USEFULNESS OF TWO COMMERCIAL ANALYSERS FOR PLASMA CHEMISTRY IN PIGEONS

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ABSTRACT

A set of two biochemical analysers which is commercially available for use in animals was evaluated for the determination of alanine aminotransferase, albumin, alkaline phosphatase, amylase, aspartate aminotransferase, calcium, cholesterol, creatine kinase, creatinine, gamma-glutamyltranspeptidase, globulin, glucose, inorganic phosphate, lactate dehydrogenase, lipase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, sodium, potassium and chloride concentrations in pigeon plasma. The results indicated that the analysers are reliable for use in this bird species. Reference values for all parameters were established through examination of healthy pigeons.

INTRODUCTION

In recent years commercial biochemical plasma analysers have become available for use in human and veterinary medicine. The greatest advantage of these analysers lies in the fact that no time is lost in transport of samples to a laboratory and practitioners are therefore sooner able to make a correct diagnosis and/or initiate an appropriate treatment.

For veterinary practice in Belgium, the VetTest 8008 (Idexx Laboratories, The Netherlands) and the VetLyte (Idexx Laboratories) are available as commercial biochemical analysers. Former studies (Little *et al.*, 1992; Mischke *et al.*, 1992, Mischke *et al.*, 1993; Tschudi, 1995) have shown that the results of the VetTest 8008 for most parameters in mammals are consistent and well correlated with results from the classic laboratory techniques. The apparatus includes reference values for a broad range of animal species, but not for pigeons. Since pigeons are the most important birds in veterinary practice in Belgium and biochemical blood investigation could contribute to the diagnosis of pigeon diseases, it was the aim of this study to test these analysers with pigeon plasma and to establish pigeon reference values.

MATERIALS AND METHODS

Experimental animals

The experimental animals for this study were racing pigeons (Columba livia domestica) aged between 4 months and 5 years. These birds were obtained from a commercial pigeon-breeding centre (Kweekstation Natural, Zoersel, Belgium) as well as from various pigeon owners. The experimental group included male and female animals in equal frequency.

None of the pigeons examined had suffered from clinical disease within at least three months before sampling and upon further examination they appeared negative for the presence of *Salmonella, Trichomonas, Ascaridia* and *Capillaria.*

Blood collection

From each pigeon, a 1.5 ml volume of blood was collected from the *vena metatarsalis plantaris superficialis medialis* using heparin (15 IE/ml) as anticoagulant. Blood samplings were always performed on fastened pigeons. Similar numbers of pigeons were tested over the different months of the year.

Analyses

All analyses were performed immediately after collection of blood. For the determination of the packed cell volume (PCV), microtubes were centrifuged at 12,000g for 120 sec at room temperature. Plasma concentrations of alanine aminotransferase (ALT), albumin, alkaline phosphatase (AP), amylase, aspartate aminotransferase (AST), calcium, cholesterol, creatine kinase (CK), creatinine, gamma-glutamyltranspeptidase (γ-GT), globulin, glucose, inorganic phosphate, lactate dehydrogenase (LDH), lipase, total bilirubin, total protein (TP), triglycerides, urea nitro-

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gen and uric acid were determined using the VetTest 8008 analyser, software version 5.8 (Idexx Europe BV, The Netherlands). Plasma was obtained after centrifugation of whole-blood samples at 12,000 g for 95 seconds. The VetTest apparatus requires ten µl volumes of plasma for each parameter. The different biochemical tests are available as dry slides that include all necessary reagents. The analyser contains calibration curves for each slide and operates with three reflectometers at six different wavelengths. The analytic methods used by the apparatus are given in Table 2. The results of the analyses can be read after a maximum of 6 minutes.

Plasma concentrations of sodium, potassium and chloride were determined using the VetLyte (Idexx Europe BV, The Netherlands) analyser. The analyser uses the Ion Sensitive Electrode measurement principle to determine electrolyte values. With this method an unknown value is compared with a known value in a reference solution to compute the sample's electrolyte level. The calibration curve is determined by two-point calibration.

