How to Interpret Cytology From Body Cavity Effusions

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Body cavity effusions are commonly sampled in clinical practice as a part of diagnostic evaluation. This presentation will review the gross appearance and common cytologic findings to aid the practitioner in rapid decision-making. Author's address: Washington State University, College of Veterinary Medicine, PO Box 646610, Pullman, WA 99164-6610; e-mail: fbain@vetmed.wsu.edu. © 2015 AAEP.

1. Introduction
Abdominocentesis and thoracocentesis are commonly used diagnostic tools in equine practice. Abdominocentesis is one of the standard diagnostic tests used in evaluating colic patients and is often performed to (1) differentiate strangulating from nonstrangulating lesions; (2) confirm gastrointestinal rupture; (3) document, evaluate, and monitor peritonitis as well as the identify-specific microorganisms involved; and (4) evaluate and determine the cell type of suspected neoplastic effusions. Pleural effusion samples may be collected when an effusion is suspected, e.g., when the patient has difficulty breathing because of a large volume of fluid or when a significant volume of effusion has been detected during diagnostic imaging. Pleuropneumonia is the most common pathologic process associated with pleural effusions in horses. Analyzing the pleural fluid is important for assessing the severity and pattern of inflammatory response as well as for detecting the presence and type of microorganisms. Cytologic evaluations are also important for detecting the presence and type of neoplastic cells in patients in which neoplasia is the underlying mechanism for the pleural effusion.

Evaluating body cavity fluids consists of an initial visual assessment, with more specific information obtained by a cytologic evaluation. The intent of this article is to provide the practitioner with information on how to perform a cytologic evaluation of a body fluid sample, specifically peritoneal or pleural fluid samples.

2. Materials and Methods
Sample Collection
Describing the techniques for collecting peritoneal fluid and pleural fluid samples is beyond the scope of this article. Fluid samples for cytologic evaluations should be collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes to preserve cellular morphology. The potential exists for false elevation of the total protein concentration of a fluid as measured by refractometry when the EDTA concentration is increased. This can occur when only a small volume of fluid is added to an EDTA-anticoagulated tube. If more than 2 hours pass before cytologic analysis, it may...
be helpful to make smears on glass slides immediately for later evaluation. Heparin anticoagulant tubes will not preserve cell morphology, and serum tubes may allow for clotting of proteins and clumping of cells, making cytologic identification impossible.

Visual Assessment

The gross appearance of the peritoneal fluid is the first stage in evaluating a fluid sample. Normal peritoneal fluid appears clear and light yellow in appearance (Fig. 1). Fluid from patients with gastrointestinal rupture will often appear green-brown in color, with varying degrees of cloudiness and possibly particulates (Fig. 2). Patients with a strangulating intestinal lesion often have serosanguinous fluid as a result of varying amounts of erythrocytes (Fig. 3). Iatrogenic bleeding into the peritoneal fluid can result from a blood vessel that penetrates the skin or abdominal wall during the procedure or from an accidental splenic puncture during the tap, which results in a blood-tinged or bloody fluid sample. Patients with a peritoneal inflammatory response (peritonitis) will have a cloudy, opaque fluid that may vary from yellow to orange or red depending on the number of erythrocytes present (Fig. 4). Enterocentesis can occur by accidental perforation of a viscus during abdominocentesis. The gross appearance of enterocentesis can vary depending on the character of the luminal content and extent of admixing with peritoneal fluid.

Pleural fluid is often evaluated when an effusion associated with infectious pneumonia (pleuropneumonia) is suspected or when there is clinical and/or diagnostic imaging that indicates a pleural effusion of unknown cause. The gross appearance of pleural fluid with pleuropneumonia can vary depending on the extent of lung injury. Many will have a suppurative (cloudy yellow) appearance, whereas others with more severe lung necrosis may have a cloudy reddish appearance. Pleural fluid samples from patients with neoplastic disease of the pleural cavity can vary in appearance depending on the process. Lymphoma that involves the cranial mediastinal lymph nodes and other thoracic lymph nodes is often associated with a serosanguinous appearance. In some lymphoma patients, pleural fluid may appear yellow with variable cloudiness depending on the degree of cellularity. In some patients with primary neoplasia, suppurative inflammation can occur from necrosis of the neoplasm or secondary infection and obscure the diagnosis; thus, cytologic evaluation is critical in making the correct diagnosis.

