Bias in feline plasma biochemistry results between three in-house analysers and a commercial laboratory analyser: results should not be directly compared

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Randolph M Baral[1,2], Navneet K Dhand[2], John M Morton[3], Mark B Krockenberger[2] and Merran Govendir[2]

Abstract

In-house analysers are commonplace in small animal practices but cannot be calibrated by the operator; therefore, any bias in the generated plasma analyte values cannot be corrected. Guidelines such as grading of renal disease and published reference intervals (RIs) in veterinary textbooks assume plasma biochemistry values generated by different analysers are equivalent. This study evaluated the degree of bias, as well as if bias was constant or proportional, for feline plasma biochemical analytes assessed by three in-house biochemistry analysers compared with a commercial laboratory analyser. Blood samples were collected on 101 occasions from 94 cats and, after centrifugation, plasma was divided into four aliquots. One aliquot was sent to the commercial laboratory and the remaining three were tested using the in-house biochemistry analysers. Results from each analyser were compared with the commercial laboratory results by difference plots and analyses, and by comparing percentages of results within provided RIs. Substantial bias was evident relative to the results of the commercial analyser for at least half of the analytes tested for each machine. In most cases, bias was proportional, meaning that the difference between the methods varied with the concentration of the analyte. The results demonstrate that values obtained from these analysers should not be directly compared and that RIs are not transferable between these analysers. Potential effects of bias on clinical decision making may be overcome by use of appropriately generated RIs, or reference change values which, for most biochemistry analytes, are more appropriate than subject-based RIs.

Introduction

In-house veterinary biochemistry analysers are commonplace in small animal practice. While such analysers provide useful information for point-of-care decision making, they cannot be calibrated by the end user (veterinary practice personnel). Analytical methodologies often differ between in-house and commercial laboratory analysers, and manufacturers of in-house analysers often provide reference intervals (RIs) that vary from published RIs[1,2] for many analytes. The fact that there is variation of RIs between analyser types suggests there are systematic differences (or 'bias') between results generated by different analyser models. Bias can be classified as constant where the average difference in results from an established method remains the same, regardless of the concentration being measured, or proportional, where the average difference in results increases(or decreases) with increasing analyte concentration. Other studies have indicated that in-house analysers show bias for several

1 Paddington Cat Hospital, Paddington, NSW, Australia
2 Faculty of Veterinary Science, The University of Sydney, Sydney,NSW, Australia
3 Jemora Pty Ltd, Geelong, Victoria, Australia

analytes compared with results determined by commercial laboratory analysers when analysing feline samples.[3–8] However, these studies have mostly been small (<26 cats)[3,4] or assessed less commonly used analysers.[5–7] None of these studies assessed multiple analysers concurrently, nor specifically assessed or categorized bias. Bias between analysers is important because, in feline veterinary practice, many recommendations assume biochemistry results generated from different analysers are equivalent. Examples of this include the International Renal Interest Society (IRIS) renal disease grading system,[9] diagnosis of diabetes mellitus,[10] the American College of Veterinary Internal Medicine proteinuria consensus statement[11] and published RIs in textbooks.[1,2] Additionally, practitioners who use a particular model of in-house analyser may be required to interpret results from another analyser (eg, when receiving results from another practice or a commercial laboratory). In this situation, anecdotally, most practitioners seem to interpret results on the basis of if they are within, above or below the provided RI.

Unlike method comparison studies, which aim to determine the amount of error that exists in the instrument being evaluated compared with that of a well-characterised instrument/method,[12] the aim of this study was to assess whether bias existed for routine feline biochemical analytes in plasma by three commonly used in-house veterinary analysers when compared with a commercial laboratory analyser, and further, if bias existed, to assess whether it was constant and/or proportional.

Materials and methods

Subjects and sampling

All plasma samples (n = 101) that were collected from 94 cats seen in a primary accession, feline-only veterinary practice (Paddington Cat Hospital, Sydney, Australia) over a 2 month period were included. Cats ranged in age from 16 weeks to 20 years; 49 cats were male (six entire) and 45 were female (five entire); 48 were domestic shorthairs, 14 were Burmese, six were Siamese, five were Russian Shorthairs and the remainder were other breeds (Devon Rex, Cornish Rex, Maine Coon, Persian or Himalayan). At the time of testing, cats were clinically healthy (63 samples) or clinically unwell (38 samples). Blood was collected from each cat by single jugular venepuncture into a lithium heparin tube. Each tube was centrifuged at 4000 rpm (1790 *g*) for 5 mins within 30 mins of collection, and then the plasma was immediately divided into four aliquots. One aliquot was sent by courier to a commercial laboratory (Gribbles Veterinary Laboratory, Rhodes, Sydney, Australia) and processed by a Cobas-Integra 400 biochemical analyte analyser; the remaining three were immediately tested on three in- house biochemistry analysers: Abaxis Vetscan VS-2 Point of Care Analyser, Heska Dri-Chem Veterinary Chemistry Analyser, and an IDEXX VetTest VT8008 and an IDEXX VetLyte (IDEXX has a separate instrument to measure electrolytes) for the following 13 biochemistry analytes: albumin, alkaline phosphatase (ALP), alanine amino transferase (ALT), total bilirubin, calcium, chloride (not available for Abaxis), creatinine, glucose, phosphate, potassium, sodium, total protein and urea. This resulted in a total of 38 analyte determinations across all three in-house analysers (three determinations for 12 analytes and two determinations for one analyte [chloride]). When a result was 'out of range', the sample was diluted with 0.9% saline and the particular analyte was reanalysed.

Laboratory methods

The methods by which each instrument determined the concentration for each analyte are summarised in Table 1.

The Cobas-Integra 400 analyser at the commercial laboratory was calibrated according to manufacturer's recommendations. The Heska and Abaxis analysers were installed to the manufacturers' specifications by their respective local agents. Although 10 years old and already present at the testing site, the IDEXX analysers had been serviced (by the manufacturer) 3 months prior to the assessment period.

The precision of all four analysers was assessed using commercial quality control materials (at two concentrations) approximately every second day over a 1 month period and was found to be similar for all four analysers.[13]

Statistical analyses

Descriptive analyses were conducted for each analyte. The results for each analyte on each analyser were compared with the commercial laboratory results using the Bland–Altman approach.[14] Difference (limits of agreement; Bland–Altman) plots (scatterplots of differences between results from each in-house analyser and the commercial laboratory for the sample plotted against the means of the two results) were assessed. The average difference was calculated as the mean of the differences in results between each in-house analyser and the commercial laboratory.

The mean and SD of the differences in results between each in-house analyser and the commercial laboratory for each analyte were then used to calculate 95% limits of agreement (mean difference ± 1.96 x SD). The mean difference in results between each in-house analyser and the commercial laboratory for each analyte was also expressed as a percentage of the mean result for each analyte on each analyser, thus enabling comparison between analytes. The range of the 95% limits of agreement was similarly expressed as a percentage of the mean result for each analyte on each analyser.

Proportional bias was assessed using both the correlation coefficient for the association between the differences and the means of the two measurements and the F-test of equality of means and variances. [15] Proportional bias was considered to be present when the correlation coefficient was greater than 0.15 (Table 2) and the *P* value for the F-test was <0.05. Where proportional bias was considered to be present, the value of the correlation coefficient was used to grade the degree of proportionality as shown in Table 2.

Proportional bias was considered as mutually exclusive to constant bias since, in a practical context, knowing the extent of constant bias is only useful when the slope is close to 1.

