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ORIGINAL RESEARCH

Canine reference intervals for the Sysmex XT-2000iV hematology analyzer

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Background: The laser-based Sysmex XT-2000iV hematology analyzer is increasingly used in veterinary clinical pathology laboratories, and instrument-specific reference intervals for dogs are not available.
Objective: The purpose of this study was to establish canine hematologic reference intervals according to International Federation of Clinical Chemistry and Clinical and Laboratory Standards Institute guidelines using the Sysmex XT-2000iV hematology analyzer.
Methods: Blood samples from 132 healthy purebred dogs from France, selected to represent the most prevalent canine breeds in France, were analyzed. Blood smears were scored for platelet (PLT) aggregates. Reference intervals were established using the nonparametric method. PLT and RBC counts obtained by impedance and optical methods were compared. Effects of sex and age on reference intervals were determined.
Results: The correlation between impedance (I) and optical (O) measurements of RBC and PLT counts was excellent (Pearson $r = .99$ and .98, respectively); however, there were significant differences between the 2 methods (Student's paired t-test, $P < .0001$). Differences between sexes were not significant except for HCT, PLT-I, and PLT-O. WBC, lymphocyte, and neutrophil counts decreased significantly with age (ANOVA, $P < .05$). Median eosinophil counts were higher in Brittany Spaniels (1.87×10^9/L), Rottweilers (1.41×10^9/L), and German Shepherd dogs (1.38×10^9/L) than in the overall population (0.9×10^9/L). PLT aggregates were responsible for lower PLT counts by the impedance, but not the optical, method.
Conclusion: Reference intervals for hematologic analytes and indices were determined under controlled preanalytical and analytical conditions for a well-characterized population of dogs according to international recommendations.

Introduction

Reference intervals are important aids for interpreting laboratory data in animal patients. For each new instrument, reference intervals must either be transferred from a previous instrument or validated from pre-existing reference intervals. When transfer or validation are not possible, reference intervals should be determined de novo following recommendations of the International Federation of Clinical Chemistry (IFCC) and Clinical and Laboratory Standards Institute (CLSI), which have been recently updated.[1] To our knowledge, there is only 1 report describing canine hematologic reference values obtained by flow cytometric analysis[2]; the analysis included 46 dogs, fewer than the minimum of 120 animals recommended when using nonparametric methods.[1,3] In addition, reference intervals have not been established for the Sysmex XT-2000iV, which uses both impedance and flow cytometry and has been recently validated for analysis of the major hematologic analytes of dogs, cats, horses, rats, and mice.[4–6]

Selection of a well-characterized reference population is the major difficulty in establishing reference intervals. Purebred dogs may provide an adequate reference population if selected based on their pre-

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valence in France.[7] The objective of this study was to establish canine hematologic reference intervals for the Sysmex XT-2000iV in accordance with IFCC-CLSI recommendations.

Materials and Methods

The experimental protocol was designed following CLSI guidelines for obtaining reference values and establishing reference intervals for a new analyte or analytical method[1] and was performed during a 1-month period in February to March 2008. To permit use of the nonparametric method of analysis, a minimum of 120 results was required; as some animals or samples might be excluded a posteriori, 137 dogs were sampled.

Inclusion and exclusion criteria

Dogs included were purebred dogs from kennels; they represented the proportion of each breed within the general canine population in France to the extent possible. Written informed consent was obtained from the owners. Dogs were ≥ 6 months and were healthy based on a questionnaire, including queries about illness within the previous month, administration of medications, fasted condition, vaccination status, and genealogy, and a physical examination, including determination of heart and respiratory rates, capillary refill time, and rectal temperature; examination of the mucocutaneous, cardiorespiratory, digestive, and musculoskeletal systems; and palpation of the abdomen and mammary glands, performed by an experienced veterinarian. Dogs were excluded a priori if any of the following were present: lactation, estrus, history of disease within the last month, history of unusual bleeding, administration of any medication except external antiparasitic agents, any abnormality found during physical examination, and nonfasted state. Samples were excluded if tubes were not filled correctly or contained clots.

Preanalytical factors

Factors were defined based on recommendations for blood collection and processing in veterinary clinical pathology.[8] Venipuncture was performed in the early afternoon on fasted dogs by an experienced phlebotomist after clinical examination. The dogs were at rest in their kennels to limit possible variability due to transport. Blood was collected from the jugular vein, as results have been shown to be similar to those obtained from blood collected from the cephalic vein,[8,9] using a 0.8×40 mm needle (Venoject, Terumo Europe N.V., Leuven, Belgium), placed in a 5 mL tube containing K_3-EDTA (Venoject EDTA K3E, Terumo Europe N.V.), and then mixed by inverting the tube 10 times, labeled, and stored at 4°C before analysis. Most analyses were performed within 4 hours of blood collection, and the maximum time before analysis was 6 hours.

Hematologic analysis

Analyses were performed using the Sysmex XT-2000iV analyzer (Sysmex, Kobe, Japan) with settings for canine blood (software version 00-09, Sysmex) after testing for trueness and precision, although this analysis had been performed previously for the most frequently measured analytes.[4–6] As canine reference samples were not commercially available, analyses were performed in duplicate in the morning and afternoon for 5 consecutive days using the manufacturer's low-, medium-, and high-level controls (e-CHECK L1, L2, and L3; Sysmex) in accordance with CLSI guidelines.[10] For canine blood samples, repeatability was determined from duplicate measurements obtained as part of the study.

