

# Stability of common biochemistry analytes in equine blood stored at room temperature

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## Summary

**Reasons for performing study:** Time delays between collection of blood samples and biochemical analysis of equine blood are unavoidably common in equine practice. The effect that delays may have on the accuracy of results of blood biochemical analyses is not well established.

**Hypothesis:** Delays in processing of blood of up to 72 h results in alterations in measured levels of common biochemical analytes that are of potential clinical relevance. Separation of serum prior to storage is protective against the effects of time delays.

**Methods:** Samples of clotted blood, separated serum and oxalate fluoride plasma from 20 horses were stored and analysed at 0, 24, 48 and 72 h. Graphical exploration of each analyte was undertaken. General linear models with fixed effects were fitted for the whole blood data. The mean bias and 95% limits of agreement were calculated, using bootstrapped data, to assess agreement between pairs of samples analysed at 0 h and other time points. Bland-Altman plots were used to explore general trends in the data. Paired *t* tests were used to compare the results from whole blood and separated serum.

**Results:** Delays in processing equine blood resulted in significant increases in measured concentrations of aspartate aminotransferase, creatine kinase, lactate dehydrogenase, total bile acids and magnesium. A significant decrease in concentration was identified for glucose (serum and oxalate fluoride preserved plasma). Separation of serum immediately following clot formation resulted in nonsignificant increases in accuracy for some analytes.

**Conclusions and practical significance:** Delays in processing of blood samples may result in biochemical changes of clinical relevance in individual cases; however, in the majority of cases, where delays are only a few days and a number of analytes are assessed concurrently, delays are unlikely to have an effect on the interpretation of results. Separation of serum following clot formation is of limited benefit. Clinical samples in which a delay in processing has occurred may be interpreted with reference to the data presented.

## Introduction

Clinical chemistry and laboratory medicine have become a fundamental part of the investigation of equine disease. The interval between sample collection and processing should be minimised to prevent *ex vivo* changes in analyte activities and concentrations. However, in ambulatory equine practice there is an inevitable delay between collection and laboratory processing with samples commonly being mailed to specialist laboratories. Specialist referral laboratories may be more experienced in processing samples from a particular species, have rigorously tested and quality controlled equipment calibrated to the species, provide established laboratory specific reference ranges and often provide interpretation by experienced clinicians. Although delays in processing occur commonly, there is little information in the literature of the influence that such delays may have on the clinical interpretation of equine clinical chemistry results.

In human medicine it is recognised that blood cell constituents can interfere directly with the measurement of analytes; for example measurements of creatine kinase (CK) activity may be falsely increased by adenylate kinase released from erythrocytes and free haemoglobin may interfere with assessment of bilirubin and optical methods of assessment of other analytes (Banfi *et al.* 2002). The concentration of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and potassium (K<sup>+</sup>) in human erythrocytes is substantially greater (20–160 times) than in plasma; and haemolysis may be expected to cause increases in these analytes (Caraway 1962). Conversely, haemolysis may result in dilution of analytes, such as sodium and calcium, that are present at lower concentrations in erythrocytes than plasma or serum (Caraway 1962). In human studies, haemolysis was found to result in increases in measured values for AST, LDH, CK, K<sup>+</sup>, magnesium (Mg) and phosphate and decreases in bilirubin (Laessig *et al.* 1976; Frank *et al.* 1978). A haemoglobin concentration of approximately 0.3 g/l is necessary before discolouration of serum may be appreciated grossly (Banfi *et al.* 2002) and it is possible that haemolysis might affect clinical chemistry results in the absence of gross changes to serum or plasma detectable to the human eye.

The results of previous studies investigating the effects of storage on biochemistry analytes in domestic animals are conflicting, vary between species and are likely to be influenced by different laboratory methods (Jones 1985a,b). Previous

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investigations have determined the effects of storage on sorbitol dehydrogenase, ammonia and ionised calcium in equine blood (Ogilvie *et al.* 1985; Sherman *et al.* 1991; Schenck *et al.* 1996). The effect of storage on a wider panel of biochemical analytes in equine blood has been investigated in one study, although only 5 horses were used and some of the analytical and statistical methods have now been superseded (Fontaine *et al.* 1987).