A system was devised to categorise the presence or absence, type and degree of bias for each analyte from each analyser (Table 2). Analyses were performed with Stata (version 11; Statacorp) and R (http://www.r-project.org/).

Although some cats contributed more than one plasma sample (seven cats contributed two samples), because the mean number of samples per cat was close to 1, results from each sample were assumed to be statistically independent of results from all other samples.

Table 1 Assay method for analyte concentrations by the Abaxis VetScan, IDEXX VetTest/VetLyte and Heska Dri-Chem analysers, and by a Cobas-Integra biochemistry analyser at a commercial laboratory Cobas-Integra (commercial)

Analyser:	Cobas-Integra (commercial)	Abaxis	IDEXX	Heska
Analyte				
Albumin	Bromcresol green binding	as for Cobas-Integra	as for Cobas-Integra	as for Cobas-Integra
Alkaline Phosphatase	p-NPP hydrolyzation	as for Cobas-Integra	as for Cobas-Integra	as for Cobas-Integra
Alanine Aminotransferase	Catalyzation (form pyruvate and N-glutamate)	as for Cobas-Integra	as for Cobas-Integra	as for Cobas-Integra
Total Bilirubin	Diazo method	Enzymatic (bilirubin oxidase)	Diazo-based dry film	Diazo-based dry film
Calcium	Spectrophotometric (CPC)	Spectrophotometric (arsenazo III)	Spectrophotometric (arsenazo III)	Spectrophotometric (Chlorophosphonazo III)
Chloride	Ion selective electrode (ISE)	Not analyzed by this instrument	as for Cobas-Integra	Potentiometric
Creatinine	Jaffe reaction	Enzymatic (creatinine amidohydrolase)	Enzymatic (creatinine amidohydrolase)	Enzymatic (creatinine deiminase)
Glucose	Hexokinase	as for Cobas-Integra	Glucose oxidase	Glucose oxidase
Phosphate	Phosphomolybdate	Enzymatic (phosphoglucomutase)	as for Cobas-Integra	Spectrophotometric (PNP)
Potassium	Ion selective electrode (ISE)	Enzymatic (pyruvate kinase)	as for Cobas-Integra	Potentiometric
Sodium	Ion selective electrode (ISE)	Enzymatic (beta-galactosidase)	as for Cobas-Integra	Potentiometric
Total Protein	Biuret	as for Cobas-Integra	as for Cobas-Integra	as for Cobas-Integra
Urea	Coupled-enzyme reaction	as for Cobas-Integra	Ammonia indicator	Bromcresol green/ammonia

'as for Cobas-Integra' indicates the same method as the commercial laboratory
p-NPP = p-nitrophenylphosphate; CPC = o-cresolphthalein complexone; PNP = purine-nucleoside phosphorylase

Additionally, to assess whether the provided RIs account for any bias, percentages of results for each analyte that were below, within and above the RIs supplied by manufacturers of each in-house analyser (for results from their analyser), or by the laboratory (for results from its analyser), were calculated.

Results

Although a total of 101 samples were collected, the number of samples processed by each analyser for each analyte varied between 80 and 101; some samples were not processed for logistical reasons such as insufficient supply of test 'slides' on individual days, insufficient sample quantity, exceeding the provided number of tests or, on one occasion, instrument failure. Results obtained by dilution of samples (one creatinine, one phosphate and one urea result from both the Heska and IDEXX equipment) were excluded since dilution introduced human error and may have influenced results. Additionally, glucose concentrations determined by the commercial laboratory in the first twelve samples collected were substantially lower than those from all three in-house analysers. These results

Table 2 Categorisation system for presence, type and degree of bias. Mean difference percentage was calculated as absolute mean difference divided by mean result for the analyte on the same analyser. Note that the mean difference has no bearing on whether proportional bias is present or not

Presence of bias	Type	Degree	Mean difference percentage	Absolute value of correlation coefficient for association between difference and mean	P-value for equality of means and variances
No bias	-	-	<5%	<0.15	-
Bias	Constant	-	≥5%	<0.15	-
Bias	Proportional	Mild	-	0.15-0.30	<0.05
Bias	Proportional	Moderate	-	0.30-0.45	<0.05
Bias	Proportional	Pronounced	-	>0.45	<0.05

were excluded because it was suspected that red blood cells were accidentally aspirated into the plasma for these samples. This may have affected the commercial laboratory results due to the delay between blood sample collection and analysis, while any effect on the in-house analyser results would have been minimal since samples were tested immediately after collection. No subsequent samples had such dramatic variation in glucose results.

Distributions of results for each analyte are summarized in Table 3, difference plots for each analyte are shown in Figure 1 and numerical analyses are shown in Tables 4 and 5. Bias is indicated by deviation of the mean difference from zero (Table 4) and/or if there is evidence of proportional bias. When assessed as a percentage of the mean result for each analyte on each analyser (Table 5), for 26/38 analytes, mean differences were within 10%, with 17 of these within 5%. This is demonstrated graphically by the deviation of the observed average agreement (dashed line) from zero; for example, in Figure 1, the difference plot of sodium values from the IDEXX analyser compared to the commercial laboratory analyser shows the in-house analyser results were, on average, approximately 12.5 mmol/L higher (8% of the mean result for sodium on the IDEXX analyser) than those from the commercial laboratory analyser.

The 95% limits of agreement were determined and also expressed as percentages of the mean result for each analyte on each analyser (Table 5) to enable comparison between analytes. Only 10/38 analytes had 95% limits of agreement that were less than 20% of the mean (five from IDEXX analyser, three from the Abaxis analyser and two from the Heska analyser) meaning that, for these analytes, 95% of the results differed from the mean difference by less than 10% of the mean.

Correlation coefficients for the association between difference and mean can vary from zero (indicating no proportional bias) to either $\pm$ 1 (indicating substantial proportional bias). Values approaching zero are associated with a near horizontal reduced major axis regression line (solid lines in Figure 1) and were found for the Abaxis analyser for albumin (-0.08), urea (0.08), glucose (-0.12), sodium (0.15), total protein (-0.15) and urea (0.08); the Heska analyser for potassium (0.02), bilirubin (0.05), and glucose (0.10); and for the IDEXX analyser for glucose (0.12) and potassium (0.12).

In contrast, for example, a large (negative) correlation coefficient (-0.90) was observed for urea measured on the IDEXX instrument (solid line in Figure 1). Urea results on this analyser were, on average, approximately 10% of the mean from this analyser lower than

Table 3 Summary of distributions of results from all analysers, showing number of samples tested (n), minimum (Min), median (Med) and maximum (Max) results, as well as lower quartile (25th percentile), upper quartile (75th percentile), mean and SD

