

Cell tube block: a new technique to produce cell blocks from fluid cytology samples

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Background: Cell blocks are widely used in human cytopathology. Several techniques have been proposed to convert fluid specimens into solid material, which after embedding in paraffin can be used for histochemistry, immunohistochemistry, or molecular testing. In contrast, only in the last few years, have cell blocks begun to be used in the veterinary field.

Objectives: The purpose of the study was to present the production and features of cell tube blocks (CTB) with veterinary liquid samples.

Methods: Liquid samples including cerebrospinal fluid, cutaneous cyst fluid, pericardial and pleural effusions, bronchoalveolar lavage, urine, and blood were centrifuged in a microhematocrit centrifuge. Capillary tubes were broken at the liquid-solid interface and fixed in 10% formalin for 24 hours. After paraffin embedding, sections of CTB were used for different stains including immunohistochemistry.

Results: The morphology and cellular detail in CTB sections were comparable to conventional histologic sections and other existing cell block techniques. The use of special stains such as Gram, Giemsa, alcian blue, and periodic acid-Schiff was straightforward, and immunohistochemistry results with antibodies to pan-cytokeratin, PAX-5, and CD3 were considered good.

Conclusions: The CTB method was easily implementable under practice conditions (up to the fixation of the microhematocrit tube), analogous to surgical biopsy submission for histology. Cell tube blocks can increase diagnostic accuracy when the technique is used in tandem after the cytologic evaluation, and the technique allows storage of fluids. Other advantages of CTB were the simplicity, low cost, and separation of erythrocytes from the nucleated cells, which was helpful in hemodiluted samples.

Introduction

Cell blocks have been used for a long time in human medicine, being first reported almost 120 years ago. However, the technique has only been widely used since the mid-20th century.¹ Cell blocks can be obtained both from fluids, such as body cavity effusions, blood or urine, and fine-needle aspirates, providing sections that can be used for histopathology, histochemistry, immunohistochemistry, as well as molecular biology analysis.² Over the years, several techniques for obtaining cell blocks have been described, such as the plasma-thrombin method, the collodion bag technique, or the use of different gels (e.g. HistoGel, albumin, gelatin, or agar).^{2,3} Despite this variety, all these methods share some common principles, like nucleated cell concentration, fixation, and paraffin wax embedding, with cell blocks being handled as any other biopsy tissue. Studies in human medicine have concluded that cell blocks may increase diagnostic accuracy from 12%⁴ up to 55%⁵, and the technique is recommended as an adjunct to the direct cytologic assessment of slides.^{2,3,6} Liquids comprise a significant proportion of the samples submitted for cytologic examination, in human as well as in veterinary medicine. However, in contrast to > 500 studies using cell blocks in human medicine², only a handful of reports have been published in veterinary medicine over the last couple of years.⁷⁻¹¹ In fact, this seems to represent an “old and forgotten” technique in the veterinary literature⁸, where cell blocks have been consolidated using agar^{8,11}, HistoGel⁷, and gelatin foam¹⁰, and/or a series of centrifugation and fixation steps.⁹

The aim of this study was to describe a new, easy to follow procedure to produce cell blocks, using routine equipment present in most practice environments, called cell tube block (CTB).

Materials and Methods

Cytologic specimens

To illustrate the technique, 8 cases with fluid specimens were selected: (1) cerebrospinal fluid from a cat with meningitis; (2) cystic fluid of a cutaneous mass from a dog with a history of mast cell tumor; (3) pericardial fluid from a dog with a history of mammary gland carcinoma; (4) pleural fluid from a cat with a mediastinal mass; (5) blood from a dog with a chronic lymphocytic leukemia; (6) cystic fluid of a sarcomatous tumefaction on the leg of a dog; (7) bronchoalveolar lavage of a dog with a history of chronic bronchitis; and (8) urine of a dog with a transitional cell carcinoma. Cytospins and/or buffy coat smears were prepared from all the samples and stained with a Romanowsky stain (Hemacolor; Merck, Darmstadt, Germany).

