Cell blocks derived from fine-needle aspirates of tumors as an alternative to surgical biopsies in cats and dogs

Celblokken verkregen via fijnenaaldaspiraten van tumoren als alternatief voor chirurgische biopsieën bij katten en honden

[1]L. Dunand, [1,2,3]H. De Cock, [1,3]H. de Rooster

[1]Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium.
[2]Zoolyx Laboratory, Groeneweg 17, B-9320 Aalst, Belgium
[3]Cancer Research Institute Ghent (CRIG), Medical Research Building, University Hospital Ghent, 9000 Ghent, Belgium

dunand.lucile@hotmail.fr

ABSTRACT

Cytology and histopathology are commonly used in the diagnosis of suspected neoplasia in companion animals. A prospective study was performed to evaluate the added diagnostic value of the cell block (CB) technique applied to fine-needle aspirate biopsy (FNAB) samples from dogs and cats with suspected neoplastic masses, compared with conventional cytology and histopathology. The study included 41 masses excised from 39 animals (35 dogs, 4 cats). Fine-needle aspirate biopsy samples were collected ex vivo for cytology and CB preparation before routine histopathologic examination, which served as the reference standard. Cytology samples were completely or partially diagnostic in 29/41 cases (70.7%), whereas CB analysis was completely or partially diagnostic in 34/41 (82.9%). The CB aided the diagnosis in 15/20 cases that were non-diagnostic or only partially diagnostic based on cytology samples. When the sample was sufficiently cellular (34/41), CB diagnoses were consistent with histopathology in 94.1% (32/34) of cases. Combining cytology and CB increased the overall diagnostic yield to 90.2% (37/41).

In this study, the CB technique substantially enhanced the diagnostic yield of FNAB, and combined with cytology, may reduce the need for more invasive surgical biopsies in selected cases.

SAMENVATTING

Cytologie en histopathologie worden vaak ingezet in de diagnostiek bij gezelschapsdieren wanneer neoplasie wordt vermoed. In deze studie werd de toegevoegde diagnostische waarde van de celblok (CB) -techniek, toegepast op monsters van fijnenaaldaspiraten bij honden en katten met een vermoedelijk neoplastische proces, geëvalueerd en vergeleken met conventionele cytologie en histopathologie. Het onderzoek omvatte 41 massa's, chirurgisch verwijderd bij 39 dieren (35 honden, 4 katten). Ex vivo werden fijnenaaldaspiraten afgenomen voor cytologie en CB-preparatie, voorafgaand aan de routinehistopathologie die als gouden standaard fungeerde. Het cytologisch onderzoek was volledig of gedeeltelijk diagnostisch in 29 van de 41 gevallen (70,7%), terwijl de CB-analyse volledig of gedeeltelijk diagnostisch was in 34 van de 41 gevallen (82,9%). De CB verbeterde de diagnostische informatie in 15 van de 20 gevallen, waarbij de cytologie geen of slechts een gedeeltelijke diagnose opleverde. Wanneer er voldoende celmateriaal aanwezig was (34 van de 41 stalen), kwamen de CB-diagnoses in 94,1% (32 van de 34) van de gevallen overeen met de histopathologie. Door cytologie en CB te combineren kon bij 90,2% (37 van de 41) een correcte diagnose worden gesteld.

De CB-techniek verbeterde de diagnostische prestaties van fijnenaaldaspiraten aanzienlijk en kan, in combinatie met cytologie, in bepaalde gevallen de noodzaak van meer invasieve chirurgische biopsieën vermijden.

INTRODUCTION

Fine-needle aspirate biopsy (FNAB)-based cytology is a well-established first-line diagnostic tool for (sub)cutaneous masses as well as for ultrasound-guided sampling of deeper lesions. When performed and interpreted by experienced clinical pathologists, it demonstrates high diagnostic performance, with reported sensitivities ranging from approximately 89% to 94% and specificities from 94% to 98% for distinguishing neoplastic from non-neoplastic lesions, depending on the study and case selection (Ghisleni et al., 2006; Emanuelli et al., 2023). The positive predictive value of cytology has been reported as high as 99.4%, while the negative predictive value is lower, approximately 68.7% (Ghisleni et al., 2006). These findings highlight that, while cytology is highly reliable for confirming neoplasia when positive, false negatives commonly occur, reinforcing the need for complementary diagnostic approaches in selected cases (Ghisleni et al., 2006). Whenever cytology yields a tentative diagnosis of neoplasia, a tissue biopsy is recommended and often required to establish a definitive diagnosis, essential for optimal treatment planning.

In contrast to cytology, histopathology is widely recognized as more informative for accurately identifying tumor type and assessing malignancy. Whereas cytology evaluates isolated cells without structural context, histopathology enables assessment of cellular arrangement and tissue structural relationships. Interpretation of tissue patterns and cellular morphology within their structural context is an essential step in determining the exact nature of a neoplasm (Sharket et al., 2014; Sabattini et al., 2017). The detailed architectural information, potentially combined with protein expression, allows the establishment of a prognosis and guides therapeutic decisions (Avallone et al., 2021). However, compared with FNAB-based cytology, routine histopathology requires surgical biopsy procedures that are more invasive, often requiring deep sedation or general anesthesia, increased clinician time and effort, and higher overall costs (Crosby, 1996; Oikonomidis and Tsouloufi, 2021; Pakdeesaneha et al., 2024).

As an intermediate approach between cytology and histopathology, several studies in human medicine have explored the use of FNAB-derived material for histopathologic evaluation, using the cell block (CB) technique (Nathan et al., 2000; Jain et al., 2014; Sharma et al., 2015; Krogerus and Kholová, 2018). This method consists of concentrating sediment, blood clots, or tissue fragments from FNAB specimens into paraffin-embedded blocks that can be processed similarly to conventional histopathology samples (Nathan et al., 2000; Jain et al., 2014; Krogerus and Kholová, 2018). In human medicine, it has been demonstrated that CB preparations at least partially preserve tissue architecture, facilitate tumor classification, and enable ancillary testing such as IHC. Depending on the specimen type and clinical setting, combining cytology with CB preparations has been reported to increase diagnostic yield by approximately 7 to 55% compared with cytology alone, while also reducing the proportion of nondiagnostic samples (Jain et al., 2014; Vance et al.; 2019; Verma et al.; 2021). Cell block preparations are particularly valuable for poorly differentiated tumors, epithelial neoplasms, cavitary effusions, and minimally invasive sampling procedures such as endobronchial ultrasound-guided FNABs (Yung et al., 2012; Vance et al. 2019; Verma et al.; 2021; Okuno et al., 2022).

In veterinary medicine, the interest in the CB technique has increased in recent years, with reported applications including bone marrow samples (Taylor et al., 2013; Valente et al., 2021), soft tissue sarcomas and mammary masses (Zanoni et al., 2012), gastrointestinal neoplasia (Marrinhas et al., 2021), lymph node aspirates (Sampaio et al., 2023), urine samples (Marcos et al., 2017), and cavitary effusions (Melega et al., 2020) in dogs and cats, among others. In veterinary studies, it has been suggested that CB preparations improve cellular preservation, enhance architectural interpretation, and allow for IHC (Kim et al., 2012; Marcos et al., 2017; Walsh and Patel, 2021; Sampaio et al., 2023), with reported improvements in diagnostic accuracy or tumor classification ranging from approximately 15 to 30%, depending on specimen type and study population (Kim et al., 2012; Çakir et al., 2014; Walsh and Patel, 2021). However, despite the growing interest, the available literature remains substantially more limited in veterinary than in human medicine. Therefore, additional prospective studies in which the diagnostic contribution of CBs are evaluated in neoplasia-suspected processes in companion animals, could accelerate their incorporation in veterinary practice. The aim of the present study was to evaluate the methodology and diagnostic contribution of CB histopathology. The authors hypothesized that combining cytology and CBs would improve the diagnostic yield compared to cytology alone in dogs and cats with different tumor types, making surgical biopsies otiose in 15-30% of the cases.

