



# Evaluation of the scil vCell 5, a novel laser- and impedance-based point-of-care hematology analyzer, for use in dogs and cats

Kim-Lina Charlotte Zelmer,<sup>1</sup>  Natali Bauer, Andreas Moritz

**Abstract.** A novel laser- and impedance-based point-of-care hematology analyzer (POCA), the vCell 5 (scil Animal Care), providing a complete blood count with 5-part leukocyte differential count has recently been introduced to veterinary laboratories. We evaluated the analyzer for use in dogs and cats including method comparison and assessment of linearity, carryover, and precision. Fresh blood samples from 192 healthy and diseased dogs and 159 cats were analyzed, and results were compared to reference methods (i.e., microhematocrit [PCV], Advia 2120 hematology analyzer). Total observed error (TE<sub>o</sub>) was calculated from CV, obtained at 3 concentrations, and bias%, and compared to total allowable error (TE<sub>a</sub>). For both species, excellent correlation ( $r_s = 0.93–0.99$ ) was seen between methods for WBC and RBC, hematocrit, hemoglobin, and platelet counts (PLT), except for feline PLT ( $r_s = 0.79$ ). Quality requirements (TE<sub>o</sub> < TE<sub>a</sub>) were fulfilled for WBC (TE<sub>o</sub> = 8.6–11.1%; TE<sub>a</sub> = 20%) and RBC (TE<sub>o</sub> = 3.5–7%; TE<sub>a</sub> = 10%), hematocrit (TE<sub>o</sub> = 5.7–9.4%; TE<sub>a</sub> = 10%), PCV (cat TE<sub>o</sub> = 7.8%; TE<sub>a</sub> = 10%), mean corpuscular volume (cat TE<sub>o</sub> = 5.1%; TE<sub>a</sub> = 7%), and PLT (TE<sub>o</sub> = 13.1–24.1%; TE<sub>a</sub> = 25%). Excellent linearity was demonstrated for WBC, RBC, and PLT, and hemoglobin. CVs of <2% for WBC, RBC, hematocrit, hemoglobin, and of <5% (dog) and 8% (cat) for PLT were demonstrated for values within the RI. Except for calculated variables and well-known species-specific deviations in feline PLT, scil POCA results were correlated favorably with reference method results and complied with quality requirements for cats and dogs.

**Keywords:** focused flow impedance; hematology; laser flow cytometry; linearity; method comparison; point-of-care analyzer; precision; total allowable error.

Blood tests serve as basic tools for assessing the health status of dogs and cats in veterinary clinical practice.<sup>6</sup> As part of screening examinations and follow-up testing to evaluate therapeutic success, complete blood counts (CBCs) are performed daily in routine practice using a variety of automated point-of-care hematology systems.

A novel in-house hematology system, developed as iVet 5 (Norma Instruments) and distributed in Europe under the name vCell 5 (scil Animal Care), is available to the veterinary market for the analysis of canine, feline, and equine blood samples. In addition to the widely established impedance technology for erythrocyte and platelet counts, this point-of-care analyzer (POCA) uses laser light flow cytometry for leukocyte differentiation, providing a CBC including a 5-part differential count. Results are displayed numerically and graphically as leukocyte scattergrams, as well as erythrocyte and platelet histograms, within 2 min.

Little information has been published about the performance of the scil POCA. Our aim was to evaluate the use of this POCA for dogs and cats including 1) a method comparison of accuracy for the variables of CBC (i.e., white blood cell count [WBC], red blood cell count [RBC], hemoglobin

[HGB], hematocrit [HCT], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red blood cell distribution width [RDW], and platelet count [PLT]) by comparing the results obtained by the POCA with those of the Advia 2120 hematology analyzer (Siemens) and the microhematocrit (packed cell volume, PCV), serving as reference methods, and 2) the evaluation of the analyzer's performance characteristics (i.e., linearity, carryover, intra-assay precision). Performance of the Advia 2120 has been evaluated previously for dogs and cats.<sup>13</sup> Our hypothesis was that the scil POCA fulfills quality requirements recommended by the American Society for Veterinary Clinical Pathology (ASVCP)<sup>31</sup> for most variables.

Small Animal Clinic, Internal Medicine (Zelmer, Moritz) and Department of Veterinary Clinical Sciences, Clinical Pathology and Clinical Pathophysiology (Bauer, Moritz), Justus-Liebig-University, Giessen, Germany.

<sup>1</sup>Corresponding author: Kim-Lina Charlotte Zelmer, Small Animal Clinic, Internal Medicine, Justus-Liebig-University Giessen, Frankfurter Str. 114, Giessen, 35392, Germany. Kim.L.Zelmer@vetmed.uni-giessen.de

## Materials and methods

### Study design

We performed our prospective study between December 2018 and October 2019. Anticoagulated blood ( $K_3$ -EDTA) samples from healthy and diseased dogs and cats submitted to the central laboratory, Department of Veterinary Clinical Sciences, Clinical Pathology and Clinical Pathophysiology, Justus-Liebig-University (Giessen, Germany) were included for the method comparison study. Inclusion criteria were the availability of a CBC performed with the Advia 2120 analyzer and the scil POCA, a duplicate measurement of a spun hematocrit, and a blood smear for each sample. Samples with macroscopic clots and incomplete measurements were excluded. Species-specific pool samples, generated from residual  $K_3$ -EDTA anticoagulated blood of the same day, as well as individual specimens, were used for performance measurements (linearity, carryover, precision). Pool samples and individual samples were concentrated by centrifugation as needed to achieve high concentrations of distinct cell populations.

Given that we analyzed residual blood samples of cats and dogs, our study was not classified as animal experimentation according to the German Animal Welfare Act (§7). Our study was approved by the competent authority (Regierungspräsidium Giessen, Dezernat 54, Wetzlar, Germany; file V 54-19 c 20-15 [1] GI18/17).

### scil POCA

During the evaluation, the scil POCA was operated with the veterinary software version 1.0.779.0 beta. For a single measurement, a sample volume of 30  $\mu$ L of  $K_3$ -EDTA anticoagulated blood is aspirated from open sample vials. Of the aspirated volume, 1.4  $\mu$ L of blood is simultaneously processed in each of the measuring units (i.e., the impedance unit and the optical-photometric unit). Within the impedance unit, RBC and PLT are counted based on hydrodynamic focusing and direct current detection. Cell populations are separated using a dynamic threshold. The results are displayed both numerically and graphically as a histogram. The HCT value and the erythrocyte indices MCH and MCHC are calculated automatically from the impedance counts by the following formulas:  $HCT = MCV \times RBC / 10$ ;  $MCH = HGB \times 10 / RBC$ ;  $MCHC = HGB \times 100 / HCT$ .

The HGB concentration is determined photometrically within the optical unit. In preparation for measurement of WBC and HGB, cells are lysed, leaving free hemoglobin and leukocyte nuclei. The lysis reagent is mixed with the sample during the passage through a thin tube system (microfluidic technology). At the end of the tube system, a light sensor registers 32 absorbance values at 540 nm wavelength as the sample liquid passes through. All measured absorbance values are summed to the total HGB absorbance value.<sup>33</sup> After passing through the photometric unit, the sample solution is

hydrodynamically focused before passing through a perpendicular laser light beam within the optical unit. The resulting low-angle (2–5°, proportional to cell size) and high-angle (7–15°, proportional to the complexity of the cells' interior) scattered light from the cells' nuclei is detected to determine WBC and differential leukocyte counts. Results of the leukocyte differential count are presented both numerically and graphically as a scattergram (dotplot) with cell size (low angle) shown on the y-axis and cell complexity (high angle) on the x-axis.

The scil POCA is capable of displaying error messages (flags), which are categorized into sample-specific (technical) and parameter-specific flags. Technical flags indicate erroneous sample handling or sample aspiration (e.g., “high pressure” [high pressure during measuring process], “low sample volume” [low sample volume or block in sampling unit]). Parameter-specific flags, marking the WBC or RBC parameter group, are labeled with different levels (#3–#9), depending on their significance and severity. Such flags indicate irregularities during the measuring process (e.g., “unintelligible histogram” [inhomogeneous sample flow in the flow cell], “RBC slice, unstable HGB” [internal homogeneity error], “WBC noise” [air bubbles in the optical unit]) or in the report (e.g., “abnormal diff” [atypical leukocyte distribution in the scattergram]) requiring troubleshooting (re-analysis, maintenance procedures), verification of results by manual methods, or if persistent, consultation with the technical service. According to the user manual, conditions causing flags do not have a major impact on results as long as the analyzer displays results. As soon as the severity of a condition causing flags impacts interpretation of results, results will not be reported.

In case of error messages displayed by the scil POCA, troubleshooting was performed according to the instructions in the user manual.<sup>43</sup> Generally, results with persistently incomplete datasets, even after troubleshooting, were excluded from our study. Flagged results after troubleshooting were not excluded when datasets were complete. In this case, measurement results after troubleshooting (i.e., the most reliable results) were included in our method comparison study. We recorded flags; however, we did not assess the diagnostic utility of flagging options of the scil POCA. During our study, maintenance procedures were performed regularly according to the manufacturer's instructions as part of troubleshooting procedures and when recommended by the analyzer's alerts. As part of quality assurance, daily quality control (QC) measurements were performed using the quality control material (QCM) provided by the manufacturer (Norma) at 3 concentrations (low, normal, high).