Quality control of analysers

The accuracy of the VetTest and the VetLyte analysers was examined every month by running a bovine reference serum for all the different parameters. Furthermore, the accuracy of the VetTest apparatus was specifically tested for the measurement of albumin in pigeon plasma. This was done since Lumeij *et al.* (1990) stated that the bromocresol green dye method, which is the basis of albumin measurement by the VetTest analyser, is not suited for pigeons. According to the same authors, pigeon albumin concentrations should be calculated on the basis of the total protein concentration obtained by the biuret method using a human standard and on the basis of the serum protein electrophoresis results. Therefore, the albumin concentration in the plasma of 40 pigeons was comparatively determined using the method of Lumeij *et al.* (1990) and the VetTest apparatus. To determine the degree to which the results of these two methods were linearly correlated, Pearson's correlation coefficient was calculated.

The determination of the degree of imprecision within a run of the VetTest 8008 for pigeon plasma was performed on a single plasma sample from one pigeon. The ammonia, TP, creatinine, urea and uric acid, AST, and AP and CK concentrations were measured as parameters to determine the imprecision of measurements performed by the apparatus at the first, second, third, fourth, fifth and sixth wavelengths, respectively. Twelve replicates of each parameter were performed simultaneously with the same analyser. The dry slides that were used for this test were all from the same batch and a single operator performed the analyses. The imprecision of the VetLyte was determined by examining one sample of pigeon plasma in 12 successive runs. For all measurements, the mean and the coefficient of variation were calculated. A variation coefficient of 20 was considered to be the upper limit for precise measurements (Büttner *et al.*, 1979).

Determination of reference values

The number of pigeons used to determine the reference values for the different parameters varied between 48 and 199 (Table 2). The reference values were established using a distribution-free method. For each variable the mean, the sample distribution and the limits of the $P_{2.5}-P_{97.5}$ percentiles within a probability range of 90% (Rümke and Bezemer, 1972; Farver, 1989) were calculated.

RESULTS

In the first quality control procedure carried out on the analysers, the measured value for ammonia in the bovine reference serum was higher than expected. It was quickly discovered, however, that this was probably associated with the use of a cleaning product containing ammonia in the environment. Once this product was no longer used, all measurements in the control serum always fell within normal ranges.

Figure 1 shows the results of comparative albumin measurements in the plasma of 40 pigeons by VetTest and the method described by Lumeij *et al.* (1990). The correlation calculated between the results of the two methods was 0.69 ($p< 0.001$).

Table 1 presents the results of the replicate analyses performed to determine the imprecision of the VetTest and VetLyte analysers. For ammonia, AP, AST, CK, calcium, creatinine, TP, urea, uric acid, sodium, potassium and chloride, the coefficients of variation were between 0 and 11.7.

The reference values for the different parameters in pigeon plasma are given in Table 2. In the course of establishing these reference values, no problems of any significance occurred, except in measuring the urea concentrations. In roughly 30% of the runs, the VetTest apparatus refused to show the concentrations for this parameter, and this remained constant, regard-

Table 1. Results of the "within run" imprecision test of 12 replicates of 11 parameters performed on pigeon plasma using the VetTest 8008 and the VetLyte analysers.

Parameter	Unit	Range	Mean	Coefficient of variation
Ammonia	µmol/l	4-7	4	11.7
AP	IU/l	90-97	93.6	2.7
AST	IU/l	100-120	107.8	6.3
Ca	µmol/l	1.90-1.99	1.92	1.21
CK	IU/l	572-602	585.5	1.4
Creatinine	µmol/l	6-8	6.8	8.4
TP	g/l	43-45	43.5	1.5
Urea	µmol/l	1.06-1.18	1.12	3.36
Uric acid	µmol/l	431-451	436.8	1.3
Sodium	mmol/l	148-149	148.92	0.19
Potassium	mmol/l	4.00-4.00	4.00	0
Chloride	mmol/l	115.0-115.0	115.0	0

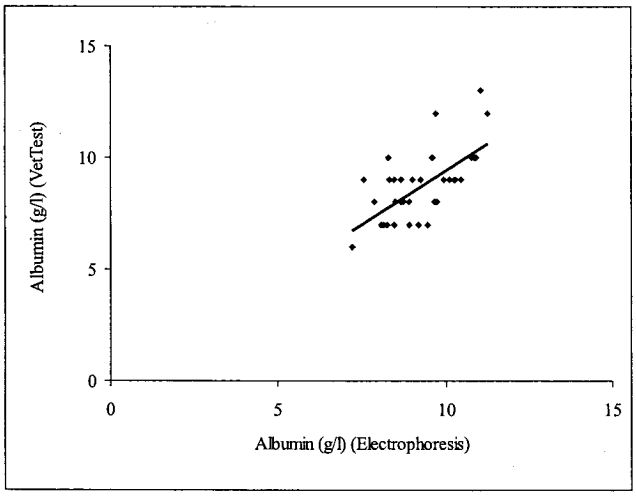


Figure 1. Albumin concentrations in pigeons determined by the VetTest 8008 and by serum protein electrophoresis.