METHODS

Study population

The study population consisted of a systematic inclusion of cats and dogs with a surgically excised mass, clinically suspected to be neoplastic, presented to the Small Animal Teaching Hospital of Ghent University between November 2023 and August 2024, whenever the first author was present to take the FNABs. The inclusion was independent of the anatomical location or size of the mass, provided that its diameter was at least 0.5 cm and histopathological evaluation was planned.

In-house sample processing

After the surgical removal of the mass, FNAB samples were harvested prior to tissue fixation in 10% buffered formalin (CellPath, Powys, United Kingdom). The FNAB sampling and needle-rinse technique were identical for all cytology- and CB samples and were always performed by the first author. Per FNAB, a 23-gauge needle

was redirected five times within the mass without applying any aspiration. The needle rinse was performed by three expulsions, using a 5-mL air-filled syringe. A fresh needle was used for every FNAB. The first two FNAB samples were used for routine cytologic evaluation, expressed onto two different microscope slides (Marienfeld, Lauda-Königshofen, Germany); smear preparations were prepared immediately after sampling, with prompt fixation and staining with Diff-Quick reagents (Merck, Darmstadt, Germany) to ensure optimal cellular preservation to minimize any potential loss of diagnostic quality. Each pair of slides was identified with an aleatory number only and this number was linked to the basic signalment of the patient, a short anamnesis and diagnostic work-up. They were subsequently stored at room temperature in a protected, labelled box until the end of the study period, after which they were submitted in a single batch to the pathologist.

The needle rinses of the following FNABs were performed by blowing the FNAB samples into an Eppendorf tube (Nerbeplus, Winsen, Germany). In the pilot phase of this study (first 15 cases), two sets of ten FNAB samples were placed in 1 mL of saline (B. Braun, Bruxelles, Belgium), one with and one without one drop of blue dye ink (Klinipath, Duiven, the Netherlands, diluted 1:10 in saline). In the second part of the study, sets of five versus ten FNAB samples were expelled into separate Eppendorf tubes, to which one drop of blue dye ink and 1 mL of 10% buffered formalin were added, to optimize the workflow and shorten processing time. The Eppendorf tubes were coded with an aleatory number, that differed from that on the cytology slides, for subsequent blinded analysis. They were submitted to the laboratory, accompanied by a dedicated research request form revealing only species, sex and age of the patient, and the tumor location.

The tissue specimens, from which the FNABs for cytology and CB originated, were fixed in 10% buffered formalin, the containers were routinely labeled with the patient's details, and a summary of the clinical history was provided, as per standard procedure and submitted to the laboratory.

Sample processing in the laboratory

In the pilot phase of the study, the Eppendorf tubes were centrifuged at 2,500 revolutions per minute (RPM) for ten minutes at arrival to the laboratory, after which the supernatant was discarded. The resulting sediment was then fixed in 1 mL of 10% formalin for 36 hours. Once fixed, the cell pellet was transferred to a small mold to which look warm liquified agar gel (Epredia™ HistoGel™ Specimen Processing Gel) was added. The gel was then allowed to harden for ten minutes at room temperature before it was transferred into a cassette. Those cell pellets were then processed identical to the tissue samples for routine histopathology.

Routine histopathological processing performed by a tissue processor (Excelsior AS Tissue Processor, Epredia, Kalamazoo, MI, USA) consists of several steps of dehydration and impregnation with paraffin wax. The paraffin-impregnated specimens were placed in a metal mold, and molten paraffins were added to form a solid block. A labeled cassette was pressed onto the mold to secure and identify the block. The mold was cooled on a cold plate (4°C) until the paraffin was fully solidified (typically within ten to thirty minutes). Once hardened, the paraffin block was removed from the mold.

Finally, 4 µm thick sections were cut from the paraffin-embedded CB or tissue, using a microtome, mounted on glass slides and stained with hematoxylin and eosin (H&E). When further differentiation of the tumor type was needed, additional immunohistochemistry (IHC) was performed with markers used for standard histopathology (supplementary Table). The immunohistochemical staining technique was performed by the DAKO Agilent Autostainer system, with 3,3'-diaminobenzidine (DAB) as the chromogen on an autostainer (Autostainer Link 48 systems). The method involves the automated detection of specific antigens in formalin-fixed, paraffin-embedded tissue sections using primary antibodies, enzyme-conjugated secondary detection system, and DAB to produce a brown-colored precipitate at the site of antigen expression. The pretreatment was performed on the PT-link system (Agilent) with pH.

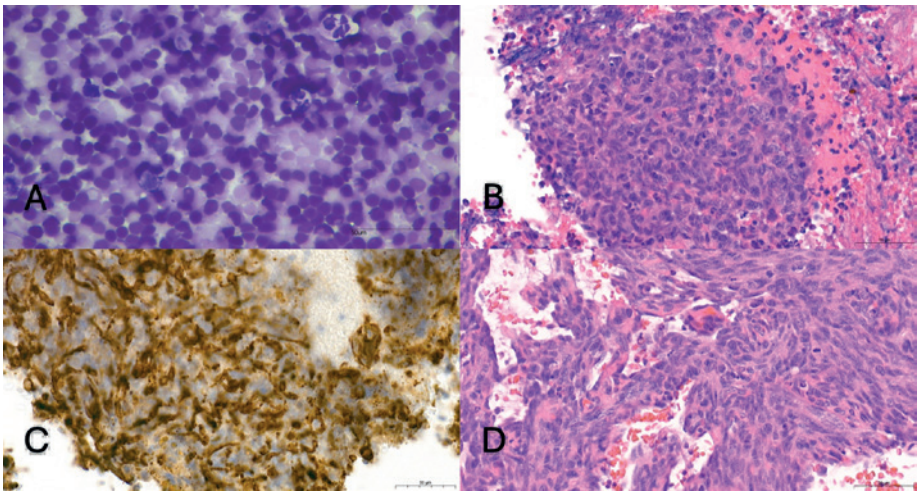

Figure 1. Microscopic images of a splenic mass in a ten-year-old, male intact Spitz (case 12). Cytology: blood contamination, non-diagnostic due to the presence of lyzed cells with naked nuclei (A); Cell block: a cellular neoplastic aggregate composed of poorly defined, collapsed vascular spaces containing occasional RBCs and lined by markedly pleomorphic endothelial cells (B); Cell block after CD31 immunohistochemistry, confirming the endothelial origin of the neoplastic cells in the neoplastic aggregate, consistent with hemangiosarcoma (C); Histopathology: hemangiosarcoma (D). Note that the scale bar of the cytology sample differs from those of the histopathology samples.