The aims of the present study were: 1) to assess the effects of storage on measurement of common clinical chemistry analytes in equine blood and whether any changes are likely to be clinically relevant; and 2) to determine whether separation of serum from clotted whole blood prior to a delay in processing was protective against artefactual changes. Study conditions were chosen to replicate those that commonly occur prior to processing in a specialist referral laboratory.

## Materials and methods

### Sample collection and storage

Blood samples were obtained from horses hospitalised at The Liphook Equine Hospital that underwent blood collection to investigate a range of medical and surgical conditions. Blood was collected from each horse into further tubes coated with micronised silica clot activator<sup>1</sup>. Once serum had separated, samples were processed within 3 h of collection (0 h). Thereafter, serum from one of the clotted tubes was removed and stored while further tubes were stored without separating the serum. In addition, blood was collected into 2 tubes containing potassium oxalate and sodium fluoride<sup>1</sup> (OxF) and each was processed in a similar manner; plasma was separated from one tube and the remainder were left as whole blood. Analysis was performed at 24, 48 and 72 h. Samples were stored in a laboratory out of direct sunlight with ambient temperature maintained at 20–25°C. Biochemical analyses were performed using a wet chemistry analyser (Synmered IR 202 model 200A)<sup>2</sup>. The methodology employed is outlined in Table 1. All data were stored and queried in an Excel (Microsoft) database and statistical analyses were undertaken using R<sup>3</sup>.

General linear models with fixed effects were fitted for each analyte measured in whole blood. For each model, outcome was

defined as the results of the analyte for time-points 24, 48 and 72 h. The model was offset against the results for 0 h and time was included as a fixed effect. Models were undertaken using linear and log-linear transformed data and model selection was based on maximum results for Akaike information criterion (AIC), a measure of goodness of fit for each model. Significance was set at  $P < 0.05$ .

Bland-Altman analyses were undertaken for all analytes with nonsignificant results to assess whether the lack of significant difference could be interpreted as 'agreement' in results at the

**TABLE 2: Results of linear and log-linear<sup>#</sup> models for 18 analytes performed on whole blood from 20 horses and stored over 72 h. Significant results are indicated ( $P < 0.05$ )<sup>\*</sup>**