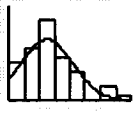
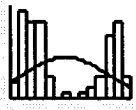
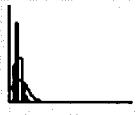
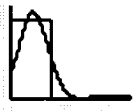
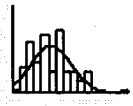
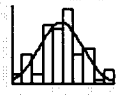
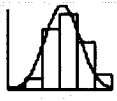
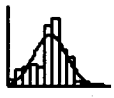
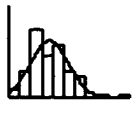
less of the batch of dry slides used. The cause of this problem has not yet been identified.

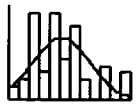
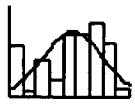
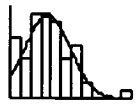
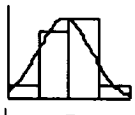
DISCUSSION

The present study demonstrates that the VetTest 8008 and the VetLyte analysers can reliably be used in the biochemical examination of pigeon plasma, provided that a few limitations are kept in mind. For a concise determination of ammonia in the plasma, it is important that no source of ammonia be present in the environment of the analyser. Analyses of urea concentrations sometimes fail. Nevertheless, these commercial analysers may serve as useful tools for the quick diagnosis and consequent treatment of diseased pigeons.

In this study, the concentration of different parameters was determined in plasma collected over different months of the year from a rather high number of pigeons of various ages, with equal numbers of males and females. These values can therefore be used as a general reference for blood chemistry analysis in pigeons, as long as the same analysers are used. Reference values for pigeons obtained through traditional laboratory techniques have been described earlier (Lumeij and De Bruijne, 1985; Lumeij and Wolfswinkel, 1988). For the most part, these values correspond very well with those established in this study, with the exception of the values for the enzymes and for creatinine. Enzyme reference values are often not comparable between laboratories since the determination of enzyme concentrations is highly dependent on temperature, pH, concentration of substrate and reaction time. The cause of differences within the nor-

Table 2. Reference values for biochemical parameters and PCV in pigeons, determined by the VetTest 8008 and VetLyte analysers.

	Units	N[1]	$P_{2.5}$[2]	$P_{97.5}$[2]	Mean[3]	Sample Distribution	Analytical basis
Enzymes							
AST/GOT	IE/l	104	17	191	79		Pyridoxal-5-phosphate activated method
ALT/GPT	IE/l	55	11	22	15		Pyridoxal-5-phosphate activated method
Amylase	IE/l	84	384	994	645		Amylopectin method
AP	IE/l	56	64	1160	292		Adenosine monophosphate method
CK	IE/l	199	166	629	349		N-Acetyl-cysteine activated, leuco dye method
gGT	IE/l	115	0	1	0		γ-Glutamyl-p-nitroanilide method
LDH	IE/l	111	133	321	217		Pyruvate to lactate method
Lipase	IE/l	65	6.2	73	39		Colipase, glycerol kinase, leuco dye method
Proteins							
Albumin	g/l	148	6	15	11		Bromocresol Green dye method
Globulin	g/l	144	18	29	22		Total protein minus Albumin
A/G ratio		108	0.27	0.70	0.51		
Total protein	g/l	149	24	41	33		Biuret method