Analysis of the 3-level controls was performed daily before analysis of the canine samples. Measurements included the following: RBC count by optical (RBC-O) and impedance (RBC-I) methods, hemoglobin (Hb) concentration, HCT, MCV, MCH, MCHC, total reticulocyte count, low-, medium-, and high-fluorescence ratios (LFR, MFR, and HFR, respectively) as grades of reticulocyte maturation, RDW expressed as RDW-SD and RDW-CV (coefficient of variation), WBC count, neutrophil, lymphocyte, monocyte, and eosinophil counts, platelet count by optical (PLT-O) and impedance (PLT-I) methods, mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR) as an index of platelet activity, and plateletcrit (PCT). MPV, PCT, PDW, and P-LCR were analyzed using the impedance method. Basophil counts were not reported as they have been shown to be unreliable in canine samples.[5,6]

For each sample, a blood smear was prepared and stained with May-Grünwald Giemsa to detect possible platelet aggregates, as described for cats.[11] Initial examination was performed at low magnification ($\times 100$ and $\times 200$) to examine the smear for platelet aggregates, especially at the feathered edge. If platelet clumps were observed, random examination of 10 fields at the feathered edge was then performed at high magnification ($\times 1000$), and the size of the aggregates was scored as follows: 5 for ≥ 30 platelets, 4 for 20–29 platelets, 3 for 10–19 platelets, 2 for 5–9 platelets, 1 for 2–4 platelets, and 0 for no aggregates. The mean score was calculated by averaging the scores obtained for 10 high-magnification fields.

Histograms of all results were visually inspected to detect possible outliers. As the CLSI recommendation

states that "emphasis should be on retaining rather than deleting" outliers,[1] only values outside the median ± the interquartile range (between the 75th and 25th percentiles) were excluded. Normality of the distributions of native or transformed values was tested using the Anderson–Darling test. Finally, reference intervals and 90% confidence intervals of the limits were determined using the Reference Value Advisor macroinstructions for Excel (Microsoft Corp., Redmond, WA, USA) and the nonparametric method.[12,13] Partitioning of reference values according to sex was based on Harris and Boyd's *z*-test.[14] Reference intervals for subgroups were estimated by parametric and robust methods from the distribution with the best fit with a Gaussian distribution.[15] Partitioning according to age was not possible owing to the low number of reference individuals in the subgroups. Therefore, possible effects of age were estimated by determining regression-based reference limits based on the 95% prediction intervals of the polynomial regression of reference values vs age.[16] Comparisons of impedance and optical PLT and RBC counts were based on CLSI guidelines[10] and general recommendations for method comparison[17,18] using Passing–Bablok regression analysis and Bland–Altman diagrams of difference with an Excel spreadsheet and macroinstructions for Analyse-It (Analyse-it Ltd., Leeds, UK).

Table 1. Comparison of the distribution of canine breeds in France and in the reference population in this study.

Canine Breeds	In France (%)[7]	In Reference Population, *n* (%)
English Setter	8.4	0
German Shepherd	7.6	11 (8.3%)
Cavalier King Charles Spaniel	6.7	6 (4.5)
Brittany Spaniel	6.6	14 (10.6%)
Golden Retriever	6.5	6 (4.5%)
American Staffordshire Terrier	5.5	6 (4.5%)
Yorkshire Terrier	5.4	9 (6.8%)
Labrador Retriever	5.2	10 (7.6%)
French Bulldog	4.9	8 (6.1%)
Cocker Spaniel	4.6	6 (4.5%)
Rottweiler	4.1	7 (5.3%)
Belgian Shepherd (Malinois)	3.7	8 (6.1%)
Beagle	3.3	0
Wire-Haired Dachshund	3.1	5 (3.8%)
Boxer	3.0	4 (3.0%)
Beauceron	2.9	2 (1.5%)
Bernese Mountain Dog	2.9	3 (2.3%)
English Pointer	2.5	8 (6.1%)
West Highland White Terrier	2.4	7 (5.3%)
German Shorthaired Pointer	2.3	0
English Springer Spaniel	2.1	0
Shih Tzu	2.1	3 (2.3%)
Australian Shepherd	2.0	9 (6.8%)
Chihuahua	2.0	0
Total	100	132 (100%)

Results

Characteristics of the reference population

After excluding 5 samples a posteriori owing to the presence of visible clots or insufficient blood volume, 132 samples were analyzed. Breed distribution was representative of the most prevalent breeds in France, except for Australian Shepherd dogs, Brittany Spaniels, and English Pointers, which were overrepresented, and English Setters, which were not represented in the study population (Table 1). There were 83 intact females (in anestrus), 2 spayed females, and 47 intact males. Ages ranged from 6 months to 14 years with a median of 43 months; 85.6% of the dogs were between 1 and 8 years of age, 9 dogs were < 1 year, and 4 dogs > 11 years (Figure 1). There was no effect of sex on age, and the mean ages of male and female dogs were 51.8 and 50.4 months, respectively (Student's *t*-test after checking the homogeneity of variances, $P = .999$).

Analytical characteristics

Trueness and precision testing using the 3 levels of control solutions yielded CVs that were below the manufacturer's specifications for within-laboratory imprecision (Table 2). For all control levels, low CVs ($\leq 1.3\%$) were obtained for RBC-O, RBC-I, Hb concentration, HCT, MCV, MCH, and MCHC, and high CVs (9.8–41.3%) for MFR, HFR, and IRF. For the repeatability study, CVs estimated from duplicate measurements using the canine samples were also lower than the manufacturer's specifications (Table 2).

Platelet aggregate scores

Platelet aggregates were observed in 73 of 132 dogs (55.3%). In most cases the mean score was low: score of < 1 for 8 samples (6.1%); score ≥ 1 and < 2 for 38 samples (28.8%). High scores (≥ 2) were observed for 27 samples (20.5%) and were considered potential sources of error for the platelet count. Thus, these samples were considered as possible outliers, and PLT-I and PLT-O counts were evaluated for the whole reference population and also for the population excluding dogs with scores of ≥ 2.

