Diagnostic Yield of Cytologic Analysis of Pericardial Effusion in Dogs

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Background: Pericardial effusion cytology is believed by many to be of limited value, yet few studies have evaluated its diagnostic utility.

Objectives: To determine the diagnostic utility of cytologic analysis of pericardial effusion in dogs and to determine if consideration of additional data could improve the diagnostic yield.

Animals: Two hundred and fifty-nine dogs with cytologic analysis of pericardial effusion performed between April 1990 and June 2012.

Methods: Electronic medical records from a university teaching hospital were retrospectively reviewed; signalment, complete blood count, serum biochemistry, cytologic analysis of pericardial effusion, and echocardiographic data were recorded. Cytology was classified as diagnostic (infectious or neoplastic) or nondiagnostic (hemorrhagic or other) and groups were compared with multiple Student’s t-tests.

Results: Cytology was grouped as nondiagnostic (92.3%) or diagnostic (7.7%) and characterized as hemorrhagic (90%), neoplastic (4.6%), infectious (3.1%), or other (2.3%). Overall cytologic analysis of pericardial effusion diagnostic utility was 7.7% and increased to 20.3% if the effusion hematocrit (HCT) <10%; echocardiographic evidence of a mass did not result in a significant increase in the diagnostic utility.

Conclusions and Clinical Importance: The diagnostic utility of cytologic analysis of canine pericardial effusion is variable depending on the underlying etiology. In this group of dogs, the diagnostic yield of cytologic analysis was greater for pericardial effusion samples in which the HCT was less than 10%.

Key words: Cardiac tamponade; Cytology; Hemangiosarcoma; Idiopathic; Mesothelioma.

Pericardial effusion has an overall prevalence of 0.43% in dogs examined at a university teaching hospital and occurs in approximately 7% of dogs with clinical signs of cardiac disease. Etiologies include septic pericarditis, coagulopathies, left atrial rupture, neoplasia, and idiopathic pericardial effusion. Idiopathic effusions and cardiac hemangiosarcoma are the most common etiologies whereas heart base tumors, mesothelioma, and lymphoma occur less frequently. Prognosis varies greatly based on the underlying diagnosis and is poor to guarded for hemangiosarcoma (median survival time 1–4 months) and good to excellent for idiopathic effusions (survival time up to 4 years).

Cytologic analysis of pericardial effusion provides an accurate and definitive diagnosis when infectious agents or lymphoma are the causative etiologies. Conversely, neoplastic diagnoses other than lymphoma (hemangiosarcoma, chemodectoma, mesothelioma) are rarely diagnosed based on cytology alone and additional diagnostic tests performed on fluid or blood samples have a poor ability to distinguish these from non-neoplastic etiologies. Pericardial effusion values for lactate, hematocrit (HCT), and urea nitrogen are significantly higher whereas effusion values for pH, bicarbonate, and chloride are significantly lower in neoplastic versus non-neoplastic effusions; although the degree of overlap between the groups resulted in limited value to this testing. The difference between peripheral and pericardial glucose values was significantly greater in dogs with neoplasia than those without neoplasia. pH is a poor diagnostic marker for differentiating neoplastic from non-neoplastic effusions.

Diagnostic utility was defined, for the purpose of this study, as the ability to obtain an etiologic diagnosis. Similar to veterinary medicine, the diagnostic utility of cytologic analysis of pericardial effusion in human medicine is variable; overall the diagnostic utility is between 24 and 26%. Although, with malignant neoplastic effusions, the sensitivity is between 67 and 92% and identifying neoplastic cells in pericardial effusion is considered to be an accurate method to distinguish malignant from benign pericardial effusions. Diagnostic yield of pericardial effusion analysis is improved with a combination of cytology as well as pericardial and epicardial biopsy via pericardioscopy.

The sensitivity and specificity of cytologic analysis of pericardial effusion in dogs is unknown and the

Abbreviations:

| Abbreviation | Description |
|--------------|-------------|
| HCT          | Hematocrit  |
| MCV          | Mean cell volume |
| PCV          | Packed cell volume |
| RBC          | Red blood cell |
| ROC          | Receiver operating characteristic |

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diagnostic utility is considered to be limited, causing some clinicians not to routinely submit effusions for cytologic analysis.3,5,8 Examination of pericardial fluid cytology diagnosed neoplasia erroneously in 4/31 (13%) and failed to identify 74% of the neoplastic cases evaluated in one population of dogs.3 It is unknown if clinical data available at initial diagnosis can aid in predicting the diagnostic utility of the pericardial effusion analysis before submission. The objective of this study was to determine the diagnostic utility of cytologic analysis of pericardial effusion in a large population of dogs and to evaluate if data available at initial presentation could increase the diagnostic utility.

Materials and Methods

Sample Identification

Electronic medical records from the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis were searched for dogs that had cytologic analysis of pericardial effusion performed from April 1990 to June 2012. Only the first cytologic analysis from a dog was included in the study. Exclusion criteria were incomplete cytologic analysis report, incomplete medical record, or a fluid analysis that was not pericardial effusion, but was billed as such. For analysis, effusion cytology was categorized as either diagnostic (infectious or neoplastic) or nondiagnostic (hemorrhagic or other).

Data Collection

Signalment was recorded along with pericardial effusion data including gross and supernatant appearance, total protein, total red blood cell (RBC) and nucleated cell counts, a differential white blood cell count, and a cytologic diagnosis. Complete blood count and serum biochemistry profiles run at the time of effusion analysis, if available, were recorded. Hematologic parameters were analyzed from April 1990 to August 2001 using a commercially available analyzera and from September 2001 to June 2012 a different commercially available analyzerb was used using the species-specific setting.8 Pericardial effusion HCT was calculated based on the measured effusion RBC count and the mean cell volume (MCV) from the CBC via a standard formula HCT (%)) = [MCV (fl) × RBC count (M/L)] / 10. If a CBC was not available from a dog, the mean MCV of 67.8 (standard error of mean = 0.4) from the CBC of all dogs was used for the calculation. Echocardiographic results performed by a board certified cardiologist, if available, were recorded. Results of echocardiography were subdivided into 3 groups: definite, suspicious, or no cardiac mass identified on echocardiogram. Histopathologic results of pericardial or cardiac tissue from either necropsy or biopsy were recorded. For statistical analysis, the histopathologic results were grouped into neoplastic and non-neoplastic diagnoses.

Cytology Review

 Archived slides of pericardial effusion samples, when available, were reviewed by a board certified clinical pathologist blinded to the results in the medical record. Slides were reviewed to determine the underlying diagnosis and to subjectively reaffirm that the previous differential leukocyte count was accurate. If a sample was not available for review, then the original cytologic diagnosis from a board certified clinical pathologist was used.

Cytologic diagnosis of the effusion, for the purpose of data analysis, was classified as hemorrhagic, infectious (bacterial or fungal) neoplastic, or other (nonhemorrhagic, non-neoplastic, and noninfectious). Neoplastic or infectious effusions may have had a hemorrhagic component, but if neoplastic or infectious cells were identified, they were not classified as a hemorrhagic effusion. Diagnosis was based off of cytology results alone, yet was subsequently compared with a histologic diagnosis, when available.

Statistics

Descriptive statistics were performed with commercially available software.a Multiple t-tests comparing dog demographics, pericardial effusion data, CBC, and serum biochemistry were performed for diagnostic versus nondiagnostic effusions. Samples were then subgrouped based on presence or absence of a mass on echocardiogram and a diagnostic or a nondiagnostic effusion. Multiple t-tests comparing 47 separate variables from patient demographics, pericardial effusion data, CBC, and serum biochemistry were performed. A Bonferroni correction was utilized and a P value of <.001 was considered significant. Receiver operating characteristic (ROC) curve analysis was performed on measured effusion RBC count and calculated HCT of the pericardial fluid and whether effusion was diagnostic or nondiagnostic on cytology. Categorical data were compared using chi-square or Fisher’s exact test where appropriate. A P value <.05 was considered significant.

Results

A total of 306 cytologic samples were identified, 33 were subsequent analyses from the same dog, leaving a total of 273 patient records identified as having cytologic analysis of pericardial effusion. A total of 14 samples were excluded from the study; 8 of these were excluded because of incomplete cytologic data, 2 were excluded because of an incomplete medical record, and 4 were excluded as no cytologic analysis of pericardial effusion had actually been performed. Two hundred fifty-nine pericardial effusion analyses remained and were included in this study with 249 slides available for re-review of cytology.

Breeds most commonly represented were mixed breed dogs (21%), Golden Retrievers (17%), Labrador Retrievers (15%), and German Shepherds (4%). Median age was 9 years (range 1–16 years). Patient weight was recorded at the time of initial presentation in 57.5% of cases, with the median weight being 32 kg (range 2–71 kg). Males represented 59% of the study population with 99/106 (93%) of the females spayed and 98/153 (64%) of the males neutered. Complete blood counts from 124 cases (47.9%) and serum biochemistries from 108 cases (41.7%) were available. Packed cell volume of the pericardial effusion was available in 19/259 (7.3%) of the cases included in this study, whereas the effusion total RBC count was available in 254/259 (98%) of the cases in this study.

Of the 259 cases included, 233 were hemorrhagic (90%), 12 were confirmed neoplasia (4.6%), 8 were infectious (3.1%), and 6 were classified as other (2.3%) based on cytologic analysis. Cytologic analysis of pericardial effusion was considered diagnostic in 20/259
(7.7%) cases. Neoplastic effusions included round cell neoplasia (n = 7), carcinoma (n = 1), atypical epithelial cells (n = 3), and hemic neoplasia (n = 1). Round cell neoplasia was further subdivided into lymphoma (n = 5), histiocytic (n = 1), and undetermined round cell neoplasia (n = 1). Infectious effusions were noted in 8 cases: bacterial (n = 7) and fungal (Candida albicans, n = 1). Six cases were classified into an “other” category: chylous effusion (n = 3), transudate (n = 1), modified transudate (n = 1), and marked neutrophilic inflammation with mixed mesothelial reactivity (n = 1).

When diagnostic versus nondiagnostic cytology was compared, there was significant differences in the pericardial effusion total nucleated cell counts, neutrophil counts, large mononuclear cell counts, total RBC counts, HCT of the effusion, and serum albumin (Table 1).

An echocardiogram was performed in 254/259 (98.1%) cases. Of those cases, 112 (44.1%) did not have a mass identified on echocardiogram, 33 (13.0%) had a definite mass noted on echocardiogram. Diagnostic and nondiagnostic effusion groups were subdivided based on echocardiographic results (no mass, suspicious for a mass, definite mass). Significant results of multiple t-tests comparing effusion and patient characteristics with no cardiac mass noted on echocardiogram are reported in Table 2. When a mass was visible only total nucleated cell count (P = .007) and large mononuclear cell count (P < .0001) remained significant. There were no differences between groups in the subgroup of patients in which a mass was considered suspicious, but not definitive on echocardiogram. No association between echocardiographic diagnosis of a mass and a diagnostic pericardial effusion analysis was noted via Fishers exact test (P = .21). Cases (n = 5) that did not receive an echocardiogram by a cardiologist had an ultrasound performed by other clinic personnel to confirm the presence of pericardial effusion, the final diagnosis in these cases included anticoagulant rodenticide, right auricular hemangiosarcoma, infectious pericarditis (n = 2), and one unknown etiology.

Receiver operating characteristic curve analysis was performed using effusion RBC count (Fig 1) and calculated HCT. The ideal cutoff for pericardial effusion RBC count was 1.35 M/μL with a sensitivity (95% CI) of 78.9% (54.4–94.0%) and specificity (95% CI) of 73.2% (67.0–78.7%). The ideal cutoff for calculated HCT was determined to be 10.1% with a sensitivity

![ROC curve of Effusion RBC Count](image)

**Table 1.** Data comparing diagnostic and nondiagnostic effusion cytology (P < .001 considered significant).

|                        | Mean ± SD |         |         | P Value |
|------------------------|-----------|---------|---------|---------|
|                        | Nondiagnostic Effusions (n = 239) | Diagnostic Effusions (n = 20) |         |
| Hematocrit (%) of effusion | 24.9 ± 17.8 | 5.7 ± 4.5 | <.0001 |
| Effusion total RBC (M/μL) | 3.6 × 10⁶ ± 2.6 × 10⁶ | 9.7 × 10⁵ ± 6.9 × 10⁵ | <.0001 |
| Effusion total nucleated cell count (cell/μL) | 1.3 × 10⁴ ± 1.9 × 10⁴ | 1.4 × 10³ ± 1.3 × 10³ | <.0001 |
| Effusion neutrophil count (cell/μL) | 8.1 × 10³ ± 1.5 × 10³ | 8.0 × 10² ± 1.3 × 10² | <.0001 |
| Effusion large mononuclear cell count (cell/μL) | 3.1 × 10³ ± 6.2 × 10² | 2.3 × 10⁴ ± 2.9 × 10⁴ | <.0001 |
| Serum albumin (g/dL) | 2.7 ± 0.5 | 2.1 ± 0.6 | .0002 |

