Veterinary Clinical Pathology ISSN 0275-6382

ORIGINAL RESEARCH

Alterations due to dilution and anticoagulant effects in hematologic analysis of rodent blood samples on the Sysmex XT-2000iV

Kaitlin A. Moorhead, Marielle L. Discipulo, Jing Hu, Roberta C. Moorhead, Jennifer L. Johns

Department of Comparative Medicine, Stanford School of Medicine, Stanford, CA, USA

Key Words
Clinical pathology, laboratory animals, methodological study, microsampling

Correspondence
J. L. Johns, 300 Pasteur Dr., Department of Comparative Medicine, Edwards Bldg. R321, Stanford, CA 94305, USA
E-mail: jljohns@stanford.edu

DOI:10.1111/vcp.12338

Background: Clinical pathology of rodents is hindered by sample volume limitations. A single whole heparinized blood sample is often submitted for hematologic and clinical chemistry analysis in exploratory research settings, and sample dilution may be required. Published information on the potential impact of sample dilution and heparin use on hematology variables in rodents is sparse.
Objectives: The purpose of the study was to evaluate the effects of sample dilution and of anticoagulant on hematologic analysis of mouse and rat blood samples on the Sysmex XT-2000iV.
Methods: Mice and rats were obtained from various ends of study research projects, and whole blood was collected via terminal cardiocentesis in lithium heparin, and additionally in EDTA when paired samples were obtained from rats. Hematology analytes were measured on the Sysmex XT-2000iV straight and diluted from ×2 to ×5.
Results: Significant differences between heparinized samples analyzed straight vs diluted were found for MCV and MCHC, with a bias for several additional variables. Significant differences between paired heparinized and EDTA-anticoagulated samples at each dilution point were found for most variables, with the largest differences found in platelet count. Evidence of platelet clumping presumably due to heparin exposure was noted in numerous samples.
Conclusions: Dilution-induced changes occur in some hematologic variables and may render dilution unacceptable in the exploratory research environment. Many variables, most notably platelet count, differ based on the anticoagulant used, and values from heparinized vs EDTA-anticoagulated samples should not be directly compared.

Introduction

Clinical pathology of rodents and other small animals is frequently hindered by sample volume limitations. In accordance with the Reduce–Refine–Replace (3 R's) philosophy[1,2], utilization of blood samples obtained from laboratory animals is optimized to limit the necessary number of animals, and nonterminal ("survival" or "interim") blood sampling is frequently performed. In the exploratory research environment, scientists often submit a single low-volume (< 200 μL) heparinized whole blood sample for both hematologic and clinical chemistry analysis. The submission of a single heparinized sample allows the diagnostic laboratory to maximize sample use for both types of analysis, but volume limitations may still necessitate dilution of samples. Published information on the potential impact of sample dilution on clinical pathology variables and analytes in rodents is sparse. Additionally, in general EDTA is preferred over heparin as the anticoagulant for mammalian hematology samples. Published data on the specific effects of heparin on hematologic analysis of rodent blood samples are inadequate for an assessment of the acceptability of heparinized samples for measurement of certain variables. In this prospective study, we evaluated the effects of

sample dilution and of anticoagulant type on hematologic variables in mouse and rat blood samples analyzed with the Sysmex XT-2000iV, and assessed the potential utility of resulting data in the exploratory research setting. In addition, platelet counts measured by impedance were compared to the optical method counts.

Materials and Methods

Sample selection and handling

Mice and rats were obtained from various ends of study research projects at the Department of Comparative Medicine, Stanford School of Medicine, Stanford, CA. A total of 19 adult (> 12 weeks of age) mice and 38 adult (> 12 weeks of age) rats, all scheduled for euthanasia in our facility within a defined period between October and December of 2014, were obtained from the Stanford University animal facility and transferred to an animal use teaching protocol approved by the Stanford University Institutional Animal Care and Use Committee, and overseen by the Veterinary Service Center. Genetic background and medical history of all mice and rats were unknown, and male and female animals were equally represented. The Stanford vivaria are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Husbandry is performed in accordance with the Guide for the Care and Use of Laboratory Animals and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. All subjects appeared healthy on initial examination. Mice and rats were euthanized by CO_2 asphyxiation following the 2013 American Veterinary Medicine Association (AVMA) Guidelines on Euthanasia. All blood samples were collected by cardiocentesis following euthanasia.

For the evaluation of dilution effects on hematology variables in heparinized blood, whole blood was collected from 19 mice and from 17 rats with a heparinized syringe into lithium heparin microtainers (BD Biosciences, Franklin Lakes, NJ, USA), by removing the needle prior to filling each tube to a maximum volume of 500 μL. For the additional evaluation of anticoagulant effect on hematology variables, whole blood from 21 rats was collected with an empty syringe and a 20 GA needle into both dipotassium EDTA and lithium heparin microtainers (BD Biosciences), alternating the filling of each type of microtainer, and removing the needle prior to filling each tube to a maximum volume of 500 μL. All blood samples were mixed by immediately inverting the microtainer 10 times with gentle flicking of the tube. In the laboratory, blood was remixed by inverting the microtainer 5 times, followed by pipetting up and down 10 times. Samples were assessed for gross clotting, and any sample with detectable clots was discarded.

CellPack diluent for the Sysmex XT-2000iV automated hematology analyzer (Sysmex Inc., Kobe, Japan) was used as the diluent. Dilutions were made from well-mixed blood in the following manner: "×2" dilution (1:1 ratio of blood to diluent); "×3" dilution (1:2 ratio of blood to diluent); "×4" dilution (1:3 ratio of blood to diluent); and "×5" dilution (1:4 ratio of blood to diluent). All samples were mixed by pipetting up and down 10 times just prior to analysis.