Routine body fluid analysis before cytologic evaluation includes a total nucleated cell count and total protein concentration (or total solids via refractometry). Most normal body cavity fluids (peritoneal
and pleural) have a total protein concentration of less than 2.5 g/dL. Total nucleated cell counts are considered normal if there are less than 5,000 cells/μL. Peritoneal fluid from normal foals will have lower total nucleated cell counts (<1,500 cells/μL). The goal of fluid analysis has traditionally been to place the fluid into a pathophysiologic grouping: transudate, exudate, or hemorrhagic effusion (Table 1). Transudates have low total protein concentration (<2.5 g/dL) and low-cellularity (<5,000 cells/μL) fluids. Exudates have an increased total protein concentration (>2.5 g/dL) and high cellularity (>10,000 cells/μL). Hemorrhagic (serosanguinous) effusions by definition contain increased numbers of erythrocytes.

Beyond the visual assessment of a fluid sample, microscopic evaluation of the cytology can provide critical information of the pathophysiologic process and aid clinical decisions. Consideration should be given to whether the cytologic evaluation will be performed in house or transported to a reference laboratory for evaluation. If transporting the samples, it is helpful to prepare direct smears to be sent along with the fluid sample. If shipping biopsy specimens, formalin-fixed tissues should be transported in a separate container because formalin fumes will alter the cytologic features of cells on glass slides, making interpretation difficult.

Techniques for Slide Preparation and Staining

For most normal body cavity fluids, the cellularity will be low (<5,000 cells/μL), and cytologic evaluation of a direct smear will be difficult (Fig. 5). Con-
The concentration of a specimen can be performed by using a regular centrifuge with low gravity (centrifuge 5–10 mL of fluid for 5 minutes at 1,000–1,500 rpm) or a cytocentrifuge (Fig. 6). For such normal fluid samples, it is rare that there will be clinically significant cytologic findings. More commonly, the identification of a transudate of low cellularity and low protein concentration implicates a particular pathophysiologic mechanism (venous or lymphatic obstruction, increased venous hydrostatic pressure, or systemic hypoproteinemia). For more cellular specimens, preparing a good-quality (cellular monolayer) smear on a glass slide is an important first step. The most common difficulty in evaluating highly cellular fluids is making a smear too thick such that staining is inadequate and observing cellular features is impossible. Slide preparation using the push technique, which is similar to what is used for peripheral blood smears, is nicely described in the literature. A drop of fluid is placed on a glass microscope slide, and the edge of a second slide is slid backward until the fluid spreads along the junction of the two slides. The spreader slide is then pushed forward to create a smear. It is useful to create a margin (“feathered edge”) that is at least 1/4 the way from the end of the slide to allow the microscope objective to reach it when the slide is placed on the microscope stage. In practice, it is helpful to prepare 3 to 5 slides so that a representative evaluation of the fluid can be made. Slides should then be air-dried and stained with a Romanowsky-type stain to allow for evaluation and observation of cellular features (Fig. 7).

Cytologic Examination Process

The cytologic examination should take into account the total nucleated cell count and total protein concentration as well as the clinical parameters of the patient. To perform a cytologic examination, one should begin with a cursory screening of the slide on the low-magnification (4×) objective of the microscope. This will allow the examiner to gain an initial impression of the overall cellularity of the smear. Once regions of cellular interest or other features are identified, the examiner can apply immersion oil and then switch to the oil objective (100×) for closer viewing of the area or object of interest. Following this pattern in evaluating a slide will ensure complete evaluation of the entire smear.