Impedance and optical methods for RBC and PLT counts

The correlation of RBC-O and RBC-I counts was high (Pearson *r* coefficient = .99), but differences between the counts were significant (Student's paired *t*-test,

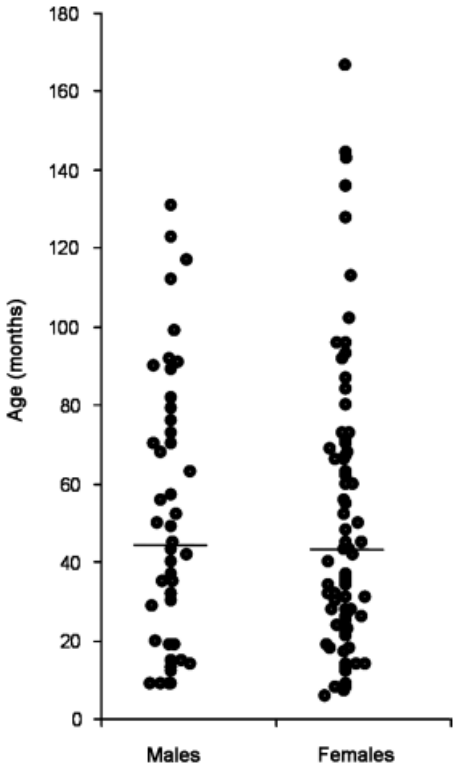

Figure 1. Distribution of ages and sexes of 132 dogs (47 males and 85 females) sampled to establish hematologic reference intervals for the Sysmex XT-2000iV. Horizontal bars indicate median ages.

$P < .0001$) and bias was proportional (Table 3). Differences (O–I) were significantly different in the 25% highest and 25% lowest values, and mean differences were -0.18 and 0.33×10^{12}/L, respectively (Student's *t*-test after checking homogeneity of variances, $P < .001$).

For PLT counts for the whole population of dogs ($n = 132$) and those from samples with platelet aggregate scores of < 2 ($n = 105$), the correlation of PLT-O and PLT-I was high (Pearson *r* coefficients of .98 and .99, respectively); however, differences between the counts were significant (Student's paired *t*-test, $P < .0001$) and bias was proportional (Table 3). Differences (O–I) were significantly different in the 25% highest and 25% lowest values, and mean differences (O–I) were -42.0 and 18.2×10^9/L, respectively, for the whole population and -43.9 and 7.3×10^9/L, respectively, for samples with aggregate scores of < 2 (Mann–Whitney's test after checking heterogeneity of variances, $P < .001$).

Reference intervals

Reference intervals were determined for analytes and indices that had been validated previously (Table 4 [excluded outliers in footnote], Figure 2) and for analytes and indices not previously validated (Table 5 [excluded outlier in footnote]). For most analytes and indices, outliers were not detected on visual inspection of the histograms or according to Tukey's criterion. Results could not be obtained for MPV, P-LCR, PCT, and PDW for 6 samples owing to poor separation of RBC and PLT by impedance. For many analytes and indices, distributions were significantly different from Gaussian (Anderson–Darling test, $P < .05$), but not after Box–Cox transformation. However, HCT distribution could not be normalized regardless of the transformation tested. For the 105 samples with platelet aggregation scores of < 2, the lower limit of PLT-I was higher than that of the whole reference population, and the upper limit was unchanged. The corresponding PLT reference intervals (90% CI) were: 115.9 (62.5–151.6)—559.4 (517.0–633.0) $\times 10^9$/L for PLT-O and 125.9 (47.0–163.5)—608.4 (535.0–661.1) $\times 10^9$/L for PLT-I.

Effects of sex, age, and breed

Spayed female dogs were not included in the analysis of sex effects on the measured variables as there were only 2 in the reference population. Sex was a partitioning factor only for HCT and PLT count, and separate reference intervals for males and females were established using the robust method on Box–Cox transformed data (Table 6); little difference between the sexes was detected. Significant decreases in total WBC, lymphocyte, and neutrophil counts were observed with increasing age (ANOVA, $P < .05$) (Figure 3); the most marked decrease was in the lymphocyte count, which decreased by about 50% between the ages of 1 year and 9–10 years. There were no significant differences in other analytes based on age (ANOVA, $P > .05$). Median eosinophil counts were higher in Brittany Spaniels (1.87×10^9/L), Rottweilers (1.41×10^9/L), and German Shepherd dogs (1.38×10^9/L) than in the overall population (0.91×10^9/L). When these 3 breeds were eliminated from the whole set of values, the estimated reference interval for the 100 remaining dogs, based on the robust method and Box–Cox transformed data, was 0–1.50×10^9/L.

Discussion

Selection of a reference population is the most difficult task in establishment of reference intervals for healthy animals. Our attempt to match the canine population according to the prevalence of the main breeds in France was limited by sampling dogs in southwest

Table 2. Trueness, within-laboratory imprecision, and repeatability of hematologic measurements for canine blood using the Sysmex XT-2000iV analyzer.

