Comparison of measurements of canine plasma glucose, creatinine, urea, total proteins, alanine aminotransferase, and alkaline phosphatase obtained with the APOLOWAKO and Vitros 250 analyzers

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Abstract

The APOLOWAKO is an entirely automatic benchtop biochemistry analyzer that uses stabilized liquid reagents. It was tested for canine blood and plasma glucose, creatinine, urea, total proteins, alanine aminotransferase, and alkaline phosphatase. The APOLOWAKO gave very similar results for whole blood and the corresponding plasma ($n = 32$). Within-laboratory imprecision was below 2.2% and 5.8% for substrates and enzymes, respectively. Comparison of results with whole blood by APOLOWAKO and with the corresponding plasma by Vitros 250 ($n = 139$) showed very good correlations. Passing–Bablok's regression slopes ranged from 0.83 to 1.12 and intercepts were close to zero, except for ALP where the results obtained by APOLOWAKO were approximately 1.5 times higher than by Vitros. The APOLOWAKO system can be a reliable instrument in veterinary practices where larger systems are not available but it should be further validated and reference intervals should be determined.

Keywords: Dog; Plasma; Whole blood; APOLOWAKO; Glucose; Creatinine; Urea; Proteins; ALT; ALP

1. Introduction

New instruments and corresponding reagents are regularly presented for in-practice animal biochemistry and haematology analyses. Most of them are based on equipment validated in human clinical pathology but not with animal specimens. The APOLOWAKO is a new entirely automatic benchtop blood chemistry analyser that uses stabilized liquid reagents and integrated calibration. The system can analyse serum/plasma or the plasma prepared from whole blood owing to a centrifugation procedure allowing to separate plasma before analysis. It is designed to produce single patient profiles of up to six different analytes. According to the manufacturer's information, this system has not yet been evaluated in veterinary clinical pathology.

The aims of this preliminary study were to test the precision of the system by repeated measurement of control sera, to compare results obtained in canine whole blood and corresponding plasma by APOLOWAKO and to compare results obtained in canine whole blood by the APOLOWAKO with those obtained in plasma with the Vitros 250 analyzer used for routine analyses at the Veterinary School of Toulouse.

2. Material and methods

The techniques of the two analysers for the following six analytes: glucose, creatinine, urea, total proteins, alanine aminotransferase (ALT, EC 2.6.1.2), and alkaline phosphatase (ALP, EC 3.1.3.1) are compared in Table 1.

Precision was tested by using two human lyophilized control sera (Wako Control Serum I and II) and making duplicate analyses of each analyte in the morning and afternoon on five consecutive days. The sera were reconstituted and stored according to manufacturer's recommendations.

Comparison studies were performed using 139 Li-heparin canine whole blood specimens. These were mostly specimens presented for routine biochemistry at the Clinical Laboratory of the Veterinary School of Toulouse. Selection was based solely on the volume of specimen available, independently of breed, age, gender, health status and on a delay between sampling and analysis of up to 6 h.

Single analyses were done of whole blood with the APOLOWAKO as done in routine practice. Analyses of the corresponding plasma with the Vitros 250 which utilizes multilayer slide technology were duplicated to avoid possible gross analytical errors with the method used for comparison. Plasmas of the first 32 cases were also used as specimens with APOLOWAKO to test the efficiency of its whole blood centrifugation process. The maximum delay between the APOLOWAKO and Vitros analyses was 2 h but most were performed within 20 min. Vitros quality control was carried out with Performance Verifier I and II (Ortho-Clinical Diagnostics, Issy-les Moulineaux, France). One abnormality was observed for total proteins which led to the rejection of 10 results.

All results are expressed in SI units as recommended by IFCCLM-IUPAC (IFCCLM and IUPAC, 1999). The following conversion factors were used for the APOLOWAKO results: 100 mg/L glucose = 5.55 mmol/L, 1 mg/dL creatinine = 88.4 μmol/L, 10 mg/dL BUN = 3.57 mmol/L urea, and 1 g/dL total proteins = 10 g/L. Within-laboratory imprecision was determined from the coefficient of variation according to NCCLS EP5-A2 (NCCLS, 2004). Results were compared by Student's paired-*t*-test, Passing–Bablok's regression and difference analysis (Jensen and Kjelgaard-Hansen, 2006; Jones and Payne, 1997) with a microsoft Excel spreadsheet and the Analyze-It set of macroinstructions (Analyze-It, Leeds, UK). The variances were heterogeneous so groups of results were compared by Mann–Whitney's test.

