *et al.* 1983, Hager *et al.* 1985, Babb & Jackman 1989, Pujani *et al.* 2016).

Given the many benefits of FNA, determining a protocol that will provide the highest chance of obtaining a diagnosis is extremely important. Currently, controversies exist in both human and veterinary literature about the influence of factors such as needle size, aspiration *versus* non-aspiration techniques and appropriate sample preparation. The aim of this report was to examine published literature on such controversies and present recommendations for successful FNA sampling of abdominal organs in veterinary practice.

RISK *VERSUS* BENEFIT OF FNA

In humans, the benefits of performing ultrasound-guided FNA of abdominal lesions have been evaluated. A study by Gani *et al.* (2011) of 67 cases concluded that the FNA procedure played a significant role in obtaining a diagnosis in the majority of

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patients, with no major clinical complications observed. In the veterinary world, the success of obtaining a diagnosis using FNA for abdominal organs is variable. Sampling of abdominal lesions is not performed with the same frequency as FNA in other sites, such as cutaneous lesions. At our institution, which receives both first-opinion and referral practice submissions, 922 FNAs from tissue samples were submitted in 2016, and of these, 429 (47%) samples were obtained from abdominal organs (unpublished data). Interestingly, Skeldon & Dewhurst (2009) looked at 945 cytology samples submitted to their laboratory and found that only 37 (3·9%) samples were obtained from abdominal organs. This difference may reflect the different proportions of submissions received from primary care and referral practices as the vast majority of veterinarians who submitted to the latter were reported to work in a primary care practice.

Diagnosis rates from abdominal organs have been found to be much lower than other sites, showing a definitive diagnosis in only 2 of 37 cases, with 27 samples described as 'moderately clinically useful' or 'clinically useful' (Skeldon & Dewhurst 2009). Other studies show more promising results; Léveillé *et al.* (1993) found that FNA specimens of abdominal organs correlated with the final diagnosis made during surgical exploration or necropsy in 59 of 70 of samples. When categorising disease according to the two criteria of 'inflammatory' or 'neoplastic', Bonfanti *et al.* (2004) observed that FNA showed an 89% success rate in differentiating inflammatory from neoplastic disease based on 132 samples from abdominal lesions. Importantly, malignancy was correctly detected in all malignant neoplasia.

The discrepancy in these results may reflect pathologist experience in cytological interpretation, differences in case load and differences in definition of agreement. Higher success rates may also be seen in referral institutions where clinicians are generally more practised at obtaining cytological samples from abdominal viscera compared with general practitioners. However, even studies conducted at referral centres may show poor diagnostic accuracy. Cohen *et al.* (2003) examined 29 liver and splenic FNA samples collected at a veterinary referral institution. FNA and impression smear techniques were used and compared with histopathology or post-mortem examination. The number of cytological samples that attained 'complete or partial agreement' with other methods was found to account for only 34% of samples. The authors also reported that impression smears had a higher level of agreement than samples obtained from FNA.

Abdominal FNA has a very low clinical complication rate, with a minor complication rate of 3·9% and no major complications observed in a study of 102 human patients with abdominal tumours (Binek *et al.* 1995). Penetrating abdominal viscera with 20 to 23 G needles has been shown to produce minimal complications, with large-scale human studies reporting a total complication rate of 0·55% and only one mortality resulting from abdominal FNA reported in 11,700 cases (Livraghi *et al.* 1983).

Similarly, in veterinary studies, medical records for ultrasound-guided FNA have been reviewed for procedural complications. Complications did not develop at all after FNAs in many studies, whilst other studies reported rare mild complications, such as pain, small haematomas, minimal bleeding from the local site and haematuria (Hager *et al.* 1985, O'keefe & Couto 1987, Léveillé *et al.* 1993, Bonfanti *et al.* 2004). Two mortalities were reported in each of two abstract reports on 307 feline liver FNAs, and 600 canine liver FNAs (Menard & Papageorges 1996, Papageorges 1996).

The safety of using an 18 G needle to obtain core biopsies of abdominal organs has been evaluated in dogs and cats with coagulation deficiencies in a study by Bigge *et al.* (2001). Findings suggested that the procedure should be delayed if there is moderate or marked thrombocytopenia because significant bleeding complications occurred in 22% of dogs and 50% of cats with platelet counts less than 80×10^9/L (Bigge *et al.* 2001). However, this may not pertain to FNA because tissue core biopsies tend to incur greater risk of haemorrhage (Léveillé *et al.* 1993, Bigge *et al.* 2001, Vaden *et al.* 2005). When 56 human patients with mild thrombocytopenia (70 to 100×10^9/L) underwent splenic FNA, no correlation between thrombocytopenia and postprocedural complications was observed, with only three cases showing complications (Civardi *et al.* 2001). In pigs treated with warfarin, there was no significant increase in bleeding compared to untreated pigs during renal and hepatic FNA (Gazelle *et al.* 1992).

Another frequently discussed complication of FNA is needle tract tumour implantation. In humans, it is considered a rare complication of FNA, occurring with an estimated frequency of 0·009%, with pancreatic neoplasia associated with most of these events (Smith 1991). In both humans and animals, pancreatic carcinoma is considered highly malignant, with most cases of needle tract seeding diagnosed within two to six months of the procedure.

In animals, rare cases of suspected tumour tract implantation following abdominal FNA have been reported; there is a single case report involving a case of renal carcinoma (Livet *et al.* 2016), but the majority of suspected needle tract implantations was suggestive of urothelial cell carcinoma (UCC) implantation in the abdominal wall following FNA of the bladder, urethra or prostate (Nyland *et al.* 2002, Vignoli *et al.* 2007, Wilson *et al.* 2007,Higuchi *et al.* 2013). UCCs (previously known as transitional cell carcinomas) are highly malignant and, so, the apparent higher risk of implantation with this tumour may be a reflection of its aggressive nature (Norris *et al.* 1992, Meuten 2008, Higuchi *et al.* 2013).

We share the opinion that in the majority of cases, the low risk of implantation is outweighed by the benefits of obtaining a minimally invasive diagnosis, although these risks should be discussed with the owner before proceeding.

