Effect of pre-warming EDTA blood samples to 37°C on platelet count measured by Sysmex XT-2000iV in dogs, cats and horses

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Short title: Effect of sample warming on platelet count

Abstract

Background: Pseudothrombocytopenia secondary to platelet clumping is a common cause of pre-analytical error for platelet counts in dogs, cats and horses. In humans, it is suggested that pre-warming blood samples to 37°C prior to haematology analysis will reduce platelet clumping.

Objectives: To evaluate the effect of pre-warming EDTA blood samples to 37°C on measured platelet counts and other haematological parameters.

Methods: EDTA blood samples from dogs, cats and horses submitted to the clinical pathology laboratory at the University of Cambridge were included. Complete blood counts (performed using a Sysmex XT-2000iV haematology analyser) were performed on samples at room temperature (approximately 22°C) and following warming of the sample to 37°C in a water bath. The Wilcoxon signed rank test was used to compare haematological parameters, including platelet count, before and after sample warming to 37°C. Data are presented as median [25th, 75th percentile] increase.

Results: Blood samples from 39 dogs, 19 cats and 10 horses were included. Sample warming to 37°C resulted in a statistically significant increase in platelet counts in dogs (11 [-2, 30] x10^9/L), cats (36 [14, 84] x10^9/L) and horses (42 [31, 79] x10^9/L). Sample warming did not significantly affect other haematological parameters.

Conclusions: Pre-warming EDTA blood samples to 37°C prior to haematological analysis reduces the severity of pseudothrombocytopenia in dogs, cats and horses and might negate the need for repeating the platelet count in animals for which determination of an accurate platelet count is essential.

Introduction

Platelet clumping is a known cause of pre-analytical error for platelet counts in dogs, cats and horses and is a common cause of pseudothrombocytopenia. Pseudothrombocytopenia is reported in 68% of feline samples,[1] and in cases for which determination of an accurate platelet count is essential (such as in cases with clinical signs of thrombocytopenia), repeat sampling is necessary to obtain a sample without platelet clumping, from which an accurate platelet count can be performed.[2] Ethylenediaminetetraacetic acid (EDTA)-dependent pseudothrombocytopenia occurs due to the presence of EDTA-dependent antiplatelet antibodies which recognise and stimulate various receptors (such as glycoprotein IIb-IIIa and thrombospondin) and trigger platelet agglutination *in vitro*. Agglutination has been reported to be associated with the γ globulin fraction and was inhibited by both anti-IgG and anti-IgM antisera.[3]

Several studies have evaluated the use of various substances which decrease platelet clumping in feline blood, such as prostaglandin E1and CTAD (citrate, theophylline, adenosine and dipyridamole).[4,5] Similar substances have also been evaluated in human patients. However in humans, it has been suggested that warming the sample to 37°C prior to analysis can also reduce platelet clumping.[6] The pathophysiological mechanism for altered platelet aggregation at room temperature has been investigated, and storage of human platelets at 20°C causes pronounced platelet activation and (reversible) morphological changes.[7] In addition, increased expression of GPIIb-IIIa and enhanced adenosine diphosphate-induced aggregation in platelets at 20-22°C has been demonstrated.[7;8] To the authors' knowledge, no studies have evaluated the effect of sample warming on platelet counts in companion animals, therefore the aim of the present study was to evaluate the effect of warming EDTA blood samples to 37°C on the platelet count measured by the Sysmex XT-

2000iV in dogs, cats and horses. Our hypothesis was that sample warming would lead to an increase in the automated platelet count. In addition, the effect of sample warming on other haematological parameters reported by the Sysmex was investigated.

