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Performance of the Sysmex XN-V hematology analyzer in determining the immature platelet fraction in dogs: A preliminary study and reference values

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Abstract

Background: Immature platelets (IPs) are newly formed platelets released into circulation that have been demonstrated as good markers of thrombopoiesis. Although many flow cytometric and fully automated-based methods are available, the latest Sysmex XN-V hematology analyzer for veterinary use is equipped with a specific fluorescent platelet channel (PLT-F) that detects platelets using a platelet-specific dye.

Objectives: The aims of this study were to evaluate the performance of the Sysmex XSN-1000V in determining the IPF (immature platelet fraction) and other selected PLT-F channel parameters and to propose IPF reference intervals (RIs) for canine blood samples.

Methods: Canine EDTA blood samples were analyzed on the Sysmex XN-1000V to assess linearity, imprecision, carryover, stability, and the effect of platelet clumping on selected platelet parameters from the PLT-F channel. We also reported the de novo generated RIs for the IPF in dogs.

Results: Imprecision was acceptable (CV <10%) for all parameters except for the absolute IPF values (IPF#), in which the reproducibility was 12.15% for the normal-low concentration samples. Linearity and carryover were excellent for all variables. Relative IPF values (IPF %) and IPF# remained stable for both storage conditions for up to 48 hours; however, a nonsignificant progressive increase in these parameters was observed from 12 hours at 4°C. We observed a statistical increase in IPF% and IPF# and a statistically significant decrease in PLT-F counts after intentional in vitro platelet aggregation. RIs were generated for all reference samples (n = 69) and for samples with (n = 25) or without (n = 44) platelet clumps.

Conclusions: The performance of the new PLT-F channel-derived variables for dogs was excellent. Specific RIs for IPF should be used when platelet aggregates are present.

KEYWORDS
canine, immature platelets, method validation, reticulated platelets

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1 | INTRODUCTION

Immature platelets (IPs) or reticulated platelets are young platelets recently released into circulation. IPs were first reported in 1969 using light microscopy and stained with methylene blue during a study of acute blood loss in dogs.[1] Therefore, they were considered analogous to reticulocytes, and they were termed "reticulated platelets."[1] IPs are larger and more reactive than mature platelets,[2,3] and contain larger amounts of RNA, mainly used for protein synthesis.[4] IPs circulate as the immature form for 24-36 hours during which their RNA is progressively degraded, and their volumes are decreased.[5,6] IP relative counts have been demonstrated as a good marker of megakaryopoiesis. Several studies have been conducted in human and veterinary medicine to support this finding.[7–10] Moreover, in human medicine, IP levels have been demonstrated to be altered in several diseases resulting in numerous potential clinical applications, such as risk assessment in patients with cardiovascular disease, response to antiplatelet drugs, predicting sepsis in critically ill patients, and recovery after marrow/stem cell transplantation or chemotherapy, among others.[11]

Detection of IPs in a laboratory setting can be achieved through (a) flow cytometric and (b) fully automated methods. Most flow cytometric methods detect IPs using the staining and detection technique with thiazole orange (TO).[7,10] However, recently, alternative flow cytometry protocols have been proposed.[12,13] Over time, methods for detecting IPs on fully automated hematology analyzers have continuously evolved. The latest Sysmex XN-1000V hematology analyzer for veterinary applications uses a novel optic-fluorescent analysis of platelets (PLT-F channel). The stained platelets are passed through a semiconductor diode laser beam. Forward scatter is recorded as platelet volume, and fluorescence intensity as platelet RNA content. From these measurements, the platelet count and immature platelet fraction (IPF) are reported.

Although numerous method validation studies for the new PLT-F channel using the Sysmex XN-1000V in human medicine have been carried out and reviewed elsewhere,[11] these are lacking in veterinary medicine. The aims of this article were (a) to study the performance (linearity, repeatability, reproducibility, carryover, interference studies, and stability) of the PLT-F channel and IPF on canine blood samples using the Sysmex XN-1000V hematology analyzer for veterinary use, (b) to assess the effect of platelet aggregation on IPF values, and (c) to propose a reference interval (RI) for canine samples.

2 | MATERIALS AND METHODS

2.1 | Study specimens

Blood samples from dogs presented to the Fundación Hospital Clínic Veterinari of the Universitat Autónoma de Barcelona between December 2020 and December 2021 were collected from the cephalic or jugular veins and placed into 0.5-1 mL ethylenediaminetetraacetic acid (K3-EDTA) (Aquisel, Abrera, Barcelona, Spain) tubes. Two cohorts of dogs were recruited, healthy dogs (Group 1) and dogs with thrombocytopenia and increased thrombopoiesis (Group 2). To be included in the study, dogs needed a complete medical history and physical examination and a minimum hematologic and biochemical assessment. Group 1 dogs were admitted for wellness care or preanesthetic examinations for elective surgical procedures. Inclusion criteria included an unremarkable physical examination and the absence of abnormalities in the complete blood count and basic biochemistry panel, the latter including a minimum of alanine aminotransferase activity, serum creatinine, total proteins, and glucose. Group 2 dogs were defined by platelet counts (PLT-F) less than 171×10^9/L and increased relative IPF (IPF % > 10.4%) based on our results.[14,15] Exclusion criteria included hemolytic, lipemic, and icteric samples. All blood samples were obtained with the dog owners' permission, and an informed consent document was obtained for each patient to use the leftover blood for possible studies.