Analyte	Analyser	n	Min	Lower	Median	Upper	Max	Mean	SD
Albumin (g/L)	Abaxis	80	28	38	39	42	46	39.3	3.4
	IDEXX	99	18	30	32	34	39	32.1	3.4
	Heska	96	21	31.5	33	35	40	32.9	3.2
	Cobas-Integra	99	19	32	35	37	44	34.3	3.9
ALP (U/L)	Abaxis	82	11	20	25	35	122	32.9	22.1
	IDEXX	101	20	32	39	52	206	53.3	37.2
	Heska	101	21	33	37	47	162	47.8	27.7
	Cobas-Integra	101	7	18	23	34	160	34.8	30.3
ALT (U/L)	Abaxis	82	32	48	62	79	258	74.9	46.6
	IDEXX	101	10	23	39	58	369	55.5	60.6
	Heska	101	27	50	64	84	416	84.8	69.4
	Cobas-Integra	101	20	41	55	76	434	74.9	66.4
Calcium (mmol/L)	Abaxis	80	2.02	2.50	2.62	2.76	3.27	2.64	0.21
	IDEXX	99	2.12	2.54	2.64	2.73	3.18	2.65	0.16
	Heska	99	2.14	2.63	2.75	2.91	3.49	2.76	0.21
	Cobas-Integra	99	2.04	2.51	2.64	2.74	3.42	2.66	0.24
Chloride (mmol/L)	Abaxis	0	-	-	-	-	-	-	-
	IDEXX	95	114	123	124	126	130	123.8	2.8
	Heska	84	96	113	116	119	126	115.0	5.9
	Cobas-Integra	96	106	116	117	119	124	117.1	3.6
Creatinine (umol/L)	Abaxis	82	40	111	133	174	277	142.5	50.1
	IDEXX	100	63	140	172	210	421	179.5	60.5
	Heska	100	38	103	129	160.5	357	137.1	53.6
	Cobas-Integra	100	40	100	121	147.5	340	128.7	47.0
Glucose (mmol/L)	Abaxis	70	4.2	5.8	6.5	7.7	16.0	7.1	2.06
	IDEXX	89	4.18	5.9	6.6	7.9	16.2	7.1	1.99
	Heska	89	4.3	5.8	6.4	7.5	16.4	7.0	2.04
	Cobas-Integra	89	2.9	5.4	6.0	7.1	15.5	6.5	1.98
Phosphate (mmol/L)	Abaxis	80	0.75	1.27	1.53	1.80	3.53	1.64	0.52
	IDEXX	94	0.65	1.14	1.33	1.57	5.73	1.49	0.66
	Heska	98	0.67	1.14	1.35	1.65	6.96	1.54	0.77
	Cobas-Integra	99	0.59	1.10	1.30	1.58	6.14	1.47	0.68
Potassium (mmol/L)	Abaxis	79	2.9	3.8	4.1	4.6	6.0	4.17	0.53
	IDEXX	97	2.7	3.6	3.9	4.2	5.7	3.94	0.51
	Heska	85	2.3	3.3	3.6	3.9	4.7	3.63	0.48
	Cobas-Integra	98	2.7	3.4	3.7	4.0	5.3	3.77	0.48
Sodium (mmol/L)	Abaxis	79	141	149	151	153	159	151.0	3.09
	IDEXX	97	144	160	161	163	167	161.0	3.30
	Heska	85	130	147	151	153	157	149.1	6.36
	Cobas-Integra	98	135	147	149	150	155	148.6	2.78
Total bilirubin (umol/L)	Abaxis	82	2	4	4	4	14	4.24	1.70
	IDEXX	100	2	2	2	2.5	8	2.48	1.10
	Heska	99	1	1	1	1	15	1.66	2.28
	Cobas-Integra	100	2	4.5	6	7	17	5.80	2.19
Total Protein (g/L)	Abaxis	80	53	67.5	74	78	94	73.3	7.70
	IDEXX	99	51	65	70	75	86	69.9	7.19
	Heska	98	52	64	70	74	89	69.6	7.34
	Cobas-Integra	99	50	67	73	78	98	72.7	8.45
Urea (mmol/L)	Abaxis	82	3.9	8.3	9.2	12.2	38.9	10.85	5.02
	IDEXX	100	3.5	7.4	8.3	10.7	29.3	9.43	4.03
	Heska	100	4.2	8.7	10.0	12.7	38.7	11.50	5.26
	Cobas-Integra	100	3.9	8.3	9.6	12.5	37	11.03	5.09

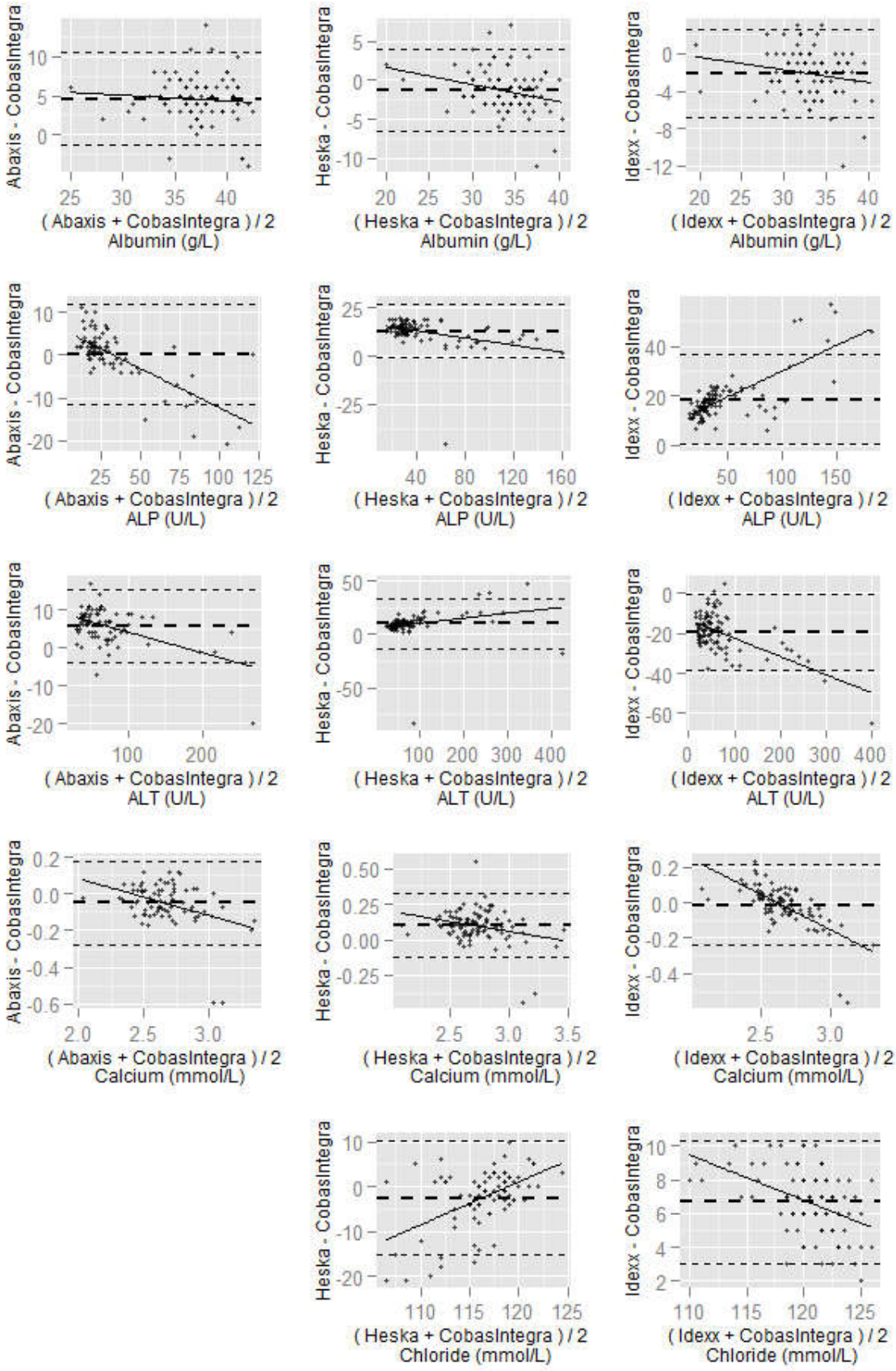
those obtained from the commercial laboratory analyser at the lower end of the reference interval, but approximately 20% lower at three times the upper limit of normal.