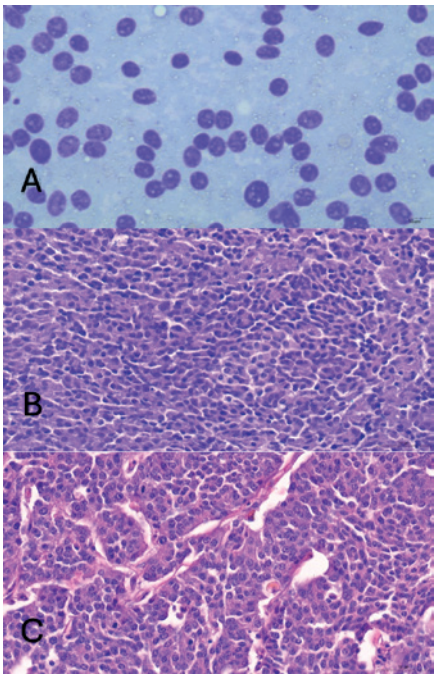

Figure 2. Microscopic images of a perineal mass in an eight-year-old, male castrated, English Cocker (case 22). Cytology: cell-rich smear of poor quality, epithelial neoplasia. The sample contains mostly naked nuclei, although often in a pseudo-acinar pattern with a proteinaceous blue background (A); Cell block: architectural information in cell clusters is preserved; anal sac adenocarcinoma (B); Histopathology: anal sac adenocarcinoma (C). Note that the scale bar of the cytology sample differs from those of the histopathology samples.

Sample analysis

All samples were read by a single anatomic pathologist (ACVP Diplomate), but on three separate occasions. The anonymized CB samples were read once available; always without being linked and prior to the routine histopathology slides from the corresponding gross specimens. The stained and coded cytology samples were provided to the pathologist in a single batch several months after the enrollment of the last dog.

Once all slides of all samples were read, the results of all three diagnostic techniques were assembled by the first author. The final diagnosis based on histopathology, served as the reference standard to evaluate the diagnostic accuracy of the FNAB-based cytology and CBs. Immunohistochemistry was incorporated into the routine diagnostic work-up whenever considered necessary and, in a subset of cases, it was essential for establishing the final diagnosis rather than merely confirming H&E findings.

Diagnoses classified as complete meant that the tumor type as well as its classification as malignant or benign could be fully established; a partial diagnosis provided some diagnostic information (confirmation of neoplasia or indication of cell origin) without enabling a full and definitive classification.

Statistical analysis

All data were quantitatively described and summarized. Since the results were ordinal in nature, nonparametric tests were performed. Whereas the Wilcoxon rank sign test was used to compare the result of five versus ten FNAB samples per CB, the Friedman test with Bonferroni correction for multiple tests was performed to compare the results from the cytology, the CB, and the combination of cytology and CB. The null hypothesis stated that the mean ranks of diagnostic accuracy compared to the histopathology diagnosis of the gross specimen were equal across those techniques.

RESULTS

Study population

Forty-one masses in 39 patients (4 cats and 35 dogs) were included. The first 15 masses were excised in two cats and twelve dogs. The following 26 masses originated from 2 cats and 23 dogs. The cohort included tumors originating from various anatomical locations and tissue origins, including splenic (n = 8), subcutaneous (n = 6), oral (n = 4), cutaneous (n = 4), anocutaneous (n = 4), bony (n = 3), urinary (n = 3), hepatic (n = 3), gastro-intestinal (n = 2), deep soft tissue (n = 2), testicular (n = 1) and pulmonic (n = 1) tissue. These details are included in Tables 1 and 2.

Sample processing and CB technique

No technical issues were encountered during sample collection nor processing. The addition of blue dye ink subjectively improved the lab tech's ability to identify and retrieve cell clusters, without interfering with diagnostic interpretation.

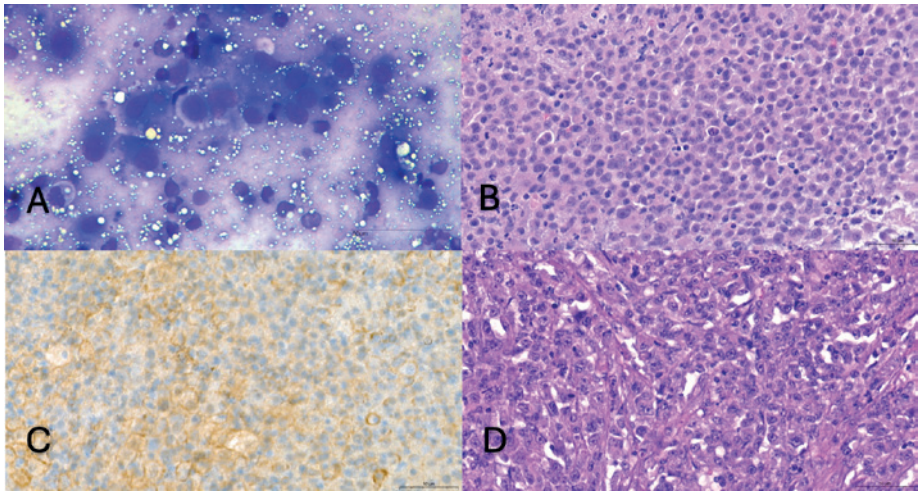

Figure 3. Microscopic images of a lung mass in a seven-year-old, male, castrated Golden retriever (case 34). Cytology: cell- rich preparation with proteinaceous blue background that contains lipid vacuoles and shows a more uniform population of oval-round to pleiomorphic cells with centrally placed nucleus, prominent nucleolus and light blue, often wispy cytoplasm. There is moderate anisocytosis and anisokaryosis with multinucleation (A); Cell block: highly pleomorphic polyglonal cells with occasional large atypical as well as multinuclear cells (B); Cell block after IHC with IBA1: histiocytic sarcoma (C); Histopathology: histiocytic sarcoma (D). Note that the scale bar of the cytology sample differs from those of the histopathology samples.

Table 1. Signalment and histopathologic diagnosis of cats and dogs included in the study.

Case number	Species	Breed	Age (years)	Sex and neutering status	Location of the mass	Size of the mass in mm (length x width x high)	Tumor type	Malignant process	Histopathological diagnosis
1	Dog	Bernese mountain dog	9	Mc	Bone	30 x 20 x 15	Mesenchymal	Yes	Osteosarcoma
2	Dog	Sheltie	7	Mc	Cutaneous	15 x 5 x 5	Non-neoplastic	No	Collagenous nevus
3	Dog	Shih tzu	15	Mc	Cutaneous	20 x 15 x 10	Epithelial	No	Sebaceous gland epithelioma
4	Dog	Sheltie	11	Mc	GI tract (colon)	112 x 53 x 46	Epithelial	Yes	Adenocarcinoma
5	Dog	Groenendael	11	Mi	Anocutaneous	40 x 20 x 20	Epithelial	Yes	Adenocarcinoma
6	Dog	Groenendael	11	Mi	Testicle	10 x 10 x 10	Mesenchymal	No	Leydig cell tumor
7	Dog	Crossbreed	15	Mc	GI tract (stomach)	10 x 7 x 10	Non-neoplastic	No	Gastric polyp
8	Dog	Bull terrier	11	Mi	Subcutaneous	50 x 35 x 20	Epithelial	Yes	Squamous cell carcinoma
9	Dog	French bulldog	9	Fc	Liver	80 x 100 x 60	Epithelial	Yes	Hepatocellular carcinoma
10	Cat	European shorthair	3	Fc	Urinary system (kidney)	Diffuse infiltrative lesion	Non-neoplastic	No	Chronic nephritis
11	Dog	Labrador retriever	10	Mc	Spleen	50 x 55 x 50	Non-neoplastic	No	Lymphoid hyperplasia
12	Dog	Spitz	10	Mi	Spleen	30 × 35 × 15	Mesenchymal	Yes	Hemangiosarcoma
13	Cat	British shorthair	7	Mc	Spleen	Diffuse infiltrative lesion	Round cell	Yes	Lymphoma (non-B non-T)
14	Dog	German shepherd	11	Fc	Oral	40 x 60 x 40	Epithelial	Yes	Amelanotic melanoma
15	Dog	Great dane	6	Mi	Anocutaneous	45 x 45 x 70	Epithelial	No	Adenoma
16	Dog	Belgian shepherd	8	Mi	Cutaneous	7 x 5 x 8	Non-neoplastic	No	Pyogranulomatous inflammation
17	Dog	Beauceron	9	Mc	Spleen	15 x 20 x 20	Non-neoplastic	No	Lymphoid hyperplasia
18	Dog	Beagle	7	Fc	Subcutaneous	10 x 9 x 5	Non-neoplastic	No	Organized hematoma
19	Dog	Border collie	7	Mc	Spleen	Diffuse infiltrative lesion	Non-neoplastic	No	Reactive nodular hyperplasia
20	Dog	Cavalier King Charles	11	Mi	Spleen	25 x 20 x 20	Non-neoplastic	No	Reactive nodular hyperplasia