	Within-Laboratory Imprecision									Repeatability	Manufacturer's Imprecision
	Level L1			Level L2			Level L3				
Analyte/Index/Measurement	Expected Range	Mean Measured	CV (%)	Expected Range	Mean Measured	CV (%)	Expected Range	Mean Measured	CV (%)	CV (%)	CV (%)
RBC-O ($\times 10^{12}$/L)*	2.16–2.64	2.33	1.3	3.87–4.73	4.16	0.8	4.69–5.73	5.05	1.0	1.00	-†
RBC-I ($\times 10^{12}$/L)*	2.24–2.48	2.36	0.6	4.27–4.53	4.38	0.5	5.04–5.58	5.29	0.5	0.58	1.5
Hb (g/L)*	56–60	58	0.9	117–125	121	0.8	156–166	162	0.6	0.53	1.5
HCT (L/L)*	0.169–0.191	0.179	0.7	0.341–0.377	0.359	0.4	0.447–0.495	0.472	0.5	0.52	1.5
MCV (fL)*	72.5–80.1	76.1	0.5	77.5–85.7	82.1	0.2	84.3–93.1	89.2	0.2	0.19	1.5
MCH (pg)*	23.4–25.8	24.7	1.0	26.1–28.9	27.6	0.8	28.8–31.8	30.6	0.7	0.58	1.5
MCHC (g/L)*	303–341	324	1.1	317–357	336	0.9	321–363	342	0.7	0.56	2.0
RDW-SD (fL)*	37.8–51.2	42.7	0.4	39.0–52.8	45.2	0.5	38.2–51.8	44.7	1.0	0.50	3.0
RDW-CV (%)*	13.9–18.7	15.8	0.9	13.4–18.2	15.5	0.7	12.3–16.7	14.5	0.6	1.20	3.0
RET (%)*	0.12–0.24	0.16	4.6	0.07–0.14	0.10	3.9	0.04–0.08	0.05	4.7	7.68	15.0
RET ($\times 10^{9}$/L)*	4.97–10.33	6.94	4.6	1.55–3.23	2.29	4.1	0.73–1.51	0.92	4.7	7.78	15.0
WBC ($\times 10^{9}$/L)*	2.65–3.25	3.02	2.5	6.45–7.27	6.89	1.6	15.78–17.80	17.17	1.2	1.32	3.0
Neutrophils ($\times 10^{9}$/L)*	0.88–1.64	1.35	3.7	2.29–4.25	3.31	2.4	6.34–11.78	9.28	1.7	1.80	8.0
Lymphocytes ($\times 10^{9}$/L)*	0.61–1.43	1.05	4.0	1.46–2.72	2.10	6.2	2.90–5.38	4.31	1.4	2.93	8.0
Monocytes ($\times 10^{9}$/L)*	0.04–0.74	0.34	8.7	0.08–1.54	0.75	7.2	0.17–3.33	1.68	4.5	5.46	20.0
Eosinophils ($\times 10^{9}$/L)*	0.14–0.42	0.29	7.1	0.34–1.04	0.73	7.3	0.92–2.76	1.90	8.6	4.98	25.0
PLT-O ($\times 10^{9}$/L)*	27–71	54	5.3	172–232	201	3.2	431–583	473	2.5	3.38	6
PLT-I ($\times 10^{9}$/L)*	33–77	53	5.1	180–244	206	2.3	432–560	474	1.6	2.16	4.0
LFR (%)‡	58.8–88.8	73.6	3.7	59.4–89.4	71.6	3.9	68.5–98.5	79.9	3.9	3.64	30.0
MFR (%)‡	8.4–34.4	21.8	9.9	7.9–33.9	23.4	10.3	1.3–27.3	16.7	12.2	22.19	50.0
HFR (%)‡	0.0–9.8	4.6	21.7	0.0–9.7	5.0	15.1	0.0–7.2	3.5	41.3	35.03	100.0
IRF (%)‡	8.2–44.2	26.4	10.2	7.6–43.6	28.5	9.8	0.0–34.5	20.1	15.5	18.14	30.0
MPV (fL)‡	7.3–9.9	9.0	2.7	7.8–10.6	9.5	1.3	7.8–10.6	9.4	0.6	1.45	4.0
P-LCR (%)‡	1.6–20.0	12.7	10.0	6.7–20.3	15.0	4.9	6.5–19.7	14.2	3.2	2.82	18.0
PCT (L/L)‡	0.0002–0.0008	0.0005	9.1	0.0014–0.0026	0.0019	3.2	0.0034–0.0056	0.0050	1.7	2.72	6.0
PDW (fL)‡	6.1–9.1	7.8	4.7	6.8–10.2	8.7	3.1	6.9–10.3	8.6	1.7	3.19	10.0

Repeatability was estimated from duplicate measurements for canine samples used in the determination of reference intervals. Trueness and imprecision were estimated in accordance with EP15-A2 using the manufacturer's human control solutions, L1, L2, and L3. For comparison, the manufacturer's imprecision is indicated in last column.

*Analytes/indices/measurements previously validated for canine blood.

†Manufacturer's precision data not available for RBC-O.

‡Analytes/indices/measurements not validated.

See text for explanation of analyte/index/measurement abbreviations.

Table 3. Passing–Bablok regression equations for RBC and PLT counts measured by impedance (RBC-I, PLT-I) and optical (RBC-O, PLT-O) methods for canine blood with the Sysmex XT-2000iV hematology analyzer.

	95% Confidence Interval		
$Y = a \times x + b$	a	b	Units
RBC-I = 1.10 × RBC-O − 0.36	1.07–1.12	− 0.51 to − 0.23	$\times 10^{12}$/L
PLT-I = 1.10 × PLT-O − 11.75*	1.06–1.13	− 21.79 to − 1.62	$\times 10^{9}$/L
PLT-I = 1.07 × PLT-O − 3.42†	1.03–1.11	− 13.51 to 9.31	$\times 10^{9}$/L

*All specimens ($n = 132$) included.

†Specimens with no platelet aggregates or aggregate scores of < 2 ($n = 105$).

France and within 200 km of Toulouse to ensure specimen stability and timely analysis. Local sampling led to a biased selection of dogs and the absence of English Setters, one of the top 10 breeds registered in France in 2006, from the reference population. For other breeds, however, the population closely resembled the distribution of breeds in France. Another bias resulted from the choice to use only purebred dogs. The proportions and types of mixed-breed dogs have not been documented; thus, a representative selection of these animals could not be established. Moreover, as major differences based on breed were not observed, it is reasonable to assume that reference intervals determined for purebred dogs would suffice for mixed-breed dogs. The distribution of breeds may be different in other countries, and reference intervals established here will have to be validated in other settings if demographic conditions are different. Such validation can be performed using a limited number of reference samples under local laboratory conditions, as recommended by IFCC-CLSI.[1] Finally, collection of samples from dogs in kennels may have limited the intraindividual factors of variation, as animals presented to private veterinary practices may have a higher degree of variation in their hematologic analytes.