PERFORMING A FINE NEEDLE ASPIRATE

Before performing an abdominal FNA, ultrasound gel should be carefully removed from the patient because the gel is observed microscopically as granular magenta debris (Fig 1). This can mimic necrotic tissue and mast cell granules and can also lead to

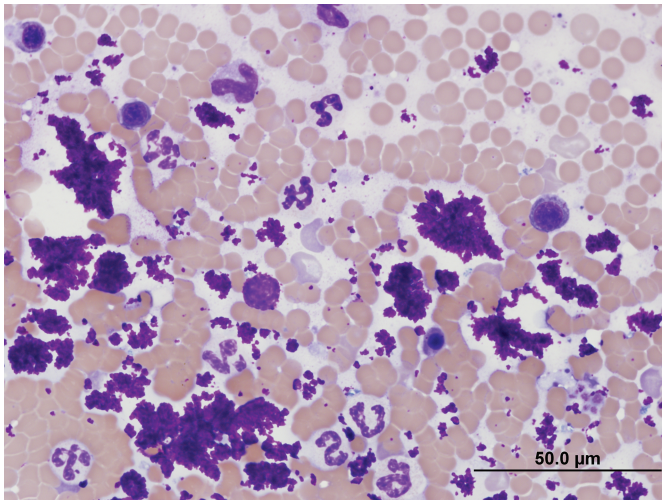

FIG 1. The magenta granular material is an ultrasound gel artefact

cell lysis and swelling that can hinder cytological interpretation (Raskin & Meyer 2015).

Needle size

The term 'fine needle aspiration' is reserved for needles smaller than 20 G or with an outer diameter of less than 1·0 mm (Livraghi *et al.* 1983, De Rycke *et al.* 1999). Disadvantages of these smaller-gauge needles are the flexibility, which can cause slight bending during sampling of deep-seated lesions, and the small diameter of the needles, which makes them more difficult to see during ultrasonography (Hager *et al.* 1985, De Rycke *et al.* 1999).

In veterinary practice, FNA is most commonly performed using a 20 to 27 G needle and a 6 to 12-mL syringe (Wypij 2011). Obtaining a diagnostic FNA from abdominal organs in smaller animals can generally be accomplished with a 2·5 to 3·8 cm needle, whereas a 6·3 to 8·9 cm spinal needle may be required for deeper internal organs or for larger animals (Raskin & Meyer 2015).

There have been many studies that aimed to determine the optimum needle gauge for FNA. In humans, 23 G needles are considered less painful than 21 G needles for sampling both head and neck (Brennan *et al.* 2007) and breast lesions (Daltrey & Kissin 2000). In a study of 100 people, there was no significant difference between needle size and diagnostic yield (Brennan *et al.* 2007). With extrapolation to veterinary medicine, the results of these studies indicate that a smaller needle gauge should be considered if the patient is likely to experience discomfort (*i.e.* is not sedated with an analgesic).

A human study by Dähnert *et al.* (1992) compared four different types of needles, all of similar gauge but with a slightly different internal diameter. The 20 G trephine, 20 G slotted, 19.5 G cut biopsy and 20 G spinal needle were compared, using a total of 551 needles for abdominal organ tissue aspiration. Although the needles were of similar gauge, the internal diameter did vary slightly (by ≤0·2 mm). Interestingly, the needle with the largest internal diameter (cut biopsy needle) yielded the largest proportion of specimens that were considered insufficient for evaluation. These results support the previous study findings that larger gauge needles do not necessarily increase the likelihood of obtaining a diagnostic sample (Dähnert *et al.* 1992).

However, other studies have refuted this, instead suggesting that larger needle diameter produced greater tissue yields (Pagani 1983, Haseler *et al.* 2011). Pagani (1983) found that, in human patients, 18 G needles retrieved a larger amount of tissue than 22 G needles when used in the aspiration of hepatic lesions using CT guidance and thus allowed the evaluation of a greater number of cells. It should be noted that CT-guided FNA is rarely used in veterinary patients for abdominal organ aspiration and is usually only reserved for lesions inaccessible by ultrasound. In Pagani's study, diagnostic accuracy was 98% when using the 18 G needle, compared to only 84% with the 22 G needle.

The correlation between increased blood loss and needle size has been evaluated in pigs. Larger needles did produce more bleeding but the differences were minimal, except when comparing needles ≤16 G with needles ≥18 G. In the kidney, no significant difference was noted between the use of 18, 20 and 22 G needles (Gazelle *et al.* 1992).

Aspiration technique

Ever since the non-aspiration technique was first described by Zajdela *et al.* (1986), many studies have evaluated aspiration *versus* non-aspiration techniques in an attempt to come to a consensus about which is preferable. A major advantage of the non-aspiration technique is reduced blood contamination when sampling highly vascular tissues, such as the spleen (Leblanc *et al.* 2009), kidney and liver (Savage *et al.* 1995, Raskin & Meyer 2015). Non-aspiration has been found to have similar or greater sensitivity to that of aspiration for a variety of tissues, including the spleen and abdominal lymph nodes (Menard & Papageorges 1995, Savage *et al.* 1995, Wallace *et al.* 2001, Sigstad *et al.* 2004, Leblanc *et al.* 2009, Sajeev & Siddaraju 2009, Jahromi *et al.* 2015). In contrast, other studies have shown an increased diagnostic yield in the liver (Haseler *et al.* 2011) and pancreas (Kinney *et al.* 1993) when using aspiration as opposed to non-aspiration techniques. In a study by Savage *et al.* (1995) that examined 95 abdominal FNA sites in humans, more diagnostic specimens were achieved when using aspiration in the pancreas and kidney, although non-aspiration showed greater diagnostic capabilities when sampling abdominal lymph nodes and pelvis masses.

If the aspiration technique is preferred, optimal syringe size should be considered. One must take into account technical control when using different-sized syringes, as well as the ideal aspiration force. Haseler *et al.* (2011) found that syringe size has a potent effect on the control of the needle, with experienced operators showing improved control when using smaller syringes than larger ones. Larger syringes limited the ability to maximally retract the plunger and create the full vacuum potential. When comparing the force capabilities of different-sized syringes, each point generated identical vacuum at the same volume displacement of the plunger. This has important implications in practice;

if the plunger of the 20 mL syringe is used to only displace the plunger to the 5 mL mark, there is an inherent disadvantage to using the 20 mL syringe compared to the 5 mL syringe as the smaller device will enable improved technical control. Some radiologists routinely attach an intravenous extension set to the needle in order to allow freedom of movement during aspiration (Menard & Papageorges 1995).