### Advia 2120

The Advia 2120 served as a reference method, using software version 5.3.1-MS. As a flow cytometer, the analyzer is based on the optoelectronic measuring principle and

provides a CBC including a 5-part differential leukocyte count and a reticulocyte count. In addition to laser light scatter, the Advia 2120 also utilizes reagent-mediated cell lysis and cytochemical staining (myeloperoxidase and oxazine 750) to differentiate cell populations.<sup>23</sup> The determination of HGB is based on a cyanide-free photometric measurement method.<sup>4</sup> Samples are analyzed within 5 measurement channels (HGB, RBC/PLT, WBC basophil/lobularity, peroxidase, and reticulocyte channel). The results of the channels are displayed as characteristic scatter plots. Detailed technical aspects of the measurement principle of the Advia 2120 have been published.<sup>22,23</sup> Daily internal QC measurements were performed using human QCM at 3 concentrations (Siemens). Calibration of the hematology instrument was performed annually by the manufacturer.

### Method comparison

We included  $K_3$ -EDTA anticoagulated blood samples from 192 healthy and diseased dogs and 159 cats that were presented to the medical and surgical service of the faculty of veterinary medicine for health checks and various internal medical and surgical conditions. The percentage of samples obtained from healthy dogs and cats presented as blood donors or for health checks was limited to a maximum of 25%. For the method comparison study, all blood samples were stored at room temperature and processed within 6 h after sampling. Measurements with the Advia 2120 served as a reference method and were conducted first, followed by the analysis with the scil POCA. Additionally, PCV, determined as the mean of a duplicate measurement by centrifugation using a standard microhematocrit procedure ( $2,376 \times g$  for 5 min, Haematokrit 200; Hettich), served as the reference method for the automated HCT. Before processing, samples were mixed properly by inverting the tubes several times.

### Statistical analysis

Statistical analysis was performed using the statistical programs MedCalc v.17.8.6, Prism v.8 (GraphPad), and Excel (Microsoft). The correlation between results was quantified using the Spearman rank correlation coefficient ( $r_s$ ). Spearman rank correlation coefficients were interpreted as excellent ( $r_s = 0.93-0.99$ ), good ( $r_s = 0.80-0.92$ ), fair ( $r_s = 0.59-0.79$ ), or poor ( $r_s < 0.59$ ).<sup>36</sup> Regression equations with y-axis intercept, slope, and 95% CIs were determined using the Passing-Bablok regression. Absolute and % bias and 95% limits of agreement were determined with Bland-Altman analysis. Percent bias was calculated as follows:  $\% \text{bias} = (\text{mean}_{\text{target}} - \text{mean}_{\text{measured}}) / \text{mean}_{\text{all}} \times 100\%$ . The statistical methods were chosen because they do not assume a normal distribution and are less affected by outliers.<sup>25</sup>

The cyanide-free HGB-measuring method of the Advia 2120 is known to be associated with a mean proportional bias of  $\sim 20\%$ .<sup>2,13,19</sup> Therefore, the statistical analysis was

performed before and after bias correction. Given that it is generally accepted to utilize standard linear regression analysis for estimation of error between 2 methods at medical decision limits in case of excellent correlation ( $r_s = 0.99$ ) and high precision of measurements,<sup>1,25,49</sup> HGB biases obtained by the cyanide-free measuring method of the Advia 2120 were corrected by using slope and intercept of the regression line for calculation of the expected HGB value using the Advia 120 cyanide-based reference method, as described previously.<sup>4</sup> Regression lines used were  $y = 0.24 + 0.79x$  for dogs and  $y = 0.18 + 0.78x$  for cats, respectively.

For objective assessment of method validation results and for comparability of the 2 hematology systems, total observed error (TEo) was determined for the main hematology parameters according to the current ASVCP guidelines.<sup>31</sup> TEo was calculated from CV as random error and bias as systematic error, applying the following formula:  $\text{TEo} = \text{bias}\% + 2\text{CV}$ .

Ideally, TEo should be less than the total allowable error (TEa) to fulfill quality requirements ( $\text{TEo} < \text{TEa}$ ). In cases of  $\text{TEo} > \text{TEa}$ , the quality goal index (QGI) was determined to evaluate whether the impact on analytical performance was a matter of unacceptably high imprecision, bias, or both. QGI was calculated according to the formula:  $\text{QGI} = \text{bias}\% / \text{CV}$ . A QGI  $< 0.8$  indicated a predominant impact of imprecision, and a QGI  $> 1.2$  indicated a predominant impact of bias. For a QGI of  $0.8-1.2$ , both imprecision and bias contributed to an observed analytical error.<sup>31,38</sup>

### Performance measurements

**Linearity.** To achieve a high concentration of RBC, species-specific pool samples were prepared from 10 mL of  $K_3$ -EDTA anticoagulated blood and centrifuged at room temperature for 25 min at  $750 \times g$  (EBA 200 centrifuge; Hettich). The supernatant was removed and discarded. After centrifugation of the sample a second time for 10 min at  $750 \times g$  and discarding the supernatant, the residual sediment served as the 100% pool. Resuspension of the 100% pool with 0.9% saline (0% pool) produced appropriate dilution levels (25%, 50%, 75%).

To achieve high concentrations of WBC and PLT, species-specific pool samples were prepared from 10 mL of  $K_3$ -EDTA anticoagulated blood and centrifuged at room temperature for 25 min at  $100 \times g$ . The supernatant and leukocyte-rich fraction (buffy coat) were removed and centrifuged again at  $750 \times g$  for 10 min. The resulting supernatant was discarded, and leukocyte-rich sediment was used as a 100% pool. Resuspension of the 100% pool with 0.9% saline (0% pool) produced appropriate dilution levels (25%, 50%, 75%).

To determine linearity for WBC, RBC, PLT, and HGB, 3 measurements of the 0% pool were made initially followed by duplicate measurements of the dilution levels in ascending order. One measurement of the 0% pool was performed between each dilution level. For 100% pool values above the analyzer's measuring range (RBC:  $> 18 \times 10^{12}/L$ ; PLT:



$>1,000 \times 10^9/L$ ), censored values (RBC:  $20 \times 10^{12}/L$ ; PLT:  $1,100 \times 10^9/L$ ) were used to allow a statistical comparison with expected (calculated) values. Linearity was evaluated using a simple linear regression analysis between the calculated (i.e., expected [calculated] values after dilution) and measured values of the dilution series.

**Carryover.** For carryover studies, 1 measurement of the 100% pool followed by 2 consecutive measurements of the 0% pool was performed. A separate sample vial was used for each measurement of the 0% pool. The procedure of measurement was repeated 3 times and the carryover was calculated for WBC, RBC, PLT, and HGB as follows:

Carryover % =  $(\bar{x}_1 - \bar{x}_2) / \bar{x}_3 \times 100\%$ ;  $\bar{x}_1$  = arithmetic mean of the first 0% pool measurements ( $n=4$ );  $\bar{x}_2$  = arithmetic mean of the second 0% pool measurements ( $n=4$ );  $\bar{x}_3$  = arithmetic mean of the 100% pool measurements ( $n=4$ ).

**Intra-assay precision.** To determine precision, 20 consecutive measurements were performed from feline and canine  $K_3$ -EDTA anticoagulated pooled blood samples with 3 concentrations (low, normal, high) within 1 h after sampling. The composition of blood samples was modified according to the experimental requirements by preparation of species-specific pool samples, centrifugation, and resuspension to provide samples with low, normal (within the RI), and high concentrations of WBC, RBC, HGB, and PLT. For each variable, the CV% was calculated from arithmetic mean and SD as follows:  $CV\% = SD/mean \times 100\%$ . A CV of  $<0.25$  TEa was considered to be acceptable as recommended by current literature.<sup>25,48</sup>

## Results

The use of the scil POCA was simple and intuitive via the 26-cm (10.1-in) touchscreen display. The analyzer had small dimensions, making it space-saving, and convenient to install and transport. Results were provided in  $\sim 1$  min, dependent on WBC, with higher WBC leading to slightly longer processing times.

Of the original 363 samples (201 dogs, 162 cats), 12 samples (9 dogs, 3 cats) were excluded because of incomplete datasets. Of these 12 samples, 4 results (all canine samples) were marked with parameter-specific flags. Three of the 4 flagged samples had distinct autoagglutination and were marked with "RBC slice, unstable HGB #6". One of the 4 flagged results, obtained from a healthy blood donor, was flagged with "WBC noise #9". The remaining 8 of 12 results (5 dogs, 3 cats) were excluded because they contained results that exceeded (PLT  $>1,000 \times 10^9/L$ ; 3 of 8) or fell below (MCV  $<30$  fL; 3 of 8) the range of the analyzer, or contained low PLT numbers ( $<12 \times 10^9/L$ ; 2 of 8) and thus interfered with the display of the calculated parameters (HCT, MCH, MCHC, PLT indices). Of the residual 351 of 363 samples

(192 dogs, 159 cats) finally considered for statistical analysis (after troubleshooting), 19 of 351 samples (8 dogs, 11 cats) were marked with "abnormal Diff #3" (1 dog, 5 cats) or "abnormal Diff #6" (7 dogs, 6 cats); 7 of 351 samples (2 dogs, 5 cats) were marked with "unintelligible histogram #6". Of the canine specimens, 11 of 192 (5.7%) samples were hemolytic (3 of 11), icteric (6 of 11), lipemic (1 of 11), or were affected by both hemolysis and lipemia (1 of 11), with 2 of 11 samples flagged by "unintelligible histogram #6" (1 of 3 hemolytic samples) and "abnormal Diff #6" (1 of 6 icteric samples). In contrast, 2 of 159 (1.3%) feline samples with apparent icterus (1 of 2) or lipemia (1 of 2) were observed but not flagged.