Hematologic analysis

A CBC from all samples was evaluated on the Sysmex XT-2000iV automated hematology analyzer. Each sample was run under the corresponding species profile (rat or mouse) on manual mode. Values were recorded for WBC and RBC count, HGB concentration, HCT, MCV, MCH, MCHC, and platelet count (PLT). The reported PLT is based on the impedance method. WBC, RBC, HGB, HCT, and PLT values were multiplied by the corresponding dilution factor for the final result. Any error messages or flags such as abnormal WBC distribution were noted.

Diluent analysis

Electrolyte concentrations in the CellPack diluent for the Sysmex XT-2000*i*V were analyzed on the Siemens Dimension Xpand Plus chemistry analyzer (Siemens Healthcare, Erlangen, Germany) to assess the difference between osmolarity of the diluent and typical osmolarity of rodent plasma.

Statistical analysis

Data were initially exported into Microsoft Excel 2013 (Microsoft Office Professional Plus; Microsoft Corp., Redmond, WA, USA). Subsequent analysis was performed in GraphPad Prism 6 for Windows (GraphPad Software Inc., La Jolla, CA, USA). Descriptive statistics (mean and SD) were performed for all datasets, and normality was evaluated via the D'Agostino and Pearson omnibus normality test. All data from samples flagged by relevant errors were removed. Outlier data were identified in datasets with Gaussian distribution by the statistical software utilizing the ROUT method with the maximum false discovery rate Q set at 1%. All data identified as outliers were individually evaluated

in combination with any instrument flags prior to removal from datasets. Data from the heparinized sample dilution study were analyzed via one-way ANOVA with Dunnett's multiple comparisons test for differences between data from samples run straight vs each dilution, and by normalized data plots and difference (bias) plots.[3] Data from the anticoagulant dilution study were evaluated by mean and SD calculation for samples collected in each anticoagulant and at each dilution level. Data were also analyzed via a 2-way ANOVA for samples processed at each dilution level (straight through ×5) with anticoagulant as the column factor. Platelet count measured via impedance vs optical method were compared on a difference (bias) plot with Bland–Altman method comparison analysis performed.[3]

Table 1. One-way ANOVA overall *P*-value, and mean differences between values for heparinized whole blood samples from 19 mice and 38 rats. Differences between each value from a sample run straight and values from the same sample run at each dilution point (×2 to ×5) were averaged to generate the mean difference values. Values in bold are significant ($P < .05$).

		Dilution Factor			
Variable	*P*-value	×2	×3	×4	×5
RBC count	.182	−0.199	−0.318	−0.480	−0.529
HB	.946	−0.06	−0.22	−0.19	−0.03
HCT	.963	0.75	0.53	0.33	0.14
WBC count	.975	0.095	0.297	0.224	0.455
PLT count	.558	11.5	46.7	86.8	117.3
MCV	**.006**	2.48	**3.36**	**3.75**	**3.88**
MCH	.397	0.20	0.44	0.52	0.84
MCHC	**.001**	**−1.18**	**−1.21**	**−1.07**	−0.68

Results

Dilution of heparinized samples and diluent analysis

There were no significant differences between the mean differences of straight and diluted heparinized blood from rats and mice, except for MCV ($P = .006$) and MCHC ($P < .001$) (Table 1). Normalized data plots were constructed to reveal overall trends in dilution-induced changes in all samples (Figure 1). Bias plots were constructed to further evaluate dilution effects for each variable at each dilution point, and to visualize potential differences based on species (mouse vs rat samples) (Figure 2). The bias plots revealed increased variability with increasing dilution in WBC, HCT, and HGB; variability that is most marked at lower concentrations in PLT; a mild constant negative bias at all dilution factors in RBC; a constant positive bias for all dilution factors in MCV; and a constant negative bias for all dilution factors with a proportionate positive bias occurring at higher dilution factors in MCHC. Patterns consistent with species-specific differences between mice and rats were noted in MCV and MCH.[4]

Comparison of dilutions in heparinized vs EDTA-anticoagulated samples

Mean and SD were calculated for values from heparinized and EDTA-anticoagulated samples from the same blood draws at each dilution level (straight through ×5) to evaluate the effect of anticoagulant on mean values and the effect of manual dilution on SD (Table 2). Comparisons were made between the values using 2-way ANOVA. Significant differences due to anticoagulant effects were found in most variables evaluated (Table 3). Platelet count means differed substantially by anticoagulant, with a marked difference in mean PLT between heparinized and EDTA-anticoagulated samples found at each dilution level (Figure 3). Mean PLT in heparinized samples averaged between approximately 58–73% of the mean PLT in EDTA-anticoagulated samples. Standard deviation values were generally similar between samples run straight and samples run at each dilution point. Platelet scatterplots and RBC and platelet volume histograms were evaluated and abnormal plots were noted (Figure 4). The platelet scatterplots and volume histograms were consistent with platelet clumping in some heparinized samples, with no evidence of clumping in the paired EDTA-anticoagulated samples.

To further evaluate the effect of anticoagulant on automated platelet counting, paired optical (PLT-O) and impedance-based (PLT-I) platelet counts were reviewed for the 21 rat whole blood samples for which paired heparinized and EDTA-anticoagulated samples were collected. A bias plot was constructed from the combined data of all samples at all dilution points (straight through ×5) (Figure 5). There was a constant negative bias (PLT-I–PLT-O) of approximately −36.4, but no substantial proportionate bias was noted in either the bias plot or the slopes of the line equations (Figure 5).