Control of preanalytical factors is essential to minimize possible effects on clinical decisions. In addition, the reliability of reference intervals is partly based on the reliability of the analytical methods used.[1] The Sysmex XT-2000iV had already been validated for analysis of most of the routinely measured analytes and indices, except for basophil counts, in canine blood.[4–6] Incomplete XT-2000iV differential counts were reported in canine blood samples, often associated with marked left shifts and toxic neutrophils.[5] However, the instrument has not been validated for LFR, MFR, HFR, IRF, MPV, P-LCR, PCT, and PDW; thus, these data may be useful in future studies. However, the clinical value of these new indices has been investigated in only a few studies in human[19–21] and veterinary hematology.[22,23] Within-laboratory precision of the analyzer with human control samples and repeatability with canine blood were satisfactory. Trueness could only be tested with human control samples; in each case, the results obtained with the analyzer were within the manufacturer's range of acceptability.

Reference intervals were established using the nonparametric method as the total number of reference individuals was > 120 and few outliers were identified in the native or transformed distributions. Establishment of reference intervals in partitioned groups based on age, sex, or breed would have required inclusion of at least 120 reference individuals in each subgroup, a laborious and expensive task. It is not required that individuals comprising the reference population be young adults, but rather should resemble the patient population as closely as possible.[1] The animals in this study spanned a large range of ages; however, most animals in kennels were young animals, 1–8 years of age, maintained for reproductive capacity. Dogs younger than 6 months were excluded a priori because previous studies have demonstrated major differences in young dogs.[24–26] Regression analysis was used to determine the effect of age, although it was known a priori that imprecision would be high owing to the low number of values available.[16] Moreover, the definition of young or old age is breed dependent: adulthood in small and large-breed dogs may be attained at 9 and 15 months of age, respectively.[27] In contrast, larger breeds are considered geriatric at earlier ages than are small-breed dogs.[28] Thus, except for 3 significant effects observed in WBC, lymphocyte, and neutrophil counts, valid conclusions about the effect of age could not be drawn. Decreases in WBC counts with age have been reported in laboratory Beagles[25,29,30] and Labrador Retrievers.[31] In the latter, a 50% decrease in the lymphocyte count was observed with increasing age, similar to the findings reported in the present study. This finding should be investigated further and considered when interpreting results from diseased dogs.

To determine the effect of sex, sufficient numbers of animals were available to permit use of parametric procedures[1]; however, large biases have been reported when small sample sizes are used.[32] It should be noted that sampling of dogs from breeding units resulted in an overrepresentation of intact females. Partitioning according to sex was relevant only for HCT and PLT count. Harris and Boyd's z criterion was selected because "any observed difference, no matter how small or how questionable its clinical significance, can be statistically significant if the sample sizes are large enough."[14] Higher HCT values in males are in

Table 4. Means and reference intervals for blood analytes/indices/measurements previously validated for canine blood using the Sysmex XT-2000iV hematology analyzer.

	Sysmex XT-2000iV Reference Intervals				Previously Reported Reference Intervals	
Analyte/Index/ Measurement	Mean*	2.5th Percentile (90% CI)	97.5th Percentile (90% CI)	Normality (*P*)	Unreported Equipment[39-41]	ADVIA 120[2]
RBC-O ($\times 10^{12}$/L)	6.3	5.1 (4.7–5.3)	7.6 (7.3–8.0)	*N*: .7153 Box–Cox: .719	–	5.68–9.08
RBC-I ($\times 10^{12}$/L)	6.6	5.2 (4.9–5.4)	7.9 (7.7–8.5)	*N*: .7103 Box–Cox: .705	5.5–8.5[39]	–
Hb (g/L)	158	124 (120–129)	192 (183–200)	*N*: .38 Box–Cox: .413	120–180[39]	137.7–203.8
HCT[b] (L/L)	0.43	0.35 (0.33–0.36)	0.52 (0.50–0.54)	*N*: .0135 Box–Cox: .016	0.37–0.55[39]	0.42–0.62
MCV (fL)	66	60 (56–61)	71 (70–73)	*N*: .8253 Box–Cox: .884	60.0–77.0[39]	62.7–74.56
MCH (pg)	24.1	21.9 (20.5–22.6)	26.3 (25.8–26.9)	*N*: .5921 Box–Cox: .516	19.5–24.5[39]	20.46–24.81
MCHC (g/L)	366	344 (328–353)	381 (379–383)	*N*: .0110 Box–Cox: .183	320–360[39]	316.1–343.5
RDW-SD (fL)	35.1	31.1 (3.5–32.3)	38.9 (38.3–41.7)	*N*: .4923 Box–Cox: .791	–	–
RDW-CV[c] (%)	16.2	13.2 (12.5–13.5)	19.1 (18.9–19.4)	*N*: .3276 Box–Cox: .328	–	12.00–13.15
Reticulocytes ($\times 10^9$/L)	58.2	19.4 (12.5–20.9)	150.1 (120.1–168.3)	*N*: < .0001 Box–Cox: .150	–	10.92–110.96
Reticulocytes (%)	0.89	0.30 (0.22–0.32)	2.37 (1.99–2.56)	*N*: < .0001 Box–Cox: .140	0.0–1.5[39]	0.14–1.48
WBC[d] ($\times 10^9$/L)	11.0	5.6 (4.9–5.9)	20.4 (19.4–21.7)	*N*: < .0001 Box–Cox: .927	6.0–17.0[40]	5.84–20.26
Neutrophils[a,e] ($\times 10^9$/L)	6.6	2.9 (2.5–3.5)	13.6 (12.3–15.5)	*N*: < .0001 Box–Cox: .106	3.0–11.5[40]	4.27–9.06
Lymphocytes[a] ($\times 10^9$/L)	2.6	1.1 (0.7–1.4)	5.3 (4.7–5.8)	*N*: < .0001 Box–Cox: .461	1.0–4.8[40]	2.04–4.66
Monocytes [f] ($\times 10^9$/L)	0.7	0.4 (0.3–0.4)	1.6 (1.4–1.7)	*N*: < .0001 Box–Cox: .522	0.15–1.35[40]	0.24–2.04
Eosinophils [g] ($\times 10^9$/L)	0.9	0.1 (0.0–0.2)	3.1 (2.7–3.4)	*N*: < .0001 Box–Cox: .154	0.10–1.25[40]	0.10–1.20
PLT-O[b] ($\times 10^9$/L)	316	108 (63–137)	562 (526–721)	*N*: .4250 Box–Cox: .791	–	173.1–486.5
PLT-I[b] ($\times 10^9$/L)	330	64 (16–137	613 (548–772)	*N*: .5017 Box–Cox: .887	200–500[41]	–