Table 1
Comparison of APOLOWAKO and Vitros 250 analytical techniques and of their characteristics according to the manufacturers

		Technique	Within-laboratory imprecision		Analytical range	Reference intervals for humans
			CV (%)	Concentration		
Glucose mmol/L	Vitros	Glucose oxidase; peroxidase	2.2 2.1	2.3 4.7	1.11–34.69	4.5–5.9
	APOLOWAKO	Hexokinase; glucose-6-phosphate dehydrogenase	0.5 0.91	4.97 10.25	0.7–33.33	3.9–6.1
Creatinine μmol/L	Vitros	Creatininase; creatine amidohydrolase; sarcosine oxidase, peroxidase	1.3 1.8	87 523	4–1238	71–133
	APOLOWAKO	Creatininase; creatine amidohydrolase; sarcosine oxidase, peroxidase	2.2 1.3	128 606	8.8–7079	53.1–97.3
Urea mmol/L	Vitros	Urease; ammonium indicator	1.8 1.5	5.6 16.4	0.71–42.83	3.2–7.1
	APOLOWAKO	Urease; glutamate dehydrogenase	0.85 0.88	5 23.45	0.57–42.8	2.9–7.1
Total proteins g/L	Vitros	Biuret reaction	2.7 2.2	43 68	20–110	63–82
	APOLOWAKO	Biuret reaction	1.2 1.09	48 73	4–120	67–83
ALT U/L	Vitros	2-Oxoglutarate; pyridoxal phosphate; lactate dehydrogenase	11.2 2.4	37 204	3–1000	11–66
	APOLOWAKO	2-Oxoglutarate; lactate dehydrogenase	3.2 0.95	43.8 262.8	5.3–1200	4–44
ALP U/L	Vitros	4-Nitrophenylphosphate; AMP; Mg^{2+}; pH 10.5	1.4 1.6	74 469	20–1500	38–126
	APOLOWAKO	4-Nitrophenylphosphate; AMP; Mg^{2+}; pH 9.9	1.7 0.8	115 332	7–1500	66–220

ALT = alanine aminotransferase; ALP = alkaline phosphatase; APOLOWAKO data converted to SI units; analytical range: upper limit is the limit of linearity; lower limit is the limit of quantification for the Vitros and of detection for APOLOWAKO.

3. Results

Some results could not be obtained. A defect in one bottle of the APOLOWAKO protein reagent led to the loss of 14 total protein results. Two APOLOWAKO ALP results were not reported and the error code indicated a non-linear reaction (means of 1540 and 1058 U/L with the Vitros). One ALT result with the Vitros was below the quantification limit and indicated "<3 U/L" for both replicates, whereas it was 72 U/L with the APOLOWAKO.

3.1. Quality control of APOLOWAKO

Within-laboratory imprecision of APOLOWAKO (Table 2) was close to 1% for all substrates, except for low concentrations of urea for which it was 2.2%. This imprecision ranged from 1.42 to 5.81% for enzymes and was lower at high activities of ALT. Means of results were within the range of acceptability of the manufacturer for all substrates and ALT, whereas results for ALP were approximately two times higher.

3.2. Comparison of APOLOWAKO results in whole blood and corresponding plasma

The results for whole blood and corresponding plasma were almost the same (Table 3). Differences, when statistically significant, were very low. Median (whole blood–plasma) differences were 0.17 mmol/L for glucose, −2.2 μmol/L for creatinine and 0.4 g/L for total proteins. Some differences i.e. glucose (24), creatinine (15) and total proteins (17) were higher than the analytical variability. The overall agreement was excellent, however, as shown by the regression equation parameters (slopes close to one and intercepts close to zero).

3.3. Comparison of APOLOWAKO whole blood and Vitros plasma results

The overall agreement between results obtained with the two systems was good (Figs. 1 and 2 and Table 4), with the main differences observed as follows.

For glucose, a constant bias was observed and the results with APOLOWAKO were 0.5 mmol/L (0.45–0.55) lower than with the Vitros. There were two grossly erroneous differences, in which the APOLOWAKO results were 1.6 and 2.4 mmol/L lower. According to the printouts, the maximum delays between the analyses with the two analysers were 15 and 60 min.