Cell tube block preparation

To obtain CTBs, samples were filled into plain type capillary tubes, sealed with one of 2 types of clay (Giotto Pongo, Fila Hispania, Barcelona, Spain; Jovi Plastilina, Rubi, Spain) and spun in a microhematocrit centrifuge (LW Scientific, Lawrenceville, GA, USA) (14,500g) for 5 min (Figure 1). With fluids of very low cellularity (< 500 cells/IL), sedimentation by centrifugation of the sample in plastic tubes in a regular centrifuge (2000g for 5 min) was required. After discarding the supernatant, the pellet was aspirated by the capillary tube (Figure 1A), which was then spun in the microhematocrit centrifuge, resulting in a longer CTB. In cases in which the CTB was located near the clay seal after centrifugation, a high-density solution such as ficoll or percoll (Sigma, St. Louis, MO, USA) was used to ensure a proper separation from the clay (Figure 2). Specifically, after filling 3/4 of the tube with the sample, an air bubble was introduced by gently rocking the tube and then 5-10 IL of the high-density solution were aspirated by capillary action and the tube was sealed with clay (Figure 1B and 2). Afterwards, capillary tubes were broken at the liquid-solid interface by using a small glass cutter, a glass-writing diamond pen (eg, Usbeck, Radevormwald, Germany) or an electric engraver (eg, Dremel 290, Breda, Netherlands) (Figure 1C). Then, the portion of the capillary tube containing the cells was fixed in 10% formalin for 24 h and routinely processed by an automated tissue processor (Leica TP1020, Nussloch, Germany). The CTB was removed from the remaining glass tube with a paper clip (in this step, cooling the capillary tube helps because the CTB hardens) (Figure 1D). Afterwards the CTB was embedded horizontally (along its long axis), so that all cellular levels could be viewed simultaneously. The paraffin-embedded CTBs were sectioned and routinely stained with H&E, and with Gram, Giemsa, alcian blue, and periodic acid-Schiff (PAS). Immunohistochemistry was tested with a pan-cytokeratin antibody (AE1/AE3; Invitrogen, Carlsbad, CA, USA) at a 1:300 dilution, and B- and T-lymphocyte-specific antibodies, respectively, PAX-5 (Leica, Newcastle, UK) at 1:40 dilution and CD3 (Dako, Glostrup, Denmark) at 1:50 dilution. Antigen retrieval was induced in a 100°C water bath for all antibodies.

Results

The CTB procedures were straightforward. Potential pitfalls were caused by the breaking of the capillary tubes and the type of clay used. Specifically, breaking the capillary tube was best accomplished by using a glass-writing pen (Figure 1C). The modeling clay Giotto tended to be dissolved during processing (clearing with xylene), prohibiting the easy removal of the cell pellet from the tube (Figure 1F), while Jovi clay worked better. The use of high-density fluids, such as percoll, facilitated the removal of the cell pellet, and neither interfered with the paraffin wax embedding nor the histo- or immunohistochemical staining (data not shown). The generated CTBs provided up to 100 routine paraffin sections (5 μ m) that could be stained with Gram (Figure 3B), Giemsa (Figure 4C), alcian blue, (Figure 8C), PAS (Figure 9C), or

immunohistochemical procedures. The cell morphology in CTBs was comparable to that of cytology smears (Figures 3-10), although cells appeared smaller due to paraffin shrinkage and cell sectioning (instead of cell spread on a slide in cytology smears). The CTB was particularly valuable for the separation of cells from erythrocytes (Figures 4,9 and 10) or the visualization of 3-dimensional cell clusters (Figures 5 and 10). In these cases, the CTBs rendered comparable details as in histopathology slides. One unique feature of CTB was the appearance of cell layers, generally with erythrocytes at the bottom (Figures 4B and 5B), clusters of neoplastic cells in the middle, and neutrophils, macrophages, and mesothelial cells at the liquid-solid interface. This selective concentration of cell types optimized the characterization of suspicious cells and reduced background in immunohistochemistry applications, such as in metastatic epithelial cells in a pericardial effusion of a dog (Figure 5C), T-cell lymphoma in the pleural effusion of a cat (Figure 6B), and B-cell chronic lymphocytic leukemia of a dog (Figure 7B).

