Effusion fluid analysis: transudates and exudates

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Introduction

Effusions are abnormal fluid accumulations in the pleural, peritoneal, or pericardial body cavities and they come in all shapes and sizes. Some are minor, some occur acutely, some are life-threatening, and some are well-tolerated by the patient. Knowing the cause of the effusion is essential for appropriate patient treatment and care.

To this end, effusion fluid analysis is an invaluable tool to have in your arsenal. Many effusions will fit into two broad categories: transudates and exudates. These two major fluid types form in vastly different ways; thus, fluid analysis and classification is an important step in the diagnostic work-up of a patient presenting with a body cavity effusion.

How do transudates form?

In health, the oncotic and hydrostatic pressures on either side of a permeable membrane (in this case, the capillary wall) are in homeostasis. Any alteration in that pressure balance, usually due to changes within the capillary, will result in fluid moving from the intravascular space to the extravascular space – this process is called transudation.

Any pathological process that increases the capillary hydrostatic pressure (e.g. cardiac disease) or decreases the oncotic pressure within the capillaries (e.g. protein-losing nephropathy) may cause a transudate. Decreased fluid removal from a body cavity due to impaired lymphatic drainage (e.g. lymphoma) may also cause a transudate. Depending on the protein concentration of the fluid, a transudate is classified as low or high protein. Some clinicians and pathologists may also call high protein transudates "modified transudates"; however, this term is a misnomer. The term "modified transudate" implies that the fluid forms by transudation and then is modified over time.

This is often not the case, and for this reason, the more accurate terms "high protein transudate" or "protein-rich transudate" should be used preferentially. Possible causes of low and high protein transudates are listed in Table 1.

When working up a patient with a low protein transudate, an important fact should be borne in mind: the oncotic pressure within the capillary needs to decrease rapidly and markedly (i.e. acute hypoalbuminaemia of < 15 g/L) to cause an effusion in isolation. This is because the body adapts to decreases in oncotic pressure over time by increasing lymphatic drainage from the affected body cavity/cavities. With a rapid, marked decrease, there is no time for such compensation to take place. For this reason, a concurrent increase in hydrostatic pressure is also present in many patients presenting with hypoalbuminaemia and a transudate.

How do exudates form?

Exudates form through active inflammation rather than by passive transudation. With the release of inflammatory mediators, there is an increase in vascular permeability. This allows plasma proteins to escape into the body cavity in a process called exudation. Leukocytes will also be activated to emigrate across the blood vessel walls and into the fluid that is now forming as a result of exudation. Exudates may be secondary to septic (i.e. infectious) or non-septic causes.

Bacteria are the most common cause of septic peritonitis and pleuritis, but other microorganisms, such as fungi and protozoa, may also cause a septic exudate. When high numbers of neutrophils are present in the fluid, especially when the neutrophils appear degenerate, careful examination for bacteria is warranted (Figure 1). Peritoneal and pleural fluid cytology has good sensitivity (82.8 – 87.5%) for detecting bacteria. In other words, most cases of a bacterial exudate can be diagnosed on cytology with false negatives expected in a low number of cases.

If a bacterial exudate is suspected (e.g., due to gastrointestinal devitalisation or bite wounds) but no bacteria are visualised, bacterial culture and antibiogram should be performed to confirm an infection. Bacterial culture and antibiogram are warranted when bacteria are visualised for appropriate antimicrobial therapy selection. Fluids submitted for culture should be placed in culture media or a sterile tube, not in an EDTA tube. This is because EDTA is bacteriostatic and will prevent bacterial growth.

In addition to fluid culture, infectious exudates may be excluded using a combination of history, clinical presentation and examination, diagnostic imaging, and clinicopathological testing (e.g. haematology, clinical chemistry). Once excluded, non-septic causes may be considered. Possible causes of non-septic and septic exudates are listed in Table 2.

April 2023

How do I analyse and classify a body cavity effusion?

Effusion fluid analysis doesn't have to be daunting; in many cases, this may be done using your in-clinic laboratory. Before sample collection, the effusion volume should be taken into consideration. In dogs and cats, fluid that can be collected by centesis is almost always pathological because, in health, these fluids are only present in very small volumes. Conversely, healthy horses and cattle may have enough normal peritoneal fluid (low protein, poorly cellular) to aspirate 5 – 10 mL easily.