Analyte	Factor	Estimate of difference over time	Standard error	P value
Gluc (plain)	Time (24 h)	-2.62	0.55	<0.001*
	Time (48 h)	-3.59	0.55	<0.001*
	Time (72 h)	-3.71	0.55	<0.001*
Glucose (OxF)	Time (24 h)	-0.72	0.31	0.03*
	Time (48 h)	-0.83	0.31	0.01*
	Time (72 h)	-1.00	0.32	0.002*
TP	Time (24 h)	0.35	0.52	0.50
	Time (48 h)	0.75	0.52	0.15
	Time (72 h)	0.90	0.52	0.09
Alb	Time (24 h)	0.35	0.32	0.28
	Time (48 h)	0.55	0.32	0.09
	Time (72 h)	0.40	0.32	0.22
AST	Time (24 h)	27.60	14.26	0.06
	Time (48 h)	49.45	14.26	0.001*
	Time (72 h)	80.65	14.26	<0.001*
CK	Time (24 h)	29.65	22.44	0.19
	Time (48 h)	37.65	22.44	0.10
	Time (72 h)	58.20	22.44	0.01*
GGT	Time (24 h)	-3.05	1.07	0.005*
	Time (48 h)	-1.70	1.04	0.11
	Time (72 h)	-2.05	1.04	0.05
LDH	Time (24 h)	136.25	37.62	<0.001*
	Time (48 h)	191.65	37.62	<0.001*
	Time (72 h)	233.35	37.62	<0.001*
tBA	Time (24 h)	1.94	1.34	0.15
	Time (48 h)	2.21	1.39	0.11
	Time (72 h)	3.04	2.26	0.03*
Urea	Time (24 h)	0.24	0.29	0.41
	Time (48 h)	0.93	0.29	0.002*
	Time (72 h)	1.82	0.29	<0.001*
Mg	Time (24 h)	0.09	0.03	<0.001*
	Time (48 h)	0.10	0.03	<0.001*
	Time (72 h)	0.12	0.03	<0.001*
Globulin	Time (24 h)	$1.7 \times 10^{-9}$	0.43	1.00
	Time (48 h)	0.2	0.43	0.65
	Time (72 h)	0.5	0.43	0.25
SAA <sup>#</sup>	Time (24 h)	-0.15	0.08	0.06
	Time (48 h)	-0.26	0.09	0.004*
	Time (72 h)	-0.34	0.09	<0.001*
GLDH <sup>#</sup>	Time (24 h)	0.02	0.07	0.80
	Time (48 h)	-0.27	0.10	0.01*
	Time (72 h)	-1.48	0.33	<0.001*
ALP	Time (24 h)	-3.45	6.51	0.60
	Time (48 h)	-3.65	6.51	0.58
	Time (72 h)	1.15	6.51	0.86
tCa	Time (24 h)	0.06	0.04	0.13
	Time (48 h)	0.06	0.04	0.16
	Time (72 h)	0.01	0.04	0.80
Creatinine	Time (24 h)	-5.00	4.75	0.30
	Time (48 h)	-1.42	4.75	2.77
	Time (72 h)	5.62	4.75	0.24
tBil	Time (24 h)	1.85	3.96	0.64
	Time (48 h)	-2.40	3.96	0.55
	Time (72 h)	-2.86	3.96	0.47

**TABLE 1: Wet chemistry methods used to determine concentrations of the analytes investigated in the present study**

Analyte	Method
Albumin	Colourimetric in the presence of bromocresol green
ALP	p-Nitrophenylphosphate hydrolysis in diethanolamine buffer
AST	Kinetic determination (Bergmeyer 1986)
tBA	Oxidation and NADH production
tBil	Controlled oxidation
tCa	Arsenazo III
CK	Adenosine diphosphate production
Creatinine	Kinetic jaffe
Globulin	Mathematical deduction
GLDH	Enzymatic
GGT	Kinetic assay. Gamma-L-glutamyl-p-nitroanilide
Glu Plain	Glucose oxidase
Glu OxF	Glucose oxidase
LDH	Lactate to pyruvate
Mg	Xylidyl blue
TP	Modified biuret
SAA	Latex agglutination
Urea	Urease

**TABLE 3: Intrasample variance and the effects of storage (for 24, 48 and 72 h) on equine whole blood and serum. Mean bias for each analyte at different time points is shown with the 95% limits of agreement (LOA) indicated in brackets. Results are shown for the 14 analytes in which no significant differences were identified at all time points using general linear models**