Materials and methods

Blood samples containing EDTA from dogs, cats and horses submitted to the clinical pathology laboratory at the Department of Veterinary Medicine, University of Cambridge, UK, between 15th January and 6th February 2014 were included in the study. Samples were submitted from all services within the Queen's Veterinary School Hospital, University of Cambridge, including our first opinion small animal and equine services, and therefore included animals under investigation for a range of conditions (medical, surgical, and neurological) and also samples taken from healthy animals for screening purposes (e.g. pre-anaesthesia). These samples were usually processed within 2 hours of sampling, although some samples (submitted on a day when the laboratory was closed, dogs n=9, cats n=2, horses n=1) were processed up to 48 hours following sampling. There was no significant difference in any haematological parameter between samples processed within 2 hours and within 48 hours of sampling (data not shown). Samples usually were refrigerated (at 4°C) prior to submission, and following sample registration (10-15 minutes duration) the samples were mixed on an automatic sample mixer for at least five minutes at room temperature (approximately 22°C). Following the mixing period, a complete blood count was performed by the Sysmex XT-2000iV haematology analyser. A blood smear was prepared before samples were placed in a pre-warmed water bath (at 37°C) for five minutes, after which the samples were gently inverted three times, and then a repeat complete blood count was performed by the Sysmex analyser. After this, a second blood smear was prepared from the

pre-warmed blood. A small pilot study determined that the five minute warming period was adequate for a 2mL EDTA blood sample to be successfully warmed from 22°C to 37°C (data not shown).

A complete blood count (CBC) included white blood cell count (WBC), five part differential cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width as coefficient of variation (RDW-CV), mean platelet volume (MPV) and reticulocyte count. Platelet counts were determined by flow cytometry (PLT-O) in cat blood, and by impedance (PLT-I) in dog and horse blood. The analyser was subject to daily quality control using the manufacturer's control solutions.

Blood smears were stained with Wright's Giemsa stain and examined by one of the authors (TW) who evaluated the feathered edge of the smears at 100x magnification for the presence of platelet clumps. No attempt was made to quantify the number and size of platelet clumps because platelet clumping is not homogeneous within blood samples, and analysis of a single blood smear will not always be an accurate reflection of the degree of platelet clumping within a blood sample.[9]

The results obtained for the CBC at approximately 22°C and 37°C were compared using the Wilcoxon signed rank test, and the proportion of smears with platelet clumping, severe thrombocytopenia or thrombocytopenia at approximately 22°C and 37°C was compared by McNemar's test. When evaluating the effect of temperature on platelet count, statistical significance was defined as $P<0.05$, since this was an *a priori* hypothesis of the study. For evaluating the effect of temperature on other haematological variables, *post hoc* Bonferroni correction was applied so that statistical significance was defined as $P<0.003$ (0.05/14) for these variables. Data are presented as median [25th, 75th percentiles].

Results

Blood samples from 39 dogs, 19 cats and 10 horses were included in the study. Measured canine platelet counts at 22°C ranged from 5-444 $x10^9$/L and at 37°C ranged from 3-485 $x10^9$/L. Severe thrombocytopenia (< $50x10^9$/L) was present in 7 dogs at 22°C and remained in 6/7 dogs following sample warming. The proportion of canine samples that were severely thrombocytopenic was not significantly different after sample warming (P=1.000). Thrombocytopenia (< $175x10^9$/L) was present in 17 dogs at 22°C and remained in 15/17 dogs following sample warming. In addition, 2 canine samples with initially normal platelet count at 22°C were thrombocytopenic after sample warming. The proportion of canine samples that were thrombocytopenic was not significantly different after sample warming (P=1.000). Warming of canine blood samples resulted in a statistically significant increase in PLT-I (Table 1 and Figure 1, P=0.005), with an increase in PLT-I of 11 [-2, 30] $x10^9$/L. Blood smears were available for review in 37/39 canine cases. At 22°C, 25/37 smears showed evidence of platelet clumping, and at 37°C, 26/37 smears showed platelet clumping (6/26 of which did not show clumping at 22°C). Only 5/25 cases which demonstrated platelet clumping at 22°C did not show platelet clumping at 37°C. There was no significant difference in the proportion of canine blood smears which demonstrated platelet clumping at 22°C and 37°C (P=1.000).