Thus, a spurious diagnosis of a low protein transudate may be made if this peritoneal fluid is sampled without assessing the volume of the fluid (e.g., by ultrasonographic imaging) or confirming a pathological effusion by thorough clinical examination.
Once collected, the fluid should be placed in an EDTA tube. If enough fluid is collected (> 2 mL), some fluid should also be placed in a plain (preferably sterile) tube. The EDTA will prevent sample clotting and will preserve the cell morphology for cytology. The plain tube will be useful if ancillary testing is needed, such as culture and chemistry testing, as EDTA may interfere with these tests.

Using the fluid in the EDTA tube, a series of simple steps may be followed for fluid analysis:

Step 1 – Examine the appearance of the fluid

Is the fluid bloody, milky, clear, brown, or green? The colour of the fluid may be useful to indicate if/what ancillary testing will be needed. For example, suppose the fluid is milky. In that case, a chylous effusion or a purulent exudate may be suspected, and plain fluid should be collected in case of fluid triglyceride measurement or culture, respectively. The colour may help to establish the cause of an effusion. For example, a very bloody effusion is probably caused (at least in part) by haemorrhage – this is the most common type of pericardial effusion in dogs. Also, brown or green fluid that smells foetid may indicate gastrointestinal contents. Any blood clots or clumps of material should be noted, as these may clog the tubes of a haematology analyser. Lastly, the fluid colour may guide early therapeutic decisions, such as in horses with colic. Serosanguinous peritoneal fluid is highly specific (up to 99%) for predicting surgical colic.

Step 2 – Measure/estimate the fluid cellularity

Fluid cellularity is traditionally used to differentiate a transudate from an exudate. Transudates typically have total nucleated cell counts (TNCCs) of < 5000 cells/µL (or 5 x 10^9/L), and low protein transudates usually have TNCCs of < 1500 cells/µL. Exudates should have a TNCC above this cut-off. Some authors prefer to use a TNCC cut-off of 3000 cells/µL to differentiate exudates and transudates. By decreasing the cut-off value from 5000 to 3000 cells/µL, more exudates can be identified, but some transudates (usually high protein) may be misclassified. Suppose a fluid TNCC is in the "grey zone" between these two cut-off values. In that case, additional testing should be undertaken to classify this fluid further (e.g. fluid culture, fluid chemistry, microscopy, diagnostic imaging). The TNCC is easily measured by many in-house haematology analysers (e.g. IDEXX ProCyte Dx, Abaxis VetScan HM5). Preparation and examination of a direct smear, in other words, a smear prepared from well-mixed and unspun fluid, is necessary to confirm the measured TNCC. An estimate of the cellularity can also be made using a direct smear if a TNCC cannot be obtained.

Step 3 – Measure the fluid total protein concentration

The total protein concentration of a fluid is used to differentiate a low-protein transudate from a high-protein transudate and an exudate. Typically, fluids with a protein concentration of < 25 g/L are classified as low protein transudates, provided the TNCC is consistent with a transudate. High protein transudates and most exudates have protein concentrations ≥ 25 g/L. Occasionally, exudates may have protein concentrations of < 25 g/L, and this protein cut-off should not be used to exclude an exudative process.
The protein concentration is measured using a refractometer and the plain or EDTA fluid supernatant (provided the EDTA tube is more than50% filled). Unspun fluid may also be used if the fluid is clear (i.e. not opaque or turbid).A quick note on feline infectious peritonitis (FIP): A classic example of fluid "misclassification" using these TNCC and protein concentration cut-offs occurs with FIP's effusive/wet form. This viral infection causes an immune-mediated vasculitis, which results in the exudation of high protein fluid of low cellularity into the extravascular space. According to traditional classification criteria, this fluid would be classified as a "high protein transudate" due to the usually low TNCC (often < 5000 cells/µL). However, as an inflammatory process causes the effusion, this fluid is referred to as an exudate.

Step 4 – Prepare a sediment smear

A direct and a sediment smear are required for a complete fluid analysis of most fluids. A direct smear alone will suffice if the cellularity is very high (e.g. > 50 000 cells/µL). The EDTA anticoagulated fluid is centrifuged at 2000 – 4000 rpm for 5 minutes to obtain a fluid sediment. The supernatant is then decanted into a plain tube by inverting the EDTA tube over the plain tube in one swift motion. In so doing, the pellet will remain "stuck" at the bottom of the tube. This pellet may be resuspended by adding a few drops of supernatant (if necessary) and tapping the EDTA tube a few times with a finger. A drop or two of the resuspended pellet is used to prepare a sediment smear.