Peritoneal Fluid Evaluation

The initial step in cytologic evaluation is to determine whether the sample is representative of the actual constituents of the body cavity effusion. Blood contamination during the procedure is often detected by observing the fluid as it is being collected. This usually results in increased erythrocyte numbers as well as platelets observed on the slide. Enteroctesis can also alter the cytologic findings and complicate the clinical evaluation when

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**Table 1. Categories of Effusion and Pathophysiologic Mechanism**

<table>
<thead>
<tr>
<th>Category</th>
<th>Total Protein</th>
<th>Nucleated Cell Count</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Transudate</td>
<td>&lt;2.5g/dL</td>
<td>&lt;5 × 10^3/uL</td>
<td>Increased hydrostatic pressure</td>
</tr>
<tr>
<td>Exudate</td>
<td>&gt;2.5g/dL</td>
<td>&gt;5 × 10^3/uL</td>
<td>Increased vascular permeability and inflammation</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>&gt;2.5g/dL</td>
<td>&lt; or &gt; 5 × 10^3/uL: RBC &gt; 1 × 10^6/uL; PCV &gt; 3%</td>
<td>Vascular injury</td>
</tr>
</tbody>
</table>

PCV, packed cell volume; RBC, red blood cells.

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Fig. 5. Direct smear of normal peritoneal fluid (200×). The low cellularity of normal peritoneal fluid will make searching for cells a difficult, tedious process, as demonstrated by the lone nucleated cell and few erythrocytes in this direct smear.

Fig. 6. Cytospin concentration of normal peritoneal fluid (200×). Concentration of low-cellularity fluid will aid in evaluating the nucleated cell population.
the patient is systemically compromised (signs of systemic inflammatory response syndrome or endotoxemia). Enterocentesis may result in the presence of protozoal organisms, plant material, and mixed bacterial organisms on the smear (Fig. 8). The task of the clinician is to differentiate enterocentesis from gastrointestinal rupture. The clinical condition of the patient combined with the discovery of some neutrophils with cellular features consistent with sepsis may be helpful in determining the presence of rupture if sufficient time has passed since the rupture for an inflammatory response to occur. In many cases of rupture, actual intact neutrophils are rare.

Cloudy peritoneal fluid samples typically have an increased nucleated cell count. The next step is determining the types of nucleated cells in the smear. Normal peritoneal (Fig. 9) and pleural fluids contain a slight predominance of nondegenerate neutrophils with lesser to almost equal numbers of mononuclear cells (which can be made up of macrophages and mesothelial cells) followed by lymphocytes. With inflammation, the percentage of neutrophils increases according to the intensity of the inflammatory stimulus (Fig. 10). Cytologic differentiation between a septic and nonseptic exudate is a traditional process based on identifying microorganisms in the cytology. An extensive microscopic search should be undertaken for evidence of sepsis in fluid samples with elevated total nucleated cell counts. Degenerative changes in the neutrophils that suggest the presence of sepsis include changes such as karyolysis, which is characterized

Fig. 7. Stain*, commonly used for rapid staining of cytology slides.

Fig. 8. Enterocentesis (100×): multiple ciliated protozoa, numerous mixed bacterial types, and abundant basophilic granular background material.

Fig. 9. Normal peritoneal fluid cytology (cytospin 200×): intact and nondegenerate neutrophils, mononuclear cells, and a few erythrocytes. Note the lightly basophilic, foamy background material associated with normal protein concentration.

Fig. 10. Peritoneal fluid sample, direct smear (200×). Markedly increased nucleated cell count, mostly neutrophils, many degenerate (indicating suppurative inflammation)—associated with peritonitis. Note the background material is lightly basophilic and finely granular, which is consistent with the presence of elevated protein concentration in the fluid and presence of free chromatin debris.
by swelling and disruption of the nuclear membrane (Fig. 11, A). When observed, it is important to perform a detailed search for microorganisms. Although this classification of septic versus nonseptic fluids is based on identifying microorganisms (Fig. 11, B) in the cytology, fluids with markedly elevated numbers of neutrophils are generally considered as potentially septic for clinical management. The presence of phagocytized microorganisms strongly supports body cavity sepsis. Neutrophils can engulf extracellular bacteria that are present from situations such as enterocentesis while in EDTA-anticoagulated tubes over time, thus creating artificial intracellular bacteria. This can happen if the fluid sample is stored for a prolonged period of time (truck or transport) prior to making a smear. Thus, it is valuable to make a smear as soon as possible after sample collection to avoid such artifactual findings. In such circumstances, it is also important to search for other evidence of degenerative changes within neutrophils to differentiate ex vivo engulfment of bacteria from true bacterial phagocytosis associated with a septic process. The type of microorganisms present may serve as a guide to the origin of the peritonitis. Mixed types of organisms suggest gastrointestinal origin or contamination from enterocentesis. Single organisms such as paired cocci may suggest an internal abscess. In postpartum mares, uterine tears are a common problem, and cytologic evaluation should include a search for meconium fragments and squamous epithelial cells that could support the diagnosis (Fig. 12).