Creatinine results were lower with the APOLOWAKO than with the Vitros and the bias was proportional. Mean bias ranged from 2.2 to 30.0 μmol/L in the 20% of specimens with the lowest and highest creatinine concentrations ($P < 0.001$). It was in the range of −10 to −20 μmol/L between 50 and 200 μmol/L, which is the diagnostically critical zone.

Urea was moderately higher by APOLOWAKO than by Vitros. The bias up to 10 mmol/L was almost constant, with a mean of 0.8 mmol/L. Two apparently aberrant differences were observed in a haemolysed and a non-haemolysed specimen, the results being 5.1 and 6.0 mmol/L higher with APOLOWAKO than the means of measurements of 3.3 and 5.1 mmol/L obtained with the Vitros.

The total protein measurements showed a proportional bias. The (APOLOWAKO–Vitros) mean difference ranged from −4.2 to −7.8 g/L in the lowest and highest 20% of results respectively ($P < 0.001$).

ALP results obtained by APOLOWAKO were approximately 1.5 times higher than by Vitros. The bias was proportional. The mean (APOLOWAKO–Vitros) difference ranged from 7 to 363 U/L in the lowest and highest 20% of results ($P < 0.001$).

ALT results by the APOLOWAKO were slightly higher than with the Vitros; in the low part of the curve (< 200 U/L) where most results were obtained, the bias was almost constant about 15 U/L. Larger differences have been observed in two specimens with high activity (1905 and 1107 U/L by the Vitros were overevaluated by 277 and 133 U/L, respectively).

4. Discussion

Pre-testing comparison of the two systems based on the manufacturer's information showed that:

Table 2
Within-laboratory precision (CV) of APOLOWAKO with low and high concentration human serum controls

		Low			High		
		Value (range)	Mean of APOLOWAKO results	CV (%)	Value (range)	Mean of APOLOWAKO results	CV (%)
Glucose	mmol/L	4.46 (3.74–5.17)	4.03	0.94	13.9 (11.7–16.1)	13.0	0.74
Creatinine	μmol/L	87 (80–126)	89	1.56	258 (201–314)	242	0.82
Urea	mmol/L	1.44 (1.12–1.75)	1.65	2.23	8.60 (6.70–10.49)	9.37	1.16
Total proteins	g/L	71 (63–79)	69	0.88	107 (95–119)	104	0.87
ALT	U/L	32 (23–39)	33	5.81	172 (132–212)	170	1.42
ALP	U/L	46 (34–57)	104	4.23	195 (146–244)	353	4.02

$n = 20$; duplicate analyses performed in the morning and afternoon of five consecutive days; for each control serum value and range between brackets according to manufacturer.

Table 3
Comparison of results obtained with APOLOWAKO using 32 specimens of canine whole blood and corresponding plasma and parameters of Passing–Bablok's equation: y[plasma] = $a \times$ [whole blood] + b, with 95% confidence interval between brackets

	Glucose mmol/L		Creatinine µmol/L		Urea mmol/L		Total proteins g/L		ALT U/L		ALP U/L	
	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma
Median	4.75	4.75	77.4	78.3	5.97	6.04	55	56	68	73	151	158
Minimum	2.72	2.22	37.1	45.1	3.28	3.28	41	43	27	26	0	38
Maximum	7.05	6.77	1611	1602	48.1	48.2	67	69	548	777	3419	3343
P	<0.001		<0.001		ns		<0.001		ns		ns	
a	1.049 (0.935/1.185)		1.000 (0.979/1.015)		1.001(0.975/ 1.032)		1.000 (0.818 /1.111)		1.000 (0.950/1.043)		0.992 (0.976/1.022)	
b	−0.420 (−1.00/0.16)		2.2 (1.0/4.9)		0.064 (−0.124/0.207)		1.000 (−4.3/11.0)		0 (−2.9/3.8)		−0.26 (−3.8/2.0)	

Comparison by Student's paired *t*-test; ns = not significant.