Step 5 – Examine the smears microscopically

As mentioned in step 3, a direct smear is used to estimate or confirm the fluid cellularity. The direct and sediment smears should also be examined to assess the degree of blood admixture, the different cell types and proportions, and any atypical findings (e.g., the presence of bacteria, foreign material, and neoplastic cells). Transudates (low and high-protein) predominantly contain low numbers of non-degenerate

neutrophils and macrophages with or without some small lymphocytes (Figure 2). The proportion of neutrophils may exceed 50% in some transudates and is not a useful marker to predict an inflammatory process (i.e., an exudate). Exudates will typically have increased numbers of neutrophils, macrophages, or a mix of macrophages and neutrophils depending on the aetiology of the exudate (Figure 3). Fluids with > 10% eosinophils are classified separately as eosinophilic effusions, regardless of the other cell types present (except for neoplastic cells) and have a specific differential list (Table 3). When examining smears microscopically, mesothelial cells may be encountered. These cells line the serosal membranes and may be seen in various numbers and states of reactivity. They are especially noted in pericardial and long-standing effusions. Mesothelial cells may be individualised or arranged in small aggregates or balls and are primarily recognised by the pink fringe on their cell margin, known as a glycocalyx (Figure 4). Reactive mesothelial cells may show significant cellular and nuclear atypia, such as multinucleation, anisocytosis and anisokaryosis, and mitotic figures. Differentiating these reactive mesothelial cells from neoplastic mesothelial and epithelial cells on cytology is challenging, and when in doubt, consultation with a veterinary clinical pathologist is advised.

What other tests can I use to classify an effusion?

Ancillary testing on fluid samples can be very useful for further classifying an effusion by providing additional information to complement the fluid TNCC and protein measurements. One such ancillary test is the Rivalta test. If FIP is suspected in a cat (e.g. young cat with a poorly cellular high protein effusion), the Rivalta test is a highly sensitive screening test that can be performed using the plain or EDTA fluid supernatant, distilled water, and acetic acid. If a Rivalta test is negative, then FIP can be excluded. For more on the Rivalta test, please refer to Fischer et al. (2012).

In some cases, fluid chemistry can provide a definitive diagnosis, such as uroperitoneum, bile peritonitis, and chylous effusions. In the case of pancreatitis, fluid lipase concentrations may be measured using the supernatant (from a plain tube). If the fluid lipase DGGR activity is < 500 U/L, then pancreatitis is a very unlikely cause of the effusion. Similarly, if the fluid Spec cPL (canine pancreatic specific lipase) concentration is < 500 µg/L, then pancreatitis is excluded. Conversely, if fluid lipase DGGR is more than double the serum lipase DGGR activity, then pancreatitis is very likely.

Lactate dehydrogenase (LDH) is a useful biomarker to distinguish transudates and exudates in dogs and cats. This is especially important when the traditional fluid classification does not fit the clinical picture or suspicions or when the TNCC is in the "grey zone" of 3000 – 5000 cells/µL. In general, fluids with increased LDH activities are classified as exudates. However, various LDH cut-offs are applied depending on the method used, so it is crucial to know the method of LDH measurement before interpreting the results.

For method-appropriate cut-off values, please refer to Smuts et al. (2016) or consult a veterinary clinical pathologist. If the method of LDH measurement is not known, then a ratio between the fluid and serum LDH activity may be calculated, provided the two samples are collected concurrently and measured using the same assay. The fluid: serum LDH ratio in dogs is typically < 0.5 in transudates, and ratios of > 4 are usually associated with septic exudates. In cats, a fluid: serum LDH ratio of ≤ 0.62 is consistent with a transudate.

Several authors have recently proposed a series of chemistry tests as an alternative to the traditional fluid protein and cellularity criteria to differentiate a transudate from an exudate. One such alternative is known as Light's criteria, which include the fluid LDH activity, fluid: serum LDH ratio (i.e. fluid LDH activity/serum LDH activity), and fluid: serum total protein ratio (i.e. fluid protein concentration/serum protein concentration). Unfortunately, Light's criteria have only been evaluated in dogs and cats with pleural effusions.