Measured feline platelet counts at 22°C ranged from 38-1376 $x10^9$/L and at 37°C ranged from 143-1394 $x10^9$/L. Severe thrombocytopenia (< $50x10^9$/L) was present in 1 cat at 22°C and resolved following sample warming. The proportion of feline samples that were severely thrombocytopenic was not significantly different after sample warming (P=1.000). Thrombocytopenia (< $200x10^9$/L) was present in 7 cats at 22°C and remained in 5/7 cats following sample warming. The proportion of feline samples that were thrombocytopenic

was not significantly different after sample warming (P=0.5). Warming of feline blood samples resulted in a statistically significant increase in PLT-O (Table 2 and Figure 2, P<0.001), with an increase in PLT-O of 36 [14, 84] $x10^9$/L. Blood smears were available for review in all feline cases. At 22°C, 14/19 smears showed evidence of platelet clumping, and at 37°C, 11/19 smears showed platelet clumping (1/11 of which did not show clumping at 22°C). Only 4/14 cases which demonstrated platelet clumping at 22°C did not show platelet clumping at 37°C. There was no significant difference in the proportion of feline blood smears which demonstrated platelet clumping at 22°C and 37°C (P=0.375).

Measured equine platelet counts at 22°C ranged from 52-233 $x10^9$/L and at 37°C ranged from 135-270 $x10^9$/L. Severe thrombocytopenia (< $50x10^9$/L) was not present in any of the horses. Thrombocytopenia (< $100x10^9$/L) was present in 2 horses at 22°C and resolved in both cases following sample warming. The proportion of equine samples that were thrombocytopenic was not significantly different after sample warming (P=0.5). Warming of equine blood samples also resulted in a statistically significant increase in PLT-I (Table 3 and Figure 3, P=0.005), with an increase in PLT-I of 42 [31, 79] $x10^9$/L. Blood smears were available for review in 9/10 equine cases. At 22°C, 4/9 smears showed evidence of platelet clumping, and at 37°C, 2/9 smears showed platelet clumping (1 of which did not show clumping at 22°C). 3/4 cases which demonstrated platelet clumping at 22°C did not show platelet clumping at 37°C. There was no significant difference in the proportion of equine blood smears which demonstrated platelet clumping at 22°C and 37°C (P=0.625).

There was also no significant change (P<0.003) in any other haematological parameters reported by the analyser after sample warming in dogs, cats and horses.

Discussion

The aim of this study was to assess the effect of warming EDTA blood samples to 37^{o}C on the platelet count reported by the Sysmex XT-2000iV haematology analyser. The results of this study demonstrate that warming EDTA blood samples to body temperature prior to analysis does result in a statistically significant increase in platelet count in dogs, cats and horses, however the clinical relevance of this increase will depend on how close the platelet count is to a clinical decision limit. If, for example, the platelet count is just below a clinically relevant decision limit (such as 50x10^{9}/L), then sample warming may increase the platelet count above this threshold, thus changing the categorisation of the animal from being severely thrombocytopenic (and at risk of spontaneous bleeding) to being mild or moderately thrombocytopenic (which has fewer clinical implications). However, if the platelet count is markedly below the clinical decision limit, then the increase in platelet count following sample warming may not change the categorisation of the thrombocytopenia (particularly in dogs since the increase in platelet count seen following sample warming is smaller). In the present study, an attempt was made to evaluate if sample warming resulted in a change in the categorisation of the platelet mass, however a change in the proportion of samples with severe thrombocytopenia or thrombocytopenia following sample warming was not evident in dogs, cats and horses. This probably reflects type II error secondary to the small sample size and larger studies would be indicated to investigate if sample warming does result in a change in the categorisation of the platelet count in dogs, cats and horses.

Although sample warming may increase platelet count, it has been suggested that the platecrit is more representative of the functional platelet mass than the platelet count.[10] Therefore, sample warming and re-evaluation of the platelet count may not be indicated in

samples with a normal platecrit, which have a low platelet count and evidence of platelet clumping, since the normal platecrit would suggest a normal platelet mass in these patients.

Evaluation of blood smears before sample warming demonstrated that the majority of samples contained platelet clumps, and it is hypothesised that sample warming may have reduced the incidence of platelet clumping leading to an increased measured platelet count. However, comparison of the proportion of samples with evidence of platelet clumping before and after sample warming did not identify a significant change in the incidence of platelet clumping before and after sample warming in dogs, cats and horses. This suggests that although sample warming might reduce platelet clumping, it does not completely abrogate it. The degree of platelet aggregation has been quantified in previous studies,[4,11] and it is possible that if platelet clumping had been quantified in such a manner in the present study, then a reduction in the degree of platelet clumping may have been apparent. However, since platelet clumping is not homogeneous within blood samples, and analysis of a single blood smear will not always be an accurate reflection of the degree of platelet clumping within a blood sample,[9] the utility of this approach is questionable. More objective methods of calculating the volume of the platelet clumps (perhaps by the use of imaging software) could be used to assess the degree of platelet clumping, although these methods would also be subject to the same limitations. Instead, we used the change in platelet count reported by the analyser as a surrogate marker of a reduction in platelet clumping, since it seems unlikely that sample warming would have resulted in an increase in platelet count for any other reason, although this cannot be definitively proven without more accurate quantification of the degree of platelet clumping.