The CellPack diluent electrolyte concentrations via automated analysis were: sodium 121 mmol/L, potassium 15.5 mmol/L, and chloride 112 mmol/L, with a total osmolarity of 248.5 mOsmol/L. This value is substantially lower than the mean plasma osmolality often reported for healthy mice and rats. For example, in healthy male CD1 mice, mean plasma osmolality was reported as 327 mOsm/kg[5], and in healthy male

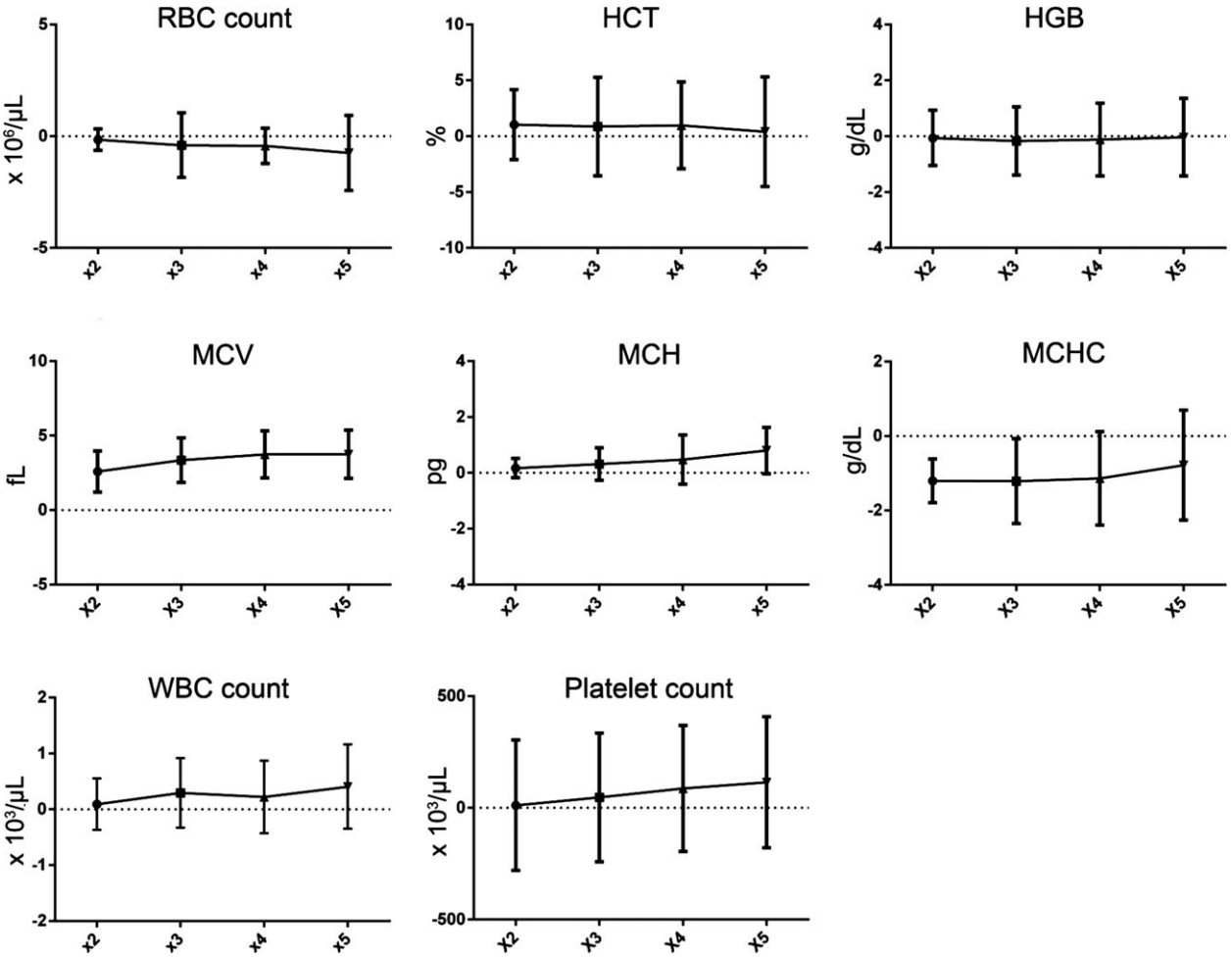

Figure 1. Mean difference and SD (normalized) between hematology variables in heparinized blood samples from 19 mice and 38 rats run straight dotted line) and at dilutions ×2, ×3, ×4, and ×5 (solid line).

Sprague–Dawley rats, mean plasma osmolality was reported as 294 mOsm/kg.[6]

Discussion

The findings from our dilution study indicate that some hematologic variables in rodent blood samples were significantly altered including proportionate bias in MCV and MCHC throughout all dilution factors. The Sysmex XT-2000iV directly measures MCV and HCT. Hematocrit is measured as the sum of RBC size measurements, and reported in proportion to the total sample volume.[7] The observed bias could be due to a difference in osmolarity between the CellPack diluent used to prepare sample dilutions and reported normal mouse and rat plasma osmolality, resulting in diluent-induced RBC swelling and consequently increased MCV.[5,6] Mean corpuscular hemoglobin concentration is calculated from HGB divided by HCT; these latter variables were not significantly altered by dilution but the trend for an increased HCT with sample dilution presumably resulted in the dilution-induced negative bias in MCHC. Increasing dilution factor resulted also in increasing variability in several variables including WBC as assessed by bias plot. One explanation for this finding is that any error introduced by manual pipetting of sample and diluent may be magnified at increasing ratios of diluent to blood; however, SD values were not substantially increased with increasing dilution. Marked variability was seen in PLT across dilutions and appeared to be associated with the use of heparin anticoagulant (see later discussion).