In a study on feline pleural effusions (Zoia & Drigo 2016), these criteria were good for transudate screening. Still, they misclassified some transudates (e.g. a high protein transudate caused by congestive heart failure was classified as an exudate). In another study on canine pleural effusions (Zoia et al. 2020), Light's criteria were simplified to only fluid LDH activity and serum total protein concentration. Transudates were identified and differentiated from exudates with a fluid LDH activity cut-off of < 100 U/L (lactate to pyruvate method).

High-protein transudates were identified and differentiated from low-protein transudates with a serum total protein concentration of ≥ 40 g/L. These criteria were able to distinguish most exudates and transudates accurately and, for this reason, show great promise in dogs. More studies on fluid classification with Light's criteria are needed in both species.

Conclusion

Although the fluid classification of transudates and exudates is not perfect, it is valuable for narrowing the list of potential differential diagnoses and, in some cases, for making a definitive diagnosis. Suppose there is still doubt after using the traditional fluid classification criteria of TNCC and protein concentration. In that case, ancillary tests such as fluid LDH activity and measurement of fluid lipase may be useful to clear some of the muddy water.

Overall, fluid analysis and classification are important pieces of the puzzle that makes up the diagnostic work-up of a patient with a body cavity effusion.

Low protein transudate	High protein transudate
Pre-sinusoidal (of the liver) portal hypertension	Congestive heart failure (Usually pleural in cats and peritoneal in dogs)
Sinusoidal portal hypertension due to liver disease	Post-sinusoidal portal hypertension (e.g., thrombosis)
Hepatic cirrhosis	Vasculitis
Protein-losing nephropathy	Organ torsion, volvulus, or ischaemia (e.g., lung lobe, intestinal, liver lobe)
Protein-losing enteropathy	Diaphragmatic hernia
Malnutrition/malabsorption	Neoplasia
Lymphatic obstruction (e.g., lymphangiectasia)	Non-neoplastic mass lesion
Neoplasia	Gastrointestinal intussusception and obstruction
Protein losing dermatopathy (Rare, e.g. massive burns)	Pneumonia
Early congestive heart failure (cats; pleural fluid)	Thrombosis (e.g., in splenic vein)
Gastrointestinal obstruction and ischaemia (horses)	Pancreatitis
	Proximal/anterior enteritis (horse)
	Trauma
	Pericardial effusion due to various causes

Table 1: Disorders and conditions associated with transudates.

Septic exudates	Non-septic exudates
Bite wounds and other penetrating injuries	Neoplasia
Rupture of an abscess (e.g. lung, liver, prostate)	Tissue necrosis (e.g., splenic torsion, intestinal volvulus)
Rupture or perforation of an infected hollow organ (e.g. pyometra)	Lymphadenitis
Bacterial pneumonia	Steatitis
Non-sterile (migrating) foreign body (e.g., grass awn)	Granulomas (e.g., sterile foreign body)
Gastrointestinal tract perforation, devitalisation, or rupture (e.g., perforation by a foreign body)	Vasculitis (including feline infectious peritonitis)*
Fungal granuloma/infection	Organ inflammation (e.g., pancreatitis, hepatitis, enteritis)
Post-operative infection	Long standing uroperitoneum, bile peritonitis, and chylous effusions
	Seminoperitoneum (spermatozoa)
	Post-operative inflammation (lasts up to 2 weeks in horses)

*Table 2: Disorders and conditions associated with exudates.****See text for more detail***

Categories	Diseases/conditions
Neoplastic disease	Lymphoma Visceral or systemic mast cell tumour
Protozoal disease	Sarcocystosis
Parasitic disease	Heartworm disease (pleural fluid) Peritoneal cestodiasis Lungworms
Eosinophilic inflammation in an organ	Interstitial pneumonia Eosinophilic pulmonary granulomatosis Eosinophil gastroenteritis
Miscellaneous	Allergic or hypersensitivity disease Eosinophilic granuloma Foreign body Hypereosinophilic syndrome (mostly cats)