*Calculated using untransformed data: [a,b], differences according to [a]age (Figure 3) and [b]sex (Table 6); [c], methods for determination of RDW differ between the Sysmex XT2000iV and ADVIA 120.

Outliers: [d], WBC (1 outliers, 27.68 $\times 10^9$/L); [e], neutrophils (2 outliers, 17.34 and 21.22 $\times 10^9$/L); [f], monocytes (1 outlier, 2.38 $\times 10^9$/L); [g], eosinophils (1 outlier, 6.34 $\times 10^9$/L).

n = 132 dogs, except when outlier were excluded; normality testing was assessed using the Anderson–Darling test on untransformed (*N*) and Box–Cox transformed values. Previously reported RI are provided for comparison. See text for explanation of analyte/index/measurement abbreviations.

–, not reported; CI, confidence interval.

agreement with the higher Hb concentration reported previously in male laboratory Beagles[24,33] and could result from sex differences or could be related to blood loss in breeding bitches, which comprised the majority of the female reference population in this study. As HCT is closely related to RBC count, MCV, and Hb concentration, the same sex-related pattern was expected for at least one of these analytes; however, these analytes and measurements did not fulfill Harris and Boyd's partition criteria.

The possible effect of breed on hematologic variables could not be evaluated owing to low numbers of animals in each breed. However, higher eosinophil counts have been reported previously for German Shepherd dogs and Rottweilers,[34] but not for Brittany Spaniels. The higher RBC count, Hb concentration,

and HCT reported for German Shepherd dogs, Boxers, and Dachshunds[35] were not observed in this study.

The analyzer provides both optical and impedance measurements of RBC and PLT counts. Although correlation between the 2 sets of measurements was excellent, the significant differences between the methods suggest that separate reference intervals should be established based on the methodology adopted. However, differences were so slight that few misclassifications were likely and comprised 2 platelet

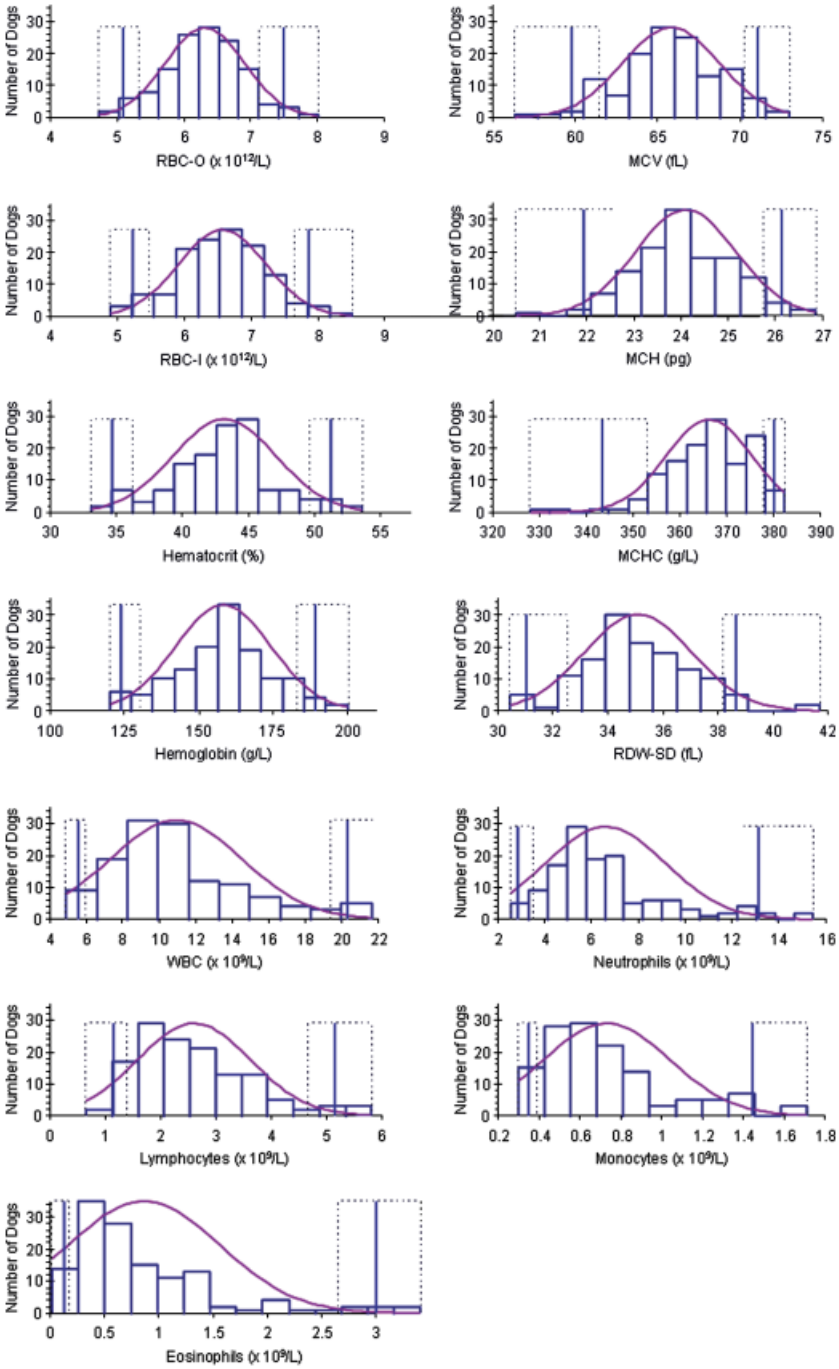

Figure 2. Observed (blue boxes) and fitted (purple line) distributions of hematologic analytes and indices for 132 healthy dogs. Blue vertical lines are the limits of the reference interval with corresponding 90% confidence intervals as dotted lines. Outliers were excluded for the WBC count (1 outlier, 27.68 × 10^9/L), neutrophil count (2 outliers, 17.34 and 21.2 × 10^9/L), monocyte count (1 outlier, 2.38 × 10^9/L), and eosinophil count (1 outlier, 6.34 × 10^9/L). See text for abbreviations.