In general, dilution of hematology samples is considered inappropriate in diagnostic and regulatory research environments. However, dilution studies uti-

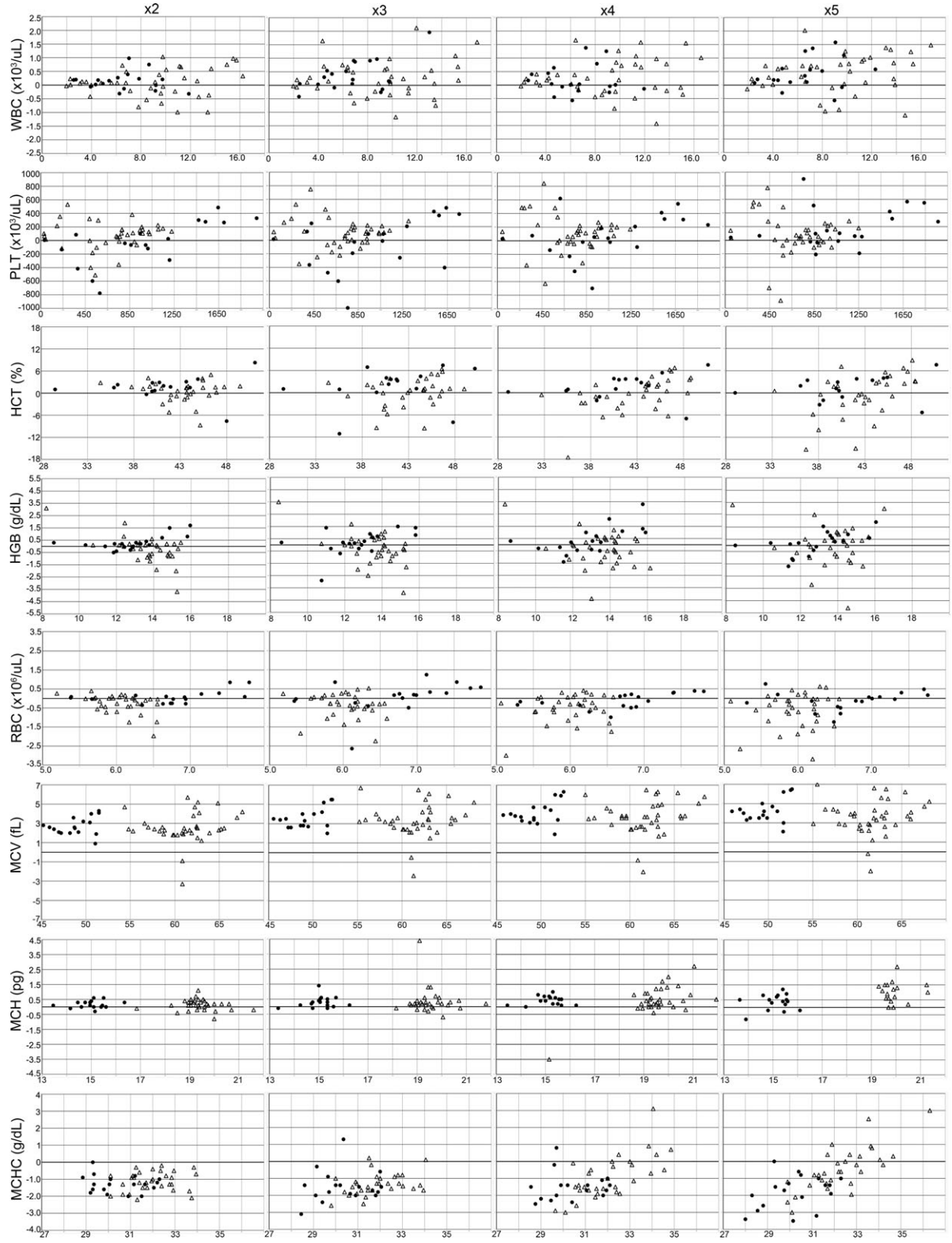

Figure 2. Composite of bias plots for hematology variables at all dilutions (×2, ×3, ×4, and x5; indicated at top of figure). Heparinized whole blood samples from 19 mice (solid circles) and 38 rats (open triangles) are represented. Units are listed on both axes with difference from sample run straight listed on each *y*-axis next to variable and mean listed on each x-axis. The straight horizontal line indicates the mean value for samples run straight.

Table 2. Mean and SD for hematology variables from paired heparinized (HEP) and EDTA-anticoagulated (EDTA) whole blood samples from 21 rats. Samples were run straight (st) and at dilutions ×2, ×3, ×4, and ×5.