Based on the categorisation system, the Abaxis analyser was determined to have no bias for three analytes (sodium, total protein and urea), constant bias for three analytes (albumin, glucose, potassium) and proportional bias for the remaining six analytes. The IDEXX analyser had no bias for potassium and phosphate, constant bias for one analyte (glucose) and proportional bias for the remaining 10 analytes. The Heska analyser had no bias for potassium only, constant bias for bilirubin only and proportional bias for the remaining 11 analytes. For two analytes, phosphate on the Heska analyser and total protein on the IDEXX analyser, proportional bias was not confirmed by a low p-value for equality of means and variances; visual inspection of the difference plots appeared to support that proportional bias was present. Proportional bias varied from mild (Abaxis: 2/6, IDEXX: 2/10, Heska: 6/11) to pronounced (Abaxis: 3/6, IDEXX: 6/10, Heska: 3/11). The categorisation of each analyte from each analyser is shown in Table 5.

The width of the limits of agreement is independent of the categorisations but a wide limits of agreement band affects the clinical interpretation of results. This can mean that an individual result on one analyte that has been graded as having no bias or constant bias but has a very wide band can have individual values that differ markedly between the in-house analyser and the commercial laboratory. For example, the total bilirubin values determined on the Heska analyser had a limit of agreement that was 600% of the mean result from that analyser, indicating that 95% of results could have a true value up to three times the mean lower or higher than that determined.

Proportional bias is less relevant for clinical practice (even if classified as pronounced) when the limits of agreement are narrow such as for calcium on the IDEXX analyser since the greater slope is within a range of only 10% above or below the average result.

Percentages of results from all analysers that were below, within and above the reference intervals supplied by the manufacturer or laboratory are shown in Table 6. Percentages of results below and above the reference interval varied markedly between analysers for all analytes. Total bilirubin had the most consistency between analysers (percentages of samples within reference intervals varied from 96% [Heska] to 100% [IDEXX]). The IDEXX analyser also had relatively fewer values above the reference interval for both total protein (all values were within reference interval compared to 9-16% for the other analysers) and urea (only 11 % of values exceeding the reference interval compared to 32-34% for the other analysers) and there were no values below the reference interval for chloride (compared to 8-14% for the other analysers). For ALP, 4% of values from the Abaxis analyser were above the reference interval compared to 9-12% for the other analysers. Large percentages of values exceeded the upper limit of the reference interval for the commercial laboratory analyser for albumin (41%) and calcium (76%), and below the lower limit of the reference interval for phosphate (50%) and potassium (52%); these were markedly different from results for these analytes from the other analysers. The Heska analyser yielded approximately twice as many results above the Heska reference interval for glucose (31%) compared to the other analysers.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Albumin (g/L)
Heska - CobasIntegra
(Heska + CobasIntegra) / 2
Albumin (g/L)
Idexx - CobasIntegra
(Idexx + CobasIntegra) / 2
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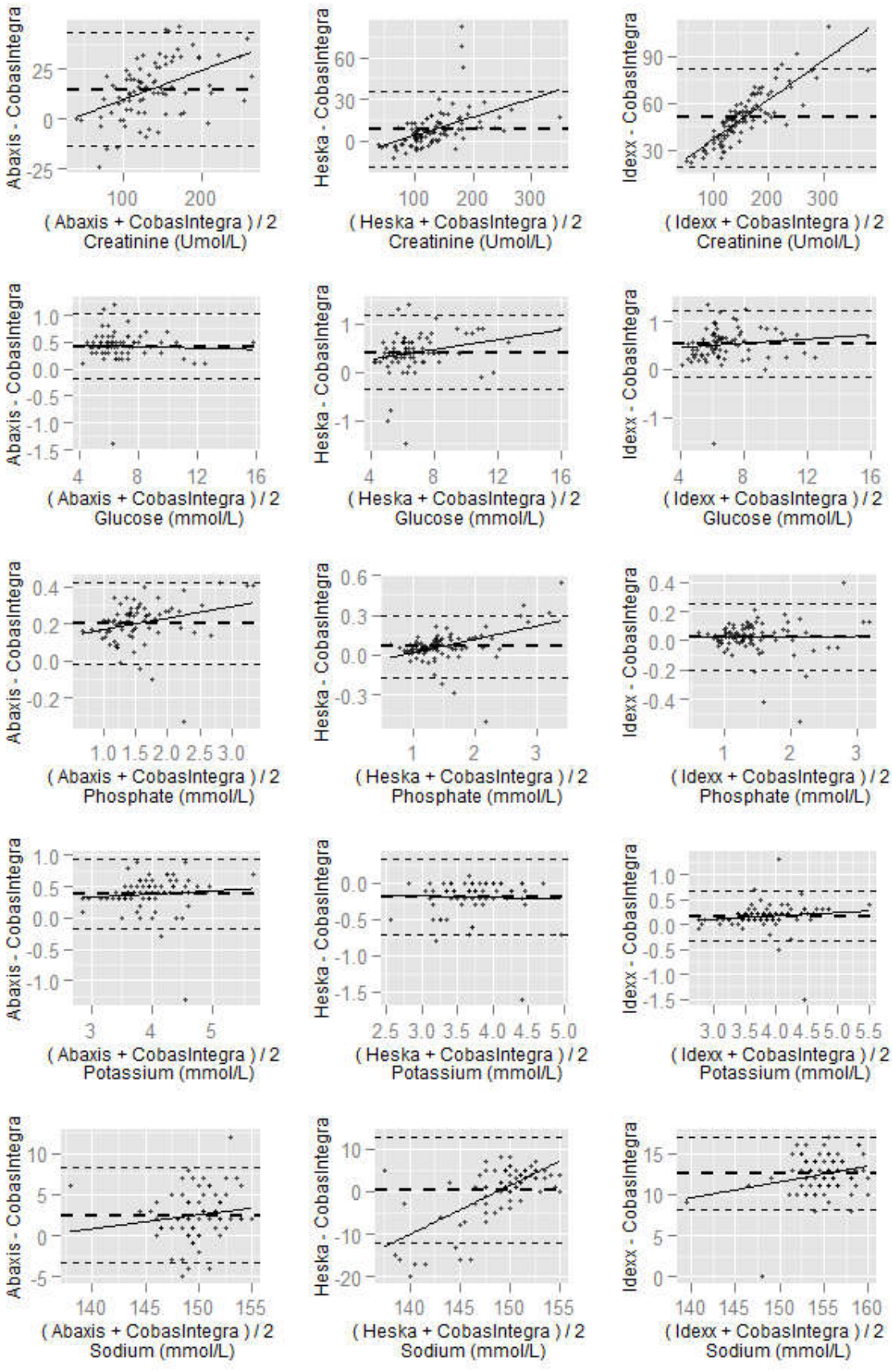
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Abaxis - CobasIntegra
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Idexx - CobasIntegra
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Phosphate (mmol/L)
Abaxis - CobasIntegra
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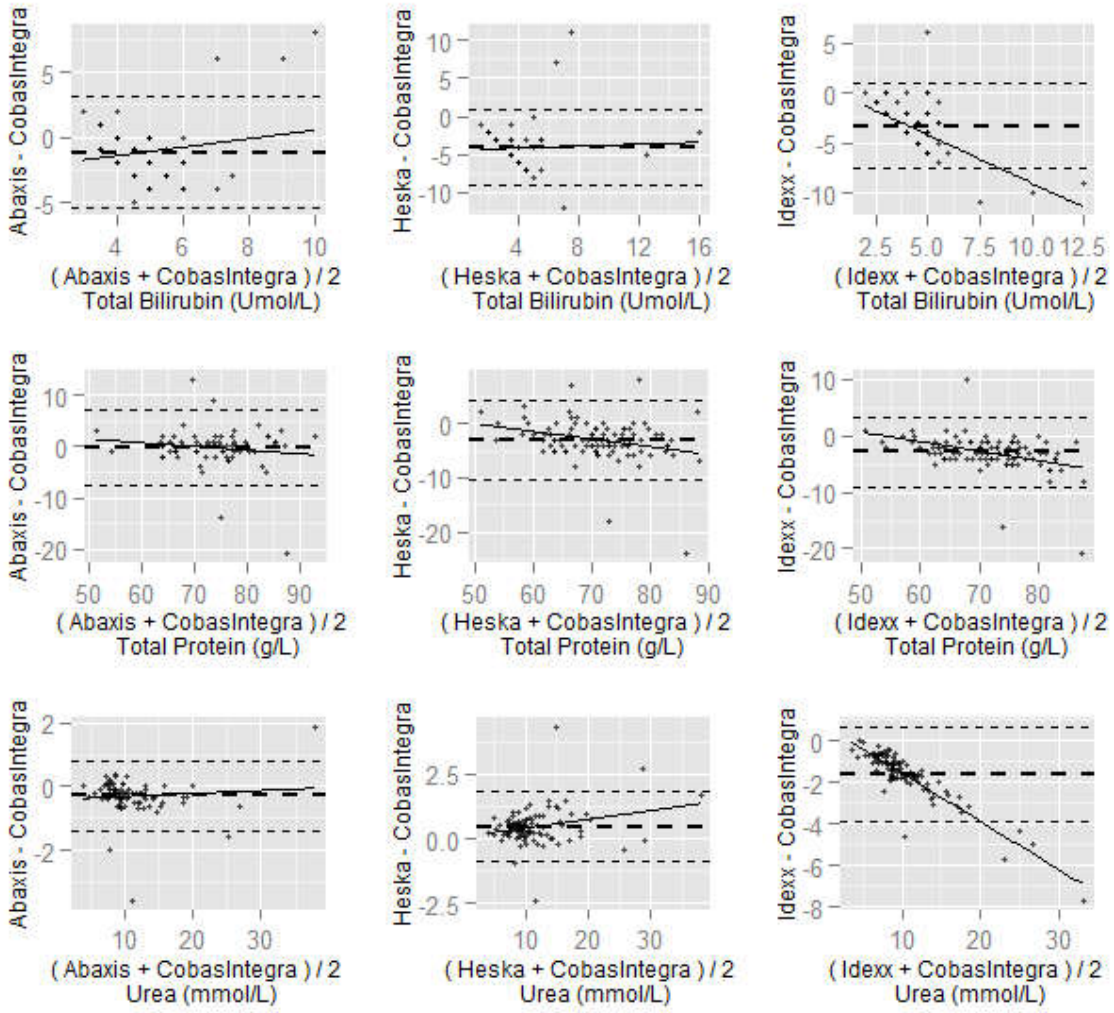