21	Dog	Alaskan malamute	11	Mc	Anocutaneous	15 x 15 x 10	Epithelial	Yes	Adenocarcinoma
22	Dog	English cocker	8	Mc	Anocutaneous	60 x 50 x 45	Epithelial	Yes	Adenocarcinoma
23	Dog	Crossbreed	10	Fc	Liver	110 x 90 x 55	Epithelial	Yes	Hepatocellular carcinoma
24	Dog	Rhodesian ridgeback	2	Mi	Subcutaneous	40 x 30 x 28	Epithelial	Yes	Carcinoma
25	Dog	Maltese	10	Fc	Liver	110 x 70 x 45	Epithelial	Yes	Hepatocellular carcinoma
26	Dog	Labrador retriever	11	Fc	Oral	12 x 10 x 10	Epithelial	Yes	Melanoma
27	Dog	English Stafford	9	Mi	Urinary system (ureter)	15 x 25 x 20	Epithelial	Yes	Urothelial carcinoma
28	Dog	Yorkshire terrier	9	Mi	Oral	5 x 5 x 8	Epithelial	Yes	Melanoma
29	Dog	Cavalier King Charles	5	Fc	Bone	24 x 20 x 20	Mesenchymal	Yes	Osteosarcoma
30	Dog	English cocker	8	Mc	Urinary system (kidney)	30 x 20 x 22	Mesenchymal	Yes	Leiomyosarcoma
31	Dog	Labrador retriever	9	Mc	Spleen	85 x 60 x 80	Mesenchymal	Yes	Fibrosarcoma
32	Dog	Crossbreed	10	Fc	Subcutaneous	100 x 80 x 40	Mesenchymal	Yes	Hemangiosarcoma
33	Dog	Golden retriever	9	Mi	Deep soft tissue (retrobulbar)	20 x 15 x 20	Mesenchymal	Yes	Histiocytic sarcoma
34	Dog	Golden retriever	7	Mc	Lung	70 x 60 x 35	Mesenchymal	Yes	Histiocytic sarcoma
35	Dog	Crossbreed	9	Fc	Bone	60 x 50 x 40	Mesenchymal	Yes	Osteosarcoma
36	Dog	Boerboel	5	Fi	Spleen	90 x 55 x 40	Mesenchymal	Yes	Hemangiosarcoma
37	Dog	Dachshund	7	Mi	Oral	45 x 45 x 55	Mesenchymal	Yes	Squamous cell carcinoma
38	Dog	Border collie	10	Mc	Deep soft tissue (periarticular knee)	18 x 20 x 15	Mesenchymal	Yes	Fibrosarcoma
39	Cat	European shorthair	14	Mc	Subcutaneous	12 x 12 x 7	Mesenchymal	Yes	Fibrosarcoma
40	Dog	Golden retriever	4	Mi	Subcutaneous	20 x 17 x 10	Round cell	Yes	Mast cell tumor low grade
41	Cat	European shorthair	12	Fc	Cutaneous	6 x 5 x 2	Round cell	Yes	Mast cell tumor low grade

Mc = male castrated; Mi = male entire; Fc = female castrated; Fi = female entire; GI = gastrointestinal.
IHC = immunohistochemistry to support the definitive diagnosis, the putative diagnosis guided antibody selection

Table 2. Results of cytology, cell block technique and routine histopathology.

Case number	Location of the mass	Cytology results	Cell block technique results			Histopathologic results	
			x5 FNB	x10 FNB and ink	IHC	Histopathologic diagnose	IHC
1	Bone	Osteosarcoma C - M	NP	Osteosarcoma C – M	NP	Osteosarcoma	NP
2	Cutaneous	Non diagnostic	NP	Collagenous nevus C - M	NP	Collagenous nevus	NP
3	Cutaneous	Sebaceous gland epithelioma C - M	NP	Sebaceous gland epithelioma C – M	NP	Sebaceous gland epithelioma	NP
4	GI tract (colon)	Non diagnostic	NP	Adenocarcinoma C – M	NP	Colorectal adenocarcinoma	NP
5	Anocutaneous	Adenocarcinoma C - M	NP	Hepatoid gland adenocarcinoma C – M	NP	Hepatoid gland adenocarcinoma	NP
6	Testicle	Leydig cell tumor C - M	NP	Leydig cell tumor C - M	NP	Leydig cell tumor	NP
7	GI tract (stomach)	Non-diagnostic	NP	Non-diagnostic	NP	Gastric polyp	NP
8	Subcutaneous	Non-diagnostic	NP	Non-diagnostic	NP	Squamous cell carcinoma	NP
9	Liver	Hepatic tumor P - M	NP	Hepatocellular carcinoma C – M	NP	Hepatocellular carcinoma	NP
10	Urinary system (kidney)	Mesenchymal proliferation, reactive P - M	NP	Non-diagnostic	NP	Chronic nephritis	NP
11	Spleen	Reactive nodule P - M	NP	Lymphoid hyperplasia C – M	NP	Lymphoid hyperplasia	NP
12	Spleen	Non-diagnostic	NP	Hemangiosarcoma C - M	CD31 +	Hemangiosarcoma	CD31 +
13	Spleen	Lymphoma C - M	NP	Lymphoid nodular hyperplasia C - NM	CD3 + CD20 + Ki67 +	Lymphoma (non-B non-T)	CD3 + CD20 + PAX-5 - Granzyme B - C-kit - MPO -
14	Oral	Amelanotic melanoma C - M	NP	Sarcoma P – NM	NP	Amelanotic melanoma	SOX10 +