	HEPst	EDTAst	HEP×2	EDTA×2	HEP×3	EDTA×3	HEP×4	EDTA×4	HEP×5	EDTA×5
RBC mean	7.398	7.406	6.944	7.149	6.825	7.020	6.691	6.840	6.675	6.594
RBC SD	0.781	0.662	0.483	0.488	0.521	0.592	0.700	0.626	0.596	0.903
HB mean	14.34	14.36	13.58	14.08	13.43	13.94	13.41	13.84	13.68	13.41
HB SD	1.33	1.07	0.75	0.89	0.98	0.95	1.25	1.03	1.21	1.71
HCT mean	43.39	44.49	42.53	45.06	42.27	44.77	41.78	43.76	41.53	42.11
HCT SD	3.29	3.39	2.48	3.27	2.89	3.16	4.26	4.20	4.40	6.398
WBC mean	9.204	9.442	9.134	9.647	9.137	9.746	9.141	9.870	9.300	9.926
WBC SD	3.923	3.891	3.990	3.786	3.804	3.747	3.836	3.970	3.967	4.055
PLT mean	525.8	757.7	598	853.7	643.2	832.6	612	850.5	595.4	857.3
PLT SD	286	361.2	276.8	368	254.4	347.9	284.9	368.7	287.4	358.8
MCV mean	59.35	60.41	61.39	62.97	62.01	63.25	62.45	63.81	63.82	62.22
MCV SD	3.06	3.42	3.10	3.34	2.94	3.78	3.09	3.15	3.16	2.95
MCH mean	19.39	19.41	19.56	19.69	19.60	19.78	20.12	20.06	20.45	20.27
MCH SD	0.60	0.60	0.59	0.68	0.62	0.58	0.89	0.86	0.68	0.82
MCHC mean	32.94	32.27	31.88	31.22	31.62	31.00	32.23	31.28	32.83	31.76
MCHC SD	0.94	1.14	0.98	1.26	1.14	1.41	1.86	1.54	1.81	1.53
PLT-O mean	567.4	764.6	665.5	860.8	708.5	855	681.2	858.8	658.1	882.3
PLT-O SD	286.5	359.7	293.3	362.4	266.6	345.8	294.9	360.5	295.8	364

PLT indicates reported impedance-based PLT count; PLT-O, optical platelet count.

Table 3. Two-way ANOVA *P*-values for comparison of hematology variables between paired data from heparinized samples and EDTA-anticoagulated whole blood samples from 21 rats diluted ×2 to ×5. Values in bold are significant ($P < .05$).

	Dilution Factor				
Analyte	Straight	×2	×3	×4	×5
RBC count	0.912	**0.022**	0.058	0.322	0.620
HGB	0.895	**0.011**	**0.036**	**0.049**	0.426
HCT	**0.023**	**0.000**	**0.001**	**0.036**	0.585
WBC count	**<0.0001**	**0.049**	**0.013**	**0.005**	**0.044**
PLT count	**0.0022**	**< 0.0001**	**0.000**	**0.001**	**0.001**
MCV	**0.009**	**< 0.0001**	0.051	**< 0.0001**	**< 0.0001**
MCH	0.642	0.204	0.259	0.805	0.198
MCHC	**0.000**	**0.001**	**0.035**	**0.010**	**0.000**

lizing human samples have been performed on Sysmex analyzers with acceptable results for some variables, supporting the cautious use of data from diluted samples.[8–10] Specific situations triggering the need for dilution of samples in these studies were volume limitations (eg, pediatric samples) and reduction of RBC aggregation by dilution. In a recent study on hematologic variables in neonatal mice, a dilution level of ×10 was utilized for all samples due to volume limitations; the authors performed a comparison of samples diluted ×5 (the 'capillary mode' dilution level for the Sysmex analyzers, as described below) vs ×10 and found no statistically significant differences caused by dilution.[11] In contrast, one study evaluating dilution of EDTA-anticoagulated hematology samples from rats on the Siemens Advia 120 analyzer found that several variables were unacceptably altered by dilution.[12] The findings of our study suggest that dilution may cause unacceptable changes in rodent hematology variables. However, the acceptability of such changes can depend on the intended use of the data. The exploratory research environment differs substantially from both the clinical laboratory and the regulatory environment, eg, investigators are frequently evaluating only a few variables, rather than the full CBC. Also, the data are generally used in statistical comparisons to matched control groups rather than in comparison to reference intervals. As such, it may be possible to develop limited-use protocols for sample dilution in this environment under strict standard protocols, such as running all samples in a study at the same dilution factor, and exclusively reporting variables with acceptable variability/bias at that dilution factor. In particular, HGB, HCT, and WBC appear less affected by dilution of lower magnitude (×2 and ×3), and results may be considered sufficiently reliable for utility in an exploratory research study.

On the Sysmex XT-2000iV, dilutions can either be made manually prior to analysis or the capillary mode can be used to perform a sample predilution of ×5 (1:4 ratio of sample to diluent). The sample path for the capillary mode is the same as that for the open/manual sampling mode. This predilution mode eliminates the potential for error induced by manually diluting samples and lessens contact time with the diluent, poten-

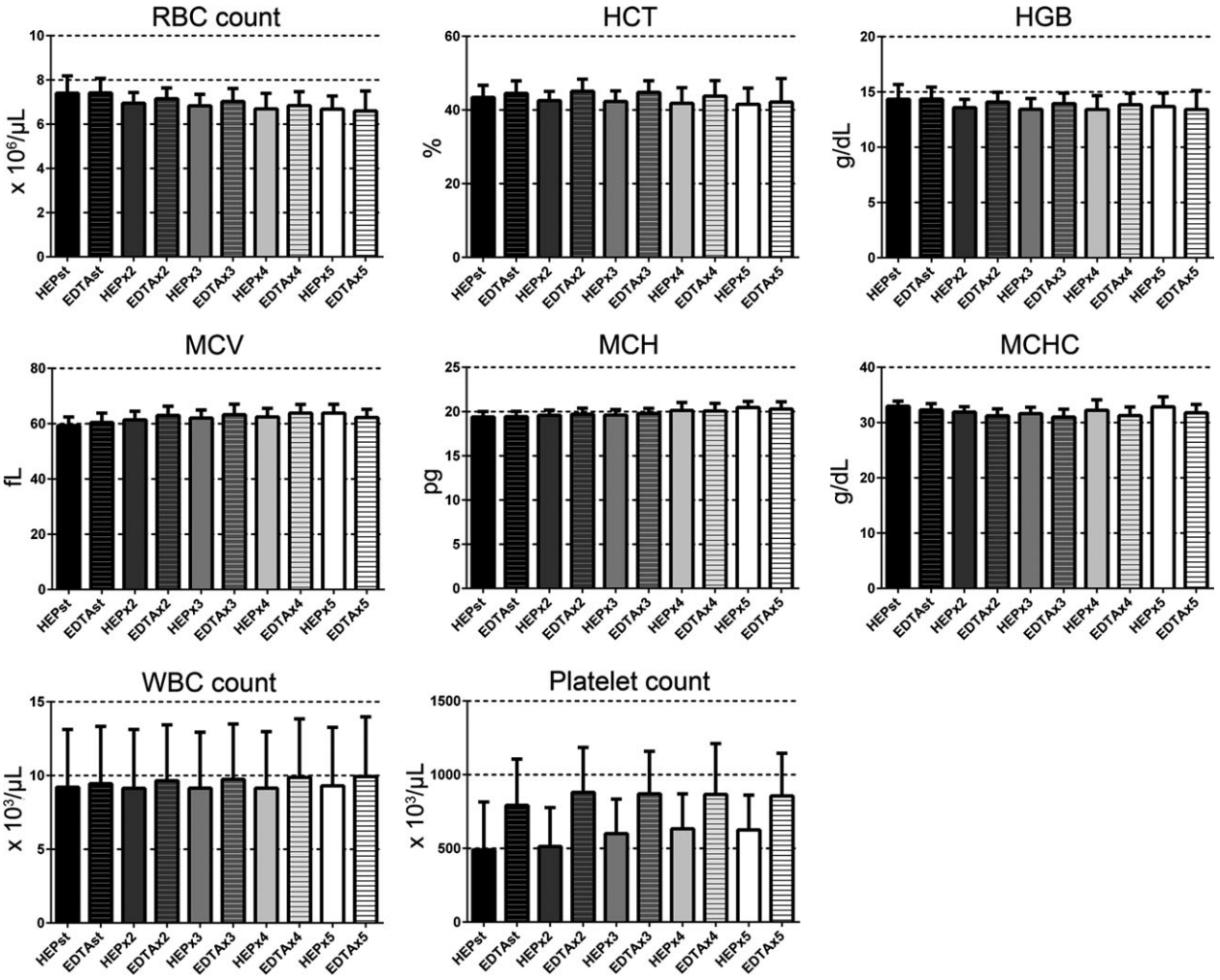

Figure 3. Mean and SD for hematology variables in 21 rat whole blood samples collected in heparin (HEP) or EDTA. Samples were run straight (st) and at dilutions ×2, ×3, ×4, and ×5.

tially reducing diluent-induced changes. However, automated dilution levels other than ×5 are not available and most available analyzer flags are suppressed in this mode. In our study, we chose to perform manual dilutions to allow evaluation throughout all dilution levels up to ×5 and use of open-mode sampling, with the understanding that manual pipetting error may increase imprecision of results. The data shown in Table 2 demonstrate that the variable SD generally remained constant throughout dilution of both heparinized and EDTA-anticoagulated samples, suggesting that manual dilution did not substantially increase the error of the measurements in this study.

Low-volume hematology analyzers can be an alternative solution to the problem of volume-limited rodent blood samples. However, the functionality of such analyzers can be limited in comparison with the Sysmex XT-2000iV and similar analyzers. If a laboratory utilizes a single hematology analyzer for all species and research demands, an analyzer with the maximum possible capability may be needed, with the resulting constraint of the relatively higher volume requirements of such analyzers. Additionally, the volume savings of a low-volume analyzer may be relatively minor, eg, in the range of 30–40 µL per sample. However, laboratories may find it beneficial to purchase a low-volume hematology analyzer if the large majority of their samples are from mice.

Significant differences due to anticoagulant (EDTA vs heparin) were noted in most variables in samples analyzed both straight and diluted, with the most striking difference found in PLT. Direct comparison between data from samples anticoagulated with EDTA vs heparin is therefore not recommended. A

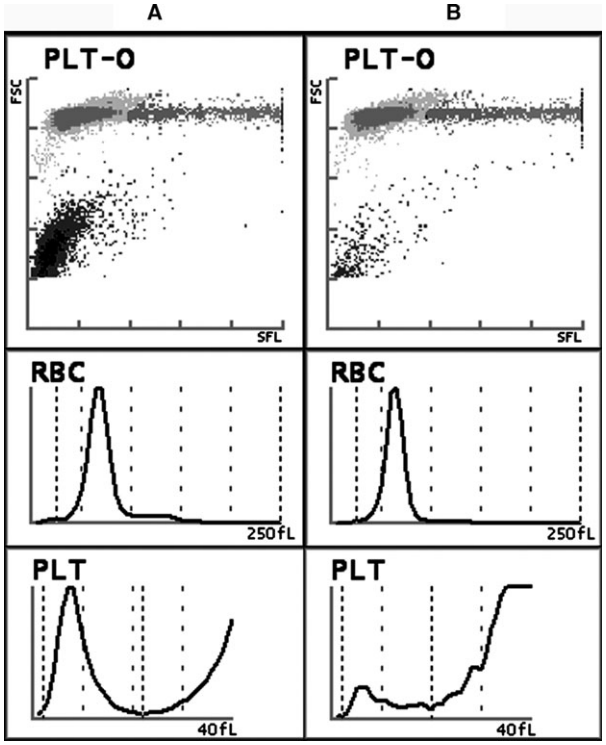

Figure 4. Representative scatterplots for optical-based platelet measurement (PLT-O) (top), RBC volume histograms (center) and platelet volume histograms (bottom) for (**A**) EDTA-anticoagulated whole blood sample, and (**B**) heparinized whole blood sample from a single blood sample from one rat.