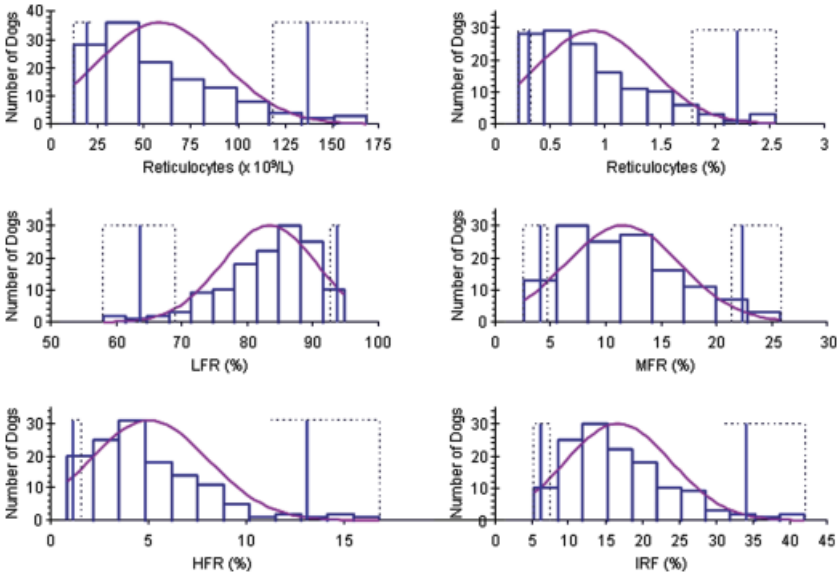

Figure 2. Continued

counts below the reference interval only with measurement by impedance. In the first case, the impedance and optical histograms did not explain the difference, but numerous PLT aggregates were observed on the blood smear. In the second case, the sample was from a Cavalier King Charles Spaniel (CKCS), and separation between PLT and RBC by impedance was not satisfactory. The optical PLT dot plot was close to the RBC cloud indicating the presence of macroplatelets; this was confirmed by blood smear examination, which also revealed PLT aggregates. Thrombocytopenia is a common laboratory finding in

Table 5. Mean and reference intervals for blood analytes/indices/measurements not previously validated for canine blood using the Sysmex XT-2000iV hematology analyzer.

	Sysmex XT-2000iV Reference Intervals			
Analyte/Index/ Measurement	Mean	2.5th Percentile (90% CI)	97.5th Percentile (90% CI)	Normality (*P*)
LFR (%)	83.3	63.7 (58.1–68.5)	93.8 (93.3–94.8)	*N*: .0001 Box–Cox: .837
MFR (%)	11.6	4.1 (2.6–4.5)	23.6 (21.9–25.8)	*N*: .0029 Box–Cox: .491
HFR (%)*	5.1	1.2 (0.9–1.5)	14.3 (11.1–16.9)	*N*: < .0001 Box–Cox: .808
IRF (%)	16.7	6.2 (5.3–6.8)	36.3 (31.5–42.0)	*N*: .0001 Box–Cox: .986
MPV (fL)†	10.62	9.05 (8.85–9.30)	12.68 (11.95–13.00)	*N*: .079 Box–Cox: .283
P-LCR (%)	30.21	16.13 (12.90–18.30)	49.16 (44.85–51.50)	*N*: .54 Box–Cox: .249
PCT (L/L)	0.0035	0.0014 (0.0005–0.0019)	0.0061 (0.0054–0.0070)	*N*: .044 Box–Cox: .260
PDW (fL)	12.45	9.30 (8.85–9.85)	18.95 (17.20–20.60)	*N*: < .0001 Box–Cox: .841

*One outlier for HFR (20.1) was excluded.

†For comparison, previously reported reference interval using the Advia 120 is 8.56–14.41 fL.

$n = 132$ dogs, except for MPV, P-LCR, PCT, and PDW, for which $n = 126$ dogs owing to insufficient PLT/RBC impedance discrimination in 6 samples; normality testing was assessed using the Anderson–Darling test on untransformed (*N*) and Box–Cox transformed values.

CI, confidence interval; see text for explanation of other abbreviations.

Table 6. Reference intervals for HCT and platelet counts partitioned by sex using the robust method for Box–Cox transformed values.

		Reference Intervals	
	Normality (*P*)	2.5th Percentile (90% CI)	97.5th Percentile (90% CI)
HCT (L/L)			
$z = 2.2296$			
Males	*N*: .2415	0.37	0.52
	Box–Cox: .4555	(0.36-0.38)	(0.50-0.54)
Females	*N*: .0293	0.34	0.50
	Box–Cox: .0871	(0.33-0.36)	(0.49-0.51)
PLT-I ($\times 10^9$/L)			
$z = 3.763$			
Males	*N*: .6257	77.1	517.9
	Box–Cox: .8681	(47.3-109.5)	460.7-573.1)
Females	*N*: .6095	100.7	619.4
	Box–Cox: .6063	(56.0-146.0)	(576.4-664.4)
PLT-O ($\times 10^9$/L)			
$z = 3.659$			
Males	*N*: .4218	85.4	506.5
	Box–Cox: .7873	(60.6-112.6)	(449.6-563.9)
Females	*N*: .7007	133.7	595.3
	Box–Cox: .8641	(105.6-167.1)	(552.0-639.4)

Males, $n = 47$; females, $n = 85$; partitioning criteria according to Harris and Boyd[14] ($z = 2.2079$); normality was assessed using the Anderson–Darling test on untransformed (*N*) and Box–Cox transformed values; platelet counts were measured by impedance (PLT-I) and optical (PLT-O) methods. CI, confidence interval.