Figure 1 Difference plots comparing analyte results by each in-house analyzer to the results found by the commercial laboratory analyzer ('CobasIntegra') from the same sample. The long dashed line indicates the (observed) mean difference; the short dashed lines indicate the 95% confidence limits for this mean. The solid lines are the reduced major axis regression lines between differences and means; with proportional bias, the slope of this line deviates from zero (horizontal). Note that the units apply to both x and y-axes.

Discussion

The findings of this study indicate that bias is common in in-house analysers, and this is commonly proportional bias. In many cases, this bias is sufficient to invalidate comparisons of biochemical results obtained from different analysers. Further, the inconsistency between results with/above/below the provided reference intervals means results cannot be compared on the basis of where they lie in reference to the provided reference intervals. In general, it may be possible to reduce biases through improvement of the assay methods and recalibration of the analyser, however, the in-house analysers assessed cannot be calibrated by the end-user and assay methods may not be able to changed for the 'dry slide' (Heska and IDEXX equipment) or 'rotor' (Abaxis equipment) technologies utilized by these analysers. Furthermore, performance may vary between analysers of the same make (even after calibration), so ideally, biochemical results should be interpreted using reference intervals determined specifically for the particular analyser being used.

Table 4 Results of Bland-Altman approach analyses. The mean difference describes the average difference between results found on each in-house analyser to those found on the commercial laboratory analyser. The subsequent column shows the difference between the upper limit of the reference interval on the commercial laboratory analyser to each in-house analyser (as provided by the manufacturer of each analyser); mean difference should agree with this result if the provided reference interval correctly accounts for the bias found. The correlation coefficients for the association between difference and mean results (second last column) give a quantitative assessment of the degree of proportional bias determined by Bland-Altman analyses. If the value is 0, there is no proportional bias and the regression line on the difference plot (solid lines in Figure 1) is horizontal. A positive value means that the average difference in results (in-house analyser minus commercial laboratory) increases with increasing analyte concentration; the regression line will slope upwards to the right. A negative value means that the average difference in results (in-house analyser minus commercial laboratory) becomes more negative with increasing analyte concentration; the regression line will slope downwards to the right. A low p-value for equality of means and variances supports rejecting the null hypothesis that the variance does not vary with the mean, and hence concluding that proportional bias is present. (n= number of observations)

Analyte	Analyser	n	Mean difference (95% limits of agreement)	Difference between upper reference limits of in-house analyser and the commercial laboratory	Correlation coefficient for association between difference and mean	P-value for equality of means and variances
Albumin (g/L)	Abaxis	80	4.56 (-1.35, 10.47)	9	-0.08	<0.001
	IDEXX	99	-2.15 (-6.89, 2.59)	5	-0.19	<0.001
	Heska	96	-1.33 (-6.54, 3.87)	0	-0.27	<0.001
ALT (U/L)	Abaxis	82	0.09 (-11.72, 11.89)	10	-0.74	<0.001
	IDEXX	101	18.50 (0.28, 36.71)	31	0.75	<0.001
	Heska	101	13.04 (-0.87, 26.95)	10	-0.37	<0.001
ALP (U/L)	Abaxis	82	5.65 (-3.98, 15.28)	10	-0.54	<0.001
	IDEXX	101	-19.35 (-38.40, -0.30)	20	-0.59	<0.001
	Heska	101	9.890 (-13.48, 33.27)	50	0.26	<0.001
Total Bilirubin (umol/L)	Abaxis	81	-1.15 (-5.40, 3.10)	0	0.17	<0.001
	IDEXX	99	-3.33 (-7.55, 0.89)	5	-0.62	<0.001
	Heska	99	-4.17 (-9.16, 0.81)	-1	0.05	<0.001
Calcium (mmol/L)	Abaxis	80	-0.05 (-0.28, 0.18)	0.45	-0.38	<0.001
	IDEXX	99	-0.02 (-0.25, 0.21)	0.33	-0.70	<0.001
	Heska	99	0.10 (-0.13, 0.33)	0.5	-0.27	<0.001
Chloride (mmol/L)	IDEXX	95	6.66 (2.00, 10.33)	6	-0.44	<0.001
	Heska	84	-2.50 (-15.29. 10.31)	2	0.51	<0.001
Creatinine (umol/L)	Abaxis	82	14.85 (-13.47, 43.17)	16	0.47	<0.001
	IDEXX	100	50.80 (19.44, 82.16)	52	0.84	<0.001
	Heska	100	8.41 (-18.42, 35.24)	-1	0.48	<0.001
Glucose (mmol/L)	Abaxis	69	0.43 (-0.17, 1.02)	1.2	-0.05	<0.001
	IDEXX	88	0.52 (-0.17 , 1.22)	1.33	0.12	<0.001
	Heska	88	0.41 (-0.37, 1.19)	-0.3	0.25	<0.001
Phosphate (mmol/L)	Abaxis	80	0.20 (-0.02, 0.43)	0.48	0.27	<0.001
	IDEXX	93	0.02 (-0.22, 0.26)	0.16	-0.24	<0.001
	Heska	95	0 .07 (-0.21, 0.35)	0.7	0.65	0.85
Potassium (mmol/L)	Abaxis	79	0.38 (-0.17, 0.94)	1.2	0.09	<0.001
	IDEXX	97	0.16 (-0.34 , 0.67)	1.2	0.12	<0.001
	Heska	85	-0.15 (-0.71, 0.42)	0.7	0.02	<0.001
Sodium (mmol/L)	Abaxis	79	2.48 (-3.25, 8.21)	12	0.15	<0.001
	IDEXX	97	12.45 (8.04, 16.87)	13	0.24	<0.001
	Heska	85	0.28 (-12.26, 12.82)	0	0.68	<0.001
Total Protein (g/L)	Abaxis	80	-0.24 (-7.65, 7.18)	2	-0.15	<0.001
	IDEXX	99	-2.90 (-9.150, 3.37)	9	-0.40	0.36
	Heska	98	-3.04 (-10.42, 4.34)	0	-0.29	<0.001
Urea (mmol/L)	Abaxis	82	-0.30 (-1.41, 0.82)	0	0.08	<0.001
	IDEXX	100	-1.60 (-3. 90, 0.70)	2.2	-0.90	<0.001
	Heska	100	0.48 (-0.88, 1.83)	0.72	0.25	<0.001