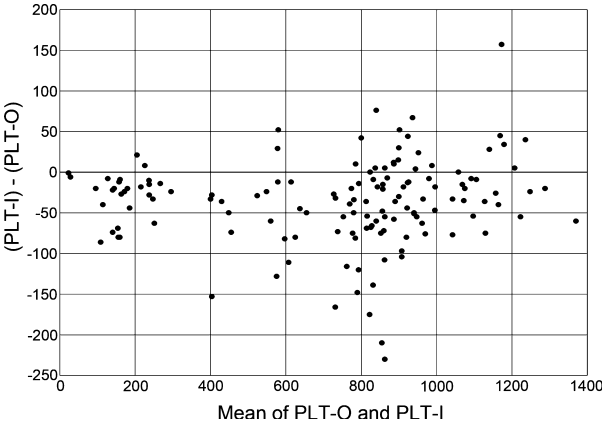

Figure 5. Bias plot for platelet count (expressed as count × $10^3/\mu L$) obtained by optical (PLT-O) or impedance (PLT-I) based methods. Mean of PLT-O and PLT-I is on *x* axis and difference between PLT-I and PLT-O is on *y*-axis. Twenty-one rat whole blood samples collected in both heparin and EDTA were run straight (st) and at dilutions ×2, ×3, ×4, and ×5; data from all dilutions are represented in the plot. There is a constant negative bias (PLT-I–PLT-O) of −36.4 but no substantial proportionate bias.

mild increase in mean PLT in heparinized samples was noted with increasing dilution, although the increases were not statistically significant. One possible explanation is a saline dispersion effect whereby increasing ratios of diluent to blood might cause a partial dispersion of clumped platelets and/or prevent further heparin-induced clumping prior to analysis. Despite this trend, mean PLT in heparinized samples remained substantially lower than mean PLT in matched EDTA-anticoagulated samples from the same animals at all dilution levels. Platelet clumping has often been reported as a problem with blood collection in mice, and evidence for platelet clumping in heparin has been reported in several studies on human blood samples.[9,13–18] However, one study on anticoagulant effects in samples from domestic animals found no alteration in PLT in heparinized samples compared to EDTA- and citrate-anticoagulated samples.[19]

Evaluation of data for evidence of platelet clumping is always advisable, but can vary by instrument.[14] Impedance-based cell counting uses the change in electrical impedance as a cell suspended in electrolyte solution passes an aperture to determine the cell's volume. In the Sysmex XT-2000iV analyzer used in this study, optical-based RBC and platelet counting is based on the combination of forward light scatter and fluorescence with an argon laser following staining of nucleic acids with a polymethine fluorophore. We utilize the platelet volume histogram and optical scatterplots on the Sysmex analyzer to assess for platelet clumping. Pseudothrombocytopenia due to platelet clumping occurred in both the optical- and impedance-based platelet counts, consistent with erroneously low platelet counts due to feline platelet aggregation that can occur with both methods.[20] In samples collected in either anticoagulant, the fluorescence-based optical platelet count had a positive constant bias over the impedance-based platelet count; in contrast, published data on hematologic analysis of EDTA-anticoagulated rat and canine blood samples on the same instrument found a negative bias in this relationship.[21] Studies on human samples describe specific situations, such as room-temperature sample storage, when either the impedance or the optical platelet count is considered more accurate on a similar Sysmex analyzer.[7,22,23] This issue warrants additional study as to the potential mechanism(s) responsible and best approaches for rodent samples.

Although the results of this study do not support use of heparinized rodent blood samples for platelet counting, it may be possible to prevent platelet clumping in heparin by addition of certain substances to the collection tube. Inhibition of platelet aggregation caused by various anticoagulants including

EDTA and heparin has been evaluated, and substances found to be effective at inhibiting platelet aggregation in human and feline samples include aminoglycosides, prostaglandin E1, and iloprost.[15,24–26] Theophylline was considered effective in some but not all studies.[24,27]

The study design was intended to reproduce the typical sample conditions in our laboratory, ie, mixed populations of mice and rats including healthy, ill and experimentally altered animals of various genetic backgrounds and induced mutations. Substantial differences in hematology variables are reported in various mutant mouse strains, and hematologic methods for rodents must take all potential variation into account.[28] Many rodent blood samples are obtained as terminal procedures such that a redraw is not possible in the case of a suboptimal or volume-limited sample, and samples are therefore analyzed to the greatest possible extent despite these limitations. These conditions are somewhat unique to diagnostic laboratories performing rodent clinical pathology in exploratory research settings, and specific data are required to better inform the decisions necessary under these circumstances.

Acknowledgments

The authors thank Elias Godoy and the Animal Diagnostic Lab in the Veterinary Service Center at Stanford University School of Medicine for technical assistance, and the Department of Comparative Medicine at Stanford University School of Medicine for support.