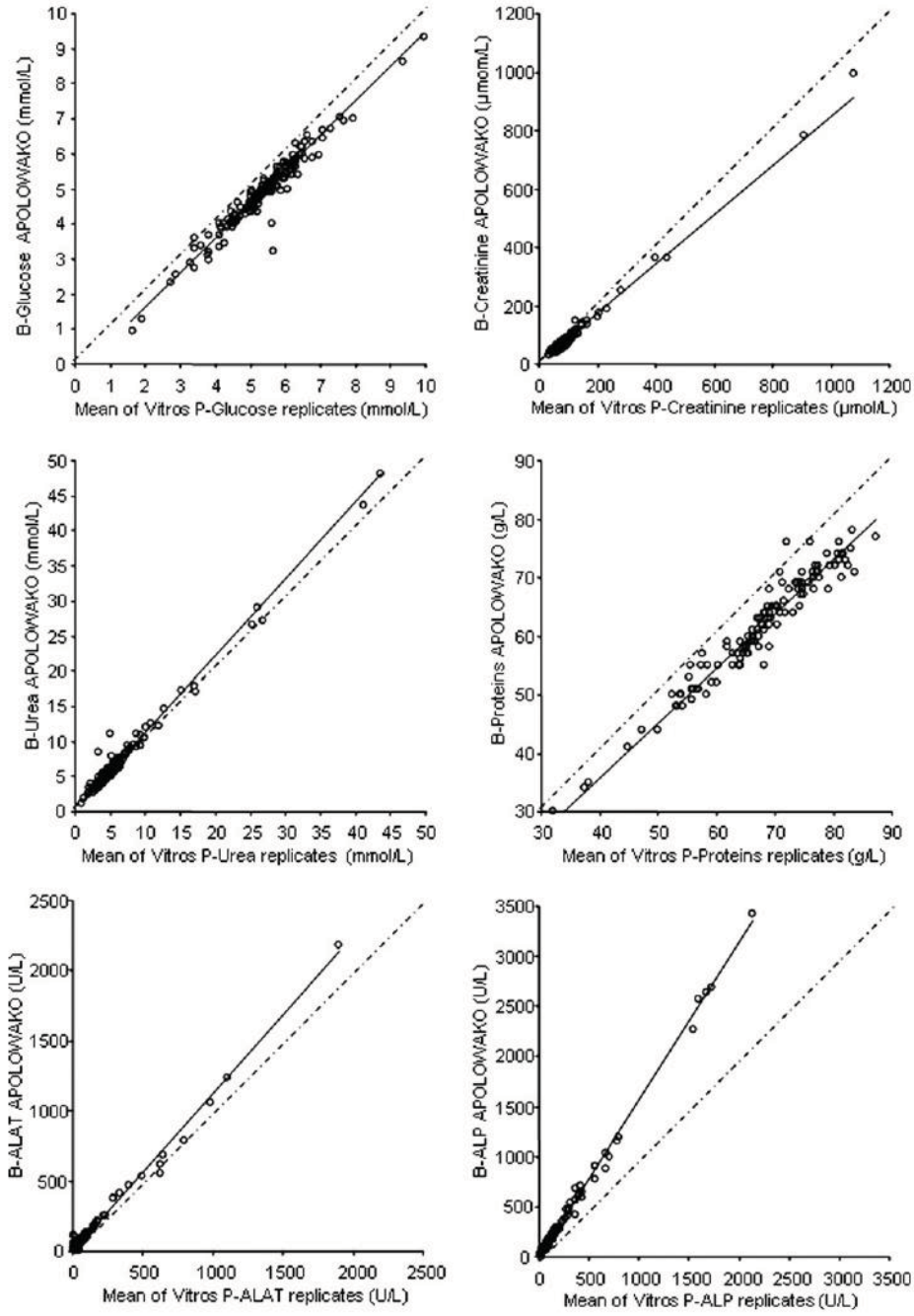

Fig. 1. Scatterplot of results obtained in canine whole blood (B) with APOLOWAKO and corresponding plasmas (P) with the Vitros analyser. Dotted line is the $y = x$ equivalence line, and black line the Passing–Bablok regression.

