Please cite as:

Baral, R. M., Morton, J. M., Dhand, N. K., Krockenberger, M. B., & Govendir, M. (2015). Repeatability of results from three in-house biochemistry analyzers and a commercial laboratory analyzer used in small animal practice. Comparative Clinical Pathology, 24(4), 755-765.

Repeatability of results from three in-house biochemistry analysers and a commercial laboratory analyser used in small animal practice

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

This chapter was accepted for publication in:

Comparative Clinical Pathology,
link.springer.com/journal/580

On: 28 July 2014

DOI: 10.1007/s00580-014-1977-8

Abstract

The repeatability (precision) of clinical pathology results is vital for confidence in the measured values. Comparison to biological variation is an accepted standard for instrument/method performance in human clinical pathology. This study aims to assess precision of biochemistry results from three in-house analysers and one commercial laboratory analyser in relation to biological variation; to compare precision between the in-house analysers and the commercial laboratory analyser. Two commercially available quality control materials (QCMs) were repeatedly tested on the same and different days. Coefficients of variation were calculated and assessed in relation to published biological variation data for cats and dogs. In-house analyser results were compared to results from the commercial laboratory. Seven of thirteen analytes assessed met desirable quality precision standards for one QCM and minimum quality standards for the other QCM on all analysers for both cats and dogs. For more than half of all determinations across all in-house analysers, precision was as good as, or better than the commercial laboratory analyser. The precision of results from the analysers assessed for most of the analytes tested is generally high, so large differences between repeated results from the same patient are probably due to biological changes rather than analyser variation.

Introduction

In-house plasma (or serum) biochemical analysis is commonplace in veterinary practice. An important aspect of clinical biochemistry analyser performance is the repeatability of the results.

The results generated by analysers are affected by inherent analytical variation (also known as total analytical error). Total analytical error consists of random error and systematic error. Systematic error (beyond the scope of this study) is also known as bias and reflects, for example, when one method for determining the concentration of a specific analyte results in a consistently higher or lower concentration than another. Random error determines the repeatability or precision of results. Technically 'precision studies' assess 'imprecision' since any variation in results reflects a lack of precision. Precision studies assess variability in repeated assessments of the same samples.[1] Knowledge of within-day precision is important clinically when assays of the same sample from a patient are repeated (for example, when a result is unexpected). In addition, between-day imprecision is important in evaluating serial results from repeated samplings from the same patient on different days in order to correctly identify variations in results that are clinically significant, and to avoid misinterpretation of changes due to analytical

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

imprecision as reflecting true changes in the patient. The overall acceptability of biochemical results obtained for clinical use is assessed on the basis of quality requirements that consider both precision *and* bias over a range of values.

Analyser method validation traditionally uses quality control materials (QCMs) to assess precision (at fixed values) and clinical samples to assess bias (across a range of values).[1] Precision (even when testing QCMs) should be assessed in relation to the biological variation (the inherent physiological variation of analytes) of each analyte[2-4] using the coefficient of variation which describes the ratio of the standard deviation to the mean. Desirable precision has been determined as the analytical coefficient of variation (CV_A) being less than half the average biological variation (the within patient coefficient of variation [CV_I]).[4] Biological variation has only recently been determined for feline biochemistry analytes,[5] and precision has not previously been assessed in relation to canine biological variation. The purpose of this study was to assess analytical coefficients of variation (CV_As) for multiple analytes in relation to biological variation values for both dogs and cats from results generated using QCMs by three in-house analysers and one commercial analyser, and to compare the CV_As for each analyte between the three in-house analysers and the commercial analyser.

Materials and methods

Study overview

CV_As were compared using two commercially available QCMs: Chemtrak® H-1 and Chemtrak® H-3 (Microgenics, California, USA). Both are unassayed and prepared from human serum with added porcine, bovine and avian tissues. Chemtrak® H-1 analyte concentrations are mostly within reference intervals, Chemtrak H-3 analyte concentrations are either above or below reference interval values (depending on the analyte). Both of these QCMs were used to assess within-day and between-day precision of an Abaxis Vetscan® VS-2 Point of Care Analyser (Abaxis, California, USA), a Heska Dri-Chem®Veterinary Chemistry Analyser (Heska, Colorado, USA) and an IDEXX VetTest® VT8008 and an IDEXX VetLyte® (IDEXX, Maine, USA) [IDEXX has a separate instrument to measure electrolytes]. The results were compared with those, using the same QCMs, generated by a commercial laboratory analyser; the Cobas-Integra 400 (Roche Diagnostics GmbH, Mannheim, Germany), operated at Gribbles Veterinary Pathology, (Rhodes, NSW, Australia). This study was performed with the co-operation of, but independent of, all companies represented.

Samples and testing frequencