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Feline Differential Leukocyte Count with ProCyte Dx: Frequency and Severity of a Neutrophil-Lymphocyte Error and How to Avoid It

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Background: Erroneous neutrophil and lymphocyte counts from analysis of feline blood samples were transferred directly into the hospital information system from the ProCyte Dx hematology instrument in our after-hours laboratory. Errors usually were not detected by the users.

Hypothesis/Objectives: To quantify the frequency and severity of errors associated with the ProCyte Dx analyzer and to identify methods to avoid the errors.

Animals: One-hundred six EDTA blood samples routinely submitted from feline hospital patients were analyzed.

Methods: ProCyte differential leukocyte counts were compared to 2 reference methods: Advia 2120 hematology instrument and manual enumeration. Limits for unacceptable deviation from the reference methods were defined as 18 for % lymphocytes and 23 for % neutrophils.

Results: Fourteen of 106 samples had unacceptable errors for both lymphocytes and neutrophils compared to both reference methods. Median % lymphocytes in those 14 samples were 11.2, 15.0, and 53.0% for Advia, manual, and ProCyte, respectively. Median % neutrophils were 85.4, 81.5, and 34.2% for Advia, manual, and ProCyte, respectively. All errors were avoided by rejecting automated ProCyte differential leukocyte results whenever the dot plot appeared clearly incorrect, but only 9 of these 14 samples had a ProCyte WBC distribution error flag.

Conclusions and Clinical Importance: Results reported by ProCyte had markedly falsely increased lymphocyte and decreased neutrophil counts in 13% of feline patient samples. Users must reject automated differential leukocyte count results when the WBC dot plot appears overtly incorrect. Rejection based only on ProCyte WBC error flag was insufficient.

Key words: Cat; Dot plot; Hematology instrument.

We noted a problem with the use of the hematology instrument IDEXX ProCyte Dx[a] in our after-hours laboratory, which is not staffed by laboratory personnel. Numerical results of ProCyte automated differential leukocyte counts were transmitted directly into the information system of the University Animal Hospital, Swedish University of the Agricultural Sciences. Some results had very incorrect increased lymphocytes and decreased neutrophils. The errors often were not detected, and results were not rejected before transfer to the patient's record in the hospital information system and became permanent data. Most users of the after-hours laboratory accepted results from the ProCyte despite abnormal appearance of instrument dot plots or instrument WBC alarms. These users seldom made a fresh blood smear that could be examined later. Erroneous results often were seen in cats with signs of severe inflammatory disease.

Abbreviations:

A-diff	automated differential leukocyte count
Advia	Siemens ADVIA 2120 hematology instrument
M-diff	manual differential leukocyte count,
ProCyte	IDEXX ProCyte Dx hematology instrument
Sysmex	Sysmex XT 2000iV
TEa	total allowable error
WBC-B	total WBC count from the instrument's basophil channel
WBC-CE	alarm indicating the instrument's 2 total WBC counts (WBC-B and WBC-P) were too different
WBC-P	total WBC count from the instrument's peroxidase channel
WBC	white blood cells: leukocytes

ProCyte is a hematology instrument with advanced technology developed for veterinary use. Previous validation studies have concluded it has good to excellent performance with veterinary blood samples.[1–3] ProCyte methods have been described previously.[1–4] ProCyte uses similar to the same technology as the Sysmex XT200iV,[b] which also has been described.[5–7]

The hypothesis of our study was that automated feline leukocyte differential count results reported by the ProCyte Dx hematology system have severe errors of undetermined frequency. The aim of our study was to identify the frequency and severity of a neutrophil-lymphocyte error of the ProCyte when analyzing feline blood. An additional goal was to determine what could be done by ProCyte users to avoid serious errors. In addition, the full comparison of 3 methods of determining feline

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differential leukocyte counts with an Advia 2120, ProCyte, and manual enumerations would allow additional observations on the performance of these methods with all leukocyte types. If documentation of frequent and marked errors is substantiated in our prospective study with >100 feline blood samples, it is important to alert ProCyte users how to detect and avoid the errors.

Materials and Methods

Blood was collected into 2 mL K_3 EDTA tubes from feline patients at the University Animal Hospital, Swedish University of the Agricultural Sciences from February to April 2016. No selection criteria for samples or method of randomization were used except that 1 researcher (VA) was available to make blood smears and analyze a patient's blood sample submitted to the laboratory with both the Advia 2120[b] and ProCyte Dx within 3 hours of collection. The goal was ≥100 blood samples. These 106 blood samples should be reflective of what type of samples our hospital's hematology laboratory analyzes routinely. Six cats had blood samples submitted twice on different days and 2 cats were sampled 3 times on different days. Blood samples were stored at room temperature and analyzed within 3 hours after collection. The blood samples were taken for hematology examination as part of routine patient care and diagnosis. Owners of hospital patients sign a consent form that information from procedures required for patient care may be used for research.

The samples were analyzed both with IDEXX ProCyte Dx automated hematology instrument (software version 00-33_51) and Siemens Advia 2120 (software version 5.3.1.-MS), both using settings for cats. Methods for the ProCyte and Advia have been reported previously.[1,3,6,7] Both instruments are flow cytometers that use laser light scatter from cells in the sample and staining of cells to identify various cell types. Forward laser light scatter (FLS) reflects the size of the cells, and side light scatter reflects complexity of the cells (SSC).

ProCyte reports a 5-part feline automated differential count (total neutrophils, lymphocytes, monocytes, eosinophils, and basophils). ProCyte uses a fluorescent polymethine stain, which binds to nucleic acids and cytoplasmic organelles in leukocytes.[1,3] Side fluorescent light (SFL) reflects polymethine staining of leukocytes. Monocytes and eosinophils stain strongly with the polymethine stain and are located higher along the *y*-axis (SFL) in the ProCyte feline WBC dot plot. Immature and toxic neutrophils have greater polymethine staining and are found higher up along the *y*-axis than normal neutrophils. Feline eosinophils and basophils with greater cell complexity are located more toward the right (greater SSC). ProCyte has a WBC abnormal distribution alarm which indicates that there was insufficient separation of clusters of leukocyte types to allow correct classification of cell types. This alarm is accompanied on the report by an asterisk or hash marks by individual results. IDEXX recommends a blood film evaluation with samples that have an alarm.[4]

Advia uses flow cytometry and a laser system to produce a 4-part feline differential count (total neutrophils, lymphocytes, monocytes, and eosinophils) using size and peroxidase staining to separate cell clusters.[6,7] Neutrophils, eosinophils, and monocytes are variably peroxidase positive. The Advia instrument has several WBC alarms. In our study, only WBC-CE was used to reject Advia A-diff results in a groomed population described later. WBC-CE indicates difference between two WBC results from WBC-B from the basophil channel, and WBC-P from peroxidase channel was unacceptably large.

Check (XS) quality control from IDEXX[c] was analyzed once a week with the ProCyte. Siemens Advia 2120 3–in-1 TESTpoint control[d] was analyzed each day at both normal and low levels.

Uniformly prepared blood smears were made with an automated blood smear-making instrument, HemaPrep.[e] Smears were stained with a modified May-Grünwald Giemsa stain.[f] Manual differential leukocyte counting (M-diff) of 100 leukocytes per sample was performed by one of the authors (HT) on all 106 feline blood smears. In the M-diff, neutrophils were divided into segmented and nonsegmented neutrophils, although the ProCyte and Advia only report total neutrophils and cannot identify band neutrophils. Therefore only total neutrophil (segmented + nonsegmented) results were compared to ProCyte and Advia A-diff neutrophil counts. Toxicity of neutrophils was subjectively classified as 0 (no toxicity) to 3+ (maximal toxicity) based on increasing basophilia and foaminess of cytoplasm seen on blood smear examination. A separate classification was the presence of Döhle bodies in neutrophils but with no other signs of toxicity.

ProCyte leukocyte differential counts were compared with both Advia and manual counts for all 106 samples. Additionally, a groomed dataset was selected for additional comparisons among the 3 methods. All samples with instrument alarms or abnormal dot plots of the ProCyte or Advia were excluded from the main groomed group. One author (IL) rejected samples based on abnormal appearance of the WBC dot plots of the ProCyte or Advia. A second "instrument alarm" groomed dataset was examined by rejecting results only based on instrument alarms and not considering if the dot plots appeared abnormal.

Guidelines were needed to identify what was unacceptable deviation of ProCyte lymphocyte and neutrophil counts from both reference methods (manual and Advia counts). The limits chosen were ≥18 for lymphocytes and ≥23 for neutrophils. These were subjectively chosen. The values are total allowable error values for lymphocytes and neutrophils reported for human blood samples (see discussion). The difference was the absolute deviation in results. For example, a ProCyte result of 50% lymphocytes was considered 25 greater than 25% manual lymphocytes. These limits are very large and include only marked deviations from both reference methods.

All leukocyte results of all 3 methods except basophils were evaluated by Bland-Altman analysis with difference plots, mean bias and 95% limits of agreement. The analysis and plots were carried out with Analyse-it Software[g]

Results

There were 14 blood samples (13% of all 106 samples) from 14 different cats in which the ProCyte A-diff results had errors in excess of the predefined criteria with both lymphocyte and neutrophil counts compared to both manual and Advia reference methods. Lymphocyte counts in % were 18–61 higher, and neutrophil counts were 23–74 lower than both the Advia and manual counts (Figures 1–4, Table 1). Median % lymphocytes in those 14 samples were 11.2, 15.0, and 53.0% for Advia, manual, and ProCyte, respectively. Median % neutrophils were 85.4, 81.5, and 34.2% for Advia, manual, and ProCyte, respectively.

The clinical decisions and descriptions also were affected in these 14 samples. ProCyte absolute neutrophil counts indicated neutropenia (<3,100 neutrophils/μL; $<3.1 \times 10^9$/L) in 9 of the 14 samples, but only 2 and 3 samples respectively had neutrophil counts indicating neutropenia by Advia or the manual method. ProCyte indicated lymphopenia (<1,300 lymphocytes/μL; $<1.3 \times 10^9$/L) in none of the 14 samples, but Advia and manual indicated lymphopenia in 11 and 8 samples, respectively. All these 14 samples could be identified as

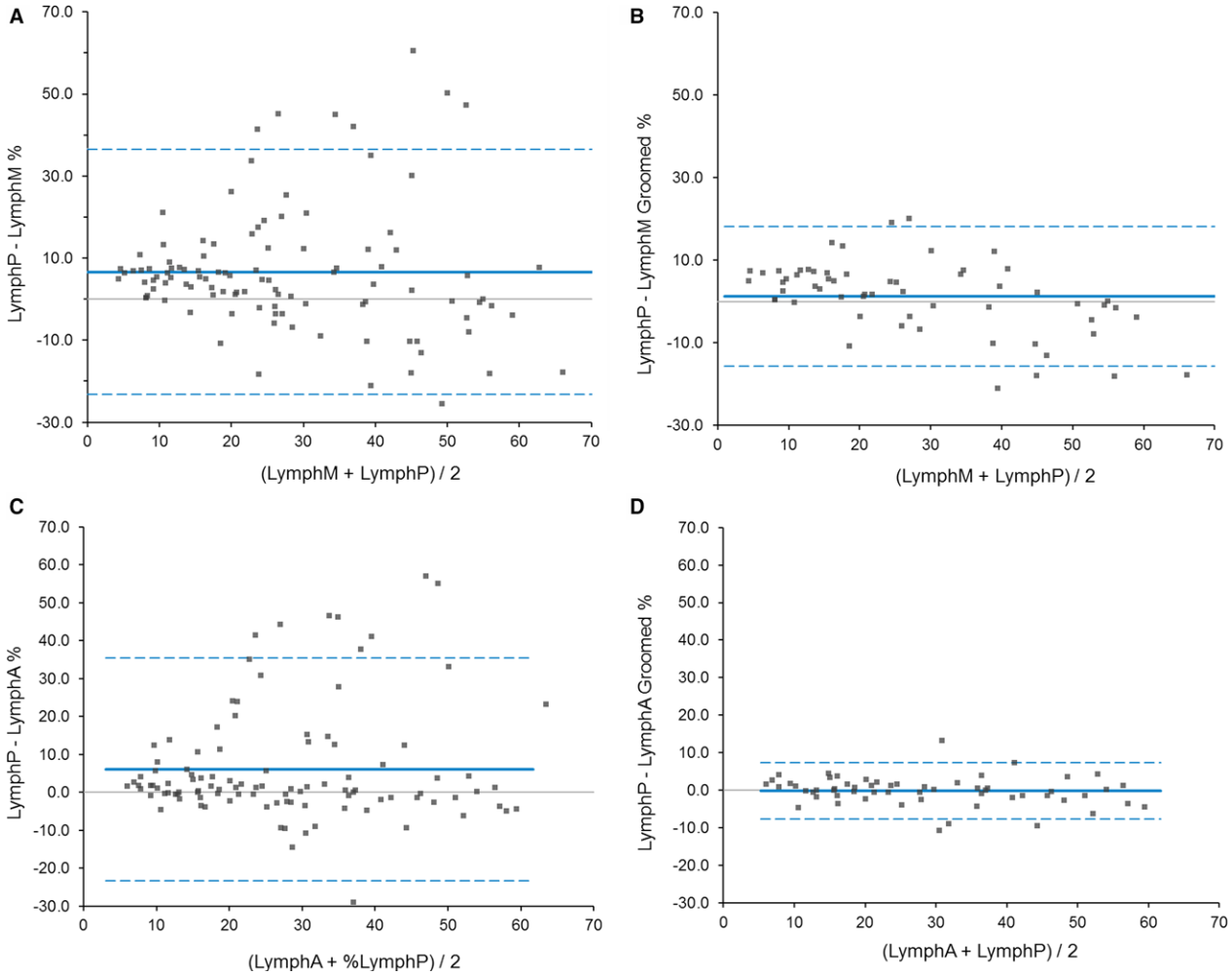

Fig 1. Bland-Altman difference plots comparing lymphocyte results from the ProCyte Dx to manual and Advia 2120 results. Manual differential (LymphM) is compared with the ProCyte Dx automated differential leukocyte counts (LymphP) in the top two figures (**A + B**). Results of all 106 feline samples are shown on the left in (**A**). Results in the groomed data group after exclusion of samples with instrument alarms or unacceptable dot plots are shown in (**B**). Similarly difference plots of % lymphocytes comparing Advia automated differential (LymphA) with the ProCyte Dx automated differential leukocyte counts (LymphP) are in the lower two figures **(C and D)** with all 106 samples on the left and groomed data on the right.

being unacceptable by inspection of the dot plots, but only 9 had ProCyte WBC abnormal distribution alarm. Five of the 14 samples had no ProCyte alarm. Five of these 14 samples also had a monocyte error (described later).

Bland-Altman results are presented both comparing all samples (106 samples) and the groomed data group (59 samples) where all samples with an instrument WBC alarm or unacceptable dot plots were excluded (Table 1, Figures 1–4). Improvement is clearly apparent in comparison with ProCyte results to either Advia or manual results in the groomed data group. The dispersion between paired results for neutrophils and lymphocytes is shown by comparing the difference plots between the group with all 106 samples and the groomed data group (Figures 1 and 2). Comparisons of the A-diff of the 2 automated instruments (ProCyte and Advia) were much tighter in the groomed data group than comparing ProCyte to M-diff results. This observation indicates that the M-diff is more imprecise than the A-diff. Bland-Altman 95% limits of agreement for the 2 instruments also reflected marked improvement in the groomed group (Table 1). Limits of agreement were decreased from −23 to +35 (absolute range of 58) down to −8 to +7 (absolute range of 15) for lymphocytes and were decreased from −44 to +8 (absolute range of 54) down to −8 to +5 (absolute range of 13) for neutrophils after grooming. No marked neutrophil-lymphocyte errors remained in the main groomed data group.

The main groomed data group had 59 samples remaining after 47 of 106 samples (43%) were excluded. The 47 samples were excluded because they had ≥1 of the following criteria: Criteria were presence of a ProCyte or Advia WBC alarm and if the ProCyte or Advia WBC dot plots had obvious classification errors (Table 2). Inspection of ProCyte dot plots indicated insufficient separation of cell clusters or incorrect

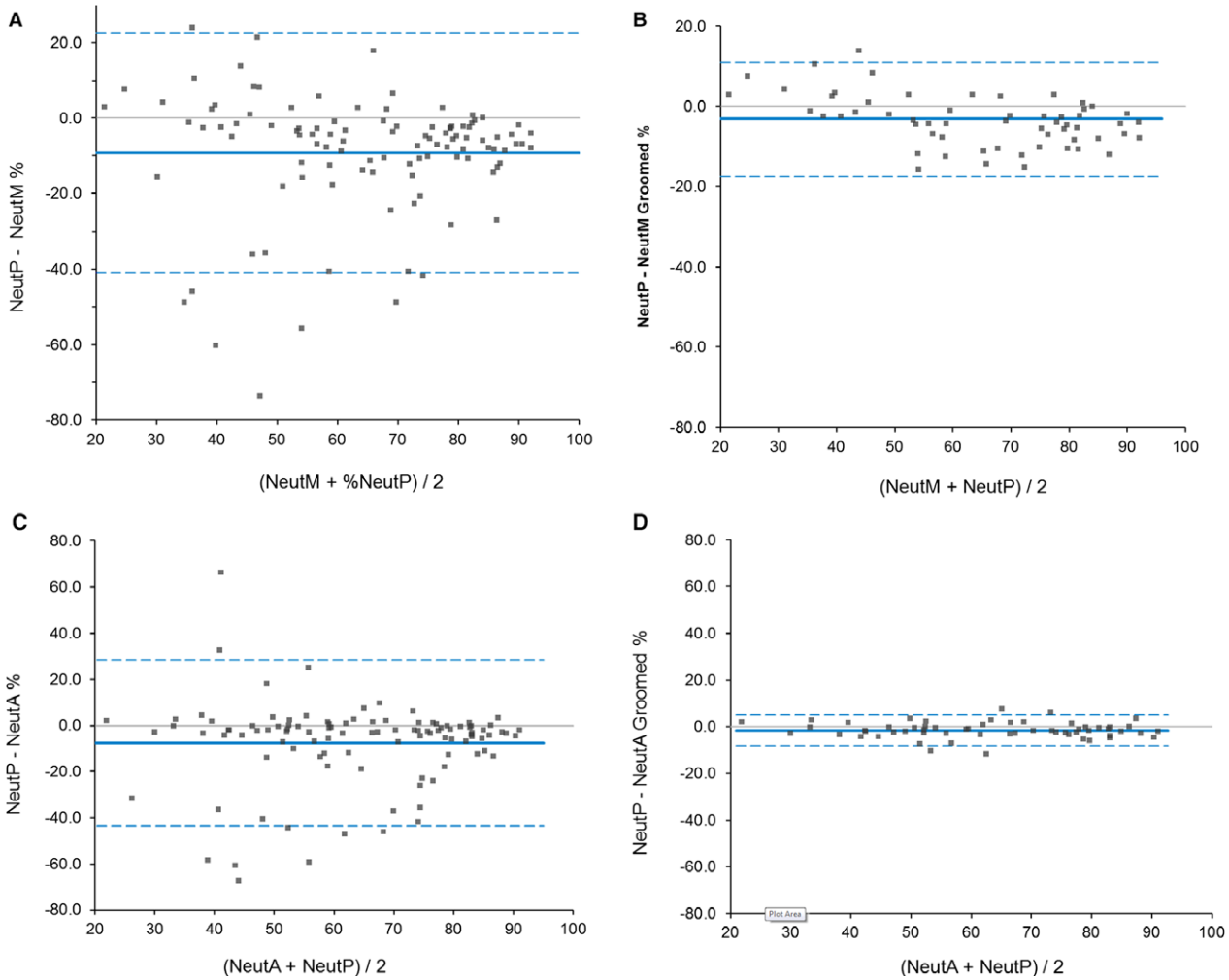

Fig 2. Bland-Altman difference plots comparing neutrophil results of the ProCyte Dx with the Advia 2120 and manual methods. ProCyte Dx neutrophil counts (NeutP) are compared to manual results (NeutM) in the top two figures **(A + B)**. All 106 feline samples are shown on the left **(A)**. Results in the groomed data group after exclusion of samples with instrument alarms or unacceptable dot plots are shown on the right **(B)**. Similarly difference plots of neutrophils comparing Advia neutrophils (NeutA) with the ProCyte Dx neutrophil counts (NeutP) are shown in the lower two figures **(C + D)**. Note a difference with the *y*-axis scale of the two upper and two lower plots.

subdivision of 1 cell cluster into ≥2 cell types in 38 samples (36%). Twenty-two of these 38 samples also had ProCyte WBC abnormal distribution and band suspected alarm, which was 21% of all 106 samples. Identification of abnormal WBC dot plots was much easier with the ProCyte than with the Advia. Advia dot plots had often large numbers of platelet aggregates than obscured cell clusters. Eosinophils are not shown with the Advia WBC dot plot.

Exclusion of samples with only an instrument WBC alarm but without consideration of the dot plots performed was not sufficient to eliminate all major errors. The instrument alarm group still had 5 of 14 samples remaining with 27–61% too many lymphocytes and 24–74% too few neutrophils. Dot plot evaluation was necessary to avoid all marked neutrophil-lymphocyte errors. Evaluation of the abnormal ProCyte dot plots indicated the neutrophil cell population extended up into the lymphocyte counting area with poor separation of neutrophil and lymphocyte cell populations. There were 35 of 106 samples in which inspection of ProCyte WBC dot plots indicated a problem with neutrophil/lymphocyte separation. Neutrophils in some samples extended even up into the monocyte counting area of the ProCyte WBC dot plot. Normal and abnormal WBC dot plots are illustrated in Figures 3A and B.

Table 3 shows that increasingly more severe toxic change subjectively noted in neutrophils was associated with increased number of samples with unacceptable neutrophil-lymphocyte ProCyte results. Thirty-nine percentage of samples with 2+ or 3+ toxicity had severe neutrophil-lymphocyte errors. Increasing toxicity also was associated with greater numbers of immature (band) neutrophils.

Monocyte results from all 3 methods (ProCyte, Advia and M-diff) showed good agreement in most cases (Table 1). In 5 samples, the ProCyte monocyte counts were higher than both Advia and manual counts. One

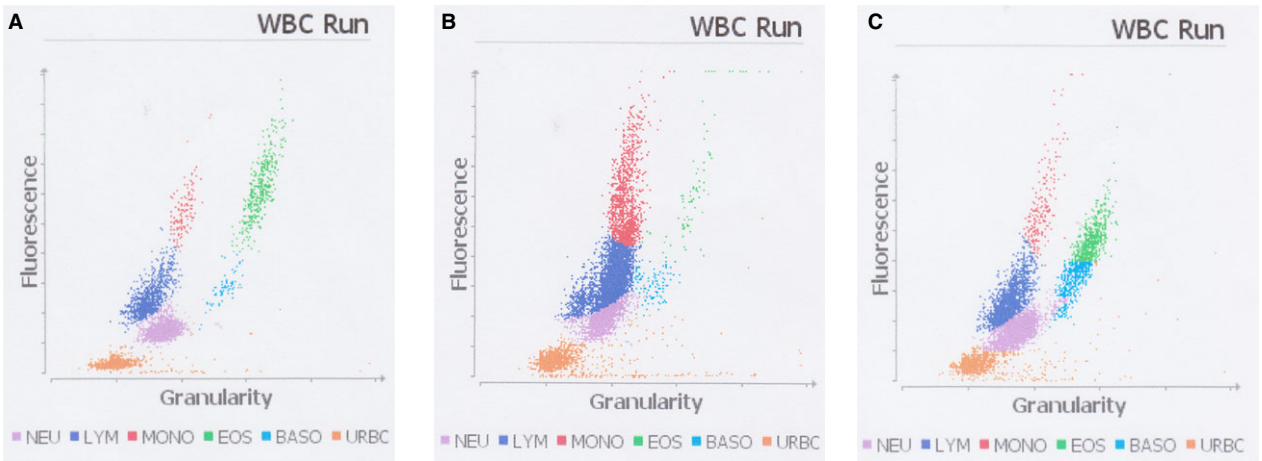

Fig 3. Normal and abnormal ProCyte Dx dot plots. **(A)** is a normal dot plot with distinct separation of cell clusters of all five cell types. Identification of each cell (dot) is indicated by the color code at the bottom (neutrophils, lymphocytes, monocytes, eosinophils, and basophils, respectively). URBC is un-lysed RBC. **(B)** is an abnormal ProCyte dot plot. There is a long oval cluster of cells extending up from the normal neutrophil area, to the right of the true, small cluster of blue lymphocytes and continuing up into the area to the lower right of the true red monocytes. This long oval cluster of neutrophils was classified as neutrophils (violet: lowest part) lymphocytes (blue: middle part) and monocytes (red: upper part). Distinct lines drawn through the middle of a cell cluster is an indication the instrument classified cells incorrectly. A cell cluster should have one cell type and one color. Dividing a cell cluster into two or more colors indicates that cluster of one cell type was classified as more than one type. The blood smear had toxic immature neutrophils which appeared to have increased fluorescence. There was no instrument WBC alarm with this cat. **(C)** is an abnormal ProCyte dot plot in which the oval cluster of cells to the far right was actually all eosinophils but about half (lower part with light blue dots) were incorrectly classified as basophils by ProCyte. The sharp line bisecting the distinct cell cluster predicts the instrument error. There was satisfactory separation of neutrophil, lymphocyte, and monocytes clusters.

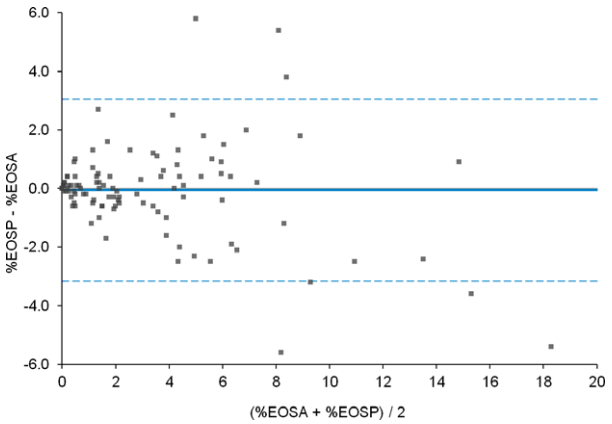

Fig 4. Bland-Altman difference plot comparing ProCyte Dx and Advia 2120 eosinophil results. The eosinophil counts varied from 0 to 22.5%. The mean difference was −0.05 (blue solid line). The dotted lines indicate 95% limits of agreement (−3.2 and +3.1). Differences were mild over the range of the % eosinophils without any proportional errors noted.

Table 1. Bland-Altman analysis of feline differential leukocyte counting: ProCyte Dx versus manual method or Advia 2120. Results within brackets are for the groomed dataset.

Comparison	Mean Difference	95% limits of agreement
Lymphocyte		
Manual	6.6 (1.2)	−23 to 36 (−16 to 18)
Advia	6.1 (0.2)	−23 to 35 (−7.6 to 7.3)
Neutrophil		
Manual	−9.2 (−3.2)	−41 to 22 (−17 to 11)
Advia	−7.6 (−1.6)	−44 to 8 (−8 to 5)
Monocyte		
Manual	2.9 (2.2)	−6 to 12 (−4 to 8)
Advia	1.4 (1.6)	−14 to 16 (−2 to 6)

Values, not in parenthesis, compare ProCyte to two reference methods, manual differential or Advia in 106 feline blood samples. ProCyte results minus reference method are reported. The mean difference shows the ProCyte had more lymphocytes and monocytes and fewer neutrophils. Values in parenthesis are based on a groomed population of 59 feline samples after exclusion of samples with instrument alarms or unacceptable dot plots. ProCyte performance was very good in the groomed population.

ProCyte monocyte result was 34% higher, and 4 ProCyte samples were approximately 10% higher than the 2 reference methods. This observation appeared to be caused by neutrophils moving up into the monocyte area as well as moving up through the lymphocyte counting area on dot plot evaluation. These 5 samples had a ProCyte WBC distribution error alarm, and the unacceptable WBC dot plots were detectable by inspection of instrument graphics.

Additionally there were 3 A-diff monocyte results with marked errors but without apparent reason. Advia had 2 samples with marked falsely high monocyte counts (39 and 59%), whereas the M-diff monocyte counts for both were 0%, and the ProCyte counts were 2, and 8%. Neutrophils were erroneously counted as monocytes with Advia in those cases, also causing

Table 2. Reasons to remove 47 of the original 106 blood samples to form a groomed dataset.

Reason to remove	Procyte	Advia	Both Procyte and Advia
Unacceptable dot plot	38 (36%)	12 (11%)	3 (3%)
Instrument alarm	22 (21%)	10 (9%)	3 (3%)

The number of samples (and % in parentheses) with unacceptable dot plots and/or instrument WBC alarms are given for each instrument.

Table 3. Association of the severity of toxic change in neutrophils with unacceptable ProCyte lymphocyte and neutrophil results and left shift.

			ProCyte neutrophil-lymphocyte error	
Toxic change classification	Number of samples	Nonsegmented neutrophils mean %	# of samples	% of samples
0	48	4.6	3	6
Only Döhle	19	2.7	1	5
1+	9	4.6	1	11
2+ or 3+	23	16.3	9	39

Toxic change was subjectively classified as 0 (no toxicity) to 3+ (greatest toxicity) by blood smear evaluation. Samples with Döhle bodies seen in neutrophils, but no other signs of toxicity were classified as a separate group (Only Döhle). Number of feline samples in each classification of toxicity is given. The mean % nonsegmented neutrophils in each classification is given. There were 14 samples with severe ProCyte lymphocyte-neutrophil classification errors. The number of blood samples with those errors is given for each classification. The percentage of samples in each toxicity classification was calculated by dividing the number of samples with severe errors in that classification by the total number of samples in that classification.

falsely low neutrophil results. Both samples had neutrophils that appeared smaller and less peroxidase positive than usual on the dot plot. No Advia instrument alarm was present in those 2 samples.

Both the 2 automated instruments and the manual method for eosinophils appeared to perform well with no or little bias. The 95% limits of agreement comparing ProCyte and Advia were between −3.2 and 3.1, and only a few results were far outside of the 95% limits of agreement (Figure 4). Similar comparisons were seen among ProCyte and M-diff or Advia and M-Diff.

A ProCyte basophil error was noted in 3 feline blood samples in which eosinophils were located lower than usual in the ProCyte WBC dot plots (Figure 3C). Eosinophils in the eosinophil cell clusters were partly or totally classified as basophils by the ProCyte. This caused both erroneously lower eosinophil counts and erroneously higher basophil counts in these 3 samples. ProCyte basophil counts were 3.0–6.6%, but no basophils were reported on the initial manual counts nor were any seen on an additional review of the 3 blood smears. One percent or more basophils were noted with M-diff in 22 of the 106 cats (20% of blood samples). The largest M-diff basophil counts were in 4 samples with 2, 2, 3, and 7% basophils. ProCyte basophil counts were 1.2, 0.5, 1.4, and 1.7% basophils, respectively. These 4 samples had a distinct cell cluster in the basophil area of the dot plot to support the presence of basophils in the sample. Four samples that had ≥1% basophils reported in the ProCyte A-diff results and a clear cell population in the basophil area in the dot plot supported the presence of basophils.

Discussion

Results of our study indicated that the routinely reported ProCyte A-diff results were markedly incorrect in approximately 13% of the 106 feline blood samples in which there were both falsely increased lymphocyte counts combined with decreased neutrophil counts. This neutrophil-lymphocyte error for both % lymphocytes and % neutrophils was noted in comparison with both reference methods (M-diff and Advia A-diff). The clinical conclusions of neutropenia and a lack of a lymphopenia usually were incorrect in these 14 feline patients. This finding should be alarming for ProCyte users who accept all instrument results as being valid.

Blind acceptance of the ProCyte A-diff lymphocyte and neutrophil counts will cause marked errors in interpretation that can greatly affect diagnosis and treatment in approximately 13% of feline patient blood samples. False neutropenia can, for example, have severe consequences for decision making in chemotherapy of oncology patients. Users of a hematology instrument must have a protocol to validate results and detect analytical errors. In our study, rejecting ProCyte A-diff results if the instrument dot plot appeared markedly in error was sufficient to avoid all 14 errors. The use of only the ProCyte "WBC distribution error alarm" to reject A-diff results was not sufficient to eliminate all errors. Five of 14 samples still had marked errors. Thus, ability to correctly interpret ProCyte WBC dot plots is necessary to avoid errors. There were 38 samples (36%) that were rejected to form the groomed data group, based on ProCyte dot plot evaluation, including 22 that also had an instrument WBC alarm. ProCyte performance appeared very acceptable in the groomed group in which samples with abnormal dot plots or instrument flags were excluded, which has been noted previously.[1]

An alternative method is needed to provide neutrophil and lymphocyte results for those samples with unacceptable dot plots or instrument alarms. The alternative method usually is a manual blood smear evaluation (M-diff). This procedure is suggested in the IDEXX ProCyte Dx manual and by others.[1,4] Blood smear evaluation is a less precise method than instrument counts but also can detect toxic changes in neutrophils and a left shift.

The Sysmex XT 2000iV is the precursor to the ProCyte. A similar problem with identification of neutrophils and lymphocytes in feline patients with toxic and immature neutrophils was noted for the Sysmex.[5,6]

The Sysmex instrument would not report complete differential leukocyte results for 18% of 65 feline blood samples.[5] The Sysmex did not report numerical results if cluster analysis by the instrument suggested a problem with cell identification. Dots on the Sysmex WBC dot plot were given a gray color and not a color that represented a type of leukocyte. This "grey out" method by Sysmex was considered good for quality control because it forced the operator to use another method to obtain leukocyte results, such as blood smear evaluation.

Very wide 95% limits of agreement were reported between ProCyte and both Advia and manual differential for neutrophil and lymphocyte counts when all feline blood samples were included in the analysis (their ungroomed data).[1] Their groomed data group after sample exclusion based on dot plot evaluation had considerably improved the limits of agreement.[1] This improvement in performance is similar to that of our study, in which the ProCyte automated differential leukocyte count performed well in the groomed sample group but not in the original group of 106 samples. This finding substantiates the need of instrument users to be able to reject samples with abnormal dot plots or instrument alarms. ProCyte and Advia A-diff results were very similar in the groomed data group. The M-diff is less precise than the A-diff of ProCyte or Advia as illustrated by more dispersion of results on difference plots and Bland-Altman 95% limits of agreement.

Arbitrary limits for unacceptable deviations of ProCyte results for lymphocytes and neutrophils from both reference methods were chosen. These limits were 18 for % lymphocytes and 23 for % neutrophils. These values originally were chosen from values reported for total allowable error for lymphocytes and neutrophils in humans.[8] The concept and principle of total allowable error (TEa) were not used for judging the accuracy of feline lymphocyte and neutrophil enumeration in our study. Despite not using the TEa principles, these values (18 and 23) functioned well to identify the most severe errors in the 106 samples. Other guidelines may have been chosen. The preliminary, unpublished draft of an American Society for Veterinary Clinical Pathology (ASVCP) recommendation on TEa for animals suggested a TEa of 15% for both neutrophils and lymphocytes. Lesser limits of unacceptable deviation from a test result obtained by either reference method could have identified a larger number of the ProCyte results in the 106 samples classified as unacceptable. The precise number of unacceptable samples in this patient population is arbitrary, but this fact does not change the major conclusion that marked errors occur too often with routinely reported results.

Absolute differences in % as units of measurement were used to indicate the magnitude of difference between ProCyte and the 2 reference methods. Relative differences could be much different. For example, the median % neutrophils in the 14 samples with marked errors were 15% for M-diff and 53% for ProCyte. The absolute increase in % units from 15 up to 53 was considered to be 38. The relative increase of 15 up to 53 is 253%. The relative increases were not used in our study. Both are substantial changes.

Our study compared the relative % of leukocyte types and not absolute numbers. The choice of % was mainly because the initial measurement in a leukocyte differential leukocyte count is the % of different leukocytes, at least with M-diff. The relative % then is multiplied by total WBC count to get the absolute differential WBC counts. The use of total WBC count adds another variable. For example, Advia determines 2 different total WBC counts.

All methods of determining the number of different types of leukocytes in our study (Advia, ProCyte and M-diff) are well accepted and useful. But all methods have limitations and can result in erroneous results. Users of in-clinic hematology instruments need to identify when the instrument is likely correct or incorrect. The ProCyte is an excellent and advanced hematology instrument but should be operated by persons able to detect errors in automated results and who do not accept all automated instrument results from all blood samples.

Increasing toxic change in neutrophils was associated with increasing numbers of unacceptable ProCyte neutrophil-lymphocyte results. Increasing toxicity of neutrophils also was associated with increased numbers of immature neutrophils. The presence of toxic and immature neutrophils was associated with upward movement of the neutrophils along the *y*-axis from their normal location in the ProCyte "WBC Run" dot plot. Neutrophils that are toxic or more immature appeared to have greater fluorescence staining and were located higher in the WBC dot plot. The lymphocyte counting area unfortunately is located directly above the normal neutrophil counting area in cats and dogs. Abnormal neutrophils move up into the lymphocyte area and can even move up into the monocyte counting area as seen in 5 of the 14 samples. These changes in feline and canine samples have been described earlier for both the ProCyte and Sysmex 2000iV.[1,5,6]

An acceptable WBC dot plot should have distinct, individual cell clusters located in the correct areas, and all dots in these clusters should have the correct color for that cell type. Examples are given here and by others.[1,4]

ProCyte monocyte results in our study agreed quite well with Advia, except in those cases in which abnormal neutrophils moved through the lymphocyte area up into the Sysmex monocyte counting area. Advia misclassified neutrophils as monocytes in 2 samples in which neutrophils were located lower and more to the left in the Advia WBC dot plot. This change caused a marked Advia monocyte-neutrophil error. Adequate evaluation of monocyte performance by the instruments was limited by the low number of monocytes in most samples. ProCyte monocyte results correlated fairly well with Advia and poorly with M-diff in a previous study.[1] Correlation, although used often in the past, is not an acceptable statistical method for validation of accuracy of a hematology instrument.[9,10]

Up to 23% eosinophils were detected in these samples, which is numerous enough to allow conclusions about eosinophil enumeration. ProCyte, Advia, and M-diff appeared to function well in identification of eosinophils (Fig 4). All 3 methods reported similar results and had essentially no negative or positive bias. Good agreement among the ProCyte, Advia, and M-diff for feline eosinophils has been reported previously.[1]

ProCyte detected basophils in 37 of 155 cats but basophils were seen in only 1 of 155 cats with M-diff in a previous study.[1] At least 1% basophils were noted with M-diff in 22 of the 106 cats (20% of cats) in our study. Twelve samples had >1% basophils reported by the ProCyte and 8 of these also had a distinct cell population in the basophil area in the dot plot, supporting the true presence of basophils in the samples. There were 5 M-diff basophil counts of 2-7% basophils. ProCyte A-diff reported fewer basophils than M-diff but the ProCyte A-diff is a flow cytometer that enumerates many more cells than M-diff and is more precise, and therefore, the ProCyte % basophil results were more likely correct. The M-diff is a method with moderate-to-high imprecision and is inappropriate to compare results of different methods with only 0-5% basophils. The ProCyte basophil count was erroneously increased in 3 samples in which eosinophils were erroneously included in the basophil counting area (Fig 4). Advia does not recognize feline basophils.[11]

Our initial concern that results produced from the after-hours laboratory frequently were markedly incorrect was substantiated. These results had gone into the patients records without proper validation of accuracy and became a permanent part of the record. It is unclear how many patients were given an incorrect diagnosis or treatment. Acceptance of ProCyte A-diff results without evaluation of the acceptability of dot plots and instrument alarms occurs in many other hospitals and is not a problem restricted to our University's after-hours laboratory. Thirteen percentage of patient blood samples were clearly unacceptable in this study. Using different criteria of what is unacceptable or a different sample population would give a value different than 13%, but it should be evident that there likely are many feline patients with very unacceptable lymphocyte and neutrophil results routinely reported by the ProCyte A-diff. There is risk in allowing use of a hematology instrument by users who cannot or will not validate the results as correct. The ProCyte had good to excellent correlation with the reference method in a previous study.[1] ProCyte results were reported to be reliable if dot plot inspection showed distinct separation of cell populations and thus used groomed data for that positive evaluation. Judging the acceptability of dot plots was considered to be a job for experts.[1] We believe that judging of acceptability of dot plots can be learned fairly quickly by most veterinarians and other instrument users.[1] It is understood that care of very ill patients in the evenings and weekends is a stressful situation, and caregivers must determine how to use the limited time available to them. Veterinarians in small clinics desire a hematology instrument that provides results without requiring the veterinarian to perform a blood smear examination or reject A-diff results based on evaluation of instrument graphical reports. Such an instrument unfortunately is not currently available.

Footnotes

[a] IDEXX ProCyte Dx, IDEXX Laboratories, Westbrook, ME
[b] Siemens ADVIA 2120, Siemens Medical Solution Diagnostics, Eschborn, Germany
[c] Check (XS) quality control reagents IDEXX Laboratories, Westbrook, ME
[d] Advia 2120 3–in-1 TEST point control reagents, Siemens Medical Solution Diagnostics, Eschborn, Germany
[e] HemaPrep, CellaVision AB, Lund, Sweden
[f] Modified May-Grünwald Giemsa stain, Merck, Darmstadt, Germany
[g] Analyse-it Software Ltd, Leeds, UK

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Conflict of Interest Declaration: One of the authors (Tvedten) has received consulting fees from IDEXX for providing advice about development of their hematology analyzers.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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