**Table 2.** Data comparing diagnostic and nondiagnostic effusion cytology in dogs with no mass seen on echocardiography (P < .001 considered significant).

|                        | Mean ± SD |         |         | P Value |
|------------------------|-----------|---------|---------|---------|
|                        | Nondiagnostic Effusions (n = 102) | Diagnostic Effusions (n = 10) |         |
| Hematocrit (%) of effusion | 23.4 ± 15.9 | 6.1 ± 4.9 | .0009 |
| Effusion total RBC (M/μL) | 3.5 × 10⁶ ± 2.3 × 10⁶ | 6.7 × 10⁵ ± 7.5 × 10⁵ | .0009 |
| Effusion total nucleated cell count (cell/μL) | 1.1 × 10⁴ ± 1.2 × 10⁴ | 1.1 × 10³ ± 1.3 × 10³ | <.0001 |
| Effusion neutrophil count (cell/μL) | 6.0 × 10³ ± 7.9 × 10² | 8.4 × 10² ± 1.1 × 10² | <.0001 |
| Effusion large mononuclear cell count (cell/μL) | 3.6 × 10³ ± 7.9 × 10² | 2.7 × 10⁴ ± 3.9 × 10⁴ | <.0001 |
Pericardial Effusion Cytology

(95% CI) of 84.2% (60.4–96.6%) and a specificity (95% CI) of 71.9% (65.7–77.6%). Area under the curve (95% CI) was 0.80 (0.74–0.86) for both effusion RBC count and calculated HCT as a discriminatory test for diagnostic versus nondiagnostic effusions on cytology.

An effusion HCT of 10% and presence or absence of a mass on echocardiogram were evaluated to see if they could increase the diagnostic utility of cytologic analysis of pericardial effusion (Table 3). The odds ratio (95% CI) was 10.8 (3.4–33.5) for a sample being in the diagnostic effusion group as compared to the nondiagnostic group when the HCT<10%.

Histopathologic assessment was available in 101/259 (39.0%) of the cases with pericardial effusion. Of the cases with histopathology available, biopsies represented 59/101 (58.4%) of the cases, necropsies represented 38/101 (37.6%), and 4/101 (4.0%) cases with histopathology had both a biopsy and a necropsy. Histopathology cases were subdivided into neoplastic (n = 24) and non-neoplastic etiologies (n = 77). Of the non-neoplastic biopsies, pericardial effusion was classified as hemorrhagic (n = 67), infectious (n = 5), neoplastic (n = 2), modified transudate (n = 1), and chylous (n = 2), resulting in a false positive rate of 2/77 (2.6%) for neoplasia. Of the nonneoplastic biopsies, the pericardial effusion was characterized as hemorrhagic in 22/24 (91.7%), resulting in a sensitivity of cytology with histologically confirmed neoplasia of 22/24 (8.3%). Pericardial effusion analysis compared to histologically confirmed neoplasia to non-neoplasia was performed and no statistically significant differences were noted between the 2 groups.

The original cytologic interpretation was compared to the reviewed cytologic interpretation and when classified as nondiagnostic and diagnostic effusions, 244/249 (98%) of the reviewed cytologic interpretations matched the initial interpretation. All cases initially classified as neoplastic, infectious, or other were confirmed on re-evaluation of the pericardial effusion slides.

### Table 3. Number of cases with diagnostic and nondiagnostic effusions based on cytology stratified by effusion hematocrit (HCT) (<10% or ≥10%) and whether or not a mass was present on echocardiogram.

| Effusion Type | Nondiagnostic Effusions (n) | Diagnostic Effusions (n) | Diagnostic Utility (%) |
|---------------|-----------------------------|--------------------------|------------------------|
| All cases (n = 254) | 165 | 4 | 7.7 |
| HCT ≥10% | 63 | 16 | 20.3* |
| HCT <10% | 8.9 |
| No echocardiographic mass (n = 108) | 73 | 3 | 3.9 |
| HCT ≥10% | 25 | 7 | 21.8* |
| HCT <10% | 6.4 |
| Suspicious/definite echocardiographic mass (n = 141) | 96 | 2 | 2.0 |
| HCT ≥10% | 36 | 7 | 16.3* |

*Indicates a difference in diagnostic utility between effusion HCT groups P < .005.

