Stability and reproducibility of ADVIA 120-measured red blood cell and platelet parameters in dogs, cats, and horses, and the use of reticulocyte haemoglobin content (CHr) in the diagnosis of iron deficiency

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SUMMARY

Modern laser-based haematology analysers such as the ADVIA 120 have species-specific software and offer the possibility of assessing new haematological parameters. These parameters have yet to be evaluated, and as these analysers are often used in referral laboratories, it is important to know whether the values of haematological parameters change during sample transport. Therefore, samples of EDTA-anticoagulated blood from nine healthy dogs and EDTA- and citrate-anticoagulated blood from six healthy horses were collected and stored at room temperature for 72 and 48 hours, respectively. In canine samples, WBC and the red blood cell parameters Hb, Hb_{cell}, Ht, MCV, and MCHC changed significantly after only 24 hours of storage. Thus if canine blood samples need to be stored for 24 hours or longer, Hb, RBC, and MCH would appear to be more reliable parameters than Ht, Hb_{cell}, MCV, and MCHC. The cytoplasmic haemoglobin content (CHr) remained stable up to 48 hours. Both dog and horse platelet numbers were stable over time when blood was anticoagulated with EDTA. Of the platelet-derived parameters, MPC was already significantly lower 2 hours after collection of equine blood samples and was also significantly lower 24 hours after collection of canine blood samples. In contrast, MPV levels were significantly higher 48 hours after sample collection. Initial platelet numbers and platelet parameters were significantly different in citrate-anticoagulated blood and EDTA-anticoagulated blood, and platelet numbers and MPM decreased significantly in citrate-anticoagulated blood samples after only 4 hours of storage.

After reference intervals for CHr had been established using samples from 53 non-anaemic dogs and 150 non-anaemic cats, the use of CHr to detect iron deficiency anaemia was tested in 63 dogs and 55 cats with different diseases. With the help of ROC curves, the optimal cut-off point was determined to be 1.22 fmol in dogs and 0.88 fmol in cats, resulting in a sensitivity of 95.2% and a specificity of 90.5% in dogs and 93.8% and 76.9% in cats, respectively.

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SAMENVATTING

Houdbaarheid en reproduceerbaarheid van erytrocyten- en thrombocytenparameters bij honden, katten en paarden gemeten op de ADVIA 120 en de toepassing van het hemoglobinegehalte in reticulocyten (CHr) voor het diagnosticeren van ijzergebreksanemie

Moderne hematologieanalysers, zoals de ADVIA 120, maken gebruik van laser flowcytometrie en toepassing van ingebouwde diersoortspecifieke software maakt het mogelijk nieuwe hematologieparameters te meten. Deze nieuwe parameters dienen geëvalueerd te worden en voor toepassing in een perifeer diagnostisch laboratorium is het van belang te weten hoe deze parameters beïnvloed worden tijdens transport van materiaal. Hiervoor werden bloedmonsters afgenomen van negen gezonde honden in EDTA en van zes gezonde paarden in EDTA en citraat en bewaard bij kamertemperatuur tot maximaal 72 uur voor verdere bepaling. Bij honden werden al significante veranderingen gevonden na 24 uur opslag bij het totaal aantal leukocyten en de erytrocytenparameters Ht, Hb_{cell}, MCV, en MCHC. Wanneer hondenbloed pas na 24 uur gemeten gaat worden, lijken Hb, RBC en MCH daarom betrouwbaardere parameters dan Ht, Hb_{cell}, MCV en MCHC. Het hemoglobinegehalte in de reticulocyt (CHr) bleef stabiel tot 48 uur. Zowel bij honden als bij paarden bleef het aantal thrombocyten betrouwbaar over de hele periode wanneer het bewaard werd in EDTA. Van de thrombocytenparameters nam de MPC al significant af na twee uur in paardenbloed en was pas significant verlaagd na 24 uur in hondenbloed, terwijl de MPV pas significant toenam na 48 uur. Vergeleken met EDTA-bloed verschillen de beginwaarden in citraatbloed al significant voor aantal thrombocyten en thrombocytenparameters, daarnaast daalden de aantallen thrombocyten en de MPM al significant na vier uur in citraatbloed.