## Method comparison

**CBC.** Based on the results obtained with the Advia 2120, WBC were  $0.7$ – $52.3 \times 10^9/L$  (median:  $9.7 \times 10^9/L$ ) in canine samples and  $2.0$ – $47.2 \times 10^9/L$  (median:  $9.3 \times 10^9/L$ ) in feline samples. RBC were  $2.0$ – $9.9 \times 10^{12}/L$  (median:  $6.3 \times 10^{12}/L$ ) in canine samples and  $2.5$ – $13.2 \times 10^{12}/L$  (median:  $8.2 \times 10^{12}/L$ ) in feline samples, and spun PCV ranged from  $0.17$ – $0.70$  L/L (median:  $0.44$  L/L) in canine samples and  $0.12$ – $0.55$  L/L (median:  $0.37$  L/L) in feline samples. PLT were  $38$ – $1,087 \times 10^9/L$  (median:  $287 \times 10^9/L$ ) in canine samples and  $22$ – $769 \times 10^9/L$  (median:  $260 \times 10^9/L$ ) in feline samples.

Passing–Bablok and Spearman rank regression analysis revealed excellent correlation of WBC and RBC results between analyzers for dogs (Table 1; Fig. 1) and cats (Table 2; Fig. 2). Bland–Altman analysis showed small negative proportional biases of 6.4% (dog) to 8% (cat) for WBC and 5.6% for feline RBC. There was excellent correlation for HCT between the analyzers and between automated results of the scil POCA and spun PCV for both dogs (Table 1; Fig. 3) and cats (Table 2; Fig. 4). Constant positive bias was seen for canine HCT results when comparing analyzers (7.3%), and between results obtained with the scil POCA and the microhematocrit method (12.9%). Smaller biases ( $-2.8\%$  and  $4.9\%$ ) between the methods for determination of HCT were observed for feline samples.

There was a significant positive proportional bias of  $\sim 20\%$  for HGB results between analyzers for dogs (Table 1; Fig. 1) and cats (Table 2; Fig. 2). HGB biases obtained by the cyanide-free measuring method of the Advia 2120 were corrected by using slope and intercept of the regression line for calculation of the expected HGB value using a cyanide-based reference method as described previously.<sup>4</sup> Using corrected values, only minimal biases between HGB results obtained with the different methods and analyzers were seen for dogs (Table 1) and cats (Table 2).

For RBC indices, good correlation was demonstrated for MCV, MCH, and RDW for dogs and cats. Results of MCHC correlated poorly in both species. Small-to-marked positive biases were present for all RBC indices, except for RDW in both species (Tables 1 and 2). Correlation between

**Table 1.** Correlation and agreement of the complete blood count obtained from the scil point-of-care and Advia 2120 analyzers for canine specimens.

Variable	Unit	<i>n</i>	$r_s$	S	I	Bias	95% LOA	%Bias	95% LOA
WBC	$\times 10^9/L$	192	0.99	0.93	-0.05	-0.8	1.7 to -3.3	-6.4	10.1 to -22.8
RBC	$\times 10^{12}/L$	192	1.00	0.96	0.31	0.07	0.32 to -0.17	1.6	6.3 to -3.2
HGB	mmol/L	192	1.00	1.28	-0.49	1.88	3.22 to 0.54	19.6	26.1 to 13.1
HGB <sub>corr</sub>	mmol/L	192	1.00	1.01	-0.14	-0.04	0.32 to -0.4	-0.7	4.8 to -6.2
HCT	L/L	192	0.98	0.95	0.04	0.03	0.06 to -0.01	7.3	18.7 to -4.1
PCV	L/L	192	0.98	1.07	0.02	0.05	0.09 to 0.01	12.9	25.8 to 0
MCV	fL	192	0.82	0.87	12.07	3.7	9.3 to -1.8	5.8	14.5 to -2.9
MCH	famol	192	0.85	1.24	-0.04	0.28	0.44 to 0.13	18	26.9 to 9.2
MCHC	mmol/L	192	-0.01	1.15	-0.06	2.9	6.3 to -0.5	12.3	26.9 to -2.4
RDW	%	192	0.82	1.00	-0.30	-0.1	3.5 to -3.7	-0.8	19.1 to -20.8
PLT	$\times 10^9/L$	192	0.96	0.93	6.09	-13.3	61 to -88	-3.4	24 to -30

Slope (S) and intercept (I) derived from Passing-Bablok regression analysis; bias derived from Bland-Altman analysis;  $r_s$  = Spearman rank correlation coefficient (rho). HCT = hematocrit; HGB = hemoglobin; HGB<sub>corr</sub> = HGB was corrected using slope and intercept of the regression line for the cyanide-based reference method<sup>4</sup>; LOA = limits of agreement; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; *n* = number of samples included; PCV = spun hematocrit; PLT = platelet; RBC = red blood cell; RDW = RBC distribution width; WBC = white blood cell.

analyzers was excellent for canine PLT with a small proportional bias (-3.4%; Table 1, Fig. 3). In contrast to dogs, only fair ( $r_s = 0.79$ ) agreement between the analyzers was observed for feline PLT, and there was a positive bias of 8% (Table 2; Fig. 4).

**Quality specifications.** Veterinary quality requirements according to ASVCP guidelines (TEa) were fulfilled for all variables of the CBC, except for canine MCV and HCT when comparing automated and manual methods (PCV) as well as for MCHC and HGB in both species because of high biases indicated by QGI (Table 3).

## Performance measurements

**Linearity.** Linearity was excellent ( $R^2 \geq 0.99$ ) for most variables (Table 4). For RBC and PLT, linearity up the reportable range given by the manufacturer was confirmed (RBC:  $18 \times 10^{12}/L$ ; PLT:  $1,000 \times 10^9/L$ ). Only for feline PLT was agreement lower between expected and measured results ( $R^2 = 0.93$ ). The slope of the regression equation was close to 1 and the y-axis intercept was close to 0, except for PLT (feline > canine).

**Carryover.** Carryover of the POCA was 0% for feline and canine WBC, HGB, and canine PLT. For feline PLT, carryover was 0.091% (for values exceeding the reportable range) to 0.097% (values within the reportable range). In dogs and cats, carryover for RBC was 0.074% and 0.075%, respectively. For feline RBC values exceeding the reportable range ( $>18 \times 10^{12}/L$ ), slightly higher carryover of 0.1% was demonstrated.