In this study, we also assessed the effect of sample warming on other haematological parameters measured by the Sysmex analyser. Our analysis indicated that no significant changes in other parameters occurred after sample warming, although it is possible that our

study may have been statistically underpowered to detect small changes. Regardless, it seems unlikely that any clinically relevant changes in other haematological parameters would have been missed due to Type II error, since the median differences in measured haematological parameters (except platelet count) between samples at 22°C and 37°C were small (<5%).

In the present study, the PLT-I was reported in dogs and horses and the PLT-O was reported in cats. It would be interesting to evaluate the effect of sample warming on the PLT-O in dogs and horses, given that PLT-O may be more accurate in canine samples containing large platelets (such as Cavalier King Charles Spaniels) and in equine samples (because small erythrocytes may be counted as platelets by the impedance method).[12] However, PLT-O data from dogs and horses were unfortunately not recorded in this study.

This study was limited by the relatively low number of samples that were evaluated (particularly in cats and horses), however significant differences in platelet counts with sample warming were noted despite the small sample size.

In conclusion, warming of EDTA blood samples to 37°C does increase platelet counts in dogs, cats and horses, however a significant change in the number of animals classified as thrombocytopenic could not be demonstrated (probably due to type II error). Sample warming might negate the need to take fresh samples from some animals if the platelet count normalises after sample warming. However, since platelet clumping remains in many samples, even after sample warming to 37°C, a new (unclumped) sample may still be needed in cases in which platelet clumping and thrombocytopenia remains (and platecrit is low), in order to provide an accurate minimum platelet count.

Acknowledgements

The authors would like to acknowledge Marie Law and Jill Free for performing the sample analyses, and Roger Powell who suggested this project.

References

1. Norman EJ, Barron RC, Nash AS, Clampitt RB. Prevalence of low automated platelet counts in cats: comparison with prevalence of thrombocytopenia based on blood smear estimation. *Vet Clin Pathol.* 2001;30(3):137-140.
2. Thomas JS. Chapter 79, Non-immune-mediated thrombocytopenia (pages 596-604). In *Schalm's veterinary hematology.* 6th ed. / editors, Douglas J. Weiss, K. Jane Wardrop. ed. Oxford: Wiley-Blackwell; 2010.
3. Shreiner DP, Bell WR. Pseudothrombocytopenia: manifestation of a new type of platelet agglutinin. *Blood.* 1973;42(4):541-549.
4. Granat F, Geffré A, Braun JP, Trumel C. Comparison of platelet clumping and complete blood count results with Sysmex XT-2000iV in feline blood sampled on EDTA or EDTA plus CTAD (citrate, theophylline, adenosine and dipyridamole). *J Feline Med Surg.* 2011;13(12):953-958.
5. Tvedten H, Johansson P. Feline platelet counting with prostaglandin E1 on the Sysmex XT-2000iV. *Vet Clin Pathol.* 2010;39(2):190-192.
6. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. *Clin Chem Lab Med.* 2012;50(8):1281-1285.
7. Maurer-Spurej E, Pfeiler G, Maurer N, Lindner H, Glatter O, Devine DV. Room temperature activates human blood platelets. *Lab Invest.* 2001;81(4):581-592.
8. Scharbert G, Kalb M, Marschalek C, Kozek-Langenecker SA. The effects of test temperature and storage temperature on platelet aggregation: a whole blood in vitro study. *Anesth Analg.* 2006;102(4):1280-1284.

9. Koplitz SL, Scott MA, Cohn LA. Effects of platelet clumping on platelet concentrations measured by use of impedance or buffy coat analysis in dogs. *J Am Vet Med Assoc.* 2001;219(11):1552-1556.