Table 5 Summary of biases. Mean differences and 95% limits of agreement, are both expressed as percentages of the mean value for that analyte from that analyser so that direct comparisons between analytes can be made. Limits of agreement (LOA) that were less than 20% are bolded; for these analytes, 95% of results differed from the mean difference by less than 10% of the mean value found on that analyser and thus proportionality is less relevant clinically. (n=number of observations)

Analyte	Analyser	n	Mean difference (%)	Width of 95% LOA (%)	Correlation coefficient for association between difference and mean	P-value for equality of means and variances	Bias	Degree of Proportionality
Albumin (g/L)	Abaxis	80	11.6	30.0	-0.08	<0.001	Constant	-
	IDEXX	99	-6.7	29.5	-0.19	<0.001	Proportional	Mild
	Heska	96	-4.0	31.6	-0.27	<0.001	Proportional	Mild
ALP (U/L)	Abaxis	82	0.3	71.8*	-0.74	<0.001	Proportional	Pronounced
	IDEXX	101	34.7	68.4*	0.75	<0.001	Proportional	Pronounced
	Heska	101	27.3	58.2*	-0.37	<0.001	Proportional	Moderate
ALT (U/L)	Abaxis	82	7.5	25.7	-0.54	<0.001	Proportional	Pronounced
	IDEXX	101	-34.8	68.6*	-0.59	<0.001	Proportional	Pronounced
	Heska	101	11.7	55.1*	0.26	<0.001	Proportional	Mild
Total Bilirubin (umol/L)	Abaxis	81	-27.1	200.5*	0.17	<0.001	Proportional	Mild
	IDEXX	99	-134.3	340.2*	-0.62	<0.001	Proportional	Pronounced
	Heska	99	-251.2	600.7*	0.05	<0.001	Constant	-
Calcium (mmol/L)	Abaxis	80	-1.9	**17.1**	-0.38	<0.001	Proportional	Moderate†
	IDEXX	99	-0.8	**17.3**	-0.70	<0.001	Proportional	Pronounced†
	Heska	99	3.6	**16.4**	-0.27	<0.001	Proportional	Mild†
Chloride (mmol/L)	IDEXX	95	5.4	**6.7**	-0.44	<0.001	Proportional	Moderate†
	Heska	84	-2.2	22.3	0.51	<0.001	Proportional	Pronounced
Creatinine (umol/L)	Abaxis	82	10.4	39.7	0.47	<0.001	Proportional	Pronounced
	IDEXX	100	28.3	34.9	0.84	<0.001	Proportional	Pronounced
	Heska	100	6.1	39.1	0.48	<0.001	Proportional	Pronounced
Glucose (mmol/L)	Abaxis	69	6.1	**16.8**	-0.05	<0.001	Constant	-
	IDEXX	88	7.3	**19.5**	0.12	<0.001	Constant	-
	Heska	88	5.9	22.3	0.25	<0.001	Proportional	Mild
Phosphate (mmol/L)	Abaxis	80	12.2	27.3	0.27	<0.001	Proportional	Mild
	IDEXX	93	1.3	32.0	-0.01	<0.001	No bias	-
	Heska	95	4.5	36.0	0.43	0.85	Proportional	Moderate
Potassium (mmol/L)	Abaxis	79	9.1	26.6	0.09	<0.001	Constant	-
	IDEXX	97	4.1	25.6	0.12	<0.001	No bias	-
	Heska	85	-4.1	30.9	0.02	<0.001	No bias	-
Sodium (mmol/L)	Abaxis	79	1.6	**7.6**	0.15	<0.001	No bias	-
	IDEXX	97	7.7	**5.5**	0.24	<0.001	Proportional	Mild†
	Heska	85	0.2	**16.8**	0.68	<0.001	Proportional	Pronounced†
Total Protein (g/L)	Abaxis	80	-0.3	20.2	-0.15	<0.001	No bias	-
	IDEXX	99	-4.2	**17.9**	-0.40	0.36	Proportional	Moderate†
	Heska	98	-4.4	21.2	-0.29	<0.001	Proportional	Mild
Urea (mmol/L)	Abaxis	82	-2.8	20.6	0.08	<0.001	No bias	-
	IDEXX	100	-17.0	48.6	-0.90	<0.001	Proportional	Pronounced
	Heska	100	4.2	23.5	0.25	<0.001	Proportional	Mild

*Independent of categorisation, clinical interpretation of results should be interpreted cautiously because of very wide limits of agreement
†Proportional bias is less relevant for clinical practice for these because of the narrow limits of agreement
‡Proportional bias not confirmed by low *P* value

Table 6 Feline reference intervals's (RI's)for biochemistry analytes (provided by manufacturers of analysers and a commercial laboratory) with percentages of results that were below, within and above the RI's supplied by that manufacturer or laboratory. A lower percentage above and/or below the RI than other analysers suggests that the RI may be too wide, which would result in reduced diagnostic sensitivity for detection of abnormal cats. A larger percentage above and/or below the RI than other analysers suggests that the RI may be too narrow, which would result in reduced diagnostic specificity of the assay. The large proportion of results from the commercial laboratory that were above the commercial laboratory's RI's for calcium and albumin and below the RI's for phosphate and potassium (without consistent clinical signs in these cats) suggests inaccuracy of the RI's for these analytes for the commercial laboratory. Inconsistencies between analysers (ie a higher or lower percentage than from other analysers) are shown in bold.