Samples were kept at room temperature and placed on an agitator for 10 minutes and then were inverted 10 times manually to ensure proper homogenization. All samples were analyzed within 2-12 hours after collection using the Sysmex XN-1000V for veterinary use (Sysmex Corporation, Norderstedt, Germany). Prior to the hematologic analysis, an air-dried blood smear was prepared and stained with an automated Wright Stainer (Hematek slide Stainer; Siemens, Erlangen, Germany). For all samples, a thorough examination of a blood smear was performed, and the presence of platelet aggregation was noted. Platelet histogram distribution was studied. When a Qflag, defined as the probability of having platelet clumps in a sample, was displayed, a value <100 indicated that platelet aggregates were unlikely to be present, and a value ≥100 indicated that platelet aggregates were most likely present. As per the manufacturer's recommendations, the presence or absence of platelet clumps was verified during the blood smear examination. The abovementioned procedures were performed by the same clinical pathology resident under board-certified supervision. If conflicting results existed, they were reviewed by both a clinical pathology resident and boarded certified clinical pathologist. Internal quality control (QC) was performed daily using three levels of commercially available QC material (Sysmex XN Check Level 1 or low range, Level 2 or normal range, and Level 3 or abnormal high range; Sysmex Corporation). The following platelet parameters were analyzed: fluorescent platelet count (PLT-F), IPF (relative [%] and absolute [#] IPF values, respectively), mean platelet volume (MPV), and PLT clump. Although other variables related to platelets, erythrocytes, and leukocytes were also analyzed, they were not included in this study.

2.2 | Repeatability and reproducibility

The repeatability coefficient of variation was studied by analyzing two selected canine group samples five consecutive times (a control animal from Group 1) with PLT-F counts within reference ranges and with low-normal IPF (%) and IPF# values, and a thrombocytopenic animal from Group 2. Normal platelet counts were established using

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previously published and validated reference ranges for the Sysmex-XT-2100iV and IPF levels based on our results (IPF >10.4%).[14,15] Due to platelet instability, reproducibility was evaluated from five replicates in two selected canine specimens, a control patient and a patient with increased thrombopoiesis, analyzed over a 12-hour period.

2.3 | Linearity

Linearity was performed using triplicates of a 6-point serial dilution (100%, 50%, 25%, 12.5%, 6.25%, and 0%) of a fresh blood specimen from a thrombocytopenic dog in Group 2. As a diluent, the Sysmex diluent solution, Cellpack-DCL (Sysmex Corporation) was used. Results below the lower limit of detection were considered as 0. Linearity was only tested on parameters expressed as counts using linear regression analysis (correlation of Pearson, r^2).

2.4 | Carryover

Carryover was assessed by measuring a healthy canine blood sample in triplicate followed by Sysmex diluent solution, Cellpack-DCL (Sysmex Corporation), in triplicate.

2.5 | Stability

EDTA blood samples from five randomly selected healthy individuals were used to evaluate the effect of storage conditions at room temperature (24°C) and stored in cold conditions (4°C). Measurements were performed in duplicate immediately, 12, 24, and 48 hours after collection. Before each run, the samples were placed on a blood mixer at room temperature for 10-20 minutes.

2.6 | Interference study

The effect of hemolysis and lipemia effect on platelet parameters, including PLT-F, IPF (%), and IPF#, was studied using previously published protocols.[16] A healthy canine blood sample with no evidence of plasma hemolysis or lipemia was used for each interference study. A 6-point dilution was used in each study, and sample aliquots were analyzed in duplicates using the Sysmex XN-1000 V. To investigate the effects of hemolysis, a fresh hemolysate was prepared by mixing distilled water with packed and washed canine erythrocytes from a healthy dog. When a final hemoglobin concentration of 100 g/L was obtained, the hemolysate was centrifuged and filtered using a syringe filter equipped with a 0.2 µm cellulose acetate filter (Sartorius AG, Göttingen, Germany) to eliminate cellular debris. Final hemoglobin concentrations in the samples were 10, 5, 2.5, 1.25, 0.62, and 0 g/L, corresponding to extreme, marked, moderate, and slight hemolysis, respectively. Lipemia was simulated by adding a fat emulsion (Lipofundina 20%; Braun Medical S.A., Barcelona, Spain) to aliquots from healthy canine blood. Final triglyceride concentrations in the samples were 20, 10, 5, 2.5, 1.25, and 0 g/L, corresponding to extreme, marked, moderate, and slight lipemia.