When using the aspiration technique, recommendations regarding the degree of suction to apply during FNA are quite variable, with reports of 'a few' millilitre of vacuum (Raskin & Meyer 2015) or one half to three fourths of the syringe (Barger 2014).

Once the needle has entered the lesion, it should be moved forward and backward three to four times in short, rapid strokes in a swift cutting motion (Raskin & Meyer 2015). Titoria *et al.* (2010) found that directing the needle in a multi-plane fashion yields significantly more tissue than single-plane yields. Using a 'coring' motion does not appear to influence tissue yield as the sample is acquired by the cutting action of the needle and is maintained in the needle core by forward motion and capillary tension (Titoria *et al.* 2010).

A 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 (Dähnert *et al.* 1992). Therefore, slides from at least four or five aspirates should be submitted from each lesion sampled. If a necrotic centre is suspected, samples should also be obtained from the margin of the lesion (Raskin & Meyer 2015). One or two slides should be stained and briefly examined to ensure they are adequately cellular before submitting to the referral laboratory (Skeldon & Dewhurst 2009, Wypij 2011). This allows for repeat FNA to be performed if the samples are inadequate (Barger 2014). If the smears appear excessively thick or if very little material is apparent, additional slides should be made.

SPECIMEN PREPARATION

Once the needle has been removed from the tissue, smears should be made without delay. A delay can lead to blood clotting within the hub, which makes expression of the specimen difficult (Mcgrath *et al.* 2008), and can also affect the ability to smear the material, which may lead to poor cell preservation.

Smears

Several smear preparations have been described in the literature, although there is scope for more case control studies. 'Shooting' the contents from a distance is not recommended as this will result in many small drops that tend to dry out before they can be spread. Instead, the plunger should be rapidly advanced to express a small droplet of the sample (2 to 3 mm diameter) onto the slide. This should be performed quickly to prevent drying of the sample (Menard & Papageorges 1995) (Fig 2).

There are several methods described to smear the aspirated material, with the aim being to achieve a monolayer of well-preserved cells. The following are descriptions of the 'squash prep', the 'blood smear', the 'line smear' and the 'starfish prep' methods.

1. The 'squash prep' is used for semi-solid material. The 'spreader' slide is placed on the specimen at right angles. The drop of material should spread only from the weight of the top slide, and downward pressure is not required. As the specimen is spreading, the top slide is gently drawn over the length of the first (Lastra *et al.* 2015) (Fig 3).

Disadvantages of the squash prep include the potential for the smear to be too thick for diagnosis, and the slight downward pressure can lead to squashing of the sample, causing cell rupture and a non-diagnostic preparation (Barger 2014).

FIG 2. A small droplet of a sample is applied to the slide

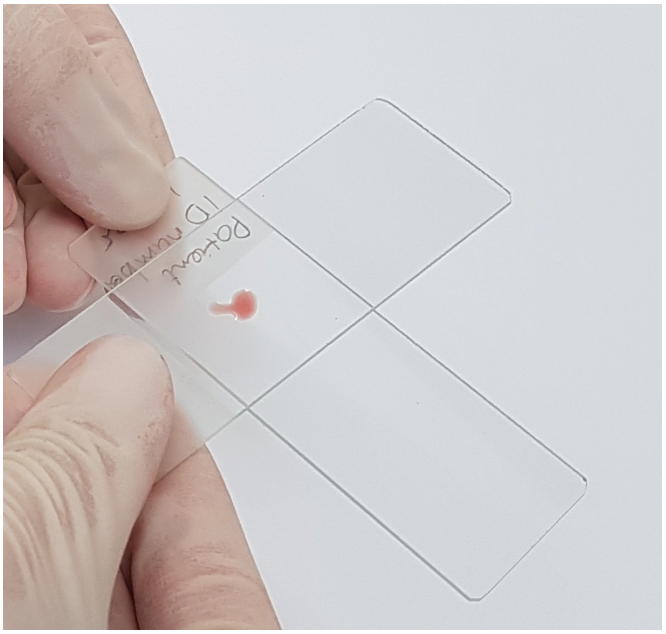

FIG 3. 'Squash prep' technique: the spreader slide is placed on the specimen at right angles

2. The 'blood smear' technique is recommended for bloody or serous samples (Barger 2014). The short edge of the slide is placed onto the flat surface of the sample slide. The spreader slide is then tilted to an angle of 45° and pulled backwards about one third of the way into the material (Fig 4).

Once the material has dispersed along the spreader slide, the slide is smoothly slid forward in one rapid motion. The finished smear should have a feather-shaped area, which is indicative of a monolayer (Fig 5).

3. The 'line smear' is recommended for serous samples with low cellularity (Barger 2014). This is very similar to the blood smear technique, except that the spreader slide is abruptly stopped and lifted off the specimen slide approximately 1 cm before reaching the end of the slide (Fig 6).
4. 'Starfish preps' involve spreading the sample peripherally with a needle tip (Sirois 2012). Many areas of the smear will be too thick for evaluation, and therefore, this method is not frequently used, and we do not recommend this technique (Fig 7).

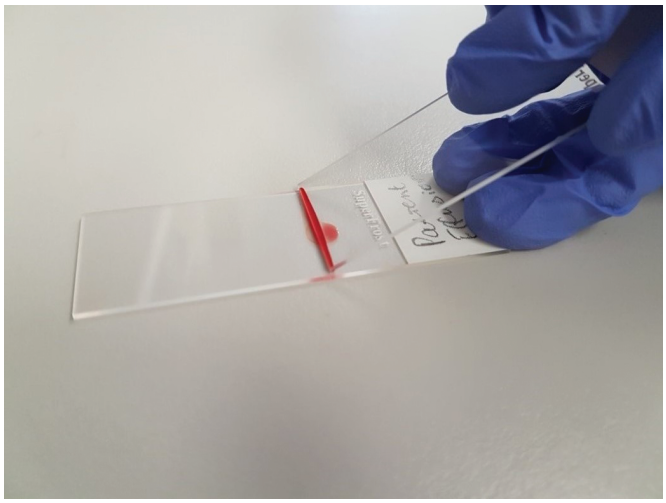

FIG 4. The 'blood smear' technique: the spreader slide is pulled into the material before spreading

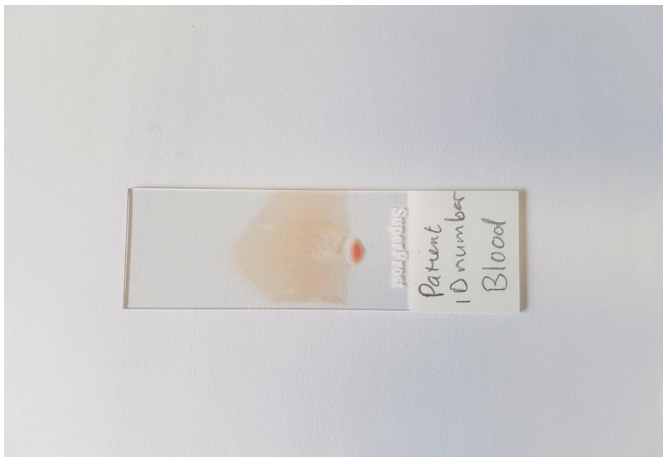

FIG 5. Blood smear technique showing a 'feathered edge'

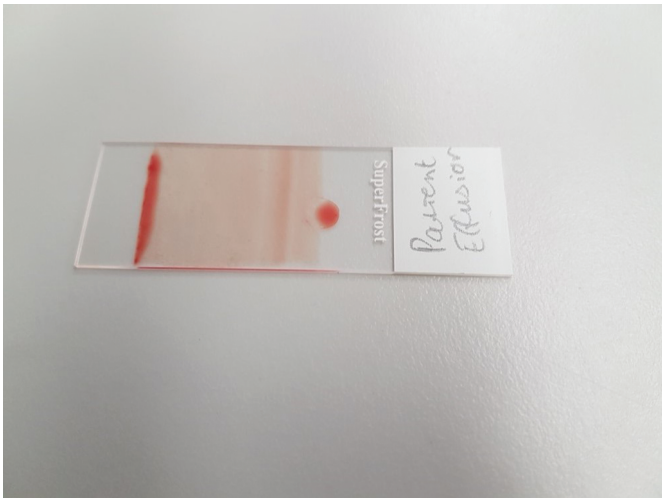

FIG 6. 'Line smear' technique

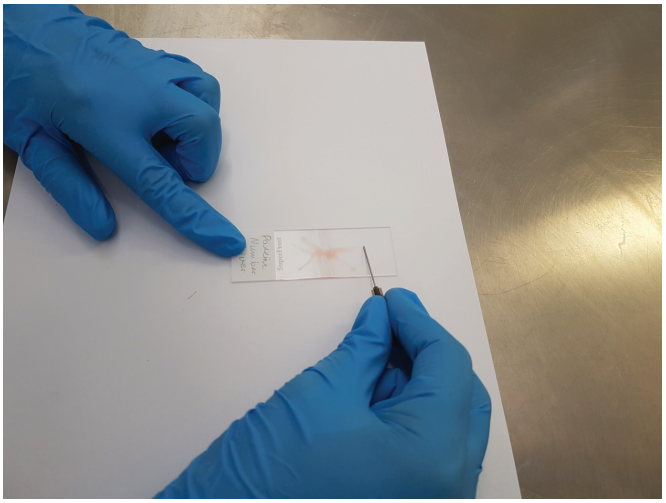

FIG 7. 'Starfish' technique

Staining

Heat fixing before staining is not recommended because it is likely to damage cell morphology (Raskin 2005). Air drying is the preferred method. Baig *et al.* (2006) found that the use of a small hand-held fan can reduce the average time needed for air drying and does not cause a reduction in stain quality.

Papanicolaou stains are commonly used in human laboratories, and Romanowsky variants are used in most veterinary laboratories, whilst Diff-Quik (a modified Romanowsky stain) is frequently used in clinics (Silverman & Frable 1990, Jörundsson *et al.* 1999). Romanowsky stains have advantages over Papanicolaou stains, including excellent depiction of cytoplasmic detail and superior visibility of bacteria, mucin and colloid, whilst limitations of Romanowsky stains include reduced nuclear and nucleolar detail (Jörundsson *et al.* 1999). Mast cell granules are not always easily seen when using Diff-Quik (Jackson *et al.* 2013).

Recommendations for Diff-Quik staining techniques in the literature suggest at least 2 minutes or 20 rapid dips in alcohol, 1 minute or 20 rapid dips in stain I and 45 seconds or 20 rapid dips in stain II (Jörundsson *et al.* 1999). In our experiences, this rec-

ommendation appears excessive and may lead to an over-stained specimen, which could hinder diagnosis. The method can be adjusted based on the thickness of the tissue and freshness of the stain, but six to eight 1-second dips in stains I and II is usually sufficient. Dipping, rather than passive immersion, enhances staining and reduces the time required in solution (Jörundsson *et al.* 1999). After staining, the slide should be gently rinsed for 20 seconds using room temperature water and be allowed to dry in a vertical position (Raskin & Meyer 2015). Avoid blotting as this can lead to removal of cell material.

The specimen should be examined with the 10× or 20× objective to assess staining quality and cell exfoliation/preservation before further examination or submission for pathologist evaluation (Raskin & Meyer 2015) (Figs 8 and 9). If staining is insufficient, slides can be stained for longer in the Diff-Quik solutions if immersion oil has not yet been applied to the slides.

The importance of assessing slides before submission for a pathologist's evaluation cannot be overemphasised. In one survey of 74 predominantly general practitioner veterinarians, Skeldon & Dewhurst (2009) found participants indicated that, on average, 51% of cytological samples were sent to an external laboratory without previous in-house evaluation. Of the total submitted, 19% of samples were considered to be of unacceptable quality to obtain a diagnosis, mostly due to low cellularity (Skeldon & Dewhurst 2009). It would follow that in-house examination would reduce the quantity of non-diagnostic slides submitted to referral laboratories. During human renal FNA evaluation, the number of needle passes and microscopic slides examined did not correlate with the adequacy or diagnostic yield of the sample. Instead, sample adequacy correlated with clinician determination of an adequate sample yield using Diff-Quik staining (Andonian *et al.* 2008) (Figs 8 and 9).

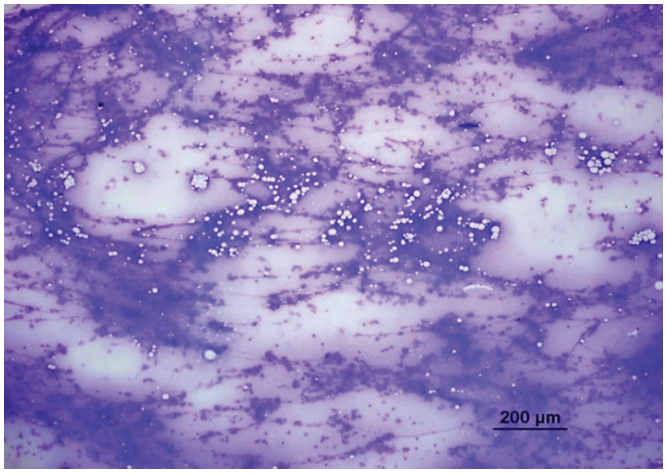

FIG 8. A non-diagnostic-quality lymph node aspirate ×10. It is highly cellular but has poor cell preservation

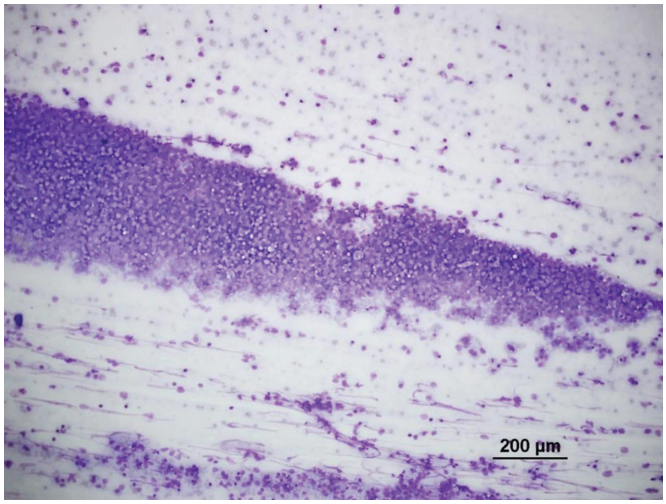

FIG 9. A diagnostic-quality lymph node aspirate ×10. It is highly cellular with good cell preservation

SUBMISSION TO THE LABORATORY

All slides should be labelled correctly (name of patient, owner and collection site) with pencil because ink will dissolve during the staining process (Mcgrath *et al.* 2008, Barger 2014). Formalin-fixed tissues should be placed in a separate bag because formalin vapour exposure often renders smears non-diagnostic (Wypij 2011, Raskin & Meyer 2015). Slides should also be protected by placing them in slide mailers and keeping separate from fluid submissions in case of leakage within the submission bag.

When sending fluid to the laboratory for testing, containers should be selected based on tests requested. Ethylenediaminetetraacetic acid (EDTA) prevents clot formation and allows accurate cell counting. Under-filling the tube may lead to haemolysis and cell damage; therefore, an adequate sample should be placed in the tube to reach the 'fill line' (Tri Ratnaningsih & Gumilang 2006). EDTA can be bacteriostatic or bactericidal and so is unsuitable for culture specimens (Banin *et al.* 2006). Placing fluid in a plain serum tube allows for biochemistry testing such as bilirubin, creatinine, triglycerides and cholesterol. A plain sterile tube should be used for culture and sensitivity testing. Avoid serum tubes with a clot activator or gel because this causes cell disruption and precipitates.

A direct or line smear of any fluid sent for analysis should accompany the sample as this will allow cell morphology to be preserved for at least several days even without fixation (Barger 2014) (Fig 10).

Immunocytochemistry (ICC) can be performed on cytological samples to determine the lineage of cells through detection of expression of a specific cellular protein or antigen (Fisher *et al.* 1995). This is useful in assisting with the determination of tumour type, including differentiating between T- and B-cell lymphomas. Several slides containing many entire, well-preserved cells are required to perform ICC stains. Polylysine-coated slides should be used if ICC may be required because this special coating helps to ensure cells bind to the slide throughout processing (Huang *et al.* 1983). Although Polylysine slides are slightly more expensive than conventional slides, the ability to immunophenotype neoplastic lesions, and thus potentially guide prognosis and treatment from a single sample submission, likely justifies the small extra outlay (Huang *et al.* 1983).

When cytology fails to confirm a diagnosis of lymphoma, more advanced techniques such as flow cytometry or PCR for lympho-

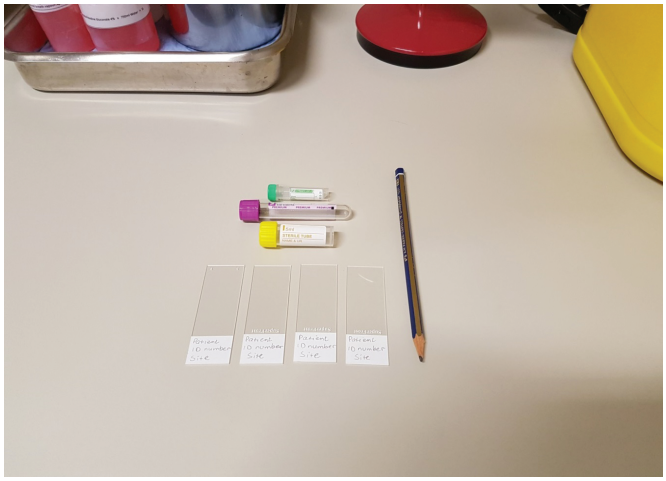

FIG 10. Recommended set-up for ultrasound-guided fine needle aspiration

cyte antigen receptor rearrangement (PARR) may be required. Flow cytometry uses fluorescent-labelled antibodies to evaluate the expression of phenotypic markers, which provide rapid identification and quantification of a large number of cells. Flow cytometry can be performed on FNA specimens and has been shown to be an accurate method for classification of cell lineage for abdominal organ samples in lymphoma patients (Lastra *et al.* 2015, Guzera *et al.* 2016). However, this method requires at least $6{\times}10^6$ cells to be mixed with serum or cell preservative and placed in a plain serum tube, and is ideally analysed within 24 hours of collection, although satisfactory results have been achieved in human samples that were several days old (Sigstad *et al.* 2004, Colorado State University 2016a,b, Guzera *et al.* 2016).