15	Anocutaneous	Adenoma C – M	NP	Non-diagnostic	NP	Hepatoid gland adenoma	NP
16	Cutaneous	Pyogranulomatous inflammation C - M	Pyogranulomatous inflammation C - M	Pyogranulomatous inflammation C – M	NP	Pyogranulomatous inflammation	NP
17	Spleen	Lymphoma C - NM	Non-diagnostic	Lymphoid hyperplasia C – M	NP	Lymphoid hyperplasia	CD3 + PAX5 + Ki67 +
18	Subcutaneous	Non diagnostic	Organized hematoma C – M	Organized hematoma C – M	NP	Organized hematoma	NP
19	Spleen	Reactive nodular hyperplasia C – M	Reactive nodular hyperplasia C - M	Reactive nodular hyperplasia C - M	NP	Reactive nodular hyperplasia	CD3 + CD20 +
20	Spleen	Non diagnostic	Reactive nodular hyperplasia C - M	Reactive nodular hyperplasia C - M	NP	Reactive nodular hyperplasia	CD31 +
21	Anocutaneous	Hepatoid gland epithelioma C – NM	Non-diagnostic	Hepatoid gland adenocarcinoma C - M	NP	Hepatoid gland adenocarcinoma	NP
22	Anocutaneous	Non diagnostic	Apocrine gland adenocarcinoma C - M	Apocrine gland adenocarcinoma C - M	NP	Apocrine gland anal sac adenocarcinoma	NP
23	Liver	Hepatocellular carcinoma C – M	Hepatocellular carcinoma C – M	Hepatocellular carcinoma C – M	NP	Hepatocellular carcinoma	NP
24	Subcutaneous	Carcinoma C – M	Carcinoma C - M	Carcinoma C – M	Vimentin - p63 - PanCK +	Carcinoma *(origin unclear)*	CD56 +/- PanCK + Synaptophysin -
25	Liver	Hepatocellular carcinoma C – M	Hepatocellular carcinoma C - M	Hepatocellular carcinoma C - M	NP	Hepatocellular carcinoma	NP
26	Oral	Amelanotic melanoma C – M	Melanoma C – M	Melanoma C – M	SOX10 +	Melanoma	SOX10 +
27	Urinary system (ureter)	Urothelial carcinoma C – M	Urothelial carcinoma C – M	Urothelial carcinoma C – M	NP	Urothelial carcinoma	NP
28	Oral	Melanoma C – M	Melanoma C – M	Non-diagnostic	NP	Melanoma	SOX-10 + Ki-67 +

29	Bone	Osteosarcoma C – M	Osteosarcoma C – M	Osteosarcoma C - M	NP	Osteosarcoma	NP
30	Urinary system (kidney)	Non-diagnostic	Non-diagnostic	Non-diagnostic	NP	Leiomyosarcoma	SMA + Desmin + Vimentin + PanCK -
31	Spleen	Sarcoma P – M	Sarcoma P - M	Sarcoma P - M	NP	Fibrosarcoma	Synaptophysin + PanCK + SMA + IBA1 + CD31 -
32	Subcutaneous	Non diagnostic	Non-diagnostic	Sarcoma P - M	CD31 +	Hemangiosarcoma	CD31 +++
33	Deep soft tissue (retrobulbar)	Sarcoma P – M	Non-diagnostic	Histiocytic sarcoma C - M	IBA1 +	Histiocytic sarcoma	IBA1 + SOX10 - PanCK -
34	Lung	Non-diagnostic	Histiocytic sarcoma C - M	Histiocytic sarcoma C - M	IBA1 +	Histiocytic sarcoma	IBA1 +
35	Bone	Sarcoma P – M	Sarcoma P - M	Osteosarcoma C - M	NP	Osteosarcoma	NP
36	Spleen	Sarcoma P – M	Non-diagnostic	Non-diagnostic	NP	Hemangiosarcoma	CD31 +
37	Oral	Squamous cell carcinoma C - M	Squamous cell carcinoma C - M	Squamous cell carcinoma C - M	NP	Squamous cell carcinoma	NP
38	Deep soft tissue (periarticular knee)	Non diagnostic	Non diagnostic	Non diagnostic	NP	Fibrosarcoma	CD31 - IBA1 - PanCK - SOX10 -
39	Subcutaneous	Sarcoma P – M	Fibrosarcoma C – M	Fibrosarcoma C - M	NP	Fibrosarcoma	NP
40	Subcutaneous	Mast cell tumor C – M	Mast cell tumor (low grade) C - M	Mast cell tumor (low grade) C – M	NP	Mast cell tumor low grade	NP
41	Cutaneous	Mast cell tumor C – M	Non diagnostic	Mast cell tumor (low grade) C – M	NP	Mast cell tumor low grade	NP

NP = Not performed. IHC = Immunohistochemistry; GI = Gastro-intestinal. C = Complete result. P = Partial result. M = Matching result. NM = Non-matching result; - = negative. + = positive. +++ strongly positive. +/- slightly positive.

Sample analysis

The results of the routine histopathology are presented in Table 1; they served as the golden standard to evaluate the diagnostic accuracy of the FNAB-based cytology and CBs. According to the histopathological classification, 15/41 cases (36.6%) were identified as epithelial tumors, 14/41 (34.1%) as mesenchymal tumors, 9/41 (22.0%) as non-neoplastic lesions, and the remaining 3/41 cases (7.3%) as round cell tumors. Of the histologic results, 12/41 (29.3%) were classified as benign processes/tumors and 29/41 (70.7%) were malignant processes/ tumors. In 16/41 (39.0%) cases, at least one IHC staining was performed in addition to routine H&E staining to support the putative diagnosis based on the gross specimen analysis.

Cytology results

The cytology results are summarized in Table 2. On cytological evaluation alone, a diagnostic result was obtained in 29/41 cases (70.7%). Among these, 21/29 samples (72.4%) provided a complete diagnosis, and the remaining 8/29 cases (27.6%) were considered to reveal a partial result. In 12/41 cases (29.3%), cytology was non-diagnostic due to an insufficient number of cells or lack of representative cellular material (Figures 1, 2 and 3).

When non-diagnostic cytology results were excluded (12/41; 29.3%), the cytology diagnosis matched the corresponding histopathologic diagnosis in 27/29 of the cases, whereas in 2/29 cases, the cytological interpretation was incorrect. A case of splenic lymphoid nodular hyperplasia (case 17) was misdiagnosed as lymphoma and an hepatoid gland adenocarcinoma (case 21) was misdiagnosed as hepatoid gland epithelioma on cytology.

CB results

All results are presented in Table 2. During the pilot phase of the study, the addition of blue dye ink to 15 specimens was assessed. In 11 of these 15 cases (73.3%), the ink improved visualization and handling of the material during laboratory processing. There were no noticeable differences in histomorphology, cellular morphology, staining intensity, or immunohistochemical labeling between inked and non-inked samples. Tissue architecture and interpretation remained unaffected by ink application.

Because blue dye ink was present in all samples and the timing of formalin fixation did not affect histological interpretation, all 41 cases were analyzed as a single cohort although they were at first splitted into two subgroups of 15 and 26 cases as mentioned above.

The CB technique yielded a diagnostic result in 34/41 cases (82.9%). Of these 34 cases, 31 (91.2%) provided a complete diagnosis, while three cases (8.8%) yielded only partial information. The remaining 7/41 cases (17.1%) were considered non-diagnostic due to insufficient cellular material.

Immunohistochemistry was performed on 7/41 CB samples. In 5/7 (71.4%), the analysis contributed to refining or clarifying of the diagnosis (Figures 1 and 3). In three additional cases, IHC could have been valuable, but there was insufficient material available to perform the analysis.

The cell block results based on the x5 technique produced diagnostic results in 18/26 cases (69.2%), including 16 complete (88.9%) and two partial (11.1%) diagnoses. The x10 technique yielded diagnostic outcomes in 22/26 cases (84.6%), with twenty complete (90.9%) and two partial (9.1%) diagnoses. Compared to the x5 approach, the x10 method allowed for a more accurate diagnosis in 4/26 cases. However, there was one case in which the X5 sample yielded a complete diagnosis, while the X10 sample was non-diagnostic due to insufficient material for analysis. The results after x5 versus x10 were not statistically different ($p = 0.194$).

When comparing the CB diagnoses with those obtained from routine histopathology, 32/34 diagnostic CB cases (94.1%) matched the final histological diagnosis (Figures 1, 2 and 3). In two cases (5.9%) however, the CB results were discordant and ultimately incorrect. In one case (case 13), the CB technique misdiagnosed a lymphoma as extramedullary hematopoiesis. In the other case (case 14), the CB technique misclassified an amelanotic melanoma as a sarcoma.