2.7 | Effect of platelet aggregation

The effect of platelet aggregation on platelet values, including PLT-F count, IPF (%), IPF#, and MPV, was evaluated by two different approaches: (a) an in vitro platelet aggregation study, where 5 µL of $CaCl_2$ was added to 250 µL of previously analyzed EDTA blood specimens from 21 healthy canine blood samples with no evidence of platelet clumping on either the blood smear or hematology analyzer evaluation. Blood samples were analyzed 1 minute after $CaCl_2$ was added, and both initial and post in vitro aggregation results were compared, and (b) selected platelets parameters from EDTA blood specimens from 69 healthy dogs used for generating "de novo" RIs were subclassified and compared according to the probability of PLT clumps determined using the Sysmex XN-1000 V (QFlag). Twenty-five dogs likely had platelet aggregates, and 44 likely had no platelet aggregates.

2.8 | Reference intervals

IPF RIs were calculated from 69 canine blood specimens.[17] Selection of healthy subjects and sample handling and evaluation were performed as previously described for this study.

2.9 | Statistical analysis

The linearity study was evaluated using the Pearson correlation coefficient. For the stability study, a repeated measurement non-parametric ANOVA (multiple effect analysis) with Dunnett's multiple comparisons test was used. The Shapiro-Wilk test was used for all samples to study normal data distributions. If parameters followed a normal distribution, a paired t-test was used, and for nonnormally distributed data, the Wilcoxon matched-pairs rank test was used to compare groups in the platelet aggregation study. One-way ANOVA with Dunnett's multiple comparison tests were used to evaluate the effect of hemolysis and lipemia on IPF values. ASVCP guidelines for the Novo RI determination were followed for IPF RI in canine blood samples.[17] Platelet parameters in the general population, dogs with platelet aggregates, and IPF (%) and IPF# values in dogs without platelet aggregates followed a nonnormal distribution; therefore, a robust method was used. However, PLT-F counts in dogs without platelet aggregates followed a normal distribution, and a parametric method was performed. The statistical program Graph Pad v 8.0.1 (Sant Diego, CA, USA) was used for statistical comparisons. $P < 0.05$ were considered statistically significant.

3 | RESULTS

3.1 | Repeatability and reproducibility

All coefficients of variation (CV%) were <13% for all parameters (Table 1). CVs were lower than 5% for all parameters at both concentration levels (normal-low and high) during the repeatability study. Furthermore, CVs for platelet parameters at high IPF levels (Group 2 dog) were also lower than 5% during the reproducibility study. However, during the reproducibility study, all CVs were between 6% and 13% at normal-low IPF levels (Group 1), presumably due to different manipulation and testing conditions and platelet stability issues.

3.2 | Linearity

The linearity of the analyzer for the assessed platelet parameters revealed excellent linearity with correlation coefficients higher than 0.99 for all variables (Figure 1; Table 2).

3.3 | Carryover

All obtained PLT-F channel values for diluent-only samples were 0 at all times; therefore, no carryover was observed for any of the evaluated variables.

3.4 | Stability

The effect of blood storage on PLT-F parameters in samples stored in K3-EDTA at 4 and 24°C is expressed as the mean and standard deviation of the animals studied (Figure 2). PLT-F count shows a progressive decrease over time. At both storage temperatures, statistically significant differences were seen at 24- and 48-hours postcollection compared with initial concentrations (24°C: $P = 0.0006$ at 24 hours; $P = 0.0003$ at 48 hours; 4°C: $P = 0.0054$ at 24 hours; and $P = 0.002$ at 48 hours) (Figure 2). By contrast, no significant differences for IPF (%) and IPF# were observed at both temperatures during the stability study. However, in refrigerated samples, a slight steady increase and great variability were observed on these parameters

TABLE 1 Repeatability and reproducibility results of PLT-F, IPF (%), and IPF# variables with the Sysmex XN-V hematology analyzer using canine blood specimens classified based on relative IPF values.

		Repeatability				Reproducibility			
		Normal-low IPF		High IPF		Normal-low IPF		High IPF	
Parameters	Units	Mean	CV %	Mean	CV %	Mean	CV %	Mean	CV %
IPF(%)	%	3.06	4.96	31.32	2.91	0.97	8.45	32.06	0.47
PLT-F	10^9/L	210.60	3.88	50.80	1.65	369.83	6.86	51.20	3.21
IPF#	10^9/L	6.46	2.08	15.92	3.59	3.58	12.15	16.44	3.06

Abbreviations: CV %, coefficient of variation; IPF (%), relative immature platelet fraction; IPF#, absolute immature platelet fraction; PLT-F, fluorescent platelet count.