10. Russell KE. Chapter 77, Platelet kinetics and laboratory evaluation of thrombocytopenia (pages 576-585). In *Schalm's veterinary hematology.* 6th ed. / editors, Douglas J. Weiss, K. Jane Wardrop. ed. Oxford: Wiley-Blackwell; 2010.

11. Norman EJ, Barron RC, Nash AS, Clampitt RB. Evaluation of a citrate-based anticoagulant with platelet inhibitory activity for feline blood cell Counts. *Vet Clin Pathol.* 2001;30(3):124-132.

12. Lilliehöök I, Tvedten H. Validation of the Sysmex XT-2000iV hematology system for dogs, cats, and horses. I. Erythrocytes, platelets, and total leukocyte counts. *Vet Clin Pathol.* 2009;38(2):163-174.

Table 1. Table showing the effect of warming canine EDTA blood samples to 37°C on haematological parameters measured by the Sysmex XT-2000i.

Parameter	Value at 22°C	Value at 37°C	n	Sig.
WBC ($x10^9$/L)	9.8 [6.9, 13.6]	9.7 [6.9, 13.6]	39	0.892
Neutrophils ($x10^9$/L)	6.6 [4.4, 10.9]	6.7 [4.5, 10.9]	37	0.735
Lymphocytes ($x10^9$/L)	1.7 [1.0, 2.6]	1.4 [0.9, 2.5]	37	0.083
Monocytes ($x10^9$/L)	0.4 [0.2, 0.9]	0.5 [0.3, 1.0]	37	0.078
Eosinophils ($x10^9$/L)	0.3 [0.1, 0.5]	0.2 [0.1, 0.5]	37	0.446
Basophils ($x10^9$/L)	<0.01 [<0.01, <0.01]	<0.01 [<0.01, <0.01]	38	1.000
RBC ($x10^{12}$/L)	6.3 [5.8, 7.0]	6.3 [5.9, 7.0]	39	0.665
HGB (g/dL)	15.2 [14.1, 16.7]	15.3 [14.2, 16.7]	39	0.198
HCT (%)	42.3 [38.5, 47.7]	42.1 [38.4, 47.6]	39	0.134
MCV (fL)	66.8 [64.1, 68.6]	66.6 [64.0, 68.7]	39	0.018
MCH (pg)	24.1 [23.3, 25.0]	24.0 [23.5, 24.9]	39	0.197
MCHC (g/dL)	36.3 [35.2, 37.2]	36.1 [35.2, 37.3]	39	0.057
RDW-CV (%)	14.9 [14.0, 15.5]	14.9 [14.1, 15.9]	39	0.052
PLT-I ($x10^9$/L)	190 [96, 266]	189 [113, 282]	39	**0.005**
MPV (fL)	11.0 [9.9, 11.5]	10.8 [9.9, 11.5]	29	0.162
Reticulocyte count ($x10^9$/L)	65.2 [36.6, 107.3]	60.6 [35.4, 100.7]	39	0.178

Data are presented as median [25th, 75th percentile]. The Wilcoxon signed rank test was used to compare haematological parameters at room temperature (approximately 22°C) and 37°C, with statistical significance defined as $P<0.05$ for platelet parameters (PLT-I) and $P<0.003$ for all other parameters. n, number, Sig., significance.

297 **Table 2**. Table showing the effect of warming feline EDTA blood samples to 37°C on
298 haematological parameters measured by the Sysmex XT-2000i.
299