**Intra-assay precision.** Generally, CVs for PLT were <5% and for the remaining CBC variables <3% at all

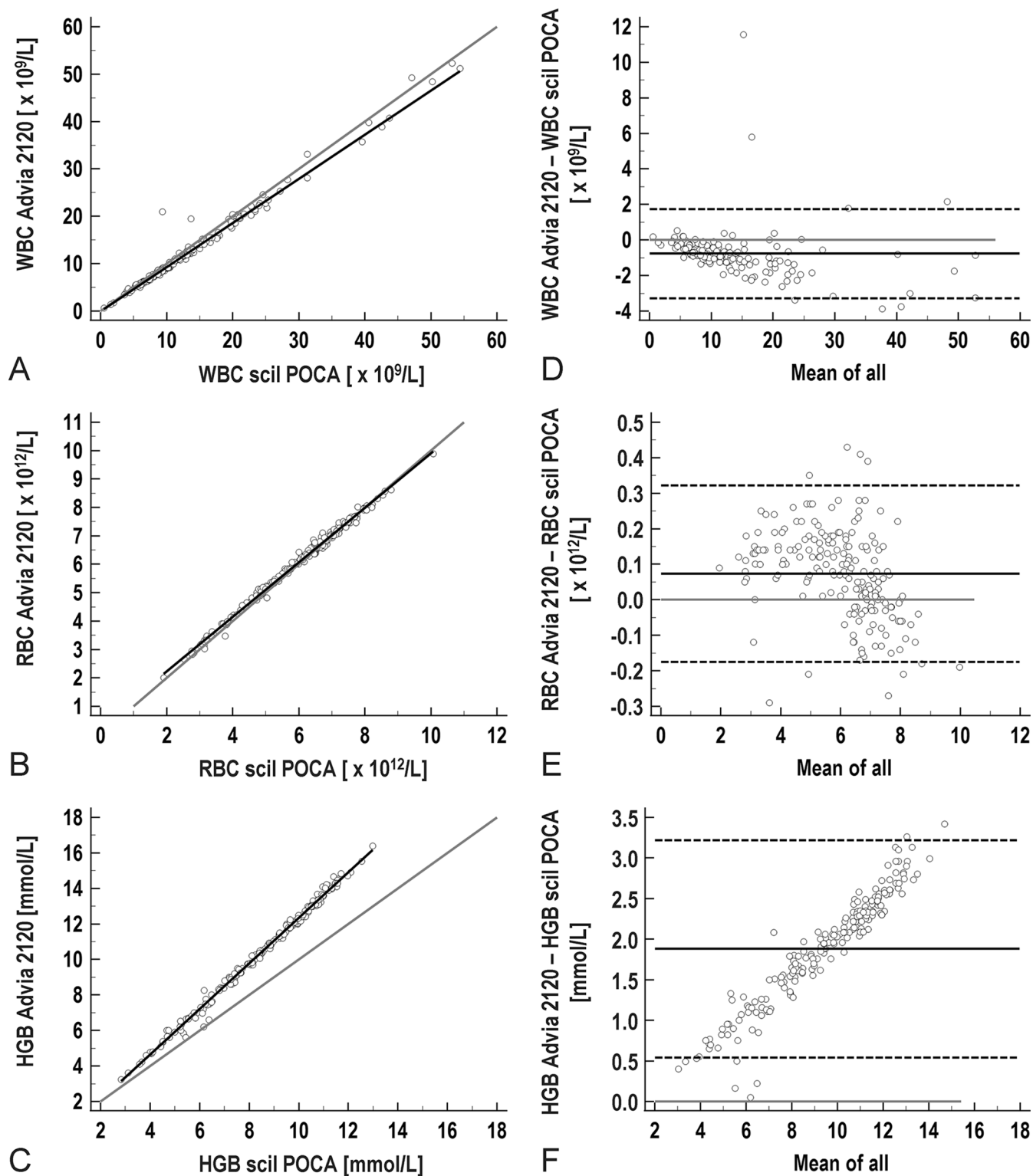
concentrations. Exceptions were feline RBC (4.7%) and HCT (4.5%) at low concentrations, feline PLT at low (15.2%) and normal (8.0%) concentrations, as well as canine PLT at a low concentration (10.6%; Table 5). The CVs of the leukocyte differential counts at normal concentrations were <2.5% for neutrophils (NEU) and up to 31% for absolute numbers of feline eosinophils (EOS). Recommended quality requirements (CV < 0.25 TEa) were fulfilled for NEU in feline samples and for NEU, monocyte (MON), and EOS in canine specimens. Calculation of CV was not possible for feline basophils given undetectable cell counts.

## Discussion

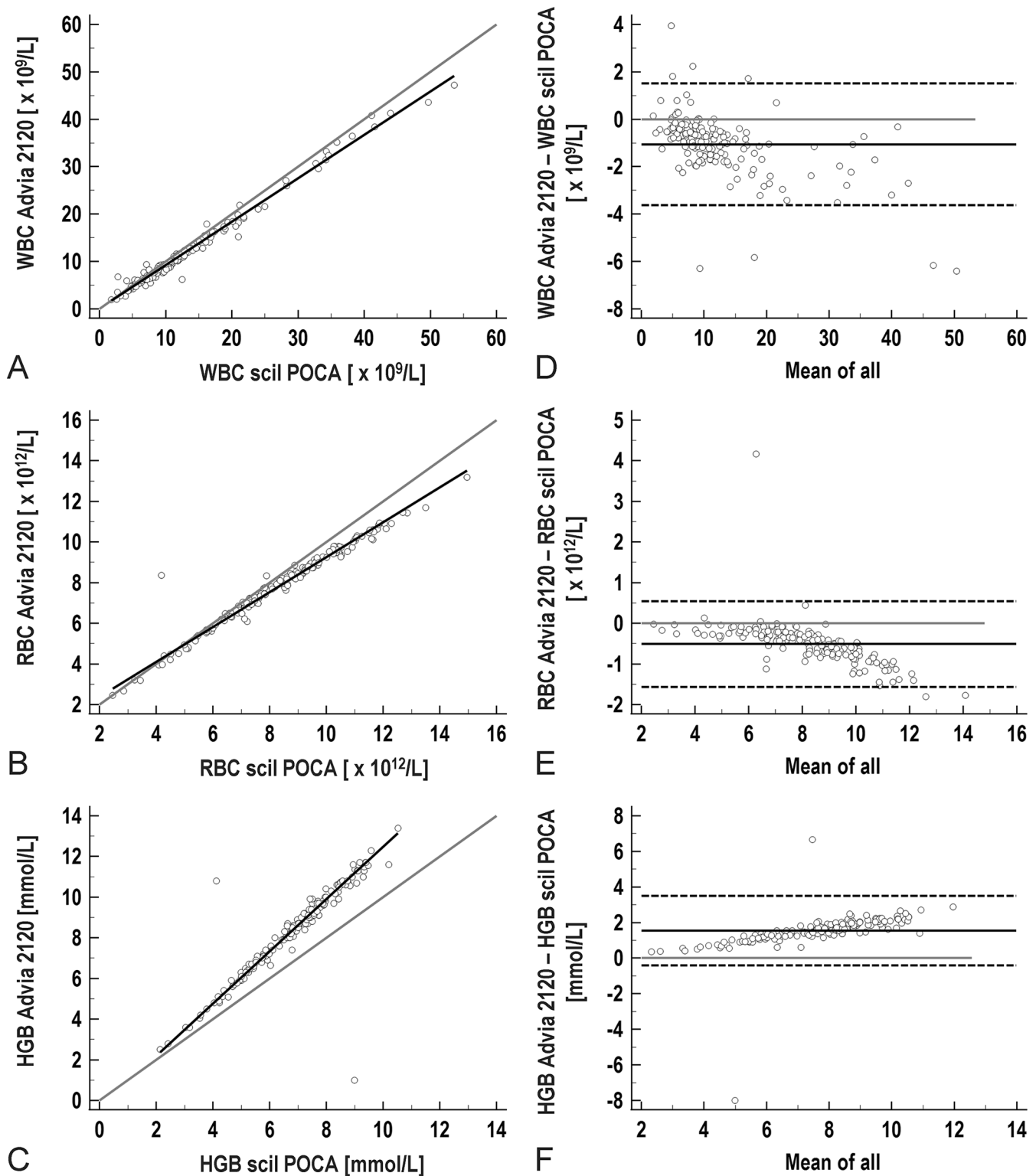
Based on our results, objective quality requirements as recommended by the ASVCP were fulfilled by the scil POCA for most measurands. Performance of comparable POCAs utilizing laser light scatter to supply a 5-part differential count has been evaluated in dogs and cats.<sup>19,37,44,47</sup> As in the analyzer that we investigated here, laser flow cytometry has been combined with impedance technology in some of these analyzers to determine particular measurands.<sup>19,44</sup>

Overall, correlation between methods was good to excellent for all variables ( $r_s \geq 0.82$ -0.98) except for calculated variables such as the canine and feline MCHC and for feline PLT. More importantly, quality requirements (i.e., TEo < TEa) were fulfilled for the main variables WBC, RBC, HCT, and PLT in canine and feline samples for normal concentrations.<sup>31</sup> Higher TEo, exceeding the recommended TEa, were demonstrated for MCV and PCV in canine samples and MCHC and HGB in both species.

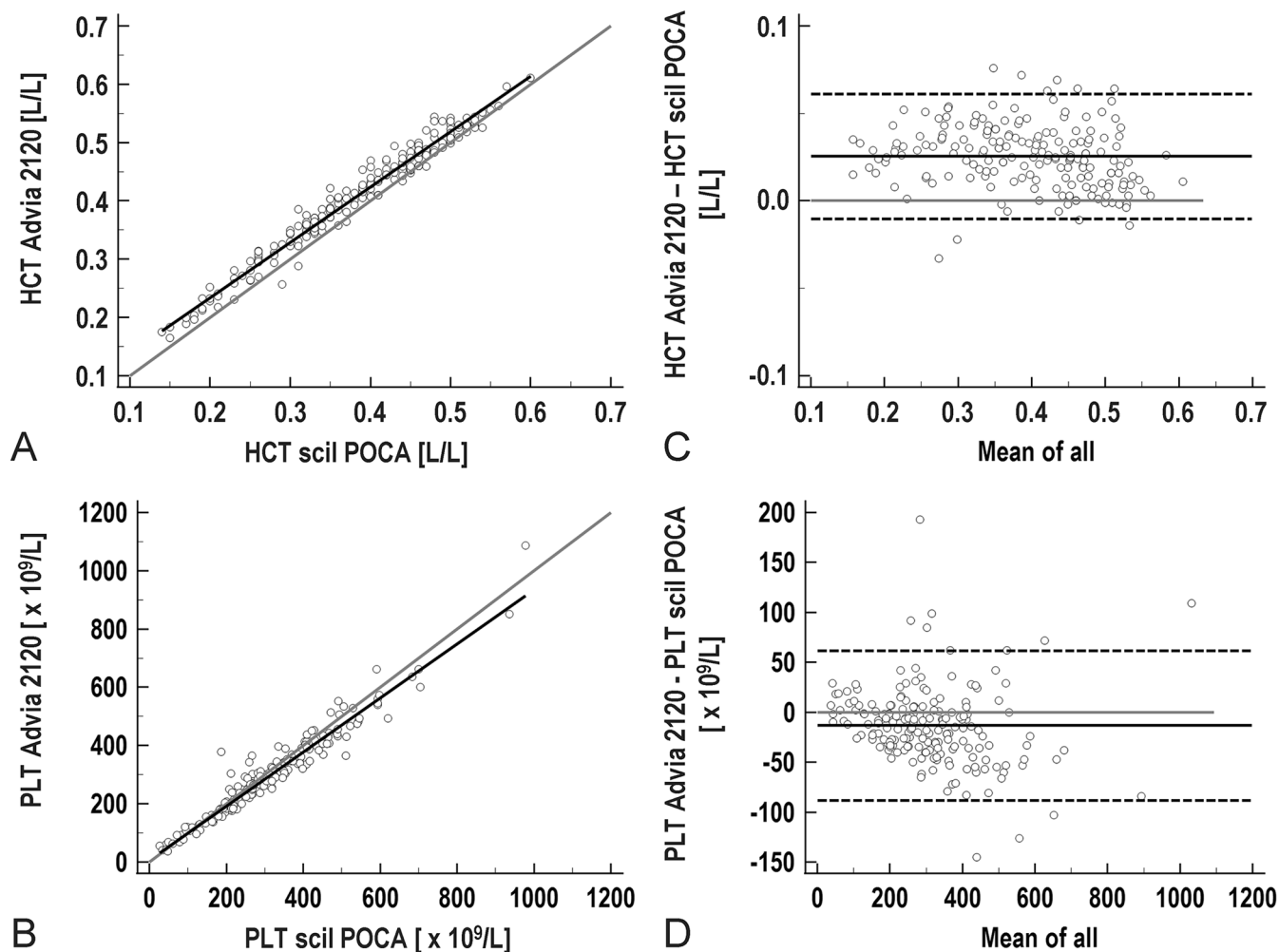
Given that TEo is substantially influenced by bias, and thus by the choice of the reference method, highly stable and validated instruments are usually chosen as reference methods in clinical laboratory medicine. Of course, the accuracy



**Figure 1.** Correlation and agreement of WBC, RBC, and hemoglobin (HGB) obtained with the scil point-of-care and Advia 2120 analyzers for 192 canine samples. **A–C.** Passing–Bablok regression analysis: gray solid line is the identity line ( $x=y$ ); solid black line is the regression line. **D–F.** Bland–Altman plots of the absolute differences against the averages of results obtained by both analyzers. Mean absolute differences (biases, solid black lines) between  $\pm 1.96SD$  (dashed black lines) are shown.



**Figure 2.** Correlation and agreement of WBC, RBC, and hemoglobin (HGB) obtained with the scil point-of-care and Advia 2120 analyzers for 159 feline samples. **A–C.** Passing–Bablok regression analysis: gray solid line is the identity line ( $x=y$ ); solid black line is the regression line. **D–F.** Bland–Altman plots of the absolute differences against the averages of results obtained by both analyzers. Mean absolute differences (biases, solid black lines) between  $\pm 1.96SD$  (dashed black lines) are shown.



**Figure 3.** Correlation and agreement of hematocrit (HCT) and platelet (PLT) obtained from the scil point-of-care and Advia 2120 analyzers for 192 canine samples. **A, B.** Passing–Bablok regression analysis: gray solid line is the identity line ( $x=y$ ); solid black line is the regression line. **C, D.** Bland–Altman plots of the absolute differences against the averages of results obtained by both analyzers. Mean absolute differences (biases, solid black lines) between  $\pm 1.96SD$  (dashed black lines) are shown.

of the new method must be judged with caution, given that the extent of bias also depends on the inherent error of the reference method that represents the “true” value.<sup>15</sup> The Advia 2120 hematology analyzer has served as the reference method in various veterinary method validation studies together with the manual methods traditionally considered as “gold standards.”<sup>2,3,5,19</sup> The Advia 2120 has been reported to fulfill objective TEa-based quality requirements for most variables evaluated here, thus justifying its use as a reference method for our study.<sup>13</sup> The only exception was the HGB measurement performed with the Advia 2120 using the cyanide-free method given the well-known large proportional bias.<sup>2,13,19</sup> The marked proportional bias demonstrated for HGB and HGB-derived variables resulting in  $TEo > TEa$  in feline and canine samples is a well-known finding in evaluation studies and attributable to methodology of the Advia 2120 as the reference method.<sup>2,13,19</sup> To exclude the impact of methodology, bias correction was performed using equation

for the slope and intercept of the cyanide-based method used by the Advia 120,<sup>4</sup> resulting in fulfilment of the quality requirements proposed by the ASVCP ( $TEo < TEa$ ) for HGB in both species.

For MCHC, the quality requirement could not be fulfilled as a result of high positive biases observed in both species. Lack of agreement for the MCHC is explainable as it is a quotient of HGB concentration and Hct. Given that the HCT is also calculated by the analyzer from MCV and RBC values, summation of errors and impact of the bias of HGB may explain the poor performance of this calculated variable.<sup>8,30</sup>

For the comparison of spun PCV and HCT obtained by the scil POCA, a high positive constant bias leading to high TEo was demonstrated in canine samples. Similar trends have been observed for other in-house hematology analyzers.<sup>6</sup> As for other calculated variables, positive bias of MCV, and for a lesser extent RBC, may have contributed to the high TEo. But also, the manual spun PCV is susceptible to



**Table 2.** Correlation and agreement of the complete blood count obtained from the scil point-of-care and Advia 2120 analyzers for feline specimens.

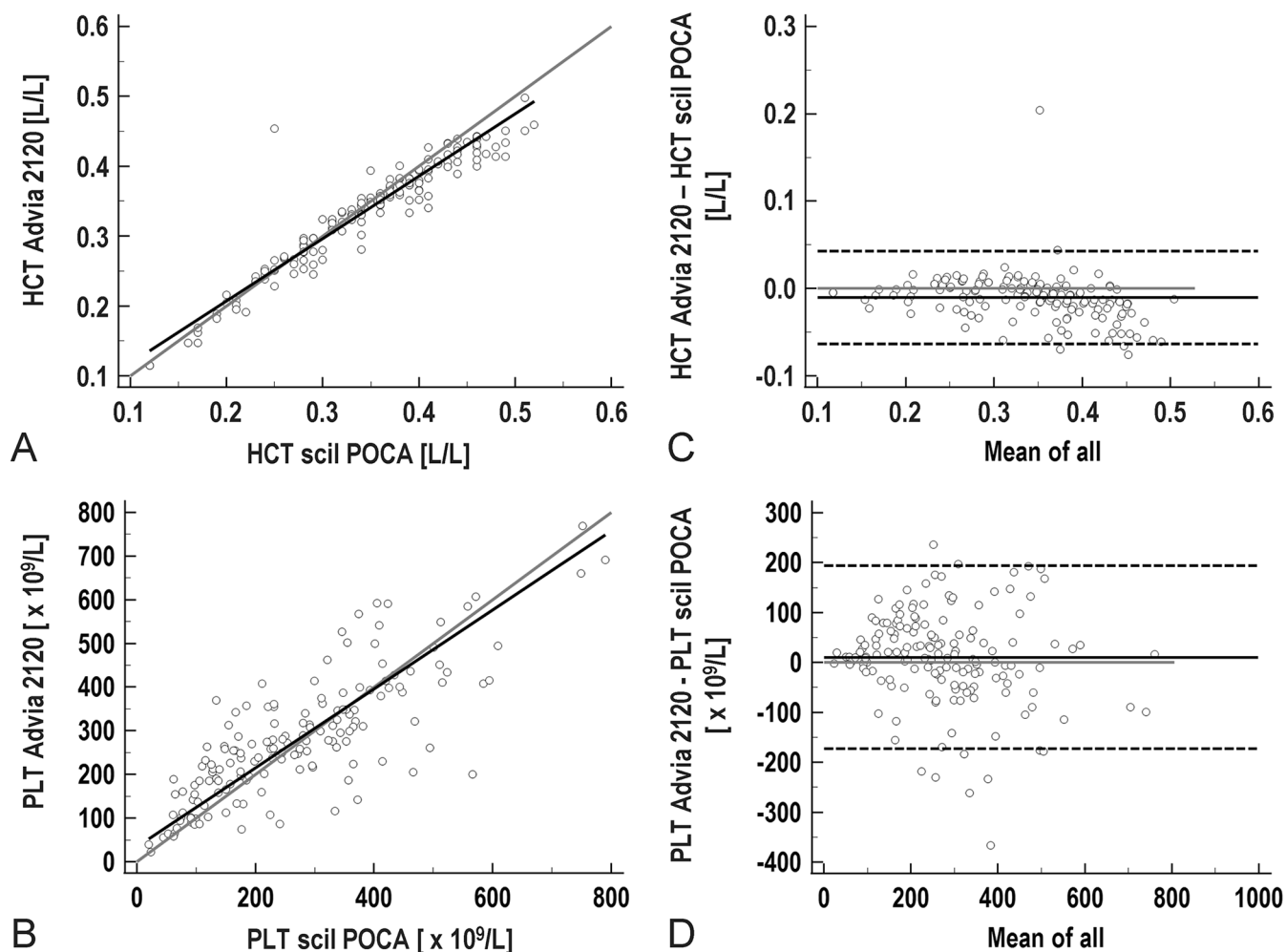
Variable	Unit	<i>n</i>	<i>r<sub>s</sub></i>	S	I	Bias	95% LOA	%Bias	95% LOA
WBC	×10 <sup>9</sup> /L	159	0.98	0.92	0.09	-1.1	1.5 to -3.6	-8	17.0 to -33.0
RBC	×10 <sup>12</sup> /L	159	0.98	0.86	0.66	-0.5	0.5 to -1.6	-5.6	7.5 to -18.8
HGB	mmol/L	159	0.93	1.29	-0.38	1.5	3.5 to -0.4	19.5	50.2 to -11.3
HGB <sub>corr</sub>	mmol/L	159	0.93	1	-0.1	-0.1	1.4 to -1.6	-2.3	25.7 to -30.2
HCT	L/L	159	0.94	0.89	0.03	-0.01	0.04 to -0.06	-2.8	12 to -17.5
PCV	L/L	159	0.96	1	0.02	0.02	0.07 to -0.03	4.9	19.8 to -10.0
MCV	fL	159	0.91	0.84	8.04	1.1	6 to -3.7	3.1	13.8 to -7.7
MCH	fmol	159	0.90	1.17	0.11	0.24	0.42 to 0.06	24.9	55.1 to -5.2
MCHC	mmol/L	159	0.32	0.58	13.59	5.1	9.7 to 0.5	22	53.5 to -9.4
RDW	%	159	0.85	0.65	5.56	0.1	3.6 to -3.5	1	19.2 to -17.1
PLT	×10 <sup>9</sup> /L	159	0.79	0.90	34.20	10.2	193 to -173	8	80 to -64

See Table 1 for explanation of abbreviations.

positive bias as a result of several well-described variables, such as plasma trapping, WBC and PLT contamination of the RBC layer, and reading errors.<sup>12</sup> Additionally, storage-induced and artifactual changes such as erythrocyte shrinkage caused by contact with an instrument's hyperosmolar diluent, or erythrocyte swelling as a result of sample aging, must be considered.<sup>3,7,9,17</sup> In addition to manual verification of automated results near clinical decision levels, modifications of software settings and calibration factors could be beneficial in minimizing the differences for automated HCT after exclusion of preanalytical errors.<sup>6</sup>

In our study, the quality goals for feline PLT were fulfilled narrowly, with imprecision and inaccuracy affecting the results (QGI 1.0). This is in agreement with previous studies demonstrating that most in-house analyzers provide reasonable results for RBC, WBC, HCT, and HGB, but TE<sub>o</sub>>TE<sub>a</sub> have been demonstrated for PLT.<sup>14,41</sup> Poor performance has been reported with high TE<sub>o</sub> for feline PLT by various hematology POCAs, with impedance-based systems tending to have higher TE<sub>o</sub> than laser-based analyzers.<sup>14</sup> In fact, most POCAs were not able to achieve TE<sub>o</sub><TE<sub>a</sub> derived from human quality specifications (e.g., CLIA recommendations<sup>29</sup>), which are comparable to ASVCP guidelines.<sup>31</sup> Compared to POCAs, lower TE<sub>o</sub> for feline PLT have been obtained for large benchtop analyzers. However, similar to the findings for POCAs, TE<sub>o</sub> for feline PLT was affected primarily by imprecision.<sup>13</sup> Preanalytical and analytical difficulties must be taken into consideration regarding discrepancies in feline PLT. In addition to the overlap of RBC and PLT cell sizes, the known tendency of feline PLT to form PLT aggregates plays a crucial role in influencing the results of impedance-based devices by increasing imprecision and leading to underestimation of the true PLT, with misclassification into other cell populations (e.g., WBC).<sup>34,35,40,50</sup> Aggregate formation is also the most likely reason for the relatively high CV and correlation coefficients seen in feline PLT, even for values within the RI in our study.

Generally, the scil POCA fulfilled analytical goals regarding linearity, carryover, and precision.<sup>11</sup> The carryover study revealed no relevant sample-to-sample carryover for all evaluated variables in both species, and results are in accordance with results obtained with other hematology bench-top analyzers and POCAs validated previously.<sup>2,6,8,19</sup> Linearity was demonstrated over a wide range for all variables with *R*<sup>2</sup> values >0.99, considered as excellent,<sup>37</sup> except for feline PLT (*R*<sup>2</sup>=0.93). The manufacturer's claimed imprecision (WBC, HCT: 3%; RBC, MCV, HGB: 2%; PLT: 5%)<sup>32</sup> was generally confirmed by our repeatability experiment for values within the RI. However, in samples with low concentrations of RBC, especially in feline blood samples, and for PLT in both species, higher CVs were observed. Regarding precision of the WBC differential count for values within the RI, the lowest CVs were seen for absolute numbers of NEU (CVs: 1–2%). Given that NEU represent the largest population of leukocytes in dogs and cats, the imprecision of the other populations is not surprising. It is well-known that the count of low cell numbers has larger variances and higher imprecision.<sup>42</sup> According to current recommendations, analyzer variability (CV<sub>A</sub>) less than or equal to half of the amount of within-individual variability (CV<sub>I</sub>) is described as “desirable imprecision.”<sup>16,24,26</sup> On the basis of these considerations, an imprecision of <0.25 TE<sub>a</sub> is acceptable for automated hematology analyzers.<sup>25,48</sup> If total imprecision exceeds this value, low bias values are necessary to achieve quality goals such as TE<sub>a</sub>.<sup>1,31</sup> Applying the previous recommendation, NEU for feline specimens and NEU, MON, and EOS in canine specimens were considered acceptable for the scil POCA. A comparatively high CV was observed for feline absolute EOS (31%), which was in accordance to results reported previously for the ProCyte Dx (Idexx).<sup>19</sup> Low cell counts, similar to the finding in our study (mean EOS: 0.12×10<sup>9</sup>/L) have been considered the most probable reason for imprecision of WBC differential counts. Highly variable intra-assay precision had been demonstrated even for frequent cell



**Figure 4.** Correlation and agreement of hematocrit (HCT) and platelet (PLT) obtained from the scil point-of-care and Advia 2120 analyzers for 159 feline samples. **A, B.** Passing–Bablok regression analysis: gray solid line is the identity line ( $x=y$ ); solid black line is the regression line. **C, D.** Bland–Altman plots of the absolute differences against the averages of results obtained by both analyzers. Mean absolute differences (biases, solid black lines) between  $\pm 1.96SD$  (dashed black lines) are shown.

populations comparing various in-house and bench-top hematology analyzers.<sup>2,5,13,14,19,44,47</sup>

A limitation of our study is that measurements with both the scil POCA and the reference methods were not performed at the same time after sampling, although always in the same order (first the Advia 2120, then the scil POCA), which might have contributed to an inherent increased systematic error. Although the time between measurements with the different methods was kept as short as possible (<6 h), the times between analyses performed with the analyzers were variable. Storage-related effects on measurands must be considered as a function of storage time and temperature, as well as of analyzer and species under investigation.<sup>3,9,27</sup> Storage-induced increase in HCT and MCV and a decrease of MCHC, attributed to RBC swelling, are changes that are commonly observed in feline and canine specimens and which are generally more pronounced when samples are stored at room temperature.<sup>3,9,17,46</sup> Increases in HCT and MCV were found to be significant as

early as 6 h (dogs) and 12 h (cat) of sampling, and changes were more distinctive in dogs compared with the other species,<sup>3</sup> supporting the differences in extent of bias between feline and canine specimens in our study. In previous studies, storage-induced changes in MCV, HCT, and MCHC of 12–15% were demonstrated for samples from healthy and diseased dogs within 24 h of storage at room temperature compared to baseline values.<sup>9</sup> However, depending on the analyzer used, relatively small (less than  $\pm 5\%$ ) changes may also be seen within the first 48 h of storage at room temperature.<sup>27</sup> In cats, differences can reach 15% for MVC and 17% for HCT within 24 h of storage for unrefrigerated samples.<sup>41</sup> With respect to canine samples, a major effect of storage-induced swelling of erythrocytes is unlikely in our study because it would have resulted in a negative rather than a positive bias between measurements obtained by both analyzers.

Storage-related changes of PLT, attributable most probably to formation and deformation of aggregates or

**Table 3.** Observed total error of the complete blood count obtained from the scil point-of-care analyzer for canine and feline specimens in 3 concentrations compared to total allowable error (TEa) and CLIA<sup>29</sup>/RiliBÄK<sup>39</sup> quality specifications.

Variable	Unit	Dogs				Cats				TEa %		
		TEo %				TEo %						
		Low	Normal	High	QGI*	Low	Normal	High	QGI*	ASVCP	CLIA <sup>31</sup>	Rili-BÄK <sup>40</sup>
WBC	×10 <sup>9</sup> /L	11.9	8.6	10.9	5.7	13.1	11.1	10.4	5.2	20**	15	6.5
RBC	×10 <sup>12</sup> /L	5.0	3.5	3.4	1.7	<b>14.9</b>	7.0	8.7	7.9	10	6	4
HGB	mmol/L	<b>21.7</b>	<b>21.1</b>	<b>23.2</b>	27.0	<b>25.3</b>	<b>21.2</b>	<b>22.1</b>	22.7	10	7	4
HGB <sub>corr</sub>	mmol/L	2.8	2.2	4.3	1.0	8.1	3.8	4.9	3.0	10	7	4
HCT	L/L	<b>13.6</b>	9.4	9.9	1.5	<b>11.7</b>	5.7	4.9	1.9	10	6	5
PCV	L/L	<b>19.2</b>	<b>15.0</b>	<b>15.5</b>	12.2	<b>13.8</b>	7.8	7.0	3.4	10	6	5
MCV	fL	NA	<b>7.5</b>	NA	6.9	NA	5.1	NA	3.0	7	NA	NA
MCHC	mmol/L	NA	<b>14.1</b>	NA	13.9	NA	<b>25.3</b>	NA	13.4	10	NA	NA
PLT	×10 <sup>9</sup> /L	24.7	13.1	12.1	0.7	<b>38.5</b>	24.1	17.1	1.0	25**	25	7.5/8.5/13.5

Bold values indicate results of TEo not fulfilling the ASVCP quality requirements (TEo < TEa). CLIA=Clinical Laboratory Improvement Amendments; HCT=hematocrit; HGB=hemoglobin; HGB<sub>corr</sub>=HGB was corrected using slope and intercept of the regression line for the cyanide-based reference method<sup>4</sup>; MCHC=mean corpuscular hemoglobin concentration; MCV=mean corpuscular volume; NA=not available; PCV=spun hematocrit; PLT=platelet; QGI=Quality goal index <0.8 indicates a predominant impact of imprecision and QGI >1.2 indicates a predominant impact of bias; RBC=red blood cell; RiliBÄK=guidelines (Richtlinie "Rili") of the German Federal Medical Council (Bundesärztekammer "BÄK"); WBC=white blood cell.

\* For normal concentration levels.

\*\* Values for in-clinic laboratory.

**Table 4.** Linearity and measuring range (MR) of the scil point-of-care analyzer for canine and feline specimens (linear regression analysis).

Variable	Unit	Dogs				Cats				
		MR	R <sup>2</sup>	I	S	MR	R <sup>2</sup>	I	S	
RBC	×10 <sup>12</sup> /L	0–13.5	>0.99	−0.02	1.00	0–17.04	>0.99	−0.37	1.01	
	×10 <sup>12</sup> /L	0–18*	NA	NA	NA	0–18*	0.99	0.10	1.01	
HGB	mmol/L	0–18.2	>0.99	0.31	1.00	0–17.8	0.99	0.11	1.00	
WBC	×10 <sup>9</sup> /L	0–118.9	>0.99	1.33	1.01	0–62.9	>0.99	2.43	1.03	
PLT	×10 <sup>9</sup> /L	0–911	>0.99	11.00	0.98	0–846	0.93	−18.01	1.00	
	×10 <sup>9</sup> /L	0–1,000*	>0.99	12.10	0.98	0–1,000*	0.99	21.00	0.97	

HGB=hemoglobin; I=intercept; MR=measuring range; NA=not available; PLT=platelet; RBC=red blood cell; R<sup>2</sup>=coefficient of correlation; S=slope; WBC=white blood cell.

\* For values of the 100% pool exceeding the reportable range of the scil POCA (RBC: >18 × 10<sup>12</sup>/L; PLT: >1,000 × 10<sup>9</sup>/L), censoring was performed. For RBC and PLT, a value of 20 × 10<sup>12</sup>/L and 1,100 × 10<sup>9</sup>/L was used, respectively.

occurrence of PLT ghosts, as demonstrated in previous studies, must also be taken into consideration.<sup>3,10,27,28</sup> These changes can be significant after 3 h of storage, resulting in an increase or decrease in PLT that is highly dependent on the storage time and species studied but not generally on analyzer technology.<sup>3,9,28</sup> However, the quality goals for PLT were still fulfilled here, thus major impacts as a result of storage are less likely. Regarding the results of PLT, especially for feline samples, exclusion of specimens with marked PLT aggregation could have resulted in lower TEo but was not performed in our study. Other strategies to improve the reliability of feline PLT have been investigated by several authors, including the use of optical methods to detect large platelets or the use of different anticoagulants to minimize aggregation, which could be addressed in future studies.<sup>20,34,40,45</sup>

Another limitation is that we did not exclude outliers from statistical analysis even if they were rare. We detected isolated deviations associated with hemolysis, icterus, and agglutination in patients with immune-mediated disease in canine specimens, mainly affecting RBC and dependent parameters. Artefactual changes are usually dependent on the concentration of the interfering substance in the sample. Although mild hemolysis is unlikely to cause a clinically significant effect on parameters of the CBC, marked hemolysis could have led to decreased RBC and HCT, and can be associated with falsely increased MCHC.<sup>1,18</sup> Lipemia, as seen in non-fasting patients, may further enhance hemolysis in vitro and influence analyzer systems based on photometric and light transmission.<sup>18</sup> Even if samples with macroscopic clots were excluded, RBC microagglutination could also have contributed to a decrease in RBC and HCT

**Table 5.** Results of 20-run intra-assay repeatability of the scil point-of-care analyzer for canine and feline samples in 3 concentrations (low/normal/high).

Variable	Unit	Dogs						Cats					
		Low		Normal		High		Low		Normal		High	
		Mean±SD	CV %	Mean±SD	CV %	Mean±SD	CV %	Mean±SD	CV %	Mean±SD	CV %	Mean±SD	CV %
WBC	×10 <sup>9</sup> /L	1.8±0.05	2.77	9.94±0.11	1.12	41.81±0.93	2.23	1.58±0.04	2.53	10.34±0.16	1.54	46.93±0.56	1.20
NEU	×10 <sup>9</sup> /L	NA	NA	7.65±0.19	2.43	NA	NA	NA	NA	9.17±0.17	1.90	NA	NA
LYM	×10 <sup>9</sup> /L	NA	NA	0.85±0.06	<b>6.98</b>	NA	NA	NA	NA	0.91±0.11	<b>12.48</b>	NA	NA
MON	×10 <sup>9</sup> /L	NA	NA	1.14±0.12	10.53	NA	NA	NA	NA	0.11±0.02	<b>20.76</b>	NA	NA
EOS	×10 <sup>9</sup> /L	NA	NA	0.2±0.02	11.18	NA	NA	NA	NA	0.12±0.04	<b>31.05</b>	NA	NA
RBC	×10 <sup>12</sup> /L	1.9±0.03	1.72	7.35±0.07	0.95	11.35±0.1	0.92	2.47±0.12	<b>4.66</b>	7.45±0.05	0.71	12.62±0.19	1.52
HGB	mmol/L	2.99±0.03	1.07	10.71±0.08	0.73	15.44±0.28	1.78	2.41±0.07	<b>2.90</b>	6.59±0.05	0.77	9.82±0.13	1.29
HCT	L/L	0.14±0	<b>3.15</b>	0.51±0.01	1.05	0.75±0.01	1.30	0.1±0	<b>4.45</b>	0.33±0	1.45	0.57±0.01	1.04
MCV	fL	NA	NA	69.6±0.58	0.84	NA	NA	NA	NA	44±0.45	1.02	NA	NA
MCH	fmol	NA	NA	1.46±0.01	0.66	NA	NA	NA	NA	0.89±0.01	0.91	NA	NA
MCHC	mmol/L	NA	NA	20.99±0.19	0.88	NA	NA	NA	NA	20.07±0.33	1.64	NA	NA
RDW	%	NA	NA	12.62±0.19	1.50	NA	NA	NA	NA	19.84±0.3	1.50	NA	NA
PLT	×10 <sup>9</sup> /L	31±3	<b>10.6</b>	314±15	4.8	628±27	4.3	48±7	<b>15.2</b>	241±19	<b>8.0</b>	692±31	4.5

Bold values indicate results of CV that do not meet the quality requirements for precision (CV < 0.25 TEa).

EOS=eosinophil; HCT=hematocrit; HGB=hemoglobin; I=intercept; LYM=lymphocyte; MCH=mean corpuscular hemoglobin concentration; MCHC=mean corpuscular hemoglobin concentration;

MCV=mean corpuscular volume; MON=monocyte; NA=not available; NEU=neutrophil; PLT=platelet; RBC=red blood cell; RDW=red blood cell distribution width; S=slope; WBC=white blood cell.

as a sequela of inappropriate sampling by the automatic analyzer.<sup>18</sup> However, in samples with marked agglutination, flagging by the analyzer and blanking of RBC variables prevented reporting of unreliable results. Icterus is considered to have a mild increasing effect on HGB.<sup>21</sup> Given that interfering factors such as hemolysis, icterus, and lipemia are not usually detected in specimens collected for hematologic testing in clinical practice because samples are usually measured immediately after collection and separation of plasma and cells is delayed, the affected specimens were not excluded from our study. However, the proportion of samples with hemolysis, lipemia, and icterus was low, hence a significant impact of potential interferences on results is unlikely.

### Acknowledgments

We thank the medical technologists of the central laboratory, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Germany for helping with the manual methods. The scil vCell5 analyzer used in our study was made available as a free loan by scil Animal Care (Viernheim, Germany).

### Declaration of conflicting interests

The authors have received funding of the study by scil Animal Care (Viernheim, Germany); however, the company was not involved in the interpretation or publication of the results.

### Funding

Our study was financially supported by scil Animal Care (Viernheim, Germany). Reagents and QCM were provided by scil Animal Care and Norma Instruments (Budapest, Hungary).