Na het bepalen van een referentie interval voor CHr in 53 niet anemische honden en 150 niet anemische katten, werd de toepassing van CHr voor het diagnosticeren van ijzergebreksanemie gemeten in 63 honden en 55 katten met verschillende ziekten. Door gebruik te maken van ROC-curven werd een

optimaal afkappunt van 1,22 fmol bij honden en 0,88 fmol bij katten gekozen. Dit resulteerde in een sensitiviteit van 95,2% en een specificiteit van 90,5% bij honden en respectievelijk 93,8% en 76,9% bij katten.

INTRODUCTION

Modern automated haematology analysers are becoming more often used in veterinary diagnostic referral laboratories (1, 2, 19, 24). Different techniques are used to assess haematological parameters, such as microscopic evaluation of blood film, capillary/buffy coat analysis, Coulter principle, and scattering flow-cytometric methods (24). The latter technique has been developed to combine laser light scattering flow-cytometry methods with different reagents to obtain more information per measurement (15, 22). Although these instruments were originally developed for human medicine, software adjustments have made them suitable for veterinary medicine (18).

Flow-cytometry instruments are not only able to differentiate different cell types, but they are also capable of measuring different cell parameters in individual cells, using high and low angle detection. Volume, size, RNA content, and haemoglobin can be measured separately, providing individual parameters for erythrocytes and reticulocytes, including the cytoplasmic haemoglobin content of reticulocytes (CHr). The CHr is an early indicator of iron deficiency anaemia (8, 23). In addition, these haematology analysers provide not only a traditional platelet count (PLT), but also platelet function parameters, such as platelet distribution width (PDW), mean platelet volume (MPV), mean platelet component (MPC), and mean platelet mass (MPM) (15, 28). However, because erythrocyte (6, 16) and platelet parameters are reported to change during the storage of blood samples (4, 9, 25), it is important to establish the stability of different haematological parameters when measured with these advanced analysers in referral laboratories.

The aim of this study was to evaluate the stability, reproducibility, and clinical use of erythrocyte and platelet parameters measured with the ADVIA 120[a] in blood samples from horses, dogs, and cats.

MATERIALS AND METHODS

Blood samples

K_3EDTA anticoagulated blood was obtained from dogs, cats, and horses by jugular venipuncture. Blood samples were stored at room temperature for less than 4 hours after collection before further analysis, unless stated otherwise.

Study 1

To determine artifactual changes in canine blood during storage, EDTA-anticoagulated blood samples from nine healthy dogs were stored at room temperature and analysed 0.5, 16, 24, 40, 48, 64, and 72 hours after collection.

a ADVIA 120 system Siemens Medical Solutions Diagnostics GmbH, formerly Bayer Health Care, Erfurt Germany.

To determine artifactual changes in platelet parameters during the storage of equine blood samples and to compare the effect of different anticoagulants on platelet variables, both EDTA and sodium citrate 3.8% were used as anticoagulants for blood samples collected from six normal, healthy horses. The samples were stored at room temperature, and analysed 0.5, 1, 2, 4, 6, 24, and 48 hours after collection.

To determine the precision of individual parameters, three equine and canine blood samples with different platelet levels were analysed 10 times, within 1 hour of collection.