Analyte	Analyser	Provided RI	Below	Within	Above
Albumin (g/L)	Abaxis	22-44	0.0%	97.5%	2.5%
	IDEXX	22-40	2.0%	98.0%	0.0%
	Heska	23-35	2.1%	**80.2%**	**17.7%**
	Cobas-Integra	22-35	1.0%	**57.6%**	**41.4%**
ALP (U/L)	Abaxis	10-90	0.0%	**96.3%**	**3.7%**
	IDEXX	14-111	0.0%	91.1%	8.9%
	Heska	0-90	0.0%	90.1%	9.9%
	Cobas-Integra	5-80	0.0%	88.1%	11.9%
ALT (U/L)	Abaxis	20-100	0.0%	84.1%	15.9%
	IDEXX	12-130	**6.9%**	84.2%	**8.9%**
	Heska	0-85	0.0%	76.2%	23.8%
	Cobas-Integra	5-80	0.0%	79.2%	20.8%
Calcium (mmol/L)	Abaxis	2.00-2.95	0.0%	93.7%	6.3%
	IDEXX	1.95-2.83	0.0%	90.3%	9.7%
	Heska	2.20-3.00	2.0%	87.9%	10.1%
	Cobas-Integra	1.75-2.50	0.0%	**24.2%**	**75.8%**
Chloride (mmol/L)	Abaxis	*	*	*	*
	IDEXX	112-129	**0.0%**	**98.9%**	1.1%
	Heska	107-125	8.3%	90.5%	1.0%
	Cobas-Integra	115-123	13.5%	84.4%	2.1%
Creatinine (umol/L)	Abaxis	27-186	0.0%	**84.1%**	15.9%
	IDEXX	71-212	2.0%	75.0%	23.0%
	Heska	71-159	7.0%	68.0%	25.0%
	Cobas-Integra	70-160	6.0%	75.0%	19.0%
Glucose (mmol/L)	Abaxis	3.9-8.7	0.0%	84.1%	15.9%
	IDEXX	4.11-8.83	0.0%	86.4%	13.6%
	Heska	3.9-7.2	0.0%	**69.3%**	**30.7%**
	Cobas-Integra	3.9-7.5	1.1%	80.9%	18.0%
Phosphate (mmol/L)	Abaxis	1.10-2.74	5.0%	91.2%	3.8%
	IDEXX	1.00-2.42	13.3%	81.1%	5.6%
	Heska	0.84-1.94	2.1%	83.0%	14.9%
	Cobas-Integra	1.29-2.26	**49.5%**	**41.4%**	9.1%
Potassium (mmol/L)	Abaxis	3.7-5.8	12.7%	86.0%	1.3%
	IDEXX	3.5-5.8	13.4%	86.6%	0.0%
	Heska	3.4-5.3	**26.1%**	**73.9%**	0.0%
	Cobas-Integra	3.8-4.6	**52.0%**	**43.9%**	4.1%
Sodium (mmol/L)	Abaxis	142-164	1.3%	98.7%	0.0%
	IDEXX	150-165	2.1%	92.8%	5.2%
	Heska	147-156	**22.4%**	**76.4%**	1.2%
	Cobas-Integra	147-156	**17.4%**	**82.7%**	0.0%
Total Bilirubin (µmol/L)	Abaxis	2-10	0.0%	97.5%	2.5%
	IDEXX	0-15	0.0%	**100.0%**	0.0%
	Heska	0-9	0.0%	96.0%	4.0%
	Cobas-Integra	2-10	0.0%	97.0%	3.0%
Total Protein (g/L)	Abaxis	54-82	1.3%	87.5%	11.3%
	IDEXX	57-89	3.0%	**97.0%**	**0.0%**
	Heska	60-80	8.2%	82.7%	9.2%
	Cobas-Integra	56-80	3.0%	80.8%	16.2%
Urea (mmol/L)	Abaxis	3.6-10.7	0.0%	65.9%	34.1%
	IDEXX	5.7-12.9	6.0%	**83.0%**	**11.0%**
	Heska	5.35-11.42	2.0%	66.0%	32.0%
	Cobas-Integra	5.4-10.7	4.0%	62.0%	34.0%

At least half the analytes tested on each machine showed substantial bias (either >5% mean difference or at least moderate proportionality or both). This was not surprising since in-house biochemistry equipment cannot be calibrated by the user and each manufacturer provides a different reference interval for each analyte, suggesting that a constant bias from the true values may be present. However, varying reference intervals can only fully account for constant bias and approximately half of all analytes tested (for each analyser) also showed proportional bias. Varying the reference interval does not fully address marked proportional bias when comparing results between analysers; and even more complex strategies may not be effective if the degree of difference changes in a non-linear way with increasing analyte concentration.

Difference (Bland-Altman) analysis can appear to indicate notable proportional bias when the calculated 95% limits of the observed average agreement are within a narrow range. Stated differently, if the values from an in-house analyser do not vary widely relative to those from the commercial laboratory analyser, the regression slope of those differences can appear to result in substantial proportional bias but this is relatively unimportant clinically because this slope is over a narrow range. An example demonstrating this is calcium as measured on the IDEXX analyser; although the correlation between difference and mean was -0.70 (poor since it is approaching an absolute value of 1.0), 95% of results were only ~0.2mmol/L above or below the average results.

Although average differences compared to the commercial laboratory analyser differed from zero for most analytes across all in-house analysers, this could be addressed by adjustment of reference intervals (provided the extent of proportional bias was minor). The average difference between results from each in-house analyser compared to the commercial laboratory analyser is shown in Table 4 (and by percentage in Table 5). The adjacent column in Table 4 shows the differences between the upper limits of the manufacturer's recommended reference intervals and the commercial laboratory's recommended upper limit for each analyte on each machine, respectively. The values in these two columns would agree if bias had been corrected for by altering the reference interval. These values are similar in some cases (for example, chloride and sodium on the IDEXX analyser, and creatinine on Abaxis and IDEXX analysers). In other cases, the values differ substantially: ALT on the Heska analyser was 9.9 mmol/L higher (on average) than the commercial laboratory analyser but the difference between upper limits of the two reference intervals was 50 mmol/L; ALT on the IDEXX instrument had an observed average difference which was 19.3 mmol/L less than the commercial laboratory analyser but the difference in reference interval upper limits was 20 mmol/L greater; sodium on the Abaxis machine was 2.5mmol/L higher than the commercial laboratory analyser, yet the reference interval difference is 12mmol/L. Such comparisons assume the reference intervals for all analytes from each analyser (including the commercial laboratory analyser) have been correctly determined. In the course of this study, for most analytes, approximately 80% of observations were within the reported normal range but some observations were challenging to interpret: for example , 78/99 (75.8%) calcium results from the commercial laboratory were above the upper limit of the reference interval, (2.50 mmol/L) despite no clinical evidence of hypercalcemia suggesting this reference interval was incorrect; additionally, many values from the commercial laboratory analyser were above the reference interval for albumin (41.4%) and

below the reference interval for phosphate (49.5%) and potassium (52.0%) without consistent clinical signs in these cats, suggesting an inaccuracy of the reference intervals for these analytes, also. These changes are shown in Table 6 which also demonstrates a lack of consistency of percentage of results within/above/below the provided reference intervals. If the reference intervals overcame the bias of the analysers, approximately the same percentage of results would have been found in each category. Where two or three analysers were consistent (and the fourth different), it suggested that those different had an incorrect reference interval. Too few results above or below the reference interval suggests that the range is too broad and pathology is likely to be missed. Too many elevated or decreased results may mean patients are incorrectly diagnosed with pathology that is not present.