PARR is used to evaluate the clonality of a lymphoid population, which can help to differentiate reactive lymphoid tissue from lymphoma. It can be performed on many sample types, including tissue, blood, bone marrow, effusions, cerebrospinal fluid (CSF) and FNA samples (Burnett *et al.* 2003, Keller *et al.* 2016). Aspirating a site at least three times and expelling the material into physiological saline and rinsing the syringe each time with saline should give sufficient material for analysis (Burnett *et al.* 2003). Smear preparations that have already been dried and stained can also be used for PARR, although at least 50,000 cells are required (usually four or five highly cellular smears) (Colorado State University 2016b).

A CLOSER LOOK AT SAMPLING SPECIFIC ORGANS

Liver

The successful diagnosis of liver lesions using FNA compares poorly to FNA in other locations (Cohen *et al.* 2003). Wang *et al.* (2004) described ultrasound-guided FNA of the liver as having 'serious limitations when used to identify the primary disease process in dogs and cats with clinical evidence of liver disease', with cytological findings showing agreement with histopathological results in only 30% of dogs and 51% of cats. Cytological evaluation appears most effective in the diagnosis of fatty change and neoplastic disease and least effective in the diagnosis of inflammatory, dysplastic or hyperplastic conditions (Cohen *et al.* 2003, Bahr *et al.* 2013). As would be expected, greater lesion size correlates positively with a successful diagnosis (De Rycke *et al.* 1999).

Interestingly, in one study, liver FNA specimens had high diagnostic sensitivity (82%) for feline infectious peritonitis (FIP) in cats, which was more impressive than the use of core biopsy (64% Se) (Giordano *et al.* 2005). Nevertheless, the cytological findings of pyogranulomatous inflammation are not specific for FIP and can be seen in many other disease processes.

Bleeding complications encountered during FNA of the liver have been reported but are rare, even with suboptimal coagulation (Gazelle *et al.* 1992, Léveillé *et al.* 1993, Savage *et al.* 1995). In a retrospective study of 85 human patients with coagulation disorders, ultrasound and laboratory results revealed no bleeding episodes after any of the 229 punctures performed (Caturelli *et al.* 1993). The complication rate when performing hepatic FNA in animals is not known, but FNA is generally estimated to be much safer than liver biopsy, with Bigge *et al.* (2001) reporting 11 major complications during 254 liver biopsies performed with 18 G needles in dogs and cats with coagulation deficiencies.

Kidney

FNA of the kidneys is used most commonly as a means of detecting highly cellular neoplasms, such as renal carcinoma or lymphoma, but it may also be used to obtain culture material if an infectious agent is suspected. Hypodermic needles of 22 to 25 G, with a length of 1·5 to 5 cm, are typically used in animals, whilst for large patients, spinal needles may be used (Osborne *et al.* 1996).

In humans, ultrasound-guided aspiration of focal renal lesions is considered to be a highly accurate method of diagnosis with minimal organ trauma (Hager *et al.* 1985). A study by Truong *et al.* (1999) found that 16% of renal FNA samples were classified as unsatisfactory when using a 22 to 25 G needle. Of the malignant lesions, cytological classification of the tumour type was correct in 44 of 46 cases, whilst 2 of 30 cases diagnosed on cytological examination as a benign cyst were actually cystic renal carcinoma and acquired cystic kidney. Of the 11 cases classified as 'suspicious of malignancy', 4 were later deemed malignant (Truong *et al.* 1999).

When assessing 22 cats with FIP for the diagnostic sensitivity of detecting FIP in renal lesions, Giordano *et al.* (2005) found that sensitivity using core biopsy (39%) was similar to FNA specimens (42%).

Using aspiration during renal FNA has proved better than non-aspiration with respect to diagnostic potential, and there was no statistically significant difference between the results when using 18, 20 and 22 G needles in healthy pigs (Savage *et al.* 1995). Complications are less common for renal FNA when compared to renal biopsy (Osborne *et al.* 1996). That being said, for all needles and at all levels of anticoagulation, greater bleeding resulted from renal biopsy when compared to liver biopsy, likely due to the highly vascular and predominantly arterial nature of the renal blood supply (Gazelle *et al.* 1992).

Spleen

FNA of the spleen is particularly useful in the diagnosis of infiltrative disorders, such as diffuse neoplasia. However, aspiration of cavitated or fluid-filled lesions, such as haematomas or haemangiosarcomas, may be inconclusive because of blood dilution (D'anjou & Penninck 2015).

Due to the high vascularity of the spleen, there tends to be a pervading fear of complications such as acute haemorrhage or splenic rupture during FNA. However, splenic aspiration is performed frequently at our institution, accounting for approximately 25% of all abdominal organ FNA submissions in 2016 (unpublished data). Major complications are rare in human literature, with large-scale studies indicating a major complication rate of less than 1% (Silverman *et al.* 1993, Keogan *et al.* 1999, Civardi *et al.* 2001, Cavanna *et al.* 2007, Gochhait *et al.* 2015). Large-scale veterinary studies are scarce, but there are many published case series with no serious complications encountered during splenic FNA (Léveillé *et al.* 1993; Watson *et al.* 2011), even in 14 thrombocytopenic animals (O'keefe & Couto 1987). However, thrombocytopenia does appear to contribute to the complication rate during core biopsy of the spleen using 18 G needles (Bigge *et al.* 2001).

Human studies have demonstrated that a definitive diagnosis could be confirmed in 88 of 130 (68%) cases using splenic FNA, with results improving with greater operator experience (Civardi *et al.* 2001, Gochhait *et al.* 2015).