Analyte	Intrasample variance	Whole blood - mean bias (95% LOA)			Serum - mean bias (95% LOA)		
		24 h	48 h	72 h	24 h	48 h	72 h
<b>Albumin</b> (g/l)	-0.38 (-1.81, 0.86)	0.35 (-1.46, 2.41)	0.55 (-1.74, 2.46)	0.40 (-1.74, 2.70)	0.06 (-2.23, 1.76)	0 (-0.99, 1.45)	0.22 (-1.45, 1.90)
<b>ALP</b> (iu/l)	-1.63 (-16.4, 12.4)	-3.45 (-32.5, 41.3)	-3.65 (-37.6, 56.3)	1.15 (-29.9, 66.4)	-16.1 (-38.9, 11.8)	-8.89 (-45.6, 30.6)	-8.72 (-39.8, 24.7)
<b>AST</b> (iu/l)	0.38 (-5.28, 3.86)	27.6 (-6.01, 115)	49.5 (-4.21, 159)	80.7 (-10.1, 233)	22.2 (-3.80, 124)	44.2 (-6.54, 167)	55.7 (-12.1, 198)
<b>tBA</b> ( $\mu$ mol/l)	0.40 (-2.35, 3.85)	1.56 (-2.53, 9.60)	1.29 (-3.26, 13.0)	2.43 (-3.36, 15.9)	1.77 (-2.10, 8.72)	2.38 (-3.29, 10.8)	1.64 (-3.99, 7.81)
<b>tBil</b> (mmol/l)	-0.13 (-6.05, 3.88)	1.85 (-8.33, 34.9)	-2.4 (-17.0, 32.5)	-2.86 (-21.0, 34.4)	0.92 (-12.4, 36.3)	0.53 (-11.2, 39.9)	-2.73 (-14.7, 31.7)
<b>tCa</b> (mmol/l)	0.04 (0.00, 0.10)	0.06 (-0.29, 0.29)	0.06 (-0.29, 0.29)	0.01 (-0.24, 0.19)	0.01 (-0.24, 0.20)	0.05 (-0.28, 0.24)	0.02 (-0.32, 0.24)
<b>CK</b> (iu/l)	14.4 (-11.7, 72.7)	29.7 (-120, 131)	37.7 (-129, 240)	58.2 (-90.0, 259)	16.8 (-123, 171)	23.3 (-124, 169)	45.5 (-139, 228)
<b>Creatinine</b> ( $\mu$ mol/l)	10.9 (-15.8, 39.4)	-5.00 (-33.7, 29.9)	-1.42 (-27.9, 39.4)	5.62 (-24.8, 48.2)	-5.33 (-25.6, 16.0)	-4.17 (-25.6, 21.1)	5.72 (-28.7, 34.2)
<b>Globulin</b> (g/l)	0.75 (-0.44, 1.59)	0 (-3.73, 2.82)	0.20 (-3.69, 2.41)	0.50 (-3.20, 2.46)	0.50 (-3.87, 4.75)	0.44 (-3.17, 3.63)	0.33 (-3.3, 1.99)
<b>GLDH</b> (iu/l)	-0.08 (-1.13, 0.70)	0.50 (-1.85, 2.96)	0.32 (-6.36, 5.63)	-1.29 (-17.3, 5.05)	0.17 (-2.26, 2.42)	0.48 (-2.64, 2.82)	-1.00 (-7.22, 1.96)
<b>GGT</b> (iu/l)	-2.38 (-5.51, -0.09)	-2.90 (-10.1, 2.53)	-1.70 (-8.53, 4.95)	-2.05 (-10.9, 7.41)	-4.61 (-11.7, 2.64)	-5.11 (-10.2, 0.89)	-4.39 (-11.7, 3.14)
<b>TP</b> (g/l)	0.38 (-0.84, 1.58)	0.35 (-3.75, 3.37)	0.75 (-3.67, 3.42)	0.9 (-4.00, 3.36)	0.56 (-3.85, 4.81)	0.44 (-3.32, 3.21)	0.56 (-4.28, 2.96)
<b>SAA</b> (mg/l)	-1.19 (-5.56, 0.00)	-4.63 (-47.5, 13.0)	-8.2 (-66.7, 10.4)	-10.5 (-97.7, 12.6)	-11.4 (-82.3, 11.0)	-12.3 (-101, 25.9)	-3.76 (-105, 112)
<b>Urea</b> (mmol/l)	-0.09 (-0.25, 0.13)	0.24 (-1.07, 1.12)	0.93 (-0.56, 3.12)	1.82 (-0.17, 4.62)	-0.04 (-0.92, 0.52)	0.87 (-0.21, 2.83)	1.33 (-0.56, 5.09)

various time points analysed. The mean bias (mean of the difference between pairs of data) and mean 95% limits of agreement (LoA) (95% confidence intervals) were calculated using bootstrapped data, to assess agreement between pairs of samples analysed at 0 h, and each of 24, 48 and 72 h, for clotted blood and separated serum (Bland and Altman 1986). The same analyses were undertaken to assess intrasample agreement (repeatability) using results of 2 analyses performed on the same blood samples at 0 h. Nonparametric bootstrapping was undertaken using @RISK (Palisade UK) over 10,000 iterations. Data were graphed using Bland-Altman LoA plots to explore general trends (Bland and Altman 1986). Each graph plotted the mean of the measurements at 2 time points against their difference for each horse.