Study 2

To establish reference intervals, CHr levels in blood samples from 53 non-anaemic adult dogs and 150 non-anaemic adult cats were measured. The male and female animals were of different breeds. The dogs were healthy, privately owned animals, and the 150 cats had been referred to the Utrecht University Clinic for Companion Animals. In all animals, haematocrit and red blood cell indices were within laboratory reference intervals, and the reticulocyte percentage was lower than 2%. Although all owners were asked not to feed their animal 12 hours before sampling, this information was not checked. To determine artifactual changes during blood storage, CHr was measured 0.5h, 16 h, 24 h, 40 h, 48 h, 64 h, and 72 h after blood samples were taken from 12 dogs. To determine the precision of CHr measurements, blood samples from three dogs and six cats with different CHr levels were measured 10 times within 1 hour of sample collection. To determine whether CHr can be used to detect iron deficiency, K_3EDTA anticoagulated blood and serum samples were collected from 63 dogs and 55 cats with different diseases, admitted to the Utrecht University Clinic for Companion Animals. In addition to the haematological parameters, serum iron and total iron binding capacity (TIBC) were measured with an automated chemistry analyser (Beckman Coulter DxC) using forrozine reagent (Biomerieux) (11). If animals had a decreased haematocrit (below 0.31 l/l in cats and below 0.35 l/l in dogs) combined with at least three of the following criteria, decreased serum iron (below 9.8 µmol/l in cats and 6.7 µmol/l in dogs), normal or increased TIBC (above 16.4 µmol/l in cats and 42.6 µmol/l in dogs), decreased MCV (less than 38.2 fl in cats and 63.5 in dogs), and a decreased MCH (less than 0.81 fmol in cats and 1.37 fmol in dogs), they were retrospectively classified as having iron deficiency. In addition, there had to be a clinical explanation for the chronic blood loss.

ADVIA 120 automated haematology analyser

All blood samples were analysed at the Utrecht University Veterinary Diagnostic Laboratory using the fully automated haematology analyser ADVIA 120[a] with species-specific software adjustments made for veterinary samples. Leukocytes, erythrocytes, and platelets are measured in

different channels. Leukocytes are counted and differentiated using peroxidase, and in the basochannel cells are lysed. Haemoglobin is measured by two methods in the ADVIA 120. In the first method cells are lysed and cell-free haemoglobin is measured by means of a colorimetric method. The second method is flow cytometry, in which isovolumetrically sphered erythrocytes and reticulocytes are passed through a laser beam. Light scattered by each individual cell is detected at two different angles: scattered laser light measured by low angle detection provides information on cell size, and high angle detection provides information on haemoglobin concentration. Reticulocytes are counted by combining this two angle laser-light scattering technique with absorbance characteristics of isovolumetrically sphered cells after staining of ribosomal RNA with chromogen, Oxazin 750, detected by a forward placed detector. Platelets are distinguished from RBC by their size and refractive index, which is linearly related to platelet density and reported as mean platelet component concentration (MPC).

The following analytes were assessed: total white cell count (WBC), red blood cell count (RBC), haemoglobin (Hb) concentration (measured colorimetrically), haematocrit (Hct; calculated), MCV, MCH, MCHC, cell Hb content (Hb_{cell} and CHr in reticulocytes), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), mean platelet component concentration (MPC), and mean platelet dry mass (MPM).

STATISTICAL ANALYSIS

SPSS 15.0 for Windows (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL, USA) was used for statistical analysis of differences between parameters. Parameters were tested for normality with a Shapiro-Wilk test. A paired t-test was used to analyse the stability of measurements at different time points. For statistical analysis of platelet parameters, a Wilcoxon signed rank test was used for comparison of EDTA and citrate; one factor repeated-measures analysis of variance and Wilcoxon 2-related test were used for changes in time. Changes were considered significant at $P<0.01$ to correct for multiple testing. Precision is reported as coefficient of variation (CV,%), which was calculated as the ratio of the standard deviation and the mean of the repeated measurements. Reference intervals were determined with the statistical software package Analyse-It 2.11 (Analyse-It Software Ltd, Leeds, UK). The Shapiro-Wilk test was used to test the data for normality. After normality was established, parametric methods were used without data transformation to calculate the reference intervals plus their 90% confidence intervals.