Discussion

The histologic assessment of CTBs offers some advantages over conventional fluid cytology. These include: (1) Maintenance of cellular architecture in the presence of cell clusters, allowing the application of criteria used in histopathology; (2) Use of histo- and immunohistochemistry, and permanent archiving allowing the later evaluation within a research setting; (3) Reduction of background staining in immunohistochemistry; (4) Storage of material from residual liquids that would otherwise degenerate and be discarded.^{2,12,13} For all these reasons, CTBs can improve diagnostic accuracy^{4,5} especially when including immunohistochemistry.² Potential drawbacks of CTBs are the suboptimal cellular recovery (in poorly cellular fluids), and increased turnaround time for the final diagnosis.² Therefore, the CTB technique should not be viewed as a replacement of the cytologic evaluation of smears of fluids.⁶ The simplicity and use of in-house technology were particularly relevant for a practice setting, as the processing of fluid samples in microhematocrit tubes by centrifugation and fixation was simple and fast. Clinicians should send CTBs in formalin, along with the fluid and direct smears. After arrival at the laboratory, the processing of CTBs is amenable by any trained histotechnician. In order to avoid potential problems, a glass-writing diamond pen is advised for breaking the capillary tube, and a vegetable modeling clay composed of vegetable matter (eg, Jovi) is preferable.

The CTB protocol described here was cheaper than cell block preparation with HistoGel (one of the first cell block methods described in the veterinary field).⁷ The rare use of high-density solutions (ficoll or percoll) is also irrelevant, as only very small volumes are needed. In our practice, one ml of percoll lasts for > 50 CTBs. In a direct comparison with a cell block using Histogel, the CTB costs up to 40 times less.

In conclusion, we demonstrate the use of CTBs with 8 different types of fluids representative of most of the liquids recovered by practicing veterinarians. This new technique can be particularly useful when only few neoplastic cells appear contaminated with large amounts of blood.

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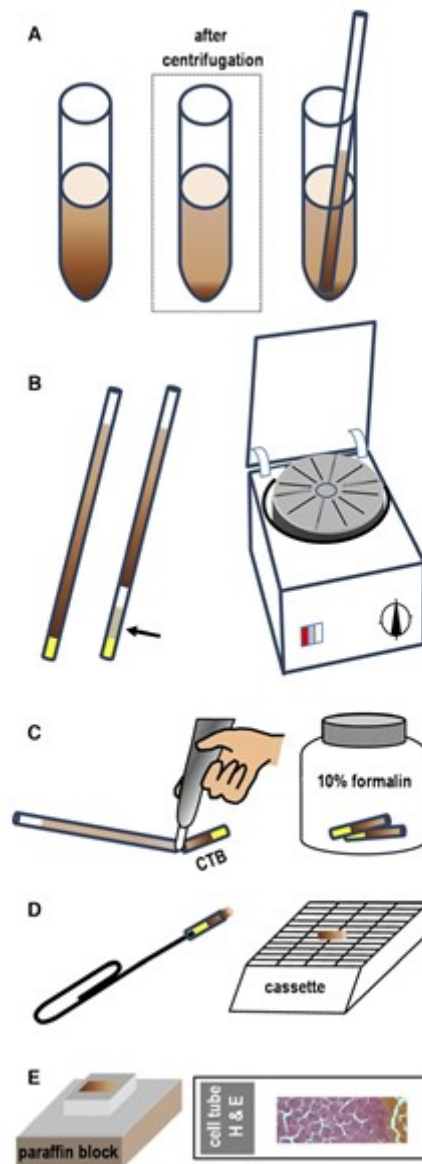


Figure 1. Schematic overview of the cell tube block (CTB) technique. (A) A capillary tube is filled with liquid or a sediment (in low cellular samples). (B) Capillary tubes are sealed with clay; in the absence of significant blood contamination, a high-density medium (eg, percoll) may be aspirated to facilitate separation of the cell pellet from the clay (arrow). (C) After centrifugation, the capillary tube is broken at the liquid-solid interface using a glass-writing diamond pen. (D) After fixation in formalin for at least 24 hours, the CTB is pushed out of the glass capillary using a paper clip and placed in a cassette for routine paraffin embedding. (E) Paraffin-embedded CTB sections are stained with H&E or other stains.