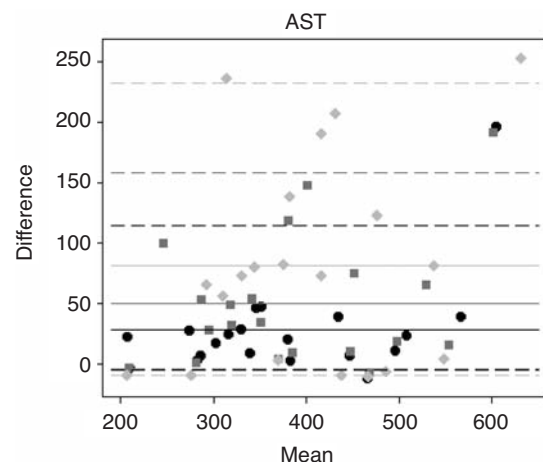
Results were interpreted with reference to the 'expected' agreement, determined from the intrasample analyses, and with consideration of predetermined levels of variation that might be of potential clinical relevance.

## Results

Linear models were used for all analytes with the exception of serum amyloid A (SAA) and glutamate dehydrogenase (GLDH), for which log-linear models were used (Table 2). Analysis of the general linear models revealed that statistically significant reductions occurred at all time points for glucose (in clotted blood and OxF plasma) while significant increases occurred for LDH and Mg at all time points. Significant reductions were found at 48 and 72 h for GLDH and SAA, significant increases were identified at 48 and 72 h for AST and urea. Significant increases were identified at 72 h only for CK and total bile acids (tBA). A significant reduction at 24 h was identified for  $\gamma$ -glutamyl

transferase (GGT); however, there were no significant differences at either 48 or 72 h.

Results for intrasample variance (ISV), the mean bias and 95% LoA for both whole blood and serum at each time point are presented in Table 3. Albumin, globulin, total protein, alkaline phosphatase (ALP), total calcium (tCa), GLDH and GGT had a



*Fig 1: Bland-Altman plot of measurement of aspartate aminotransferase (AST) in whole blood at different time points (24, 48, 72 h) compared with results at 0 h. Circles indicate the results at 24 h compared to 0 h, squares the results at 48 h compared to 0 h and diamonds the results at 72 h compared to 0 h. The plot therefore demonstrates that the differences increase over time (the diamonds representing 72 h results are generally spread further from 0 difference on the y axis than the circles representing results at 24 h). Solid lines represent mean bias and dashed lines 95% limits of agreement.*

decrease in accuracy of measurement with the 95% LoA indicating both increases and decreases in measured values beyond what was expected from the ISV results. For all of these analytes there was progressive divergence from the 0 h values with time. The results for GLDH indicated reduced agreement at higher initial values.

Increases in measured values with storage time were identified for AST, BA, CK, and urea supporting the results of the general linear models. Aspartate aminotransferase (Fig 1) and urea increased progressively with time. In contrast, tBA increased markedly within the first 24 h with lesser increases occurring between 24 and 72 h. Whereas changes in CK were greater in a positive direction, LoA expanded in both directions over time indicating a general loss of accuracy.

Reductions in measured values over time were identified for bilirubin and SAA. For all of these analytes, decreases were marked over the first 24 h with smaller reductions at 48 h and 72 h. The dataset for SAA was smaller than for other analytes as SAA was not detected in blood or serum from 7 of the horses in the study. From the plots it was apparent that SAA levels changed very little in the majority of subjects; however, large changes in 3 samples resulted in wide LoA.