In a small study by O'keefe & Couto (1987), 14 dogs and cats underwent both splenic FNA and histological evaluation of splenic lesions; all cytological diagnoses correlated well with their final histological diagnoses. However, other studies show a poorer level of accuracy when performing splenic FNA. In 32 dogs and cats with splenic lesions, there was a diagnostic correlation of 61% between cytological and histological interpretation (Ballegeer *et al.* 2007). In another study of diagnostic samples from 35 dogs, cytopathological and histopathological diagnoses agreed completely in 18 of 35 dogs (51%), partially in 3 of 35 dogs (9%) and were in disagreement in 14 of 35 dogs (40%) (Watson *et al.* 2011). Non-aspiration techniques appear to be superior during FNA of the spleen in both cats and dogs because they produce samples of greater cellularity and less blood contamination (Leblanc *et al.* 2009).

Pancreas

In humans, endoscopic, ultrasound-guided FNA of the pancreas is frequently utilised, especially in the diagnosis of pancreatic neoplasia, and although this method has been described in animals, ultrasound-guided FNA is a more common technique (Gaschen *et al.* 2003, Gaschen *et al.* 2007, Zamboni *et al.* 2009, Kook *et al.* 2012, Lewitowicz *et al.* 2012). In Bonfanti's report of 102 FNA samples submitted from abdominal masses, only one was from the pancreas (Bonfanti *et al.* 2004). At our institution, 17 pancreatic FNA samples were submitted in 2016, of which five were considered non-diagnostic (unpublished data).

In a large-scale human study, ultrasound-guided pancreatic FNA samples were diagnostic in 509 of 545 cases (93·4%), although it should be noted that these procedures benefited from an on-site cytopathologist, which allowed immediate assessment of sample adequacy (Zamboni *et al.* 2009). If the 36 non-diagnostic samples were excluded, the procedure achieved 99·4% sensitivity, 100% specificity and 99·4% accuracy, with no major complications observed.

In a large-scale veterinary study, pancreatic FNA samples obtained via both non-aspiration and aspiration methods from 94 dogs undergoing pancreatic FNA for clinical diagnostic evaluation were reviewed. A total of 25 samples (27%) were non-diagnostic, predominantly because of low cellularity. Cytological and histological findings agreed in 10 of 11 cases (91%). Clinical complications following pancreatic FNA in this case series were considered uncommon (6·3%). The majority of dogs with complications had concurrent diagnostic procedures performed and significant comorbidities, and in most cases, pancreatic FNA was considered unlikely to be the primary cause of the complications (Cordner *et al.* 2015). Interestingly, in another study by Cordner *et al.* 2010, 20 healthy dogs had ultrasound-guided pancreatic FNA performed, which revealed that none of the 20 samples had sufficient cellularity to be considered diagnostic quality. Ultrasound-guided FNA fared much worse than intraoperative aspirates (67% of diagnostic quality) and touch impressions from biopsy samples (78% of diagnostic quality). The authors of this study suggested two possible reasons for these findings: the poor cellular samples obtained via ultrasound-guided FNA of normal pancreatic tissue may be poorly exfoliative due to a tightly associated fibrovascular network when compared to diseased organs, as well as limited operator experience during FNA sampling. A more encouraging finding of this study concluded that ultrasound-guided FNA did not lead to significant increases in serum concentrations of pancreatic enzymes, suggesting minimal trauma to the pancreas (Cordner *et al.* 2010).

In a study involving 73 cats with pancreatic disease, 24 of 73 (67%) of the cytological samples obtained via both aspiration and non-aspiration ultrasound-guided FNA of the pancreas were diagnostic. No cats developed complications within 48 hours of the pancreatic FNA procedure, and there was no difference in complication incidence or survival to discharge when compared to cats with pancreatic disease that did not undergo pancreatic FNA. Pancreatic cytology and histopathology were in agreement in seven of the nine cases (Crain *et al.* 2015).

Bladder

UCC is easily the most common bladder tumour in both dogs and cats (Osborne *et al.* 1968, Norris *et al.* 1992, Mutsaers *et al.* 2003, Meuten 2016) and is one of the most malignant tumours in domestic animals, with only 16% of treated dogs surviving for one year or more and metastases present in 50 to 90% of dogs and 50% of cats at necropsy (Norris *et al.* 1992, Meuten 2016).

Options for collection of cytological samples from bladder masses include urine cytology, traumatic catheterisation, cystoscopy and ultrasound-guided FNA. The quality of samples obtained via urine cytology or traumatic catheterisation varies (Fulkerson & Knapp 2015), and urine samples are reported to be poorly sensitive for the diagnosis of neoplasia, with malignant cells detected in only 29 of 96 (30%) urinary sediments in dogs with bladder tumours (Norris *et al.* 1992, Raskin & Meyer 2015).

Bladder FNA is considered a relatively reliable method of diagnosing bladder tumours in both cats and dogs (Walker *et al.* 1993, Wilson *et al.* 2007, Wypij 2011). Bladder tumours were successfully diagnosed by FNA in 20 of 22 (91%) dogs and in 10 of 13 (77%) dogs using urethral wash methods (Norris *et al.* 1992). In a study by Bonfanti *et al.* (2004), eight of nine canine and feline urinary bladder FNAs correlated well with histopathological diagnoses, and included various types of bladder neoplasms and inflammatory conditions, with one of nine cases proving to be non-diagnostic.