Table 3: Disorders and conditions associated with eosinophilic effusions

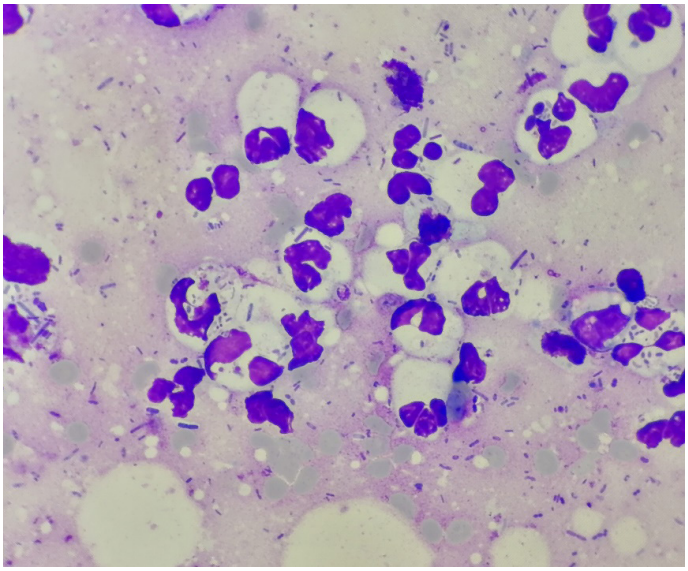

Figure 1: Degenerate neutrophils containing phagocytosed rod-like bacteria

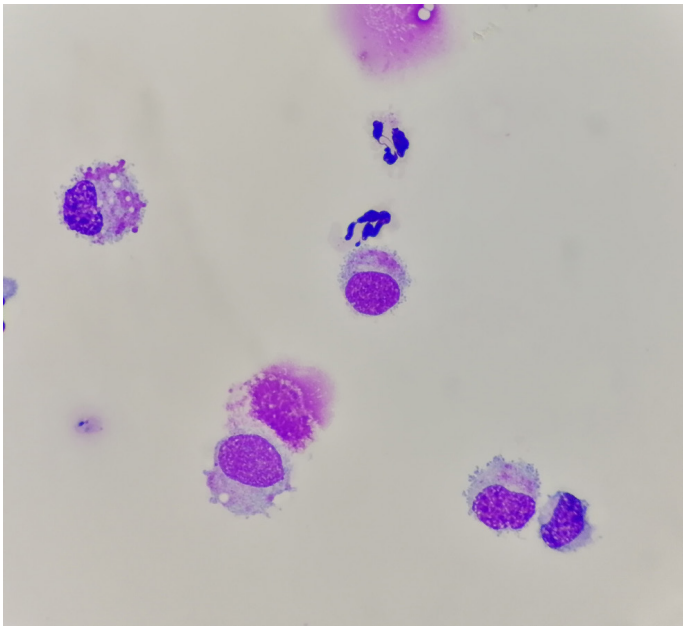

Figure 2: Transudate with a low number of neutrophils and macrophages bacteria

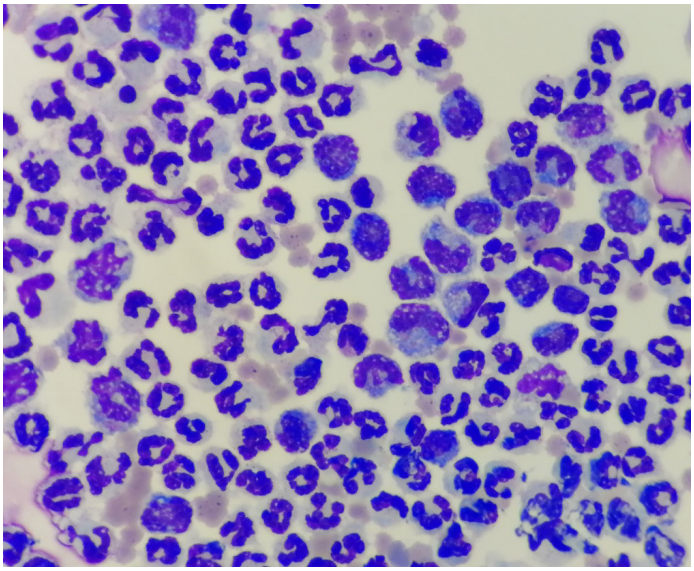

Figure 3: Exudate with an increased number of non-degenerate neutrophils and macrophages