When compared to cytology alone, the CB technique improved diagnostic outcomes in 15/41 cases (36.5%), either by upgrading a non-diagnostic result to a partial or complete diagnosis (8/15; 53.4%), or by improving an incorrect or partial diagnosis to a complete one (7/15; 46.6%). On the other hand, cytology outperformed the CB based on x10 blow in five cases (12.2%).

Finally, when the results from cytology and CB techniques were combined and then compared to the histopathology diagnosis, a diagnostic conclusion, either partial (4/37; 10.8%) or complete (33/37; 89.2%), was reached in 37/41 cases (90.2%) (Figure 3). Among these, only four cases showed contradictory results: in two of them, cytology agreed with histopathologic results but the CB did not, while in the other two, the CB technique matched histopathologic results whereas cytology did not. Overall, the combination of cytology and CB represented a statistically significant increase in diagnostic yield compared to cytology alone ($p < 0.039$).

DISCUSSION

In this study, the added value of the CB technique when combined with conventional cytology, was demonstrated, using histopathology of the surgical specimen as the gold standard, across a diverse cohort of canine and feline tumors. Both neoplastic and non-neoplastic processes from various sites were included, reflecting the heterogeneity of cases encountered in clinical practice. Malignant tumors represented over two thirds of the cases. Whereas the CB technique is hardly more invasive than traditional cytology, it offers the opportunity to evaluate architectural details and perform IHC. Furthermore, general anesthesia can be avoided, and CB has a shorter turnaround

Supplementary Table. Panel of immunohistochemical parameters tested.

Primary antibody	Clone	Company	Dilution
CD31	D CD31/PECAM-1 (JC/70A)	Yvsolab (Vitro Master Diagnostica)	1:50
CD3	Polyclonal	Agilent Technologies	1:100
CD20	Polyclonal	Thermofisher Scientific	1:100
PAX-5	DAK-Pax5	Agilent Technologies	1:30
Granzyme B	GrB-7	Agilent Technologies	1:50
c-kit	EP10	Yvsolab (Vitro Master Diagnostica)	1:50
MPO	Polyclonal	Yvsolab (Vitro Master Diagnostica)	1:800
SOX-10	EP268	Yvsolab (Vitro Master Diagnostica)	1:150
Ki-67	SP6	Yvsolab (Vitro Master Diagnostica)	1:50
CD56	123C3	Agilent Technologies	1:50
PanCK	AE1/AE3	Agilent Technologies	1:50
Synaptophysin	DAK-SYNAP	Agilent Technologies	RTU
SMA	1A4	Agilent Technologies	1:100
Desmin	D33	Abcam	1:150
Vimentin	V9	Abcam	1:1000
IBA1	Polyclonal	FUJIFILM Wako Pure Chemical Corporation	1:500

time than routine histopathology, both important advantages of CBs, particularly in geriatric animals. In addition, CB preparation may be an acceptable procedure to owners who are reluctant to pursue surgical biopsies. It can provide further characterization of a mass and help justify subsequent therapeutic decisions while remaining minimally invasive. However, owners should be informed of its limitations, including the possibility that a traditional surgical biopsy may still be required if CB results prove inconclusive or insufficient for clinical decision-making.

The ability to highlight architectural patterns in tissue fragments or cell clusters represents a major advantage of CB preparations compared with conventional cytology, alongside the added possibility of performing IHC (Jain et al.; 2014; Sharma et al.; 2015; Marcos et al., 2019; Melega et al.; 2020; Shidham and Layfield, 2021; Verma et al.; 2021; Sampaio et al., 2023). The selection of IHC markers used in this cohort was tailored to each individual case and based on the clinical history, anatomical location, lesion morphology, and relevant differential diagnoses. Consequently, IHC served different purposes depending on the case: in some instances, it was primarily used to confirm a diagnosis that was strongly suspected based on H&E morphology, whereas in others it was essential to distinguish between competing differential diagnoses when morphology alone was insufficient. Obviously, cytology smears can undergo immunocytochemistry (ICC), in analogy with IHC on CBs. Since in ICC, cells on quality smears typically form a monolayer, a greater resolution can be obtained, and moreover, ICC is a fairly quick technique. Nevertheless, there are some distinct inferiorities of ICC compared to IHC. First, there are some technical aspects. The preparation of cytology smears by unexperienced clinicians holds the risk to result in technically poor slides, obscuring blood, cellular overlap, forceful smearing, or air-drying artifacts (Valenciano and Cowell, 2007; Qiu et al., 2008). In addition, background staining is more often observed in ICC samples than in IHC samples. Furthermore, it is known that the cellularity in different cytology slides is often highly variable, whereas multiple consecutive sections from CBs allow for various IHC stains on a comparable amount and type of material (Sharma et al., 2015). Furthermore, a smaller amount of antibody is usually required to perform IHC compared to ICC (Shidham and Layfield, 2021). Second, ICC is also less available. Although ICC and IHC rely on the same detection reactions, antibodies need to be optimized for each method individually, and validated ICC protocols for a wide array of antibodies are not readily available in most clinical diagnostic veterinary pathology services, resulting in a broader and more tailored immunophenotypic work-up in CB samples when indicated. Both techniques can be applied to small amounts of material; however, neither can be performed when the available sample is insufficient for processing and evaluation. A limited number of cells was the reason why cytology outperformed CB in two cases. One CB sample revealed moderate neutrophilic inflammation but contained only a small amount of cellular material. Consequently, a diagnosis could not be made with confidence based on the corresponding cytology sample alone. In the second case, where splenic hemangiosarcoma was diagnosed, there were only occasional large, atypical spindle cells noticed in the CB, while several of them were seen by cytology. In retrospect, IHC using CD31 would potentially have revealed a complete diagnosis. Likewise, an oral amelanotic

melanoma was suspected by cytology, whereas it was misdiagnosed as sarcoma (no IHC was performed due to insufficient material), and a non-B non-T lymphoma was diagnosed as lymphoid nodular hyperplasia on the CB, because of the absence of signs of malignancy and KI-67 labeling not being diffuse as expected in case of lymphoma.

Although a higher number of expulsions generally resulted in improved diagnostic yield, the number of sample-taking must be balanced against potential risks such as bleeding or discomfort in an awake patient. Increasing the needle size potentially could yield higher material load without increasing the number of FNABs but also that needs to be outbalanced with the potential disadvantages for the patient (Fleming et al., 2019; Arai et al., 2019; Llanos et al., 2023). During their clinical activities, the authors normally would not apply aspiration for cytology samples, and therefore also opted not to do so while preparing samples for the CB technique. Yet, aspiration during fine-needle biopsy sampling and its effect on the number of cells recovered should be considered and outweighed against the introduction of blood contamination and the integrity of the cells recovered (Fleming et al., 2019; Arai et al., 2019; Whitlock et al., 2021; Llanos et al., 2023).

Blue dye ink was added to the sample processing, based on veterinary surgery pathology guidelines (Kamstock et al., 2011). It proved advantageous as it facilitated retrieval of the clustered cells on the slices and slides; without interfering with H&E nor IHC staining. Blue dye ink is also a routinely used and well-established technique in histopathology for margin identification and is not known to interfere with histological or immunohistochemical interpretation. When other protocols such as the CB technique using a microhematocrit tube for fluid specimens (Marcos et al., 2017) would have been used, the ink would likely not have been of any added value. In retrospect, the use of high-speed centrifugation as described by Marcos et al. (2017) could have been of added value in blood-contaminated samples since it separates the red blood cells from the nucleated cells in the final CB sample (Marcos et al., 2017; Marrinhas et al., 2021). On the other hand, centrifugation at high speed might dislodge cell clusters into individual cells and therefore result in less architectural detail, despite being prepared as a CB. Furthermore, it is the authors' recent experience that high-speed centrifugation results in a higher number of damaged cells, decreasing the quality of the CB.