Metabolites							
Ammonia	µmol/l	90	3	52	11		Bromophenol Blue method
Cholesterol	mmol/l	64	5.4	9.8	7		Enzymatic reaction
Creatinine	µmol/l	82	0	20	11		Enzymatic reaction
Glucose	mmol/l	48	12	22	17		Glucose oxidase method
Total bilirubin	µmol/l	73	0	9	2.2		Diazo dye method
Triglycerides	mmol/l	60	1.2	3.7	2.1		Glycerol kinase, leuco dye method
Urea	mmol/l	68	0.77	1.63	0.9		Ammonia indicator method
Uric acid	µmol/l	114	191	663	336		Uricase, leuco dye method
Ions							
Calcium	mmol/l	56	1.76	2.87	2.1		Arsenazo III dye method
Chloride	mmol/l	105	106	135	116		Ion sensitive electrode
Sodium	mmol/l	117	128	166	144		Ion sensitive electrode
Anorganic phosphate	mmol/l	60	0.42	2.33	1.6		Ammonium molybdate method
Potassium	mmol/l	111	3.03	5.00	4		Ion sensitive electrode
PCV	%	72	43	63	52		

[1] Number of pigeons sampled (N)

[2] Upper ($P_{97.5}$) and lower ($P_{2.5}$) reference values

[3] Mean values (Mean)

mal ranges of creatinine concentrations measured in the present and earlier (Lumeij and De Bruijne, 1985) studies are probably also due to the different techniques that were used. Indeed, the VetTest measurements for creatinine are based on an enzymatic reaction, while Lumeij and De Bruijne (1985) determine the concentration of this parameter by the Jaffé kinetic reaction.

Lumeij *et al.* (1990) postulated that the bromocresol green dye method, as it is used by the VetTest analyser, is not suited for the determination of albumin in pigeon blood. Nevertheless, in the present study a rather high correlation was calculated between the results of albumin measurements obtained by VetTest analysis and an alternative method proposed by Lumeij *et al.* (1990). This may indicate that the VetTest can be rather reliably used for the determination of albumin. Slight fluctuations due to the measurement method used are probably of limited clinical significance.

The present experiments indicated that the VetTest and VetLyte analysers carry out precise measurements for all tested parameters. Earlier studies with canine plasma also reported good precision in the VetTest analyses, except for the calcium measurements (Little *et al.*, 1992; Mischke *et al.*, 1993). Imprecise measurements of plasma calcium concentrations were also observed for pigeon samples when earlier software versions were being used (data not shown). After the introduction of the 5.8 version, this problem no longer occurred.

The study establishes the basis for the regular use of plasma chemistry as a diagnostic tool in pigeon medicine. The interpretation of changes in the concentration of plasma parameters is extremely difficult at the current time since the factors influencing the plasma profile in these birds have scarcely been studied. Further research on this subject is thus required.

REFERENCES

Büttner J., Borth R., Boutwell J.H., Broughton P.M.G., Bowyer R.C. (1979) Approved recommendation on quality control in clinical chemistry, *Clinica Chimica Acta 98*, 129-143.

Farver T.B. (1989) Concepts of Normality in clinical biochemistry. In: Kaneko J.J. (editor) *Clinical Biochemistry of Domestic Animals,* 4th edition, Academic Press, San Diego, 1-7.

Little C.J.L., Gettinby G., Downs J., Marshall C.B.M. (1992) Consistency of results from the VetTest 8008 clinical biochemistry analyser. *The Veterinary Record,* 419-424.

Lumeij J.T. and De Bruijne J.J. (1985). Blood biochemistry reference values in racing pigeons (*Columba livia domestica*). *Avian Pathology 14*, 401-408.

Lumeij J.T., De Bruijne J.J and Kwant M.M. (1990) Comparison of different methods of measuring protein and albumin in pigeon sera. *Avian Pathology 19,* 255-261.

Lumeij J.T. and Wolfswinkel J. (1988). Blood biochemistry reference values for use in columbine hepatology. *Avian Pathology 17*, 515-817.

Mischke R., Meinhof B., Wirth W. (1993). Messung ausgewählter klinisch-chemischer Mebgröben beim Hund mit VetTest 8008. *Monatshefte für Veterinarmedizin 48*, 493-500.

Mischke R., Schossier N., Wirth W. (1992). Trockenchemische Blutanalysen bei Hund und Katze mit dem VetTest 8008 im statistischen Vergleich mit naßchemischen Methoden. *Kleintierpraxis 37*, 183-200.

Rümke C.L. and Bezemer P.D. (1972). Methoden voor de bepaling van normale waarden II Nieuwe methoden, *Nederlands Tijdschrift voor Geneeskunde 116*, 1559-1568.

Tschudi P.R. (1995). Evaluation des Trockenchemie-Analysengerates VetTest 8008. *Schweizer Archiv für Tierheilkunde 137*, 381-385.