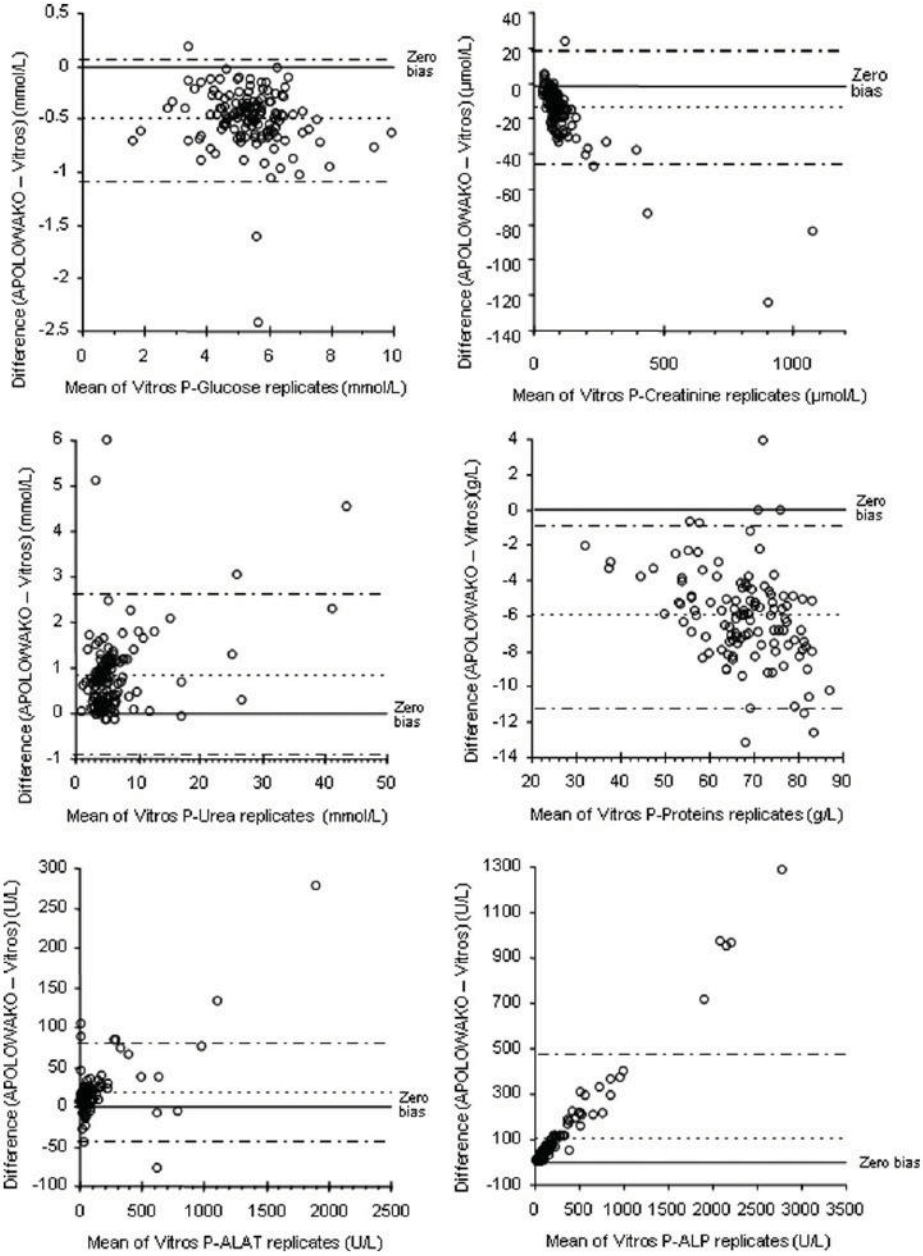

Fig. 2. Difference plot of results obtained in canine whole blood with APOLOWAKO and corresponding plasmas (P) with the Vitros analyser. Dotted lines are mean and 95% confidence interval of bias.

Table 4
Comparison of results obtained with APOLOWAKO in canine whole blood and corresponding plasma with Vitros: Spearman correlation coefficient, parameters of Passing–Bablok's equation

		n	*r*	*a*		*b*	
				mean	95% CI	mean	95% CI
Glucose	mmol/L	139	0.96	0.982	0.946 to 1.020	−0.381	−0.586 to −0.183
Creatinine	µmol/L	139	0.90	0.838	0.796 to 0.870	4.510	1.567 to 8.514
Urea	mmol/L	139	0.94	1.095	1.057 to 1.129	0.276	0.104 to 0.461
Total proteins	g/L	115	0.96	0.937	0.896 to 0.9805	−1.812	−4.637 to 0.943
ALT	U/L	138	0.88	1.117	1.079 to 1.152	5.821	4.037 to 7.841
ALP	U/L	137	0.95	1.575	1.542 to 1.607	−12.360	−16.208 to −8.397

$y = ax + b$, with $x =$ mean of replicates with the Vitros and $y =$ results of APOLOWAKO; CI = confidence interval.