MedCalc 9.2.1.0 (MedCalc Software, Mariakerke, Belgium) was used to calculate Receiver Operating Characteristic (ROC) curves. In a ROC curve, the true positive rate (Sensitivity) is plotted as a function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision

Parameter	Initial Sample 0.5 hr		Repeated Samples 16 hr		24 hr		40 hr		48 hr		64 hr		72 hr	
	Mean	%	%	P	%	P	%	P	%	P	%	P	%	P
WBC (10^9/l)	9.6	100	91.8	0.113	94.8	0.001	92.0	<0.001	86.3	<0.001	83.8	<0.001	80.5	<0.001
RBC (10^{12}/l)	6.95	100	101.2	0.043	101.4	0.060	100.8	0.387	100.9	0.217	100.6	0.597	99.4	0.226
Hb (mmol/l)	10.1	100	101.9	0.001	102.2	0.009	101.7	0.052	101.7	0.012	102.8	0.026	101.7	0.012
Hb_{cell} (mmol/l)	10.0	100	99.6	0.373	97.5	0.002	96.6	0.001	95.1	<0.001	94.2	<0.001	92.6	<0.001
Haematocrit (l/l)	0.462	100	108.6	<0.001	110.3	<0.001	115.6	<0.001	117.5	<0.001	119.5	<0.001	118.8	<0.001
MCV (fl)	69.1	100	106.4	<0.001	105.5	0.103	111.3	0.005	113.0	0.002	115.8	<0.001	116.4	<0.001
MCH (fmol)	1.49	100	101.4	0.034	101.0	0.063	101.0	0.116	100.7	0.387	102.2	0.154	102.1	0.010
MCHC (mmol/l)	21.62	100	95.2	0.001	95.5	0.106	91.1	0.003	88.9	0.001	88.1	0.001	87.5	<0.001
CHr (fmol)	1.55	100	100.4	0.698	98.1	0.100	97.2	<0.001	93.6	<0.001	94	<0.001	91.8	<0.001
platelets (10^9/l)	306	100	97.1	0.353	99.9	0.987	97.9	0.818	103.6	0.760	103.3	0.759	105.2	0.713
MPV (fl)	11.9	100	102.7	0.110	103.2	0.058	103.8	0.116	108.8	0.008	106.3	0.049	108.9	0.016
MPC (g/dl)	19.7	100	96.0	0.017	94.0	0.004	95.8	0.01	97.7	0.001	94.8	0.010	94.1	0.003
MPM (fg)	1.96	100	102.0	0.087	100.1	0.952	102.9	0.054	102.3	0.067	102.8	0.020	1.1.6	0.101
PDW (%)	64.8	100	96.3	0.063	95.7	0.054	100.3	0.887	99.7	0.872	103.7	0.162	104.2	0.106

Table 1. Mean haematological data for whole blood collected from 9 dogs and analysed within 0.5 hour and again after 16, 24, 40, 48, 64, and 72 hours of storage at room temperature, expressed as percentage of the original mean value combined with significance level (P).

	N	PLT mean (cells10^9/l)	PLT cv (%)	MPV mean (fl)	MPV cv (%)	MPC mean (g/dl)	MPC cv (%)	MPM mean (fg)	MPM cv (%)	PDW mean (%)	PDW cv (%)
Canine											
– Sample 1	10x	79	3.6	13.1	4.2	18.8	6.2	2.03	3.4	74.9	2.9
– Sample 2	10x	131	3.8	15.0	4.0	20.8	2.8	2.53	1.2	71.4	2.9
– Sample 3	10x	345	4.5	11.3	2.7	19.5	1.1	1.89	2.6	75.7	2.2
Equine											
– Sample 1	10x	33	9	7.3	16.3	25.6	6.1	1.98	2.2	36.6	72.7
– Sample 2	10x	133	2.7	7.5	1.7	27.0	1.1	1.82	1.1	54.3	3.2
– Sample 3	9x	210	3.6	6.9	1.8	28.9	0.4	1.82	1.4	52.5	2.2

Table 2. Precision of measurements of platelet parameters was calculated for 3 canine and equine blood samples and expressed as coefficient of variation.