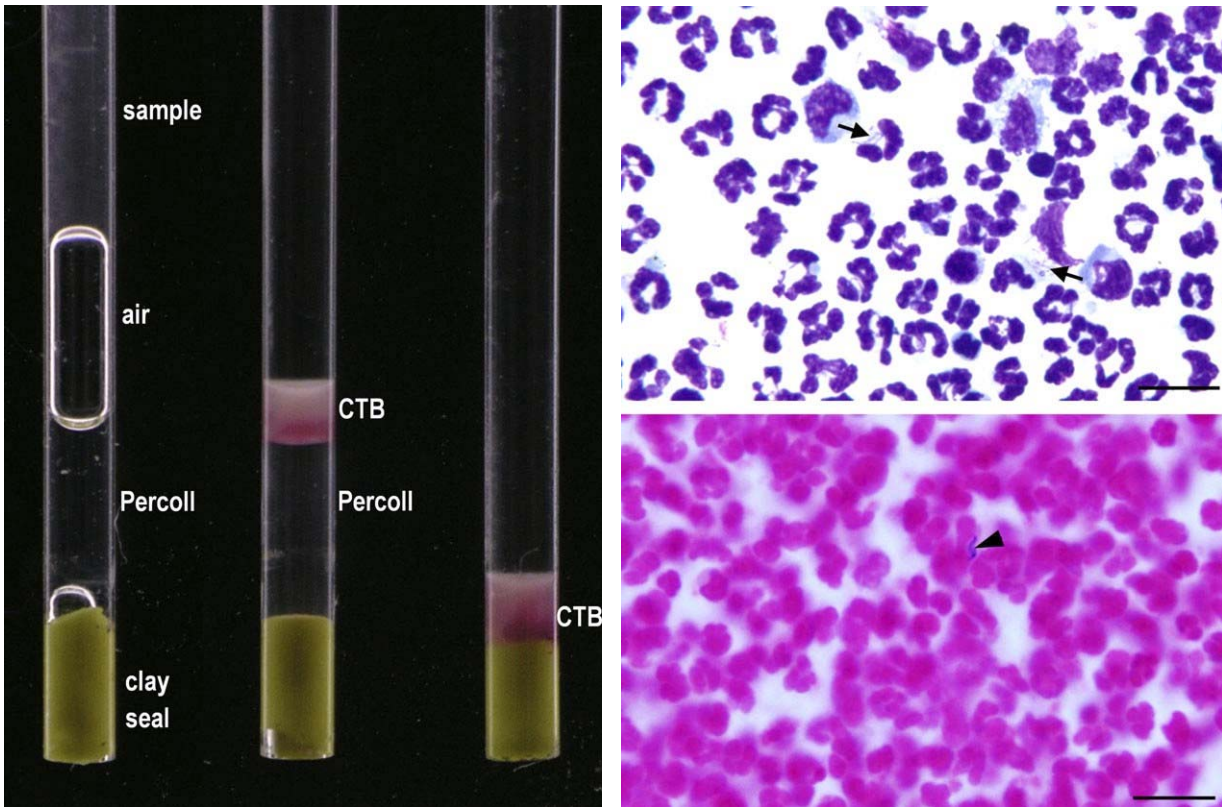


Figure 2. Use of high-density fluids (eg, percoll) for the cell tube block (CTB). (A) The capillary tube is filled with the sample up to three-fourths of the tube length. The tube is gently rocked for formation of an air bubble. A small volume of percoll is slowly aspirated and the tube is sealed with modeling clay. (B) After centrifugation at 14,500g, the CTB remains separated from the clay. (C) Without percoll, the CTB may be difficult to separate from the clay.