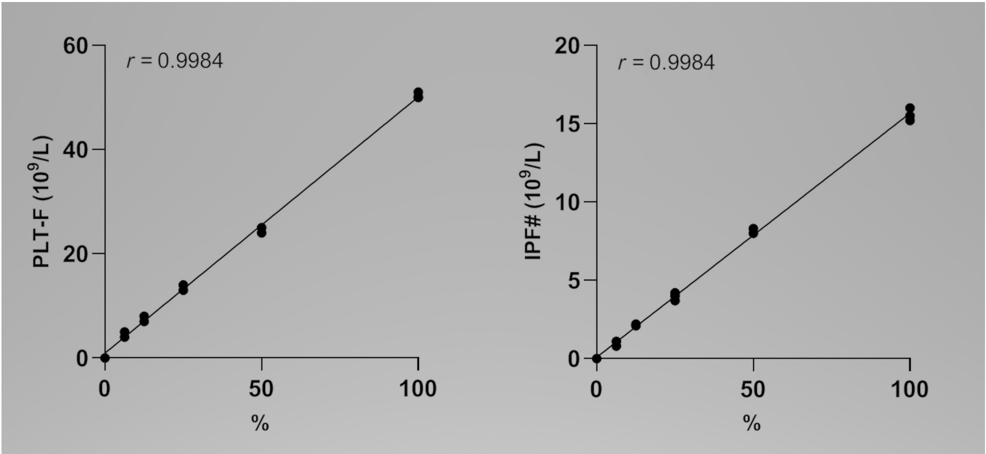

FIGURE 1 Visual linearity results for PLT-F and IPF# of a 6-point serial dilution in a dog from Group 2. IPF#, absolute immature platelet fraction; PLT-F, fluorescent platelet count.

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from 12 hours (Figure 2). Furthermore, at 24°C, IPF (%) and IPF# values remained stable during the 48-hour duration, although a slight decrease in IPF# was observed from 24 hours.

3.5 | Interference study

Hemolysis interfered significantly with all platelet parameters. PLT-F counts showed a constant increase, reaching maximum values at higher hemoglobin concentrations with significant interferences at 5 and 10 g/L hemoglobin concentrations. On the contrary, IPF (%) and IPF# values decreased progressively, reaching minimum values at 10 g/L hemoglobin concentration (Figure 3).

Increased triglyceride concentrations did not cause significant changes in PLT-F values, although we observed a slight trend in decreasing PLT-F values at higher lipid concentrations (5 g/L). However, IPF (%) and IPF# values progressively increased with increasing concentrations and reached significant differences at higher lipid concentrations (5 g/L) (Figure 3).

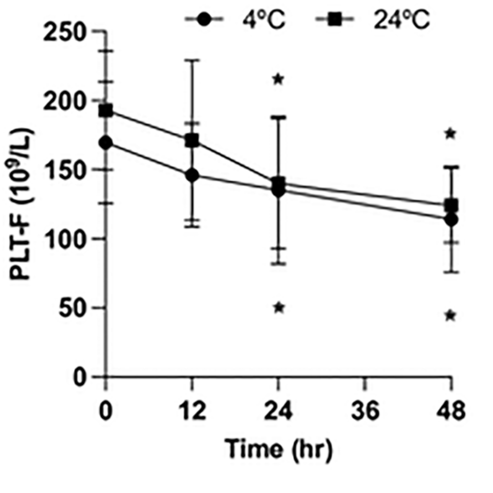
TABLE 2 Linearity results for PLT-F and IPF# variables in a dog from Group 2.

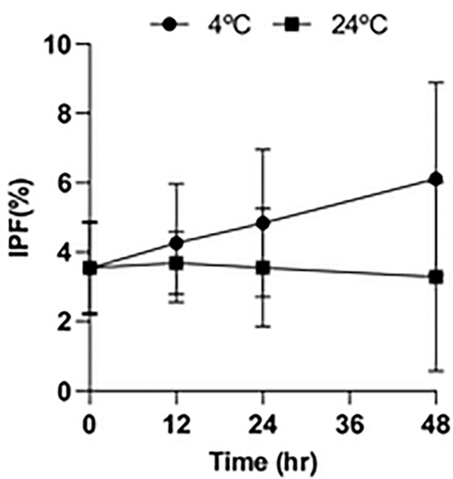
Parameters	Range	*R*
PLT-F 10^9/L	4.67-50.33	0.999
IPF# 10^9/L	1.00-15.57	0.999

Abbreviations: IPF#, absolute immature platelet fraction; PLT-F, fluorescent platelet count.

3.6 | Effect of platelet aggregation

After the addition of $CaCL_2$, an increase in platelet aggregates was observed during the blood film examination, and the Sysmex XN-1000V analyzer was able to report a flag regarding platelet clumps in all except two of the animals (91%). Furthermore, platelet counts decreased, and IPF (%) and IPF# increased considerably after in vitro





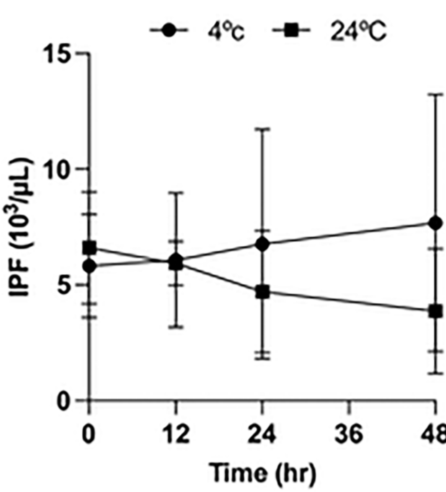


FIGURE 2 Changes in PLT-F, IPF (%), and IPF# parameters after storage at room temperature (24°C) and 4°C in canine blood samples with the Sysmex XN-1000V analyzer. Data are shown as mean and standard deviation. Statistical significance is indicated by an asterisk (*$P < 0.05$). IPF (%), relative immature platelet fraction; IPF#, absolute immature platelet fraction; PLT-F, fluorescent platelet count.