Anticoagulant	Time (hr)	PLT (x10⁹/l)	%	MPV (fl)	%	MPC (g/dl)	%	MPM (fg)	%
EDTA	0.5	107.7	100.0	6.5	100.0	29.4	100.0	1.83	100.0
	1	108.3	100.6	6.7	103.1	28.8	98.0	1.85	101.1
	2	116.7	108.4	7.1	109.2	27.5*	93.5	1.80	98.4
	4	114.7	106.5	7.2	110.8	27.0*	91.8	1.78	97.3
	6	111.7	103.7	6.9	106.2	26.6*	90.5	1.80	98.4
	24	100.7	93.5	7.3	112.3	25.7*	87.4	1.84	100.5
	48	104.8	97.3	7.8	120.0	23.6*	80.3	1.95*	106.6
Sodium citrate	0.5	93.5	100.0	8.4	100.0	24.8	100.0	1.79	100.0
	1	90.7	97.0	8.1	96.4	23.7	95.6	1.79	100.0
	2	89.7	95.9	8.2	97.6	22.9	92.3	1.77	98.9
	4	84.2	90.1	8.1	96.4	23.7	95.6	1.74*	97.2
	6	76.0*	81.3	8.0	95.2	23.4	94.4	1.73*	96.6
	24	69.3*	74.1	7.7	91.7	23.4	94.4	1.67*	93.3

Table 3. Platelet parameters measured in blood from 6 horses, analysed after 30–60 minutes and following storage of samples at room temperature. Parameters are listed as absolute values and as percentages of initial value. * Value significantly (P < 0.01) different from initial value.

threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test (30). The 95% confidence intervals (95% CI) for proportions (P) were calculated as P±(1.96x S.E.)

RESULTS

Study 1

In canine samples, the WBC (P<0.001) decreased significantly from 24 hours up to 72 hours after sample collection, the Hb increased significantly after 16 hours (P<0.001) and 24 hours (P= 0.009), Hb_{cell} decreased significantly from 24 hours (P= 0.002) up to 72 hours (P<0.001), Ht increased significantly, by 10%, from 16 hours up to 72 hours (P<0.001), CHR decreased from 48 hours onwards (P<0.001), MPV increased significantly after 48 hours (P=0.008), MCV started to increase after 16 hours (P<0.001), MPC decreased after 24 hours (P<0.004), 48 hours (P<0.001), 64 hours (P=0.001), and 72 hours (P<0.003), and MCHC decreased after 40 hours (P=0.003). No significant change was seen in platelet count (Table 1).

The precision of measurements was calculated for canine and equine blood samples and expressed as coefficient of variation (Table 2). In canine and equine samples, measurements of PLT (≤3.6% and ≤9%, respectively), MPV (≤4.2% and ≤16.3%, respectively), MPC (≤6.2% and ≤6.1%, respectively), and MPM (<3.4% and ≤2.2%, respectively) showed acceptable precision. Although in canine samples, PDW measurements had a good precision (CV ≤2.9%), the measurements were highly variable in one equine sample (CV=72.7), and for this reason PDW was considered an unreliable parameter in horses.

Artifactual changes in platelet parameters during storage of equine blood are listed in Table 3. Platelet numbers and MPC were significantly higher in EDTA-anticoagulated blood, whereas MPV was significantly lower in EDTA-anticoagulated blood compared with citrate-anticoagulated blood. In EDTA-anticoagulated blood, PLT and MPV were stable up to 48 hours, MPM up to 24 hours, but MPC