**Discussion**

The overall diagnostic utility of cytologic analysis of pericardial effusion was 7.7% in all cases and increased to 20.3% in cases with an effusion HCT<10%, yet an echocardiogram did not significantly alter the diagnostic utility. Although performed at a university teaching hospital, these results are likely comparable to other populations as our study population and etiologies of pericardial effusion are similar to those previously reported.10,12,13,23

When nondiagnostic and diagnostic effusions were compared, multiple differences were found. Effusion total nucleated cells and neutrophil counts were higher in the diagnostic group as the infectious effusion cases had a significantly higher population of neutrophils associated with that disease. Numbers of large mononuclear cells were increased in the effusions of the diagnostic group as expected in the infectious and the nonneoplastic effusion cases. Serum albumin concentration decreased in the diagnostic group likely because of the chronicity of the primary disease and an inflammatory response.24 Dogs with neoplasia have been reported to have a higher HCT in the pericardial fluid, which is in contrast to our study in which patients with a diagnosis based on cytology had a lower RBC count and HCT.13 The difference is explainable by the method of diagnosis, which was by echocardiography, whereas ours was based on cytology. Cases in the previous study diagnosed with a right atrial mass (21/28 [75%] of enrolled cases) were considered to have a neoplastic diagnosis and hence the high effusion HCT associated with a neoplastic etiology. In contrast, only cases with a pericardial effusion analysis were analyzed in this study and animals with a cardiac mass on echocardiogram were unlikely to have had an effusion submitted and therefore did not contribute substantially to the diagnostic group.

The effusion HCT value in our study was calculated based on a measured RBC count of the effusion and the MCV from the peripheral blood. HCT was calculated as a post hoc value after the finding of a difference of RBC count in diagnostic effusions was obtained. This post hoc analysis was performed, as one of our goals was to identify a value at presentation that could increase the diagnostic yield of cytologic analysis of pericardial effusion. In practice, measurement of effusion PCV would be more readily available than effusion HCT. Limitations to this analysis are that the PCV and HCT are not directly interchangeable with the PCV being approximately 2–3% higher than HCT and only 48% of our patients had an MCV available.25 As the mean MCV was used for HCT in roughly half of our population if any severe micro- or macrocytosis existed in those dogs, it would diminish the accuracy of our results. Additionally, the
MCV of the peripheral blood might not have matched the MCV of the effusion if the effusion formation was not acute because chronicity could affect the MCV. It should be noted that the difference still existed for both measured effusion RBC count and calculated HCT in statistical analysis \( P < .0001 \) so the results are likely valid. Calculation of the effusion HCT was necessary because no cases in this study had a measured effusion HCT.

The ideal cutoff for effusion HCT based on ROC curve analysis was 10.1%, with lower HCT values having an increased sensitivity for cytologic diagnosis. A significant AUC \(_{\text{ROC}}\) value of 0.80 indicates that this is a good discriminatory test for whether cytologic analysis will be diagnostic or nondiagnostic. Ideally, measured RBC count from an automated hematology analyzer would be used in clinical practice, but as this test is not always available, and because of the differences in PCV and HCT mentioned above, the authors propose the submission of any fluid with a PCV <12% to 13% as it is likely to have an increased diagnostic yield relative to more hemorrhagic samples.

Comparing the cytologic diagnosis of neoplasia to the histopathologic diagnosis, when available, resulted in a false positive rate of 2.6% (2/77) and no cases with a histopathologic diagnosis of neoplasia had an equivalent cytologic diagnosis in our study. Both cases that were considered a false positive had a cytologic diagnosis of neoplasia. The first case had cytology highly suggestive of hemangiosarcoma whereas the second case had a diagnosis of hemorrhagic effusion with atypical epitheloid cells and was interpreted as a malignant epitheloid neoplasia. As cases with a definitive diagnosis of neoplasia on cytology were unlikely to have histopathology submitted, because a diagnosis was already known, the true false positive rate remains unknown.