Figure 3. Neutrophilic pleocytosis in a cerebrospinal fluid from a cat. (A) Cytospin smear with mildly degenerate neutrophils and intracellular suspected bacteria (arrows), Hemacolor. (B) Section of the cell tube block with Gram-positive bacteria (arrowhead), Gram stain; bar = 12 μ m.

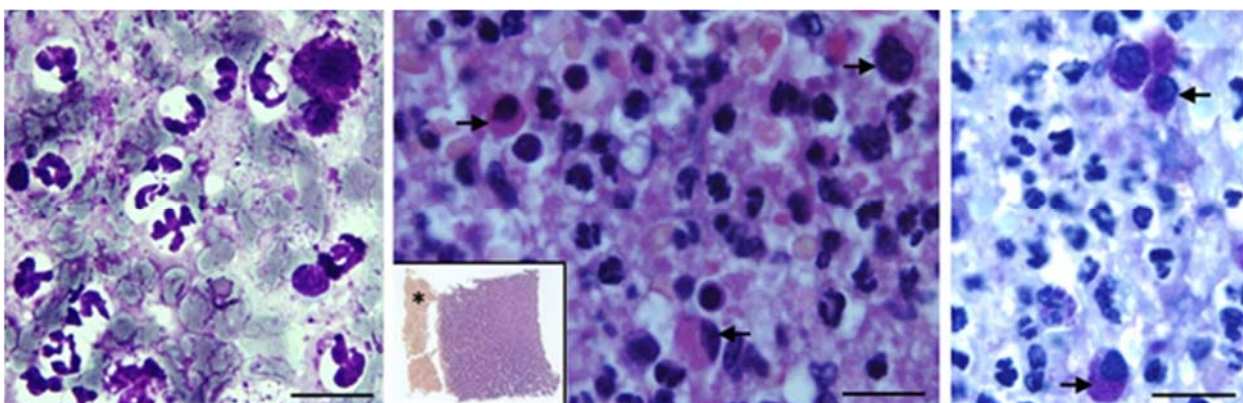


Figure 4. Cystic fluid from a cutaneous mast cell tumor in a dog. (A) Cytospin smear with neutrophils and a mast cell in a hemodiluted background, Hemacolor. (B) Section of cell tube block (CTB) with mast cells (arrows); inset: CTB with a clear separation between erythrocytes (asterisk) and nucleated cells, H&E. (C) Metachromasia of mast cells in a section of the CTB, Giemsa; bar = 12 μ m (inset = 1000 μ m).