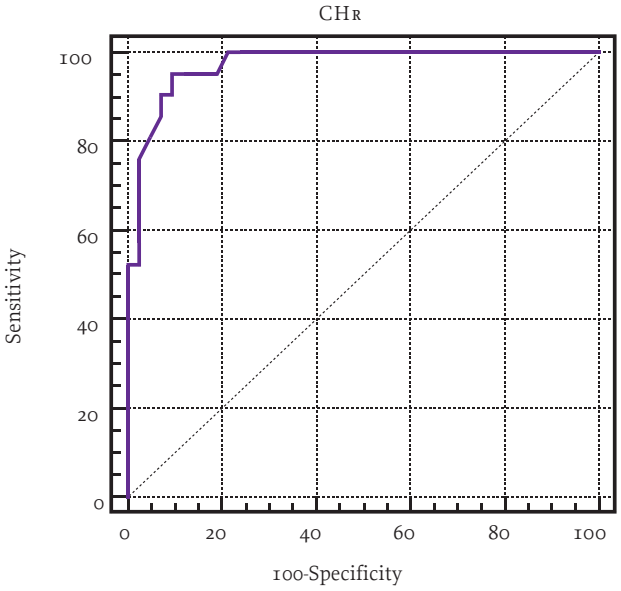

Figure 1. Receiver Operator Characteristic curve to determine optimal cut-off point for CHR in dogs (n=63).

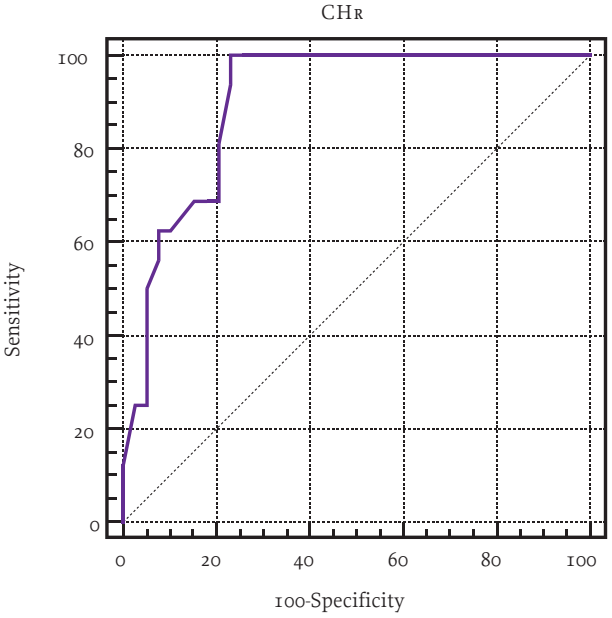

Figure 2. Receiver Operator Characteristic curve to determine optimal cut-off point for CHR in cats (n=55).

only up to 1 hour. In citrate-anticoagulated blood, PLT decreased after 6 hours, MPV and MPC were stable up to 48 hours, and MPM decreased after 4 hours.

Study 2

The reference interval for CHR was 1.43 fmol (90% CI 1.41–1.46) to 1.71 fmol (90%CI 1.68–1.74) in dogs and 0.88 fmol (90% CI0.86–0.90) to 1.22 fmol (90% CI 1.21–1.25) in cats. Parametric tests were used to calculate these intervals. The coefficient of variation was less than 1% in dogs and varied from 1.3% to 3.8% in cats. When blood was stored, CHR levels started to decrease significantly after 40-48 hours, although the difference was less than 0.08 fmol (i.e., 4.5–5.5%). On the basis of serum iron and haematological parameters (Ht, MCH, MCHC, and MCV), 21 of the 63 dogs and 16 of the 55 cats were classified as having iron-deficiency anaemia. Analysis of the ROC curves yielded an optimal cut-off point of 1.22 fmol in dogs (Figure 1) and 0.88 fmol in cats (Figure 2), resulting in a sensitivity of 95.9% (95% CI 76.1–99.2) and a specificity of 90.5% (95% CI 77.4–97.3) in dogs, and 93.8% (95% CI 69.7–99.0) and 76.9% (95% CI 60.7–88.8) in cats, respectively.