### ORCID iD

Kim-Lina Charlotte Zelmer  <https://orcid.org/0000-0001-7856-7291>

### References

1. Arnold JE, et al. ASVCP guidelines: principles of quality assurance and standards for veterinary clinical pathology (version 3.0), developed by the American Society for Veterinary Clinical Pathology's (ASVCP) Quality Assurance and Laboratory Standards (QALS) Committee. *Vet Clin Pathol* 2019;48:542–618.
2. Bauer N, et al. Evaluation of the automated hematology analyzer Sysmex XT-2000iV™ compared to the Advia® 2120 for its use in dogs, cats, and horses: part I—precision, linearity, and accuracy of complete blood cell count. *J Vet Diagn Invest* 2011;23:1168–1180.
3. Bauer N, et al. Evaluation of the automated hematology analyzer Sysmex XT-2000iV™ compared to the Advia® 2120 for its use in dogs, cats, and horses. Part II: accuracy of leukocyte differential and reticulocyte count, impact of anticoagulant and sample aging. *J Vet Diagn Invest* 2012;24:74–89.
4. Bauer N, Moritz A. Evaluation of three methods for measurement of hemoglobin and calculated hemoglobin parameters with the Advia 2120 and Advia 120 in dogs, cats, and horses. *Vet Clin Pathol* 2008;37:173–179.
5. Bauer NB, et al. Evaluation of the impedance analyzer PoCH-100iV Diff for analysis of canine and feline blood. *Vet Clin Pathol* 2012;41:194–206.
6. Becker M, et al. Comparative clinical study of canine and feline total blood cell count results with seven in-clinic and two commercial laboratory hematology analyzers. *Vet Clin Pathol* 2008;37:373–384.



7. Boisvert AM, et al. Artfactual effects of hypernatremia and hyponatremia on red cell analytes measured by the Bayer H\*1 analyzer. *Vet Clin Pathol* 1999;28:91–96.
8. Bollinger PB, et al. The Technicon H\*1—an automated hematology analyzer for today and tomorrow. Complete blood count parameters. *Am J Clin Pathol* 1987;87:71–78.
9. Bourgès-Abella NH, et al. Changes in hematology measurements in healthy and diseased dog blood stored at room temperature for 24 and 48 hours using the XT-2000iV analyzer. *Vet Clin Pathol* 2014;43:24–35.
10. Bourgès-Abella NH, et al. Reference intervals, intraindividual and interindividual variability, and reference change values for hematologic variables in laboratory beagles. *J Am Assoc Lab Anim Sci* 2015;54:17–24.
11. Briggs C, et al. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. *Int J Lab Hematol* 2014;36:613–627.
12. Bull BS, et al. International Council for Standardization in Haematology (ICSH) recommendations for “surrogate reference” method for the packed cell volume. *Lab Hematol* 2003;9:1–9.
13. Cook AM, et al. Quality requirements for veterinary hematology analyzers in small animals—a survey about veterinary experts’ requirements and objective evaluation of analyzer performance based on a meta-analysis of method validation studies: bench top hematology analyzer. *Vet Clin Pathol* 2016;45:466–476.
14. Cook AM, et al. Objective evaluation of analyzer performance based on a retrospective meta-analysis of instrument validation studies: point-of-care hematology analyzers. *Vet Clin Pathol* 2017;46:248–261.
15. Flatland B, et al. Differentiating between analytical and diagnostic performance evaluation with a focus on the method comparison study and identification of bias. *Vet Clin Pathol* 2014;43:475–486.
16. Fraser CG, Petersen PH. The importance of imprecision. *Ann Clin Biochem* 1991;28:207–211.
17. Furlanello T, et al. Artfactual changes in canine blood following storage, detected using the Advia 120 hematology analyzer. *Vet Clin Pathol* 2006;35:42–46.
18. Gilor S, Gilor C. Common laboratory artifacts caused by inappropriate sample collection and transport: how to get the most out of a sample. *Top Companion Anim Med* 2011;26:109–118.
19. Goldmann F, et al. Evaluation of the IDEXX ProCyte Dx analyzer for dogs and cats compared to the Siemens ADVIA 2120 and manual differential. *Comp Clin Pathol* 2014;23:283–296.
20. Granat F, et al. Comparison of different anticoagulant associations on haemostasis and biochemical analyses in feline blood specimens. *J Feline Med Surg* 2017;19:394–402.
21. Grebert M, et al. Validation of the Sysmex XN-V hematology analyzer for canine specimens. *Vet Clin Pathol* 2021;50:184–197.
22. Harris N, et al. Performance evaluation of the ADVIA 2120 hematology analyzer: an international multicenter clinical trial. *Lab Hematol* 2005;11:62–70.
23. Harris N, et al. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. *Lab Hematol* 2005;11:47–61.
24. Hyltoft Petersen P, et al. Proposed guidelines for the internal quality control of analytical results in the medical laboratory. *Eur J Clin Chem Clin Biochem* 1996;34:983–999.
25. Jensen AL, Kjelgaard-Hansen M. Method comparison in the clinical laboratory. *Vet Clin Pathol* 2006;35:276–286.
26. Kjelgaard-Hansen M, Jensen AL. Is the inherent imprecision of manual leukocyte differential counts acceptable for quantitative purposes? *Vet Clin Pathol* 2006;35:268–270.
27. Lee J-M, Kang JS. Changes of hematological references depends on storage period and temperature conditions in rats and dogs. *Lab Anim Res* 2016;32:241–248.
28. Mathers RA, et al. Platelet measurements in rat, dog and mouse blood samples using the Sysmex XT-2000iV. *Comp Clin Pathol* 2013;22:815–821.
29. Medicare, Medicaid and CLIA programs; regulations implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA)—HCFA. Final rule with comment period. *Fed Regist* 1992;57:7002–7186.
30. Moritz A. Der Einsatz lasergestützter Multiparameter-Hämatologiesysteme in der Veterinärmedizin [Use of laser-based multiparameter hematology systems in veterinary medicine]. Justus-Liebig-Universität Giessen, 2000. German.
31. Nabity MB, et al. ASVCP guidelines: allowable total error hematology. *Vet Clin Pathol* 2018;47:9–21.
32. Norma Instruments. Operator’s manual, iVet5. 2017.
33. Norma Instruments. Service manual, Norma iVet5: automated 5-part hematology analyzer for veterinary use. Version 1.3, 2019.
34. Norman EJ, et al. Evaluation of a citrate-based anticoagulant with platelet inhibitory activity for feline blood cell counts. *Vet Clin Pathol* 2001;30:124–132.
35. Norman EJ, et al. Prevalence of low automated platelet counts in cats: comparison with prevalence of thrombocytopenia based on blood smear estimation. *Vet Clin Pathol* 2001;30:137–140.
36. Papsouliotis K, et al. Analysis of feline, canine and equine hemograms using the QBC VetAutoread. *Vet Clin Pathol* 1999;28:109–115.
37. Papsouliotis K, et al. Comparison of white blood cell differential percentages determined by the in-house LaserCyte hematology analyzer and a manual method. *Vet Clin Pathol* 2006;35:295–302.
38. Parry DM. Quality Goal Index. [cited 2021 Apr 16]. <https://www.westgard.com/guest34.htm#QGI>
39. Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (Rili-BÄK) [Guideline of the German Medical Association for quality assurance laboratory medical examinations]. Bundesärztekammer, Dec 2019. German. <https://www.bundesaerztekammer.de/aerzte/qualitaetssicherung/richtlinien-leitlinien-empfehlungen-stellungnahmen/richtlinien-leitlinien-empfehlungen-zur-qualitaetssicherung/labor/>
40. Riond B, et al. Effective prevention of pseudothrombocytopenia in feline blood samples with the prostaglandin I2 analogue Iloprost. *BMC Vet Res* 2015;11:183.
41. Rishniw M, Pion PD. Evaluation of performance of veterinary in-clinic hematology analyzers. *Vet Clin Pathol* 2016;45:604–614.
42. Rümke CL. Laboratory aids. Variability of results in differential cell counts on blood smears. *Triangle* 1960;4:154–158.
43. scil Animal Care. User manual, scil vCell 5. Version 20180220, 2018.
44. Thongsahuan S, et al. Precision and accuracy of the Mindray BC-5000Vet hematology analyzer for canine and feline blood. *Vet Clin Pathol* 2020;49:207–216.

45. Tvedten H, Johansson P. Feline platelet counting with prostaglandin E1 on the Sysmex XT-2000iV. *Vet Clin Pathol* 2010;39:190–192.
46. Weissenbacher S, et al. Evaluation of a novel haematology analyser for use with feline blood. *Vet J* 2011;187:381–387.
47. Wenger-Riggenbach B, et al. Evaluation of the LaserCyte: an in-house hematology analyzer for dogs and cats. *Comp Clin Pathol* 2006;15:117–129.
48. Westgard JO. The replication experiment. [cited 2021 Apr 16]. <https://www.westgard.com/lesson22.htm>
49. Westgard JO. *Basic Method Validation and Verification, Training in Analytical Quality Management for Healthcare Laboratories*. 4th ed. Westgard QC, 2020.
50. Zelmanovic D, Hetherington EJ. Automated analysis of feline platelets in whole blood, including platelet count, mean platelet volume, and activation state. *Vet Clin Pathol* 1998;27:2–9.