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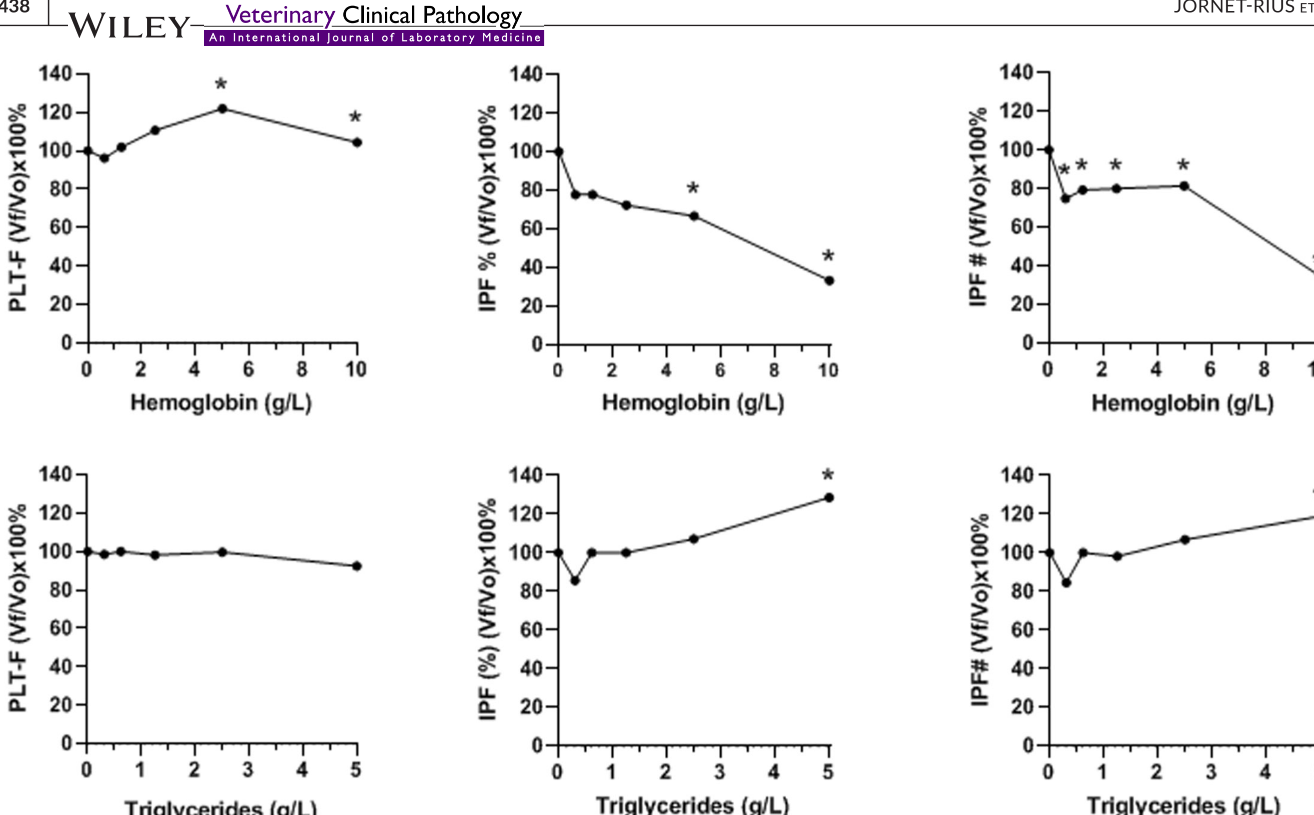

FIGURE 3 Effects of hemolysis and triglycerides on canine PLT-F count, IPF (%), and IPF# parameters with the Sysmex XN-1000V analyzer. *x*-axes show increasing concentrations of hemoglobin and triglycerides; *y*-axes show the mean percentage change of each parameter expressed as the final value/initial value × 100 (final value = enriched value; initial value = value without interferent). Each data point represents the mean of duplicate determination. *$P < 0.05$. IPF (%), relative immature platelet fraction; IPF#, absolute immature platelet fraction; PLT-F, fluorescent platelet count.

platelet aggregation. Statistically significant differences were found in all variables studied, and none were observed for MPV (Figure 4).

When comparing animals according to the probability of PLT clumps, as determined by the Sysmex XN-1000V (QFlag), animals with platelet clumps had lower PLT-F counts and higher IPF (%) and IPF# than animals without evidence of platelet aggregate, as shown in Table 3, matching the in vitro study.