DISCUSSION

CBC, PCV, and blood smear analyses are routine laboratory tests in veterinary medicine. With the introduction of modern haematology analysers, these tests have become more accurate and new parameters have become available. The ADVIA 120 is a modern analyser that uses laser light-scattering flow cytometry to assess cells by both size and refractory index. The advantage of this machine is that it can be adapted for use in veterinary medicine by using species-specific software (18). However, the accuracy and reliability of several of these 'new' parameters still need to be evaluated in several species. Moreover, because these analysers tend to be used in referral laboratories, the stability of these parameters needs to be assessed.

In canine blood, RBC, WBC, and levels of platelet parameters changed within 24 hours of sampling. The decrease in WBC was clinically relevant, namely, 5% after 24 hours and 20% after 72 hours. Although such a decrease has been described before in the dog (9), the decrease observed in our study was greater. The decrease in WBC has been attributed to lysis of WBCs during storage. The effect is less pronounced if samples are stored at 4°C (8). The WBC of equine blood was stable during storage of blood samples at either 4 or 24°C (6).

Of the red cell parameters, the most dramatic and significant changes were noted in Ht, MCV, and MCHC. As the RBC and MCH remained stable, the changes are due to erythrocyte swelling and consequently increased cellular water content, as reported in other studies with dogs (9, 16), horses (6), and other species (12). These changes were seen within 16 hours. The best temperature for the storage of blood samples appears to be species related. In dogs, red blood cell parameters remain more stable at a storage temperature of 4°C (9), whereas in horses red blood cells parameters change more when samples at stored at 4°C than at 24°C (6). The ADVIA 120 uses both a colorimetric cyanmethaemoglobin method, which does not distinguish between cellular Hb and extracellular Hb, and a flow cytometry method to measure cellular Hb (Hb_{cell}). The Hb_{cell} has the advantage of not being influenced by haemolysis or lipaemia. Although Hb measured by the colorimetric method significantly increased during sample storage, this effect was less than 2% and was not clinically important. The Hb_{cell}, however, decreased with storage time, to maximally 5% after 48 hours and thus would seem to be less practical for a referral setting. If canine blood samples need to be stored for 24 hours or longer, Hb, RBC, and MCH would appear to be more reliable parameters than Ht, Hb_{cell}, MCV, and MCHC.

In contrast, platelet numbers showed less variation during the storage of canine and equine EDTA-anticoagulated blood samples. However, platelet numbers decreased rapidly if equine blood samples were anticoagulated with citrate. This decrease was not observed with EDTA-anticoagulated blood, as reported earlier (6). However, the storage temperature influenced platelet counts, with platelet counts in EDTA-anticoagulated blood stored for 48 hours at 4°C being higher than the initial count, which was probably due to misclassification of ghost RBCs as platelets or to different mechanisms of stabilization of platelet aggregates during their formation at low temperatures (17). Moreover, intial platelet counts were already lower in citrate-anticoagulated blood samples than in EDTA-anticoagulated blood samples, as has been reported for human (15), feline and canine (9, 25) blood samples, and is probably due to greater platelet aggregation in citrate-anticoagulated blood.

Of the different platelet parameters, MPV and especially MPC have been associated with platelet activation. For this reason, it is unfortunate that these parameters were the most sensitive to change during storage, in both equine and canine blood samples. MPV decreased significantly over 48 hours. A time-dependent increase in MPV in different anticoagulants has been reported before in humans (21). MPV varies with platelet count, anticoagulant, time to analysis, and method (13). Platelet activation is associated with shape change, swelling, and degranulation. In a canine model of endotoxaemia, MPV increased as a result of platelet swelling (27). During platelet activation, the MPC decreases due to the uptake of fluid, rather than due to granule loss, which is supported by the finding that the MCV increased without a concurrent increase in MPM. Artificial platelet swelling complicates the interpretation of MPV and MPC findings (29). One study reported a decrease in MPC accompanied by an increase in the expression of P-selectin, a marker of secretion, in dogs with inflammatory disease (20). From our experiments, it can be concluded that canine and equine platelet numbers remain stable during the storage of EDTA-anticoagulated blood, but that platelet-derived parameters, such as MPV, MPC, and MPM, should be measured within 2 hours of blood sampling. Both our results and those of others (19)

indicate that EDTA-anticoagulated blood is to be preferred to citrate-anticoagulated blood for measuring platelet parameters. Reference intervals for platelet parameters should be calculated for each individual anticoagulant and laboratory variables must be standardized.