Oncologic patients are often older and carry a higher prevalence of comorbidities, which significantly increases anesthetic risk (Pouchelon et al., 2015). Multimorbidity is particularly common in senior dogs: over 70% with one condition have three or more concurrent diseases, often including cardiovascular and renal disorders (Pouchelon et al., 2015; Sordo et al., 2020; Fang et al., 2025). These factors highlight the importance of minimally invasive diagnostic options, such as cytology followed by CB preparation, that avoid the added anesthetic risks associated with surgical biopsies. The results of this study are promising and in line with the previous literature. Yet, the design of the present study was ex vivo and therefore in vivo studies to validate the results under clinical conditions remain to be performed.

In terms of turnaround time, cytology results provided by external laboratories in practices without an in-house cytopathologist are usually available within one to two days (Batt Laboratories, 2025; Idexx Laboratories, 2025). In human medicine, CB preparations have been reported to add only a modest delay, with results typically accessible within two-three days, depending on the necessity for IHC (Jones and Novis, 2001; Carter et al., 2016). By contrast, routine histopathology often requires five or more days (Sharma et al., 2025). At the Small Animal Teaching Hospital of Ghent University, cytology samples of clinical patients are not submitted to an external laboratory but interpreted in-house, in most instances on the day of collection. This approach may substantially reduce the turnaround time compared with external submission; however, this advantage should be interpreted in the context of the expertise and experience of the interpreting clinician, which also play an essential role in diagnostic accuracy. In the present study, the turnaround times obtained for CB and histopathology results were consistent with those reported in the human literature, namely approximately three and five working days or more, respectively.

While the CB technique offers many advantages, its diagnostic yield varies depending on tumor type and localization. Several factors likely contribute to these differences. First, tissues with cohesive architecture and higher cellular exfoliation (e.g. liver, lung) are easier to sample and interpret (Taylor et al., 2013; Sabattini et al., 2017), whereas organs with limited exfoliation or complex architecture (e.g. urinary system, deep soft tissues) yield less material and lower diagnostic accuracy (Young et al., 2014; Brisson and Regan, 2022). Second, tumor differentiation and morphology influence results: well-differentiated tumors are more readily diagnosed, while poorly differentiated or mixed-origin tumors often require additional histologic context (Brisson and Regan, 2022, Davidson et al., 2023; Brilhante-Simões et al., 2025). Third, overlapping histologic features in certain tumors, particularly oral masses and spleen, can lead to misinterpretation or partial diagnosis. Finally, sampling challenges in deep or less accessible tissues (GI tract, urinary system) can limit representative material (Marrinhas et al., 2021). Due to the limited number of cases per category, no definitive conclusions on positive or negative predictive factors could be drawn.

This study had several limitations. From a technical standpoint, the study reflects idealized sampling conditions that do not fully reproduce routine clinical practice because all FNABs were obtained ex vivo after surgical excision. Factors, such as tissue perfusion and blood contamination, native tissue tension and consistency, patient movement, or potential discomfort and pain responses were therefore not taken into account. Consequently, the diagnostic performance observed here might overestimate the yield achievable under in vivo minimally invasive sampling conditions. In

addition, the interpretation by a single ACVP pathologist may limit the generalizability and reproducibility of the findings, as cytologic and histopathologic interpretation can be influenced by individual experience and subjective assessment. The absence of interobserver variability assessment also precluded the evaluation of diagnostic consistency between different pathologists. Future studies incorporating independent evaluations by multiple pathologists and formal assessment of interobserver agreement would strengthen the evaluation of the reproducibility and generalizability of the findings. Furthermore, the cohort was relatively small. While studies with limited cases can provide valuable preliminary insights, their findings must be interpreted with caution. Given the very low number of cats in the current cohort, interspecies differences could not be evaluated. In future studies, not only should a clear distinction between species be maintained, also a more balanced and stratified representation of tumor types and anatomical locations is strongly recommended. Finally, the authors acknowledge also that the cytology slides were stained exclusively using a Diff-Quik-type stain, in line with the routine diagnostic workflow at the Veterinary Teaching Hospital (Faculty of Veterinary Medicine, Ghent University). Alternative or additional staining methods, such as other Romanowsky-type stains, may in some cases improve cytomorphologic detail and thus the diagnostic yield of cytology, in particular in case of mast cell tumors. In the present cohort, only two of the latter cases were included and both were readily diagnosed after Diff-Quick staining of the cytology samples.

Overall, the results of this study underscore the possible clinical relevance of CB preparation in addition to cytology as a feasible, reproducible, and diagnostically valuable ancillary tool in veterinary oncology, but warrant larger, future studies. In comparison with conventional histopathology, the combination of cytology and CB diagnoses demonstrated a very high level of reliability. The ability to enhance diagnostic accuracy, to allow for ancillary testing such as IHC, reducing diagnostic uncertainty, supports its broader integration into routine veterinary diagnostic workflows. Cell block preparation may represent a valuable option to discuss with owners, provided they understand its limitations, including the fact that a traditional surgical biopsy may still be required if results would turn out to be inconclusive or insufficient for clinical decision-making. When results from CB and cytology are discordant, histopathology should be performed for definitive diagnosis.

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REFERENCES

Arai S., Rist P., Clancey N., et al. (2019). Fine-needle aspiration of cutaneous, subcutaneous, and intracavitary masses in dogs and cats using 22- vs 25-gauge needles. *Veterinary Clinical Pathology 48*, 287-292.

Avallone G., Rasotto R., Chambers J.K., et al. (2021). Review of histological grading systems in veterinary medicine. *Veterinary Clinical Pathology 58*, 809-828.

Batt Laboratories Ltd. (2025). Cytology. Available at: https://battlab.com/cytology/ (Accessed October 25, 2025).
Brilhante-Simões P., Delgado L., Martins Â., et al. (2025). Association between cytological and histopathological diagnoses of neoplastic and non-neoplastic lesions in oral cavity from dogs and cats: an observational retrospective study of 103 cases. *Veterinary Sciences 12*, 75.

Brisson B.A., Regan D. (2022). Diagnostic challenges in canine soft tissue sarcomas: a review of cytology and histopathology. *Journal of Veterinary Diagnostic Investigation 34*, 185-193.

Carter M.D., Moore D.P., MacIntosh R.F., et al. (2016). Impact of routine cell block preparation on results of head and neck fine needle aspirates. *Diagnostic Cytopathology 44*, 880-887.

Çakır L., Çolakoğlu E.C., Tunç A.S., et al. (2014). Cytological evaluation of pleural effusion with cell block technique in a dog. *Ankara Üniversitesi Veteriner Fakültesi Dergisi 61*, 73-77.

Davidson B., Harris K., Cantrell T. (2023). Diagnosis and management of canine soft tissue sarcomas. *Veterinary Practice*. Available at: https://www.veterinary-practice.com/article/diagnosis-and-management-of-canine-soft-tissue-sarcomas (Accessed July 10, 2025).

Emanuelli Mello C.B., Engelmann A.M., Kommers G.D., et al. (2023). Fine needle aspiration cytology: high accuracy in diagnosing cutaneous and subcutaneous neoplasms in dogs. *Comparative Clinical Pathology 32*, 155-164.

Fang A., Kumar L., Creevy K.E., et al. (2025). Constructing the first comorbidity networks in companion dogs in the Dog Aging Project. *PLOS Computational Biology 21*.