These inconsistencies may result in either unnecessary clinical management or missing a diagnosis. For example, if albumin alone was used as a measure of dehydration based on the commercial laboratory analyser and using the Gribbles upper limit of reference interval, approximately 40 additional cats may have received unnecessary treatment such as fluid therapy compared to if the IDEXX and Abaxis analysers were used. Conversely, using urea as an indicator of dehydration, the approximately 20% less cats over the reference limit on the IDEXX analyser may mean that if this analyser alone was used, approximately 20 cats may not have receive appropriate management of azotaemia. ALP is regarded as a sensitive indicator of hepatopathy in cats since it has a short half of approximately 6 hours in this species.[16] The mean and median results for ALP are similar for Abaxis and Cobas-Integra analysers yet the reference interval is 10U/L lower for the Abaxis analyser resulting in 5-8% less cats having an elevated level. This 5-8% of cats may not receive appropriate further diagnostics. Inaccurate diagnosis of hypercalcaemia (as appears likely for approximately 65 cats when assessed by the Gribbles reference interval) could lead to unnecessary investigations to determine ionised calcium and parathyroid hormone levels as well as the potential for unnecessary treatment.

No proportional bias was recognised for any analyser for potassium (demonstrated by the straight lines showing the average in Figure 1 for this analyte) and the mean average differences were only 4% (though in opposite directions) for each of the Abaxis and IDEXX analysers. Therefore, for this analyte, reference interval adjustments alone should be able to provide direct comparisons between analysers yet >50% of results from the commercial analyser and >25% of results from the Heska analyser were recognised as hypokalaemic which may result in unnecessary supplementation of these cats.

IRIS stages chronic kidney disease based on creatinine levels and ‘Stage 2’ for cats is defined as creatinine concentrations of 140-250 µmol/L.[9] The mean differences in creatinine concentrations for the in-house analysers were 8 µmol/L (Heska), 15 µmol/L (Abaxis) and 51 µmol/L (IDEXX) compared to the commercial laboratory. The result of these differences means that cats assessed by these in-house analysers (particularly IDEXX) will be staged with more advanced kidney disease than if they were assessed by the commercial laboratory analyser. This may have implications on management for anaesthesia, ongoing monitoring and long term prognostication.

Reference interval adjustments may not overcome substantial proportional bias but any assessment of proportional bias assumes that the commercial laboratory analyser results are correct. Commercial laboratory analyser analysis was chosen as the ‘gold standard’ for this

study since this is generally considered the 'highest standard' routinely available to practitioners. However, commercial laboratory analyser results may not always be the most accurate. For example, the method for creatinine analysis used by the commercial laboratory in this study was the Jaffe reaction. Compared to high performance liquid chromatography (HPLC), the Jaffe reaction has a strong positive proportional bias.[17] By inference, the negative proportional bias to the commercial laboratory analyser found by the enzymatic method of creatinine assessment used by all three in-house analysers may be due to better agreement with 'true values' determined by HPLC.

Of the published studies assessing veterinary in-house analysers, only three have assessed the analysers assessed in this study with feline samples.[3-4,8] Mischke *et al* assessed eight of the analytes assessed in this study, with results of plasma samples from 22 cats tested using an IDEXX VetTest compared to results from a Hitachi 704 commercial laboratory analyser.[3] Sutton *et al* (1999) assessed ten of the analytes assessed in this study on an Abaxis Vetscan analyser compared to a commercial laboratory Hitachi 911 analyser with 26 feline serum samples.[4] Flatland *et al* (2014) assessed all 13 analytes in this study on a Heska SpotChem analyser compared to an Hitachi 911 analyser with plasma results from 53 cats.[8] Across these studies, similar results to the current study were seen for half to three-quarters of the analytes but different methodologies preclude direct comparisons. Potential reasons for differences in findings may be due to one or more of the following: smaller sample sizes, different ranges of concentrations, assessing serum instead of plasma and different reference laboratory equipment.

This is the first study to assess the correlation and bias of multiple in-house veterinary biochemistry analysers. These results show that, for many analytes, results from in-house analysers are not directly comparable to those from the commercial laboratory or other in-house analysers and universal reference intervals (such as those published in text books) may not be appropriate. Additionally, practitioners should not compare results from different analysers only on the basis of if results are within, above or below the provided reference interval. The potential affect on clinical decision making may be overcome by the use of appropriately generated reference intervals, or reference change values which have recently been described as more appropriate for most feline biochemistry analytes.[18] Further, universal guidelines such as grading of renal disease or diagnosis of diabetes mellitus cannot be made on the basis of absolute values; however guidelines could be made on the basis of percentage increases from a cat's prior results or above the upper limit of a reference interval.

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References

1 Kaneko JJ, Harvey JW and Bruss ML. Clinical biochemistry of domestic animals. 6th ed. San Diego, CA: Academic Press, 2008, pp 873–904.

2 Norsworthy GD, Grace SF, Crystal MA, et al (eds). The Feline Patient, 3rd ed, 2006. Blackwell Publishing, Iowa, USA.

3 Mischke R, Schossier N and Wirth W. Blood testing with dry reagents (VetTest 8008) in dogs and cats in comparison with standard methods [in German]. *Kleintierpraxis* 1992; 37: 183–199.

4 Sutton A, Dawson H, Hoff B, et al. Analyte comparisons between 2 clinical chemistry analyzers. *Can Vet J* 1999; 40: 255–260.

5 Grosenbaugh DA, Gadawski JE and Muir WW. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc* 1998; 213: 691–694.

6 Papasouliotis K, Dodkin S, Murphy K, et al. Analysis of canine and feline blood samples using the Kuadro inhouse wet-reagent chemistry analyser. *J Small Anim Pract* 2006; 47: 190–195.

7 Papasouliotis K, Dodkin S, Murphy KF, et al. Comparison of measurements of 18 analytes in canine and feline blood samples using the in-practice Falcor 350 and the reference KoneLab 30i analysers. *J Small Anim Pract* 2008; 49: 494–501.

8 Flatland B, Breickner LC and Fry MM. Analytical performance of a dry chemistry analyzer designed for in-clinic use. *Vet Clin Pathol* 2014; 43: 206–217.

9 Elliott J and Watson ADJ. IRIS staging system. (2013, accessed August 13, 2014).
http://www.iris-kidney.com/_downloads/N378.008%20IRIS%20Website%20Staging%20of%20CKD%20PDF.PDF

10 Rand JS and Marshall RD. Diabetes mellitus in cats. *Vet Clin North Am Small Anim Pract* 2005; 35: 211–224.

11 Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). *J Vet Intern Med* 2005; 19: 377–385.

12 Jensen AL and Kjelgaard-Hansen M. Method comparison in the clinical laboratory. *Vet Clin Pathol* 2006; 35: 276–286.

13 Baral RM, Morton JM, Krockenberger MB, et al. Repeatability of results from three in-house biochemistry analyzers and a commercial laboratory analyzer used in small animal practice. *Comp Clin Pathol*. In Press, 2014. Epub ahead of print 9 August 2014. DOI: 10.1007/s00580-014-1977-8.

14 Bland MJ and Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement.
Lancet 1986; 327: 307–310.

15 Bradley EL and Blackwood LG. Comparing paired data: a simultaneous test for means and variances. *Am Stat* 1989; 43: 234–235.

16 Hoffmann WE, Renegar WE and Dorner JL. Serum halflife of intravenously injected intestinal and hepatic alkaline phosphatase isoenzymes in the cat. *Am J Vet Res* 1977; 38: 1637–1639.

17 Le Garreres A, Laroute V, De La Farge F, et al. Disposition of plasma creatinine in non-azotaemic and moderately azotaemic cats. *J Feline Med Surg* 2007; 9: 89–96.

18 Baral RM, Dhand NK, Freeman KP, et al. Biological variation and reference change values of feline plasma biochemistry analytes. *J Feline Med Surg* 2014; 16: 317–325.