In companion animals, laboratory variables used to distinguish iron-deficiency anaemia due to chronic gastrointestinal blood loss from that due to other diseases include MCV, MCHC, serum ferritin, and transferrin. In humans also transferrin receptors are measured. The gold standard, iron staining of bone marrow aspirates, is, however, a very invasive method and not sensitive enough to detect iron-deficiency anaemia in an early phase (7, 14). The disadvantage of measuring ferritin and transferrin is that they are both also acute-phase reactins and have biological variability (7). Measurement of serum iron concentration, TIBC, and percent saturation of transferrin also provide unreliable results for the diagnosis of iron deficiency anaemia because levels change during inflammation, corticosteroid administration, and consumption of meat (5, 10). The modern haematology analysers can routinely provide additional parameters that will help in the diagnosis of iron deficiency. Reticulocytes are the earliest erythrocytes released into the blood and circulate for only 1 to 2 days, and for this reason CHR is a measure of the availability of iron to red cells recently produced by the bone marrow. In human patients, a normal CHR value, a parameter for normal haemoglobinization, is used as a direct marker to identify functional iron deficiency (26), with a sensitivity of 57% in blood donors (22) and 90% in apparently healthy young women (14).

This study validated the measurement of CHR to detect iron deficiency anaemia in dogs and cats. The ADVIA 120 measurements of CHR were highly reproducible, in both dogs and cats. The slightly higher coefficient of variation seen in cats in this study can be explained in part by the lower number of reticulocytes in these cats. The reference interval 1.43–1.71 fmol found in this study is similar to that reported in another study of dogs (23). No reference interval has been reported in cats previously. Thus CHR offers a fast and easy method to diagnose iron deficiency anaemia in dogs and cats. Reticulocyte indices were more time sensitive indicators of iron-deficiency anaemia than conventional haematological or biochemical indicators in dogs with experimentally induced nutritional iron deficiency (8). It is measured routinely by the ADVIA 120 in combination with the reticulocyte count. With an optimal cut-off point of 1.15 fmol in dogs and 0.88 fmol, the acceptable sensitivity (78.9%) and high specificity (91.9%) in dogs and the high sensitivity (93.8%) and acceptable specificity (76.9%) in cats make it a good test as an early indicator of iron-restricted erythropoiesis. Our results are consistent with those of an earlier study of dogs with experimental nutritional iron deficiency (8). Although in that study cut-off values and sensitivity and specificity were not calculated, the areas under the curve (AUC) of the ROC curve for the different parameters of iron deficiency anaemia were comparable (0.869–0.905) to our AUC (0.901). In a study of pregnant women at term, the AUC of the ROC curve for CHR to test iron-deficiency anaemia was even lower (0.790) (7). The lack of a good reference method for diagnosing iron deficiency anaemia, however, hampers all studies, including ours. CHR levels decreased during the storage of blood only after 40-48 hours, and the decrease was less than 0.08 fmol. Thus the stability of CHR levels over time makes it possible to send blood samples by the post to referral laboratories.

In conclusion, the ADVIA 120 haematology analyser is a valuable extension to our diagnostic panel. However, it is important to take changes in analyte levels during storage into consideration. These changes are different for the different parameters, species, storage temperature, and type of coagulant. In addition, the reticulocyte parameter CHR can be used as a fast and relatively reliable parameter for detecting iron-deficiency anaemia in both cats and dogs.

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