3.7 | Reference intervals

Sixty-nine healthy dogs meeting the RI inclusion criteria were comprised of 53 purebred (76.8%) and 16 crossbred dogs (23.2%). The most representative breeds were Labrador Retriever (n = 6), German Shepherd dog (n = 5), and Maltese dog, Golden Retriever, and Pinscher with n = 3 each. Age ranged from 6 months to 16 years, with a median age of 5 years. Females represented 65.2% of the population, while males accounted for 34.8% of all dogs. All dogs included in this study were intact.

Given the influence of the presence of platelet aggregates on platelet variables, RIs were calculated for all healthy dogs (n = 69) independent of platelet aggregation and for the subgroups of "dogs with platelet clumps" (n = 25) and "dogs without platelet clumps" (n = 44). In the subgroup of "dogs without platelet clumps," the median age was 6 years (range from 6 months to 16 years); intact females represented 65.9%, whereas intact males represented 34.1% of the population. Dogs in the subgroup "with platelet clumps" had a median age of 4 years (range from 1 to 14 years), and 64% and 36% were represented by intact females and males, respectively. Table 3 shows the obtained RIs. RIs for the general population, independent of the platelet aggregation, had wider ranges for all variables (PLT-F [10^9/L]: 171.0-491.5; IPF (%): 1.24-10.42; and IPF# [10^9/L]: 1.68-25.56), and dogs without platelet clumps had the narrowest ranges (PLT-F [10^9/L]: 176.1-480.3; IPF (%): 1.04-6.85; and IPF# [10^9/L]: 1.31-20.59). Furthermore, dogs with platelet clumps have higher ranges for IPF (%) and IPF# and lower ranges for PLT-F counts (PLT-F [10^9/L]: 151.5-466.63; IPF (%): 2.33-10.44; and IPF# [10^9/L]: 4.47-25.56).

4 | DISCUSSION

The IPF parameter is an important part of the hematology diagnostic profile in human medicine due to its utility for diagnosis, prognostication, follow-up, and clinical decision-making of many pathologic conditions.[11] Its applicability in veterinary medicine remains largely unknown, but there is some evidence that IPF could be a marker for the thrombopoietic rate in canine samples.[7,18,19] This study evaluates

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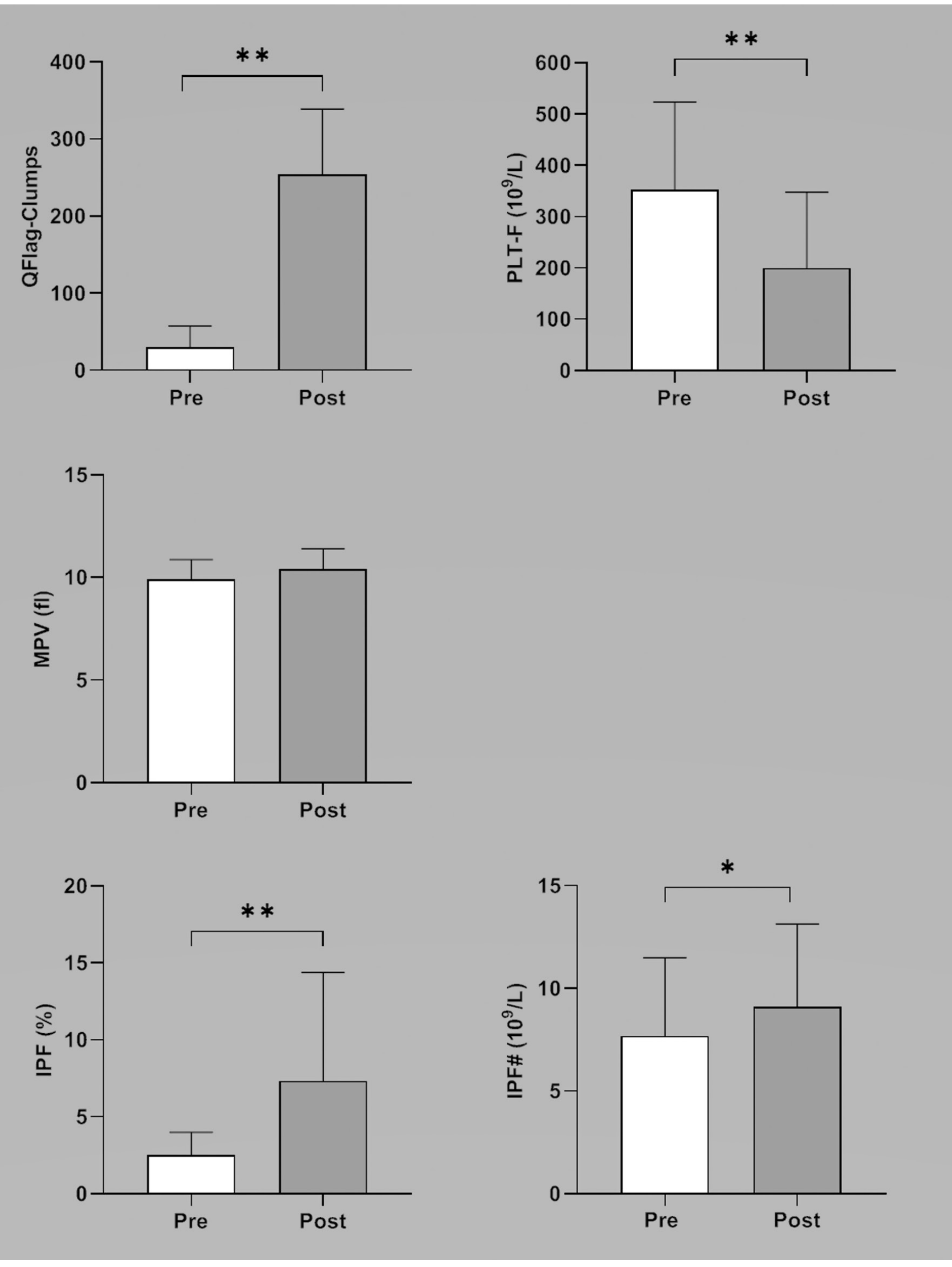