Despite the reliability of FNA in the diagnosis of bladder masses, there is some hesitance in performing the procedure because of the perceived risks of tumour seeding. Abdominal wall metastasis following FNA and core biopsy and surgical implantation associated with cystotomy and laparotomy has been reported in dogs, although some of these cases had concurrent evidence of other metastatic disease, such as involvement of lungs and lymph nodes, at diagnosis, in accordance with the highly aggressive nature of these tumours (Nyland *et al.* 2002, Higuchi *et al.* 2013). In a study of 544 UCC cases at one institution, 24 cases of abdominal wall metastasis were identified (4·4% of UCC cases), including 18 associated with cystotomy or laparotomy (3·3%); 2 cases associated with FNA of the bladder or prostate (0·36%); and 4 cases with no evident inciting cause, including 1 case with evidence of transmural spread of the tumour (0·72%) (Higuchi *et al.* 2013). In another report of 12 dogs with cutaneous metastasis of UCC without abdominal muscle involvement, all dogs had evidence of other sites of metastasis at diagnosis; most had evidence of lymphatic invasion, suggesting lymphatic spread was a potential mechanism for the cutaneous metastases, and interestingly, 11 of these cases had metastases in close proximity to the vulva. The authors proposed the implantation of neoplastic cells from urine into erosions of urine-scalded skin as another potential mechanism for metastasis (Reed *et al.* 2013). In our opinion, the uncommon to rare risk of needle tract metastasis associated with this tumour is outweighed by the ability to make a rapid, minimally invasive, cytological diagnosis through FNA cytology, although the risk should be discussed with the owner because once UCC becomes established in the abdominal wall and clinically detectable at that site, it appears that medical treatment is rarely effective (Higuchi *et al.* 2013).

Prostate

Disorders of the prostate gland occur commonly in older dogs. Although various methods of obtaining prostatic samples have been described, including prostatic wash, semen evaluation and urine sediment examination (Barsanti & Finco 1984, Raskin & Meyer 2015), preparations obtained by these methods typically include contaminants from the reproductive and urinary tract, and so, direct FNA is considered a superior method of collection (Thrall 1985, Cowell & Tyler 2002). Indeed, at our institution, all prostatic cytological samples submitted to the laboratory in 2016 (n=17) were obtained via ultrasound-guided FNA (unpublished data).

Apart from the possible complications associated with needle tract seeding, other reported contraindications include the potential for contamination of the abdominal cavity, leading to septic peritonitis or bacteraemia following FNA of prostatic abscesses (Thrall 1985, Raskin & Meyer 2015). However, drainage of prostatic abscesses has been described in 53 dogs without any post-operative complications and can provide a minimally invasive method of diagnosis and an important adjunct to treatment (Bussadori *et al.* 1999, Boland *et al.* 2003).

Of 25 cytological samples collected by various methods from the canine prostate, including 16 by ultrasound-guided FNA, the primary diagnoses consisted of the following: benign prostatic hyperplasia (BPH) (n=8), neoplasia (n=8), inflammation (n=4), non-diagnostic (n=2), squamous metaplasia (n=2) and prostatic cyst (n=1) (Powe *et al.* 2004). Another study found similar proportions of diagnoses during cytological examinations of canine prostatic disease (Thrall 1985).

Ultrasound-guided prostatic FNA samples were concordant with the final diagnosis in 12 of 16 cases in one study (Powe *et al.* 2004) and 11 of 12 samples in another (Thrall 1985) and showed a sensitivity of 77% and specificity of 100% for the diagnosis of BPH and 100% sensitivity/specificity for the diagnosis of chronic prostatitis in a study of 16 dogs (González 2010). Interestingly, in the study by Powe *et al.* (2004), there was good agreement between prostatic massage/wash samples and histological diagnosis, with eight of nine samples showing concordance; however, in the study by Thrall (1985), FNA (n=15) was found to produce greater yields and more reliable results than prostatic wash (n=11).

Cytology was found to be a more sensitive method for the diagnosis of septic prostatitis (n=5) than biopsy (Powe *et al.* 2004).

Abdominal lymph nodes

The results of ultrasound-guided FNA from various lymph nodes in 179 human patients were obtained from samples that were sufficient for cytological analysis in 97% of cases. Correct benign and malignant cytological diagnoses were achieved in 97% (33/34) of deep abdominal and retroperitoneal lesions (Tikkakoski *et al.* 1991).

Many studies have demonstrated increased diagnostic capabilities using a non-aspiration technique in lymph nodes because suction often produces a haemodiluted sample. In 34 human patients, an average of 2·4 needle passes (range 1 to 5) were used for abdominal and pelvic lymph nodes, with a correct specific diagnosis made in 86% of cases (Fisher *et al.* 1997).

In a study of 1274 canine lymph node aspirates from various locations, the median number of slides submitted per patient following lymph node aspiration was three, and in dogs, the number of slides was significantly associated with an increased probability of obtaining both a diagnostic specimen and a cytological diagnosis (Amores-Fuster *et al.* 2015). In feline samples, the association was not significant, although this may be due to a smaller sample size (Amores-Fuster *et al.* 2015). Of the canine samples, 73% were diagnostic, and of the 199 feline lymph nodes aspirates evaluated, 86% were considered diagnostic. The most common reasons for a non-diagnostic sample included the absence of nucleated cells, cell disruption and a low yield (Amores-Fuster *et al.* 2015). It is important to note that the lymph node samples in this study were not specifically from the abdomen, and in our experience, obtaining samples from abdominal lymph nodes is more likely to be non-diagnostic compared with subcutaneous

lymph nodes. Review of lymph node submissions to our institution in 2016 found that 30% of 64 abdominal lymph node samples were non-diagnostic, compared with 23% of 138 lymph node samples submitted from other anatomic locations (unpublished data). Obtaining a minimum of three non-aspiration FNAs is therefore recommended for each abdominal lymph node sampled, and ideally more. We also recommend extra care when extruding aspirated material onto slides and when making smears to avoid rupture of the cells because lymphocytes are fragile, particularly neoplastic lymphocytes.

The most common diagnosis from canine lymph node FNAs is lymphoma (27·5%) (Sapierzyński & Micuń 2009, Amores-Fuster *et al.* 2015), whilst in cats, reactive hyperplasia is most commonly diagnosed (32%) (Amores-Fuster *et al.* 2015).

CONCLUSION

Ultrasound-guided FNA of abdominal organs can provide a minimally invasive assessment of underlying disease, with a low complication rate. Although a final diagnosis may not be possible for all lesions by cytology alone, it can be used as a tool to help narrow the differential diagnosis list, thus guiding treatment and prognosis, and is therefore an invaluable diagnostic tool for veterinary clinicians. The use of optimal techniques to obtain the highest-quality FNA samples will increase the chance of obtaining a diagnostic quality sample.

Conflict of interest

No conflicts of interest have been declared.

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