The results of paired *t* tests did not indicate any significant differences at any time points between whole blood and serum samples. The results of the Bland-Altman analyses indicated greater agreement over different time points for measurements of GLDH in serum compared to whole blood. Lesser, but improved, agreement over time in serum compared to whole blood was noted for albumin, BA, bilirubin, creatinine, globulin, glucose, LDH, TP and urea. For the remaining analytes, there was no apparent difference in agreement over time for serum compared to whole blood.

## Discussion

The results of this study indicate that changes in blood biochemical analytes may be clinically relevant when delays in processing occur. Increases in concentrations of intracellular constituents, such as AST, LDH, CK and Mg, occurred in whole blood over time. In human studies haemolysis results in increases in measured values for AST, LDH, CK, K, Mg and phosphate and clinical chemistry results may be affected prior to identifiable colour changes in serum or plasma (Laessig *et al.* 1976; Frank *et al.* 1978). Changes in concentrations of potassium and phosphate were not investigated in the present study because of financial constraints. Results from the linear models allow prediction of average increases over time; at 72 h AST increased on average by 81 iu/l, LDH by 233 iu/l, CK by 58 iu/l and Mg by 0.12 mmol/l. While increases in concentrations of these analytes in separated serum were lower in magnitude the differences between whole blood and serum did not attain statistical significance. Release of these analytes from erythrocytes and leucocytes as a result of transmembrane leakage or cell lysis prior to separation of serum may have occurred. Leakage of analytes from platelets following separation is an alternative explanation and has been observed to occur in human, ovine and murine serum (Friedel and Mattenheimer 1970; Caisey and King 1980; Jones 1985a).

Increased blood concentrations of AST, CK and LDH are indicative of damage to myocytes; delays in sample processing may therefore result in false positive diagnosis of myopathy. The authors have noted that increases in these enzymes occur more commonly in warmer weather in mailed samples (D. I. Rendle, unpublished

observations). The effect of temperature on blood biochemistry analytes in stored samples merits further investigation.

Total protein, albumin and globulin concentrations both increased and decreased over time. The changes at all time points were not statistically significant and unlikely to be of clinical relevance. When protected from denaturation by heat, ultraviolet light, chemicals or enzymes, blood proteins remain stable for several days at room temperature (Banfi *et al.* 2002).

The biochemical analytes used as markers of hepatic disease were sensitive to delays in processing. Statistically significant increases in aspartate aminotransferase (80 iu/l at 72 h) and bile acids (3.04  $\mu$ mol/l at 72 h) occurred with time while bilirubin decreased, although this change did not attain significance. Decreases in bilirubin concentration identified may also be attributed, at least in part, to the dilutional effect of haemolysis. In the present study, measures were taken to protect samples from direct sunlight; however, the samples were not kept in total darkness and photo-oxidation may have contributed to the reduction in bilirubin over time in both whole blood and in separated serum.

A significant decrease in GGT occurred at 24 h; however, the validity of this result is questionable as the trend did not continue and there were no significant decreases at either 48 h or 72 h. The presence of both increases and decreases in GGT in the present study is in accordance with disparate results reported in studies of human and canine whole blood (Thoresen *et al.* 1992; Heins *et al.* 1995). However, in canine, bovine and ovine serum, concentrations of GGT are reportedly stable for up to 3 days (Jones 1985a,b; Thoresen *et al.* 1992). Changes in GLDH followed a more consistent trend with significant reductions occurring at 48 h and 72 h. Glutamate dehydrogenase was the only analyte for which accuracy was clearly reduced with higher initial concentrations.

Intuitively, analytical errors would be expected to be magnified with higher analyte concentrations and it was surprising that this trend was not identified for more of the analytes. Fortunately, most clinicians assess the activity of a number of enzymes derived from hepatocytes and biliary epithelial cells in addition to bile acids and bilirubin and the likelihood of all of these results being affected significantly due to delays in processing would seem very small.