FIGURE 4 Differences in PLT-F, IPF (%), IPF#, MPV variables, and QFlag after inducing in vitro platelet clumping with the addition of $CaCL_2$ in canine blood samples with the Sysmex XN-1000V analyzer. Data are shown as mean and standard deviation. Statistical significance is indicated by an asterisk (**P* < 0.05; ***P* < 0.01). IPF (%), relative immature platelet fraction; IPF#, absolute immature platelet fraction; MPV, mean platelet volume; PLT-F, fluorescent platelet count.

the performance of the fluorescent platelet channel (PLT-F) of the Sysmex XN-1000V hematology analyzer. Imprecision was found to be acceptable for the selected platelet parameters (PLT-F, IPF [%], and IPF#) for canine samples when compared with the human literature.[11,20,21] The CV was less than 10% for all parameters except for IPF# during the reproducibility study for normal-low samples (Group 1). This result is anticipated because variability tends to be higher at lower concentrations, especially when using patient samples. In contrast to our study, a previous study using the same hematology analyzer showed a lower CV during the reproducibility study; however, they used quality control material to calculate both repeatability and reproducibility CV for PLT parameters.[15] On the other hand, another study using a Sysmex XT-2000iV analyzer and the PLT-O channel to calculate IP showed higher CV on patient samples.[17,22] The performance of the Sysmex XN-1000V is expected to be high because of the exclusive channel for the detection of platelets (PLT-F channel). This channel uses reagents that specifically bind platelet mitochondrial and ribosomal RNA, leading to a more intense labeling signal and, therefore, better identification of platelets than with the reagents used in the RET/PLT-O channel on the Sysmex analyzer

TABLE 3 Reference intervals for PLT-F, IPF (%), and IPF# in healthy dogs using the Sysmex XN-1000V analyzer. Platelet parameter RIs are determined for all samples and for samples with and without platelet clumps.

		All samples (n = 69)					Samples with platelet aggregates (n = 25)					Samples without platelet aggregates (n = 44)					
Variable	Units	Mean	SD	Median (min-max)	Percentile 2.5%	Percentile 97.5%	Mean	SD	Median (min-max)	Percentile 2.5%	Percentile 97.5	Mean	SD	Median (min-max)	Percentile 2.5%	Percentile 97.5%	*P-value*
PLT-F	10^9/L	302.68	199.40	343.00 (156-596)	171.00	491.50	274.85	32.53	179.00 (156-596)	151.50	466.63	318.76	64.35	438.50 (176-514)	176.10	480.30	0,0334*
IPF (%)	%	3.77	1.91	5.45 (0.3-11.6)	1.24	10.42	4.83	1.77	5.55 (2.2-10.4)	2.33	10.44	3.19	1.84	2.80 (0.3-11.6)	1.04	6.85	0,0016*
IPF#	10^9/L	10.25	4.31	16.75 (1.2-29,2)	1.68	25.56	12.19	4.95	10.20 (5.9-29.2)	4.47	25.56	9.11	9.83	12.85 (1.2-24.2)	1.31	20.59	0,0065*

Abbreviations: IPF (%), relative immature platelet fraction; IPF#, absolute immature platelet fraction; max, maximum; min, minimum; PLT-F, fluorescent platelet count; SD, standard deviation.

*$P < 0.05$.