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

References

1. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. London: Methuen; 1959.
2. Kilkenny C, Browne WJ, Cuthi I, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *Vet Clin Pathol*. 2012;41:27–31.
3. Jensen AL, Kjelgaard-Hansen M. Method comparison in the clinical laboratory. *Vet Clin Pathol*. 2006;35:276–286.
4. Ameri M, Schnaars HA, Sibley JR, Honor DJ. Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4 degrees C in EDTA using the ADVIA 120 hematology analyzer. *Vet Clin Pathol*. 2011;40:188–193.
5. Bekkevold CM, Robertson KL, Reinhard MK, Battles AH, Rowland NE. Dehydration parameters and standards for laboratory mice. *J Am Assoc Lab Anim Sci*. 2013;52:233–239.
6. Barney CC, Schanhals EM, Grobe JL, Andresen BT, Traver M. Heat acclimation and thirst in rats. *Physiol Rep*. 2015(epub ahead of print);3.
7. Hill VL, Simpson VZ, Higgins JM, et al. Evaluation of the Performance of the Sysmex XT-2000i Hematology Analyzer With Whole Bloods Stored at Room Temperature. *Lab Med*. 2009;40:709–718.
8. Wang H, Lu L, Zhou Y, et al. Use of RBC-O and S-MCV parameters of SYSMEX XE-2100 in a patient with RBC cold agglutination. *Clin Lab*. 2013;59:217–219.
9. Freise KJ, Schmidt RL, Gingerich EL, Veng-Pedersen P, Widness JA. The effect of anticoagulant, storage temperature and dilution on cord blood hematology parameters over time. *Int J Lab Hematol*. 2009;31:496–504.
10. Boer K, Deufel T. Workflow restrictions on hematology analyzers XE-2100 and XS-800 when assaying pediatric samples with limited volume. *Clin Chem Lab Med*. 2009;47:607–611.
11. White JR, Gong H, Colaizy TT, Moreland JG, Flaherty H, McElroy SJ. Evaluation of hematologic composition in newborn C57/BL6 mice up to day 35. *Vet Clin Pathol*. 2015; (epub ahead of print).
12. Zamfir M, Prefontaine M, Copeman C, Poitout F, Provencher A. Dilution of blood for hematology and biochemistry analysis in rats. *Vet Clin Pathol*. 2014;43:E10.
13. Sakoguchi T, Fujii S, Inuzumi K, et al. [Verification of complete blood cell count (CBC) data from heparinized blood gas samples]. *Rinsho Byori*. 2014;62:139–146.
14. Lombarts AJ, de Kieviet W. Recognition and prevention of pseudothrombocytopenia and concomitant pseudoleukocytosis. *Am J Clin Pathol*. 1988;89:634–639.
15. Zhou X, Wu X, Deng W, Li J, Luo W. Amikacin can be added to blood to reduce the fall in platelet count. *Am J Clin Pathol*. 2011;136:646–652.
16. Schrezenmeier H, Muller H, Gunsilius E, Heimpel H, Seifried E. Anticoagulant-induced pseudothrombocytopenia and pseudoleucocytosis. *Thromb Haemost*. 1995;73:506–513.
17. Kawamoto T, Hiino M, Takubo T, Tatsumi N. Low-molecular-weight heparin as a multipurpose anticoagulant for laboratory testing. *Osaka City Med J*. 2000;46:37–53.
18. Reinhart WH, Haeberli A, Stark J, Straub PW. Influence of blood withdrawal and anticoagulant on clotting

activity, hematologic data, and certain rheologic measurements. *J Lab Clin Med*. 1990;115:98–103.

19. Bauer N, Nakagawa J, Dunker C, Failing K, Moritz A. Evaluation of the automated hematology analyzer Sysmex XT-2000iV compared to the ADVIA (R) 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.
20. Lilliehook I, Tvedten H. Interpretation of Graphical Reports from the Advia 2120, Sysmex XT-2000iV and Cell-Dyn. Paper Presented at: Joint Meeting of the ACVP and ASVCP; November 16–20, 2013; Montreal, Canada.
21. Mathers RA, Evans GO, Bleby J. Platelet measurements in rat, dog and mouse blood samples using the Sysmex XT-2000iV. *Comp Clin Pathol*. 2013;22:815–821.
22. Briggs C, Kunka S, Machin SJ. The most accurate platelet count on the Sysmex XE-2100. Optical or impedance? *Clin Lab Haematol*. 2004;26:157–158.
23. Dadu T, Sehgal K, Shaikh A, Khodaiji S. Comparison of platelet counts by sysmex XE 2100 and LH-750 with the international flow reference method in thrombocytopenic patients. *Indian J Pathol Microbiol*. 2013;56:114–119.
24. Lombarts AJ, Zijlstra JJ, Peters RH, Thomasson CG, Franck PF. Accurate platelet counting in an insidious case of pseudothrombocytopenia. *Clin Chem Lab Med*. 1999;37:1063–1066.
25. Tvedten HW, Backlund K, Lilliehook IE. Reducing error in feline platelet enumeration by addition of Iloprost to blood specimens: comparison to prostaglandin E1 and EDTA. *Vet Clin Pathol*. 2015;44:179–187.
26. Tvedten H, Johansson P. Use of prostaglandin E1 to obtain more accurate platelet counts in cats. *Vet Clin Pathol*. 2011;40:3.
27. Ohnuma O, Shirata Y, Miyazawa K. Use of theophylline in the investigation of pseudothrombocytopenia induced by edetic acid (EDTA-2K). *J Clin Pathol*. 1988;41:915–917.
28. Nagarajan P, Shailendra A, Venkatesan R, Kumar MJM, Majumdar SS, Juyal RC. Sex and strain-related differences in the peripheral blood cell values of mutant mouse strains. *Comp Clin Pathol*. 2012;21:1577–1585.