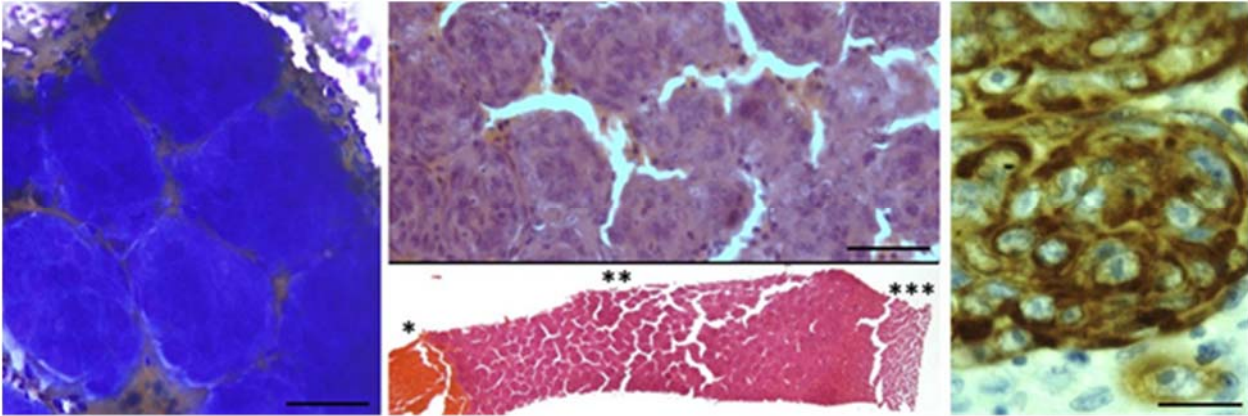


Figure 5. Pericardial fluid from a dog with a metastatic mammary tubulopapillary carcinoma. (A) Cytospin smear showing highly cohesive epithelial clusters, Hemacolor. (B) Section of the cell tube block (CTB); cellular details in neoplastic epithelial cell clusters, such as anisokaryosis and prominent nucleoli are easy to recognize (inset: layered appearance of the CTB, with erythrocytes [asterisk], neoplastic cells [double asterisk], and macrophages and mesothelial cells [triple asterisk] next to liquid-solid interface), H&E. (C) Immunohistochemistry of CTB section with positive reaction to pan-cytokeratin antibody AE1/AE3. Diaminobenzidine chromogen, hematoxylin counterstain; bar = 12 μ m (A), 20 μ m (B) (inset 1000 μ m), 8 μ m (C)

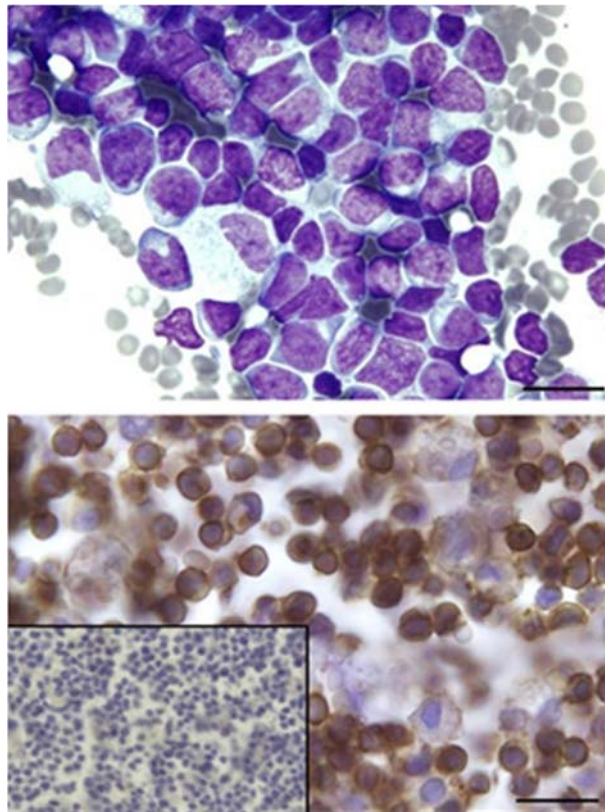


Figure 6. Pleural effusion in a cat with a T-cell lymphoma. (A) Cytospin preparation with medium to large lymphocytes displaying monocytoid features, Hemacolor; (B) In cell tube block sections, a positive immunohistochemistry for CD3 on cell surface was observed, with negative immunohistochemistry for PAX-5, in the inset. Diaminobenzidine chromogen, hematoxylin counterstain; bar = 12 μ m (inset 100 μ m).