Mesothelial cells are occasionally seen in body cavity fluids as individual cells or in cohesive sheets. Reactive mesothelial cells are frequently seen in inflammatory effusions and often require differentiation from neoplastic cells and have thus been called the “great impersonator” (Fig. 13, A–C). Reactive mesothelial cells usually have intense cytoplasmic basophilia, large nuclear-to-cytoplasmic ratios, and binucleate and multinucleate forms—all features similar to those of neoplastic cells. Ruffled cytoplasmic borders are also often seen in reactive mesothelial cells.

Neoplastic effusions may appear as exudates or hemorrhagic effusions. Cytologic evaluation is important in attempting to make a diagnosis before exploratory surgery. Lymphoma is common in the horse; however, neoplastic lymphoid cells may not appear in the peritoneal fluid as commonly as they do in the pleural fluid (Fig. 14). In some cases, there is a secondary inflammatory response with a predominance of neutrophils. Squamous cell carcinoma of the nonglandular region of the stomach (Fig. 15) is another neoplasm that may involve the abdominal cavity and can be diagnosed while cytologically evaluating the peritoneal fluid. Because

Fig. 11. A, Septic peritoneal fluid with marked degenerative changes (karyolysis) in the neutrophils and scattered free chromatin material; paired bacterial cocci are also seen (100×). B, Intracellular bacterial rods in peritoneal fluid from a horse with septic peritonitis (200×).
of the tendency of these neoplasms to penetrate the gastric wall and metastasize, the peritoneal fluid may be seen as an exudate, sometimes septic. Malignant squamous cells can be identified by cytoplasmic keratin formation and often appear as large, bizarre cells that are asymmetrical in size with multiple nuclei. Nuclear features of malignancy are abnormal nuclear chromatin patterns and multiple nucleoli. Mitotic figures, some of which appear atypical, also indicate neoplasia.

Serosanguinous fluids can occur with strangulating intestinal lesions as well as with exudates. Cytologic evaluation can aid in differentiating an active hemorrhage from an iatrogenic one, in which

Fig. 13. A, Peritoneal fluid (500×): reactive mesothelial cells. Note the cytoplasmic basophilia, the large nuclei with clumped chromatin, and the ruffled cytoplasmic margins. B, Peritoneal fluid (500×): binucleate reactive mesothelial cell with ruffled cytoplasmic margin. C, Peritoneal fluid (1,000×): mesothelial cells molding against each other. Note the magenta-ruffled margins characteristic of mesothelial cells.

Fig. 14. Pleural fluid from a patient with cranial mediastinal lymphoma (500×). Most of the nucleated cells are neoplastic lymphocytes with single, large, round nuclei with clumped chromatin and thin rims of darkly basophilic cytoplasm. A single macrophage is present in the upper right with foamy, lightly basophilic cytoplasm. Scattered erythrocytes are also present. The fluid was serosanguinous in its gross appearance.

Fig. 15. Malignant squamous cells in peritoneal fluid (200×) of a horse with gastric squamous cell carcinoma. Note the large variation in nuclear size and atypical cytoplasmic keratinization as features of malignancy.
case platelets may be seen on the smear. A longer-standing hemorrhage may result in erythrophagocytosis (Fig. 16).