Cytologic analysis of pericardial effusion has been evaluated throughout the veterinary literature, but the sensitivity and specificity of cytologic analysis are currently ill defined. Unfortunately, without histopathology being available in all cases, specificity and sensitivity in a large group of dogs could not be addressed in this retrospective study. Cytologic analysis of pericardial effusion does not appear to be highly sensitive for achieving a diagnosis with primary cardiac neoplasms in people. However, it appears to have a moderate to high sensitivity for metastatic neoplasia (61–100%) and specificity for metastatic neoplasia in human patients (93.3–100%).\(^{18,20}\) This variable diagnostic yield in human patients is explained by the differences in the underlying etiology of pericardial neoplasia. Human patients more commonly have metastatic carcinoma effusions, which can be diagnosed on cytology. Cytology is considered the gold standard for detection of malignant neoplastic effusions in human medicine because of the high sensitivity of cytologic analysis of pericardial effusion.\(^{26–28}\) Cardiac angiosarcoma is the most common malignant cardiac neoplasm in adult human patients. Unlike metastatic carcinomas, diagnosis of angiosarcoma is based on open cardiac biopsy or surgical resection of a right atrial mass with no data showing an ability to diagnose this tumor based on effusion cytology alone. The diagnostic capabilities of cytology for cardiac angiosarcoma appear to be similar to what is seen in dogs with hemangiosarcoma resulting in differing diagnostic yields.\(^{22,28–31}\)

The prevalence of a cardiac mass noted on echocardiogram was comparable to previous reports in the literature with 30 and 49.5% of pericardial effusion cases reported to have a cardiac mass compared to 42.9% with a definite or 13% with a suspicious mass on echocardiogram in this study.\(^{4,23}\) With the increasing availability of ultrasound in veterinary practice, the presence or absence of a cardiac mass was a potential variable that could alter the diagnostic value of pericardial effusion analysis. This, however, failed to reach statistical significance as a sole preanalytical variable. When effusion HCT is combined with the absence of a mass on echocardiography, the combination of the two does not result in a significant change in the diagnostic utility. The maximal diagnostic yield of 21.8% found in this study was in the subgroup of dogs with no identifiable mass on echocardiography and an effusion HCT <10% compared to the overall yield of 7.7%. This yield represents an increase in the cost–benefit ratio to the client suggesting that if the effusion and dog fit these criteria, submission of pericardial effusion to a clinical pathologist gives a 3-fold increased chance of receiving a specific diagnosis.

Limitations in this study include those inherent in all retrospective studies; the data evaluated were incomplete with regard to age, weight, complete blood counts, and serum biochemistry data. Variability exists within the cytologic diagnosis because of different clinical pathologists interpreting the slides. Slides were re-evaluated in a blinded fashion to reduce this variability, yet a small percentage of slides were not available for re-evaluation. Echocardiograms were performed by a variety of individuals. Each scan was performed by either a board-certified cardiologist or a supervised cardiology resident, yet variation will be present because of examiner skill level variability. Confirmation of a cytologic diagnosis was limited based on histopathology as only 39% of the cases included in this study had concurrent histopathology available precluding true sensitivity and specificity analysis. Cases available for inclusion in this study are not representative of the entire population of pericardial effusion in dogs because not all cases with pericardial effusion had a sample submitted for cytology.

Predicting the diagnostic utility based on the preliminary data (effusion HCT/PCV) and a noninvasive diagnostic modality (echocardiogram) helps the clinician to decide if sample submission is appropriate. The overall diagnostic utility improves from 7.7 to 20.3% with an effusion HCT <10%, yet an echocardiogram did not significantly improve this diagnostic utility, suggesting that submitting fluid for cytologic analysis if the PCV <12 to 13% will have an increased yield. Despite previous reports concluding a poor diagnostic utility, we conclude that the diagnostic value of
Pericardial effusion cytologic analysis is variable depending on the etiology.

Footnotes

a Baker Systems 9110 Plus Hematology Analyzer; BioChem ImmunoSystems Inc, Allentown, PA
b ADVIA 120 Hematology System; Siemens Healthcare Diagnostics Inc, Tarrytown, NY
c MultiSpecies System Software; Siemens Medical Solutions Diagnostics Inc, Tarrytown, NY
d GraphPad Prism 6.0; Graph Pad Software, La Jolla, CA

Acknowledgment

Conflict of Interest Declaration: The authors disclose no conflict of interest.

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