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XT-2000iV.[23] The imprecision results obtained in our study agree with what has been previously published in human medicine.[20,24,25]

The linearity study was performed using blood from dogs with thrombocytopenia and increased IPF (Group 2) to increase the range of values across multiple dilutions. Linearity and carryover were excellent for all platelet variables studied and similar to those reported in a recent study on PLT-F counts.[15] Also, our results agree with what has been previously published in the human literature.[20,21]

PLT-F counts were stable for up to 24 hours at both 24 and 4°C; however, a progressive decline in its values was observed right after extraction. By contrast, IPF (%) and IPF# remained stable during the stability study for both storage conditions for up to 48 hours. However, due to the variability observed and a progressive increase in these parameters from 12 hours at 4°C, these parameters would lack clinical utility for samples stored at 4°C for 24 hours. A recent study evaluating the performance of the Sysmex XN-1000V for canine blood samples revealed similar stability results for PLT-F counts.[15] This progressive increase in IPF values has been associated with the nonspecific binding of the dye to RNA released from disintegrated platelets and to other membrane systems.[26,27] Moreover, cold-induced platelet aggregation could play an important role in this finding, as previously demonstrated.[28]

To the authors' knowledge, the effect of hemolysis and lipemia on PLT-F channel parameters in canine blood samples using the Sysmex XN-1000V has not been previously reported. Hemolytic samples impaired the detection of both mature and IPs at higher hemoglobin concentrations. PLT-F counts increased, and IPF (%) and IPF# decreased with increasing hemoglobin concentrations. Although the fresh hemolysate used during this study was filtered to avoid the interference of red blood cell fragments, the remaining fragments could have been counted as mature platelets and, therefore, increasing PLT-F counts and decreasing IPF (%) and IPF# values. By contrast, a slight decrease in PLT-F counts and an increase in IPF (%) and IPF# values with increasing triglyceride concentrations were seen, but only significant differences were observed with extreme lipemia.

We previously noticed a proportion of dogs with pseudothrombocytopenia due to platelet aggregation had moderately high IPF values. These dogs did not have diagnoses or clinical signs that would correlate with platelet consumption or destruction, such as petechiae, ecchymoses, mucosal bleeding, or other bleeding sources. Thus, we hypothesized that IPF values would be affected by platelet aggregation. After promoting platelet aggregation in vitro, we observed a statistical increase in IPF (%) and IPF# values and a decrease in PLT-F counts. A single report in human medicine evaluating the effect of platelet aggregation on IPF values found that after platelet aggregation during cold storage, IPF (%) increased significantly in a time-dependent manner and resolved after the addition of an excess of EDTA. Moreover, they found a positive correlation between IPF and MPV, suggesting that these changes were related to a false appearance of larger platelet sizes in the samples with platelet aggregates.[28] However, our study suggests that these erroneous high IPF values could also be related to a higher fluorescence intensity by platelet aggregates compared with individual platelets since MPV was not affected after the in vitro platelet aggregation study. To the author's knowledge, this report is the first in veterinary medicine to demonstrate a clear effect of platelet aggregates on IPF (%) and IPF# values.

In our study, RIs for platelet parameters consider the presence of platelet clumps in the samples, especially for IPF. This strategy is not used in human or veterinary medicine, and further studies concerning the clinical utility of this approach are warranted. Also, the IPF%, which predicts adequate bone marrow responses to platelet consumption or destruction, is not defined in veterinary medicine, and the determination of such a decision limit will need to consider the presence of platelet aggregates. Moreover, a study evaluating the effect of platelet clumping on platelet counts when measured using impedance and buffy coat methods observed that the presence and degree of platelet aggregation were variable and not homogeneous within blood samples.[29] Thus, there is no consensus in the literature on how to evaluate the effect of platelet clumping on platelet counts. Consequently, RIs for dogs with platelet clumps reported in this study should be interpreted with caution and may not have clinical significance. Independent of the presence of platelet clumps, the RIs for IPF (%) in our study are not in concordance with those previously published using either the Sysmex XT-2000iV or flow cytometry with the TO method.[7–9,17,22] Sysmex XN-V detects a higher percentage of IPF compared with those previous studies; a possible explanation for the discrepancies observed among RIs for IPF (%) using different techniques could be caused due to variations of staining protocols and gating strategies. Discrepancies between studies that compared methodologies have been found. While a study performed in veterinary medicine found that the results of IPs obtained using the Sysmex XT2000iV were comparable to those obtained with flow cytometry using the TO methodology, only a modest correlation was found between IPs obtained with flow cytometry using TO and IPF obtained with the Sysmex XE-2100/5000 in the human literature.[24,30] Possible explanations for the differences between results obtained with the different methods available may be related to the stains, with different affinities to bind to different cellular components between dyes and different gating strategies used.[23,24]

Limitations of our study include the absence of a comparison study with other methodologies and the lack of assessment of interference by icteric plasma on platelet variables derived from the PLT-F channel. Further studies will be needed to evaluate the effect of icterus interferences and whether the determination of a decision limit would work better for estimating the rate of thrombopoiesis.

In conclusion, the new PLT-F channel can be confidently used in canine blood samples for detecting IPs in samples up to 24 hours postextraction samples at both storage conditions (24 and 4°C). Furthermore, samples with platelet clumps and hemolytic and lipemic samples should be interpreted with caution.

DISCLOSURE STATEMENT

The authors have stated that they have no affiliation to, financial interest in, or financial competition with the subject matter or materials discussed in this article.

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