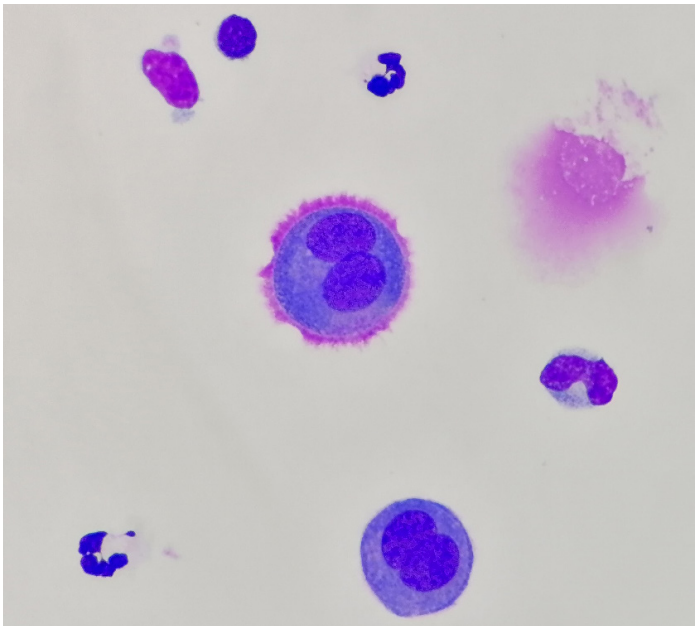

Figure 4: Binucleated mesothelial cells with neutrophils, a small lymphocyte, and a macrophage. The top mesothelial cell has a pink fringe-like glycocalyx

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QUESTION 1

Exudation is a process that:
a. Is caused by inflammation.
b. Results in high protein fluid entering the extravascular space
c. Is often, but not always, accompanied by leukocyte emigration
d. May be caused by neoplasia
e. All of the above

QUESTION 2

Microscopic examination of direct fluid smears is useful to:
a. Confirm the TNCC measured by a haematology analyser is correct.
b. Estimate the fluid cellularity
c. Perform a leukocyte differential count
d. Determine the degree of blood admixture
e. All of the above

QUESTION 3

You collect pleural fluid from a dog and see the following cytological picture on the sediment smear: 67% non-degenerate neutrophils, 8% small lymphocytes, 12% activated macrophages, and 13% eosinophils with a low number of mesothelial cells, some of which are multinucleated. Your diagnosis is:
a. Neutrophilic exudate
b. Eosinophilic effusion
c. Exudate with mixed inflammation
d. Mesothelioma
e. High-protein transudate

QUESTION 4

Which pathomechanism is not involved in transudate formation?
a. Lymphatic obstruction
b. Hypoalbuminaemia
c. Vascular permeability
d. Thrombosis
e. Increased pressure in the capillaries

QUESTION 5

Bacterial culture of peritoneal effusion fluid is indicated when:
a. Mostly degenerate neutrophils are seen microscopically
b. Bacteria are visualised on fluid cytology
c. A septic process is suspected (e.g. bacterial pneumonia)
d. The fluid: serum LDH ratio is >4
e. All of the above

QUESTION 6

Normal peritoneal fluid:
a. Is poorly cellular and low in protein
b. Cannot be aspirated in horses
c. Can be aspirated easily in cats
d. May contain low numbers of bacteria
e. May contain >10% eosinophils

QUESTION 7

Bloody or blood-tinged fluid:
a. May indicate a haemorrhagic effusion
b. Is usually associated with medical colic in horses
c. Is uncommon in pericardial effusions
d. Is a normal finding when aspirating effusion fluid
e. All of the above

QUESTION 8

Effusion fluid in a syringe is not an appropriate sample for:
a. Fluid lipase DGGR activity
b. Fluid cellularity or TNCC
c. Refractometric protein concentration
d. Rivalta test
e. Fluid LDH activity

QUESTION 9

The term modified transudate:
a. Inaccurately describes how many high-protein transudates are formed
b. Is a transudate with a protein concentration ≥ 25 g/L
c. Is a transudate with a TNCC of > 1500 cells/uL
d. Can be used interchangeably with the term "high-protein transudate"
e. Is accepted amongst all clinicians and pathologists

QUESTION 10

Fluid LDH activity:
a. Alone can be used to identify a low-protein transudate
b. Has been assessed in canine and feline pleural effusions
c. Has specific cut-off values in dogs regardless of the LDH method
d. Can replace microscopic examination of effusion fluid
e. All of the above

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