Biochemical analysis of peritoneal fluid creatinine and electrolyte concentrations is the common method for confirming uroperitoneum in neonatal foals and, less commonly, in adults. Cytology is occasionally useful in supporting the diagnosis. The key cytologic feature of urine contamination of the peritoneal space is the finding of calcium carbonate crystals (Fig. 17).

Pleural Fluid Evaluation

Evaluating pleural fluid most often involves animals with effusions that have been detected with diagnostic imaging techniques (ultrasonography or radiography). Horses with pleuropneumonia should have thoracocentesis to (1) remove the fluid if excessive volume is present such that lung expansion is compromised and (2) differentiate septic effusion from nonseptic parapneumonic effusion. Nonseptic, noninflammatory effusions can arise from neoplasia and cardiovascular disease. Cytologic evaluation can be useful in further defining the pathologic process.

Pleural fluid from horses with pleuropneumonia can change in gross appearance over time with maturation of the pleural inflammatory process. In the early stages, a parapneumonic effusion of a slightly cloudy, yellow appearance can be seen. Cytologic evaluation of this stage may characterize the effusion as a nonseptic exudate. Changes may differ between pleural spaces that depend on the inflammatory process within that lung (Fig. 18). With more chronicity, pleural effusions can become a thicker, purulent exudate (Fig. 19). In cases of a thick purulent exudate from the pleural space, a direct smear would be appropriate for cytologic evaluation. With chronic fibrinopurulent pleuropneumonia, it is also important to evaluate the pleural space with diagnostic imaging for the presence of loculation, because this could result in variable cytologic changes between fluid pockets. Detecting pleural abscess during diagnostic imaging might guide the fluid collection process for cytologic evaluation.

Pleural effusions associated with lymphoma within the thoracic cavity are often serosanguinous (Fig. 20). Neoplastic lymphoid cells can vary in size, but a predominance of large lymphoid cells or premature stages suggests lymphoma (Fig. 14).
3. Discussion

Cytology of body fluids is a valuable technique for clinical practice. Although often associated with personnel trained in pathology, it is also something clinical practitioners can perform themselves. Practice in slide preparation and learning the technique for slide scanning will allow for obtaining quality information. Performing cytology in house allows for a real-time assessment of a fluid instead of the time required to transport it to a reference laboratory. The time required for an assessment will become shorter as the practitioner gains experience evaluating cytologic smears. When beginning to evaluate cytology of fluid samples in practice, it may be useful to refer fluid samples to a clinical pathologist when there is some question as to the cellular inflammatory patterns or when there are cells that appear atypical or possibly having features consistent with neoplasia. Comparing the in-house observations to the pathologist’s descriptions can be a useful learning process. Currently available reference texts provide excellent photos of common cytologic findings that can help practitioners assess the smear.3,5 The goal of the practitioner is to classify the type of effusion based on total nucleated cell count and total protein concentration and then to pursue further information regarding the cytologic evaluation. As with other clinical examinations (lameness, reproductive, etc.), developing a routine procedure for evaluating a smear will give the clinician a sense of completeness. In certain situations, the smear will confirm clinical suspicions (e.g., suppurative peritonitis associated with intra-abdominal abscessation with *Streptococcus equi* subspecies *equi* or *Rhodococcus equi*, where the microorganism might be observed during the cytology). In some patients, the cytologic evaluation will provide the critical piece of information in making a correct diagnosis, such as a horse with cranial mediastinal lymphoma with voluminous serosanguinous pleural effusion, ventral edema, and weight loss. Finer details of smear evaluation of septic processes include

![Fig. 19. Thick, opaque, purulent exudate from a horse with pleuropneumonia with pleural abscess formation.](image1)

![Fig. 20. Serosanguinous pleural fluid from a horse with cranial mediastinal lymphoma.](image2)
differentiating granular chromatin from ruptured neutrophils, stain precipitates, and other debris from bacterial structures. The process of evaluating a body cavity fluid from the initial clinical examination to collecting the fluid sample and visually assessing and then cytologically evaluating it can provide a more complete understanding of the pathophysiologic mechanisms underway and guide the medical or surgical treatment process more directly.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnote

*Jorvet Dip Quick Stain, Jorgensen Laboratories, Inc., Loveland, CO 80538.*