- The assay principles were very similar. The main difference was for glucose: both analysers use enzymatic techniques but APOLOWAKO uses the hexokinase/glucose-6-phosphate dehydrogenase coupled reactions, which are often considered as a reference method (Pelletier and Arratoon, 1987), whereas Vitros analysis is based on the glucose oxidase/catalase reaction which allows more interference (Sacks, 1999). It can also be noted that Vitros ALT reagents include the coenzyme pyridoxal phosphate, although this had little effect on the results obtained in this study.
- The human reference intervals provided by the manufacturers were quite similar except for ALP which were much higher with APOLOWAKO. This difference probably does not result from the pH of the buffers, as an optimum of 10.40 has been reported for human serum, which would cause higher results by the Vitros (Tietz et al., 1983).

Results were thus expected to be very similar with both systems for glucose, urea and total proteins, moderately lower with the APOLOWAKO for creatinine and ALT, and notably higher with the APOLOWAKO for ALP.

A quality control of the APOLOWAKO could not be done with whole blood because the required controls are not commercially available. Home-made specimens would not have been stable, as shown previously (Thoresen et al., 1992). Quality control of the APOLOWAKO results with control sera was very good and performances were within the recommendations for total allowable error indicated in the clinical laboratory improvements amendments (CLIA) (Koch and Peters, 1999). Within-laboratory imprecision in this study was moderately higher than indicated by the manufacturer, especially for enzymes and low concentrations of urea. However, it was lower than the imprecision of other systems such as Spotchem (Trumel et al., 2005) or the Vitros (Table 1).

Centrifugation by the APOLOWAKO proved satisfactory as the results with whole blood and plasma were either identical or very similar. The slightly lower glucose concentration measured in plasma could not have resulted from the fact that the whole blood was analyzed first, so that a moderate delay occurred before centrifugation and permitted continued in vitro glycolysis. This delay was very short, usually <15 min. The average glycolysis in heparinized canine blood stored at room temperature was reported to be about 2.5% h^{-1} (Thoresen et al., 1992). So, even if the delay had been as long as half an hour, the decrease in P-Glucose would have been only approximately 0.05 mmol/L. No explanation could be found for the higher urea and creatinine concentrations measured in plasma than in whole blood. However, they were so slight that they have no clinical relevance.

Comparison of results obtained by APOLOWAKO and Vitros showed very low biases for urea and glucose but slightly higher than would be expected from comparison with the human reference intervals. The differences were also low for ALT and creatinine, except at very high concentrations. The differences were higher for total proteins and ALP. The protein measurement bias was unexpected and unexplained. This proportional bias was approximately 10% of the measured value and cannot be ignored: it was close to the estimated critical difference of 6.3 g/L for the clinical interpretation of plasma total protein concentration (Jensen and Aaes, 1993). It is similar to the difference observed between biuret and refractometry (Briend-Marchal et al., 2005) and warrants similar caution in the transferability of results between results of protein measurements obtained with different methods.

A proportional bias for ALP was not unexpected as measurement of the activity of this enzyme greatly depends on measuring conditions and buffers. The magnitude was of the same order as expected from comparison with the human reference values, i.e. the APOLOWAKO results were approximately 1.5 higher than the Vitros results.

As no canine reference intervals were provided by the APOLOWAKO manufacturer, it was impossible to determine whether the two analysers would give the same classification of the results as normal or abnormal. According to the Passing–Bablok's equations calculated for each analyte, it was possible to calculate canine reference intervals for APOLOWAKO from the Vitros canine reference interval (Anonymous, 1990). However, the latter cannot be a valid substitute to the determination of reference intervals according to recommended procedures (NCCLS, 2000).

In this preliminary study, where conditions were similar to veterinary routine in-practice use, the APOLOWAKO provided very good quality results in canine whole blood or plasma for the 6 analytes tested in this study. However, due to some analytical biases animal reference ranges will have to be determined and further validation of other analytes are needed before it can be extensively used in veterinary practice.

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