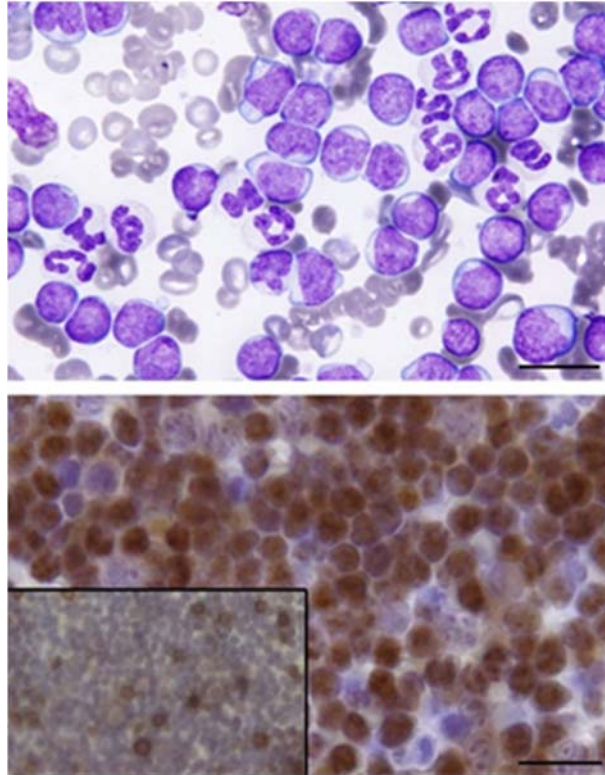


Figure 7. Blood cells from a dog with B-cell lymphocytic leukemia with blastic transformation (A) Buffy coat smear with predominance of immature lymphocytes, Hemacolor; (B) In cell tube block sections, positive immunohistochemistry to PAX-5, with negativity to CD3 (inset) were observed. Diaminobenzidine chromogen, hematoxylin counterstain; bar = 12 μ m (inset 100 μ m).

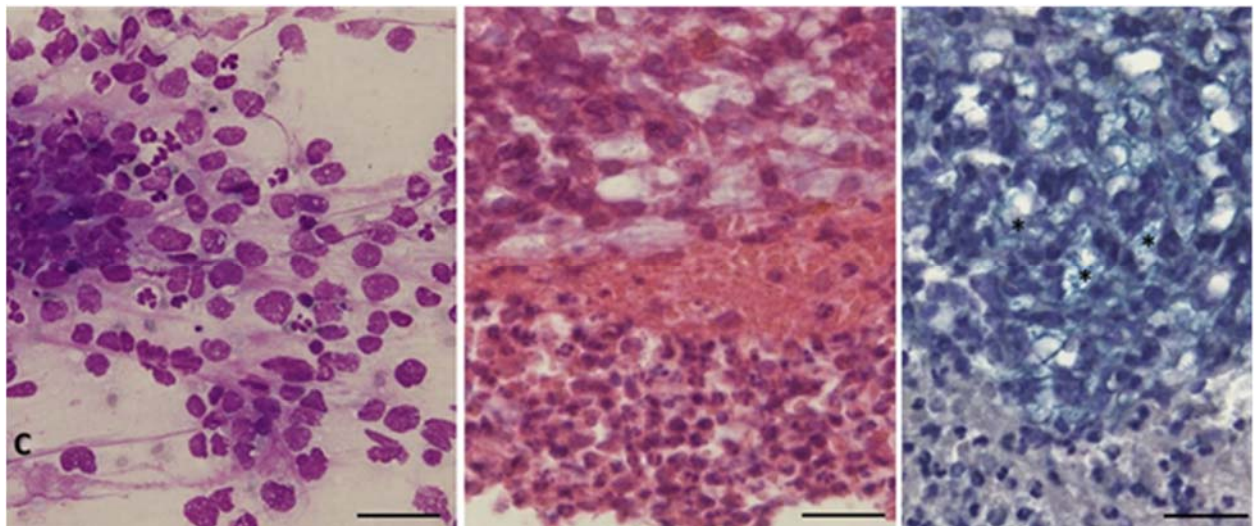


Figure 8. Cystic fluid from a myxosarcoma in a limb of a dog. (A) Cytospin smear with a mixed population of neutrophils, macrophages, and clumps of mesenchymal cells with extracellular matrix, Hemacolor. (B) In cell tube block sections, mesenchymal cells were separated from inflammatory cells, H&E. (C) Alcian blue staining confirmed the mucinous nature of the ground substance (asterisks), supporting the diagnosis; bar = 50 μ m.