Fleming K.L., Howells E.J., Villiers E.J., et al. (2019). A randomized controlled comparison of aspiration and non-aspiration fine-needle techniques for obtaining ultrasound-guided cytological samples from canine livers. *Veterinary Journal 252*, 105372.

Ghisleni G., Roccabianca P., Ceruti R., et al. (2006). Correlation between fine-needle aspiration cytology and histopathology in cutaneous and subcutaneous masses from dogs and cats. *Veterinary Clinical Pathology 35*, 24-30.

Idexx Laboratories. (2025). Pathology clinical cytology. Available at: https://www.idexx.co.uk/en-gb/veterinary/reference-laboratories/pathology/clinical-pathology/ (Accessed October 25, 2025).

Jain D., Mathur S.R., Iyer V.K. (2014). Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies. *Cytopathology 25*, 356-371.

Jones B.A., Novis D.A. (2001). Nongynecologic cytology turnaround time: a Q-Probes study. *Archives of Pathology & Laboratory Medicine 125*, 1279-1284.

Kamstock D.A., Ehrhart E.J., Kiupel M. (2011). Recommended guidelines for tumor biopsy specimens in veterinary surgical pathology. *Veterinary Pathology 48*, 19-31.

Kim Y., Cho Y.M., Lee S.H., et al. (2012). Application of the cell block technique to diagnostic cytology in veterinary medicine. *Veterinary Clinical Pathology 41*, 307-308.

Krogerus L., Kholová I. (2018). Cell block in cytological diagnostics: review of preparatory techniques. *Acta Cytologica 62*, 237-243.

Llanos C., Holmes E., White C., et al. (2023). Diagnostic quality of ultrasound-guided fine-needle aspirates from canine liver and spleen. *Veterinary Radiology & Ultrasound 64*, 966-972.

Marcos R., Marrinhas C., Malhão F., et al. (2019). Cell tube block technique and immunohistochemistry panel in cavitary effusions. *Veterinary Clinical Pathology 48*, 50-60.

Marcos R., Santos M., Marrinhas C., et al. (2017). Cell tube block: a new technique to produce cell blocks from fluid samples. *Veterinary Clinical Pathology 46*, 195-201.

Melega M., Santos M., Caniatti M., et al. (2020). Cell blocks in veterinary medicine: comparison of two methods. *Veterinary Clinical Pathology 49*, 632-639.

Marrinhas C., Oliveira L.F., Sampaio F., et al. (2021). Needle rinse cell blocks as an ancillary technique. *Veterinary Clinical Pathology 50*, 47-54.

Nathan N.A., Narayan E., Smith M.M., et al. (2000). Cell

block cytology: improved preparation and diagnostic efficacy. *American Journal of Clinical Pathology 114*, 599-606.

Oikonomidis I., Tsouloufi T. (2021). Diagnostic accuracy of cytology for canine osteosarcoma. *Veterinary Evidence 6(2)*. doi:10.18849/VE.V6I2.399.

Okuno M., Mukai T., Iwata K., et al. (2022). Cell block method using bile for malignant biliary stricture diagnosis. *Cancers (Basel)*1(11), 2701. doi:10.3390/cancers14112701.

Pakdeesaneha T., Chankow K., Techarungchaikul S., et al. (2024). Fine-needle aspiration vs core needle biopsy in mammary tumors. *Veterinary and Comparative Oncology 22*, 566-573.

Pouchelon J.L., Atkins C.E., Bussadori C., et al. (2015). Cardiovascular–renal axis disorders consensus statement. *Journal of Small Animal Practice 56*, 537-552.

Qiu L., Crapanzano J.P., Saqi A., et al. (2008). Cell block as an ideal method for hemorrhagic thyroid aspirates. *Acta Cytologica 52*, 139-144.

Sabattini S., Renzi A., Buracco P., et al. (2017). Accuracy of cytology and histology in canine bone lesions. *Journal of Veterinary Internal Medicine 31*, 864-871.

Sampaio F., Marrinhas C., Oliveira L.F., et al. (2023). Immunophenotyping in dogs and cats using cell blocks. *Veterinary Sciences 10*, 157.

Sharkey L.C., Seelig D.M., Overmann J. (2014). Diagnostic cytology in veterinary medicine. *Diagnostic Cytopathology 42*, 544-552.

Sharma R., Nagaish N., Shubha G., et al (2015). Role of cell block in diagnostics - a new paradigm in diagnosis. *International Clinical Pathology Journal 1(5)*, 113-118.

Sharma A., Nishadham V., Gupta P., et al. (2025). Evaluation of turnaround times in diagnostic biopsies. *International Journal of Surgical Pathology 33*, 344-352.

Shidham V.B., Layfield L.J. (2021). Cell-blocks and immunohistochemistry. *CytoJournal* 18, 2.

Sordo L., Breheny C., Halls V., et al. (2020). Disease prevalence and age-related behavioral changes in cats. *Veterinary Sciences 7*, 85.

Taylor B.E., Leibman N.F., Luong R., et al. (2013). Detection of carcinoma micrometastases using cell block cytology. *Veterinary Clinical Pathology 42*, 85-91.

Tripathi R.C. (1974). Comparative physiology of aqueous outflow pathway. In: Dawson H., Graham L.T. (editors). *The Eye*. Second edition, Vol. 5. Academic Press, New York, 163-336.

Valenciano A.C., Cowell R.L. (2020). Sample collection and preparation. In: Valenciano A.C. and Cowell R.L. (editors). *Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat*. Fifth edition, Mosby, St. Louis, Missouri, 1-17.

Valente P.C.L.G., Peleteiro M.C., Pissarra H., et al. (2021). Accuracy of minimally invasive techniques in canine lymphoma diagnosis. *Veterinary and Comparative Oncology 19*, 512-523.

Vance J., Durbin K., Manglik N., et al. (2019). Diagnostic utility of cell block in fine needle aspiration cytology of thyroid gland. *Diagnostic Cytopathology 47(12)*, 1245-1250.

Verma V., Khan A., Rao R.N., Nath A., Hashim Z. (2021). Role of endobronchial ultrasound guided transbronchial needle aspiration with cellblocks in diagnosis and subtyping of intrathoracic lesions: Two year experience from a tertiary care center. *Journal of Cytology 38(3)*, 120-126.

Walsh K.A., Patel R.T. (2021). Cell block preparation techniques in veterinary medicine. In: Sharkey L.C., Radin M.J., Seelig D. (editors). *Veterinary Cytology 8*, 73-78.

Whitlock J., Taeymans O., Monti P. (2021). Cytological quality of FNA techniques in lymph nodes. *Veterinary Record 188*, e25.

Yung R.C.W., Otell S., Illei P.B., et al. (2012). Improvement of cellularity using tissue coagulum clot method. *Cancer Cytopathology 120*, 185-195.

Young B., Lowe J.S., Stevens A., et al. (2014). Mesenchymal tumors and their origins. In: *Histology for Pathologists*, 4th ed. Lippincott Williams & Wilkins, 347-351.

Zanoni D.S., Grandi F., Rocha N.S. (2012). Agarose cell block technique in veterinary cytopathology. *Veterinary Clinical Pathology 41*, 307-308.

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DIEREN LITERAIR

OOK RUITERS

Zondag. Een hoed, een broek en tussen bei
De suikerbakker op zijn hakkenei.

(…) Te paard reed Attila, te paard Napoleon.
Tot wat dient, schimmel, uw beslagen hoef?
Is 't daarom dat uw oog zo nat is en zo droef?

Richard Minne