Reductions in the concentration of glucose were expected in whole blood as glycolysis is known to occur in stored blood in as little as 10 min (Banfi *et al.* 2002). Glucose concentration in whole blood decreased, on average, by 2.6 mmol/l after 24 h and by 3.7 mmol/l after 72 h. Glycolysis is more pronounced if cellular concentrations in blood are increased (for example during leucocytosis) or storage temperatures are increased (Sunderman *et al.* 1956). While laboratory temperature was controlled in this study, cell counts of blood samples were not recorded. Reductions in the concentration of glucose in separated serum between 0 h and 24 h and between later time points were unexpected. The magnitude of these changes was larger than the ISV for glucose so this trend was considered relevant and may have been due to a failure to separate all cellular constituents from the serum. While the reductions in glucose concentrations were smaller in the OxF samples compared to the reductions that occurred in both whole blood and in separated serum, they still attained statistical significance from 24 h. In OxF samples concentration of glucose decreased, on average, by 0.7 mmol/l after 24 h, 0.8 mmol/l after 48 h and 1 mmol/l after 72 h. In human medicine it is recognised that preservatives do not completely inhibit glycolysis in blood (Waring *et al.* 2007).

Blood concentrations of urea and creatinine, indicators of nephropathy, were susceptible to both increases and decreases over time, although none of these changes was statistically significant.

Intrasample variance was greater than expected for many of the analytes. While the mean bias for all the analytes, except creatinine and magnesium, was within the acceptable limits as defined by the WHO (Banfi *et al.* 2002), the upper and lower 95% LoA for many of the analytes were beyond these limits. This inaccuracy may be largely explained by the small dataset used to determine the ISV. Had a larger number of horses been used, the validity of values may have been more robust. Furthermore, for ethical reasons, the samples used to determine the ISV were not collected from clinically healthy animals but determined from blood from clinical cases. Previous studies have identified increased levels of biological variation when subjects with different pathologies are used rather than healthy subjects (Ricos *et al.* 1999). Day-to-day variations may have reduced the accuracy of the results for changes with time as precision is expected to be lower day-to-day than within-run and within-day because of a greater number of variables such as reagent decay and operator idiosyncrasies (Kramer and Hoffmann 1998).

The results of the present study illustrate that, although separation of serum results in greater accuracy, especially in the measurement of GLDH, this effect was not statistically significant for any of the analytes. The reason for the lack of stability of analytes within separated serum in the present study is unclear. The results appear valid as group trends in the serum data are apparent and reflected the trends seen in whole blood. A logical explanation would be an excessive serum-clot contact time; however, serum was separated within a few hours, which is in accordance with recommendations derived from investigations performed with human blood (Zhang *et al.* 1998). If contact time is prolonged, the biological activity of the cells and transmembrane diffusion can change the concentrations of certain analytes. Minimum clotting time for human blood is suggested to be 20–30 min (Zhang *et al.* 1998); the authors are unaware of specific recommendations for horses. The maximum permissible delay between time of collection and separation is dependant upon the stability of individual analytes. While many analytes may be stable for 24 h a maximum limit of 2 h is commonly recommended (Zhang *et al.* 1998). Species variations may exist and further work investigating the effects of serum-clot contact time in equine blood may be warranted.

In conclusion, delays in processing equine blood resulted in significant increases in AST, CK, LDH, tBA, Mg and decreases in glucose (plain and OxF) that may affect clinical interpretation in individual cases. However, in the majority of cases changes of the magnitude identified are unlikely to alter the interpretation. The likely influence of *in vitro* changes on clinical interpretation should be considered on a case-by-case basis with reference to the data presented. Separation of serum immediately following clot formation resulted in nonsignificant increases in accuracy for many of the analytes. However, changes that might affect clinical interpretation still occurred and hence separation prior to delays processing appears of limited value.

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#### Manufacturers' addresses

<sup>1</sup>Vacurette, Greiner Bio-One Ltd, Stonehouse, Gloucester, UK.

<sup>2</sup>Synermed Europe Ltd, Burgess Hill, West Sussex, UK.

<sup>3</sup>R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>

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