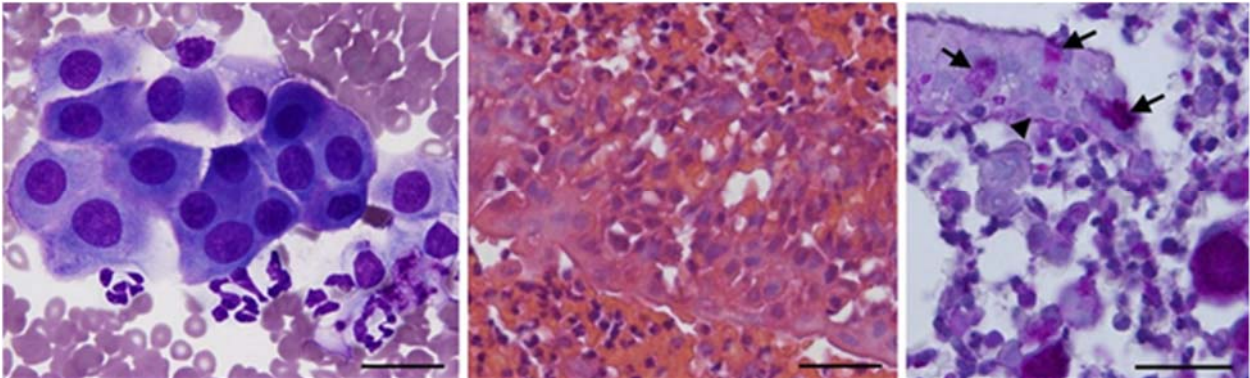


Figure 9. Bronchoalveolar lavage from a dog with chronic bronchitis. (A) Hemodiluted cytospin smear with neutrophils and basophilic ciliated epithelial cells, Hemacolor. (B) Section of cell tube block with ciliated epithelium and inflammatory cells, H&E. (C) Positive staining mucous in hyperplastic goblet cells (arrows) and in basal membrane (arrowhead), periodic acid-Schiff; bar = 50 μ m.

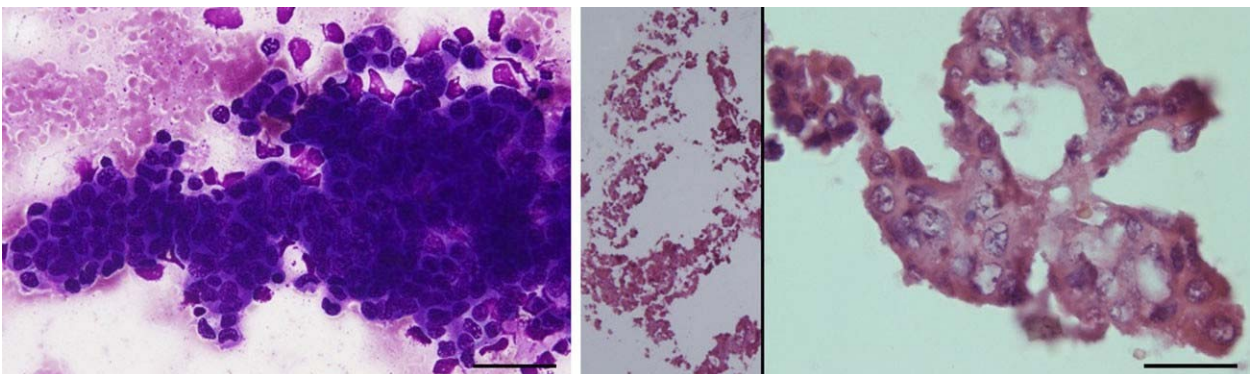


Figure 10. Urine from a dog with a transitional cell carcinoma; (A) Hemodiluted cytospin with large cohesive three-dimensional epithelial clusters in which nuclear details can be only viewed at the periphery, Hemacolor. Bar = 40 μ m. (B) Section of the cell tube block in which epithelial clusters appear separated from blood (left inset) and nuclear features can be easily detailed, H&E; bar = 50 μ m (inset 450 μ m).