CKCS, resulting in discordant measurements between impedance and optical methods.[36–38] Nevertheless, as reported previously in samples with numerous large platelets, the PLT-O count appeared to be more accurate, and this reference interval would be optimal even though there was good agreement between both methods.[4]

The most frequently cited canine hematologic reference intervals originate from textbooks published in 1961 and 1965 for most analytes[39,40] and in 1975 for platelets[41] and have been repeated in most textbooks, including recent ones.[42,43] However, these reference intervals do not meet the currently accepted international standards as preanalytical and analytical conditions, population characteristics, and statistical procedures are not reported.[1,3] Moreover, analytical methods have greatly improved. Thus, establishment of new reference intervals is warranted. In a recent report using the Advia 120, reference intervals were based on a limited reference sample that was too small for the recommended nonparametric method to be used.[2] However, most of the reference intervals established in the present study were similar to those reported in textbooks[39–41] and the recent report using the Advia; the 3 major differences from previous reports were for reticulocytes, HCT, and platelet

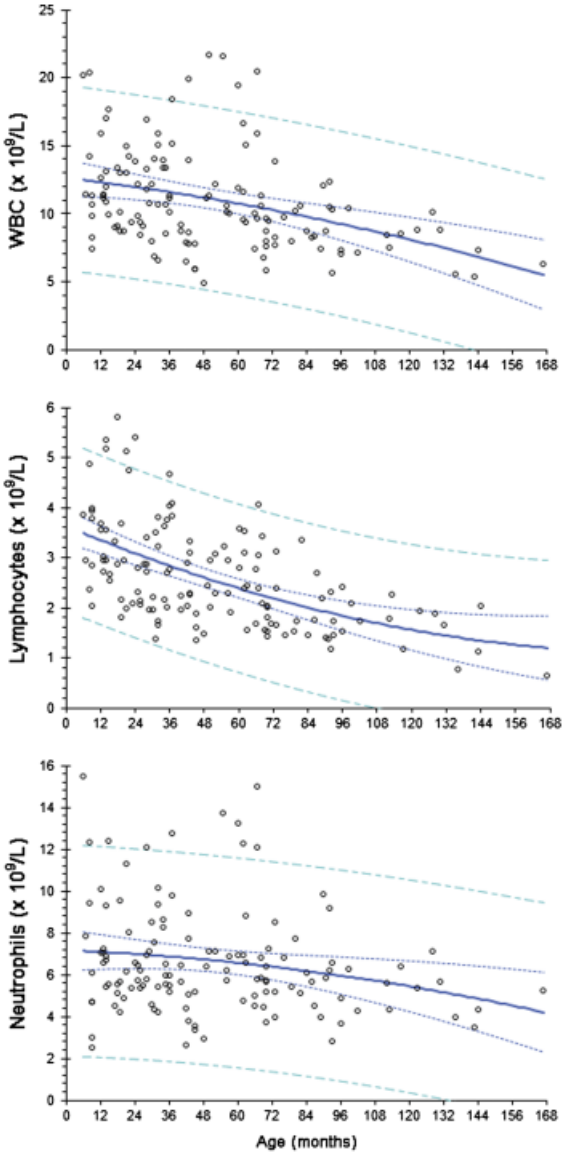

Figure 3. Effect of age on canine reference values for WBC ($n = 131$), lymphocyte ($n = 130$), and neutrophil ($n = 132$) counts. Scatter plots with polynomial fits (solid line) and 95% prediction interval (dotted line). The outliers are the same as those listed in Table 4.

counts. For some analytes, such as MCHC, MCV, and HCT, their dependence on adjustments specific to each instrument, species settings, and the software version mean that it is expected that results will differ even between 2 instruments from the same manufacturer.

As reported previously, reticulocyte counts obtained with Sysmex XT-2000iV were higher than with the Advia 120.[6] These analyzers use different methodologies for detecting reticulocytes and different reference values should be expected and used. Higher reticulocyte counts in this study could have resulted

from active erythropoiesis related to blood loss occurring during annual to biennial whelping in breeding bitches; however, there was no difference between males and females. The reference interval for HCT obtained with the Sysmex XT-2000iV was lower than with Advia 120, but similar to that reported in textbooks.[39] The latter was obtained by centrifugation,[44] whereas the Sysmex XT-2000iV sums individual volumes of RBCs counted and the Advia 120 calculates HCT from the RBC count and the MCV. Thus, observed differences could result from differences in the methods. The platelet reference intervals established in this study with impedance and optical methods were wider than the frequently used interval of $200–500 \times 10^9$/L,[41] although more recently the interval published in a textbook is $166–575 \times 10^9$/L.[45] The lower limit obtained by impedance in this study was especially low, but was higher when only samples with no platelet aggregates or low levels of aggregates were evaluated. On the other hand, platelet aggregation had little effect on the PLT-O reference intervals. Detection of platelet aggregation is critical to interpretation of low PLT counts, especially if an impedance analyzer is used.

In conclusion, reference intervals for hematologic analytes and indices were determined under controlled preanalytical and analytical conditions for a well-characterized population of dogs according to international recommendations. These reference intervals can be adopted by laboratories using the same equipment with similar analytical performance and with a canine patient population similar to the one evaluated in this study. For laboratories serving a patient population with different demographic characteristics, validation of these reference intervals should be performed before use.

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