Parameter	Value at 22°C	Value at 37°C	n	Sig.
WBC ($x10^9$/L)	8.66 [7.82, 12.63]	8.53 [6.62, 10.05]	19	0.469
Neutrophils ($x10^9$/L)	5.22 [4.13, 6.90]	5.31 [4.20, 6.71]	15	0.280
Lymphocytes ($x10^9$/L)	2.08 [1.65, 2.59]	1.86 [1.68, 3.07]	15	0.285
Monocytes ($x10^9$/L)	0.23 [0.12, 0.49]	0.35 [0.21, 0.52]	15	0.037
Eosinophils ($x10^9$/L)	0.4 [0.15, 0.56]	0.35 [0.16, 0.54]	15	0.346
Basophils ($x10^9$/L)	0.01 [<0.01, 0.01]	0.01 [<0.01, 0.01]	15	0.025
RBC ($x10^{12}$/L)	7.4 [6.5, 7.8]	7.3 [6.5, 7.9]	19	0.005
HGB (g/dL)	9.7 [8.0, 12.2]	9.8 [7.9, 12.2]	19	0.285
HCT (%)	27.3 [23.2, 42.3]	27.0 [23.1, 42.3]	19	0.775
MCV (fL)	39.1 [36.2, 46.3]	38.6 [36.1, 46.6]	19	0.014
MCH (pg)	13.7 [12.8, 14.6]	13.7 [12.7, 14.5]	19	0.098
MCHC (g/dL)	32.7 [31.5, 35.8]	33.1 [31.1, 35.8]	19	0.736
RDW-CV (%)	20.8 [19.6, 23.0]	21.1 [19.8, 23.2]	19	0.004
PLT-O ($x10^9$/L)	249 [153, 382]	274 [186, 393]	19	**<0.001**
Reticulocyte count ($x10^9$/L)	104.4 [46.7, 121.1]	87.3 [40.0, 107.4]	19	0.809

300
301 Data are presented as median [25th, 75th percentile]. The Wilcoxon signed rank test was used
302 to compare haematological parameters at room temperature (approximately 22°C) and 37°C,
303 with statistical significance defined as $P<0.05$ for platelet parameters (PLT-O) and $P<0.003$
304 for all other parameters. n, number, Sig., significance.
305

306 **Table 3**. Table showing the effect of warming equine EDTA blood samples to 37°C on
307 haematological parameters measured by the Sysmex XT-2000i.
308

Parameter	Value at 22°C	Value at 37°C	n	Sig.
WBC ($x10^9$/L)	8.5 [5.6, 10.3]	8.5 [5.8, 10.1]	10	0.203
Neutrophils ($x10^9$/L)	5.4 [3.5, 7.3]	5.5 [3.6, 7.0]	10	0.919
Lymphocytes ($x10^9$/L)	2.2 [1.6, 2.5]	2.3 [1.8, 2.6]	10	0.017
Monocytes ($x10^9$/L)	0.33 [0.24, 0.40]	0.33 [0.26, 0.40]	10	0.812
Eosinophils ($x10^9$/L)	0.07 [0.02, 0.17]	0.06 [0.02, 0.21]	10	0.546
Basophils ($x10^9$/L)	0.03 [0.02, 0.20]	0.02 [0.02, 0.21]	10	0.336
RBC ($x10^{12}$/L)	7.8 [6.6, 8.4]	7.5 [6.6, 8.2]	10	0.959
HGB (g/dL)	13.1 [11.5, 13.7]	12.2 [11.5, 13.7]	10	0.380
HCT (%)	33.6 [30.0, 35.5]	31.9 [30.1, 35.0]	10	0.153
MCV (fL)	44.5 [41.4, 45.5]	44.4 [41.3, 45.4]	10	0.028
MCH (pg)	16.9 [16.6, 17.5]	16.8 [16.6, 17.5]	10	0.280
MCHC (g/dL)	38.4 [38.1, 39.3]	38.6 [38.1, 39.5]	10	0.797
RDW-CV (%)	23.6 [22.8, 24.9]	23.5 [22.6, 25.0]	10	0.297
PLT-I ($x10^9$/L)	141 [106, 195]	196 [150, 234]	10	**0.005**
MPV (fL)	8.1 [7.5, 8.2]	7.4 [7.2, 7.9]	7	0.017

309

310 Data are presented as median [25th, 75th percentile]. The Wilcoxon signed rank test was used
311 to compare haematological parameters at room temperature (approximately 22°C) and 37°C,
312 with statistical significance defined as $P<0.05$ for platelet parameters (PLT-I) and $P<0.003$
313 for all other parameters. n, number, Sig., significance.
314

315 **Figure 1**. Line chart showing the change in platelet count (PLT-I in dogs and horses, or PLT-
316 O in cats) when canine (A, left), feline (B, middle) and equine (C, right) EDTA blood
317 samples were warmed from 22^{o}C to 37^{0}C. The dotted lines represent the limits of the
318 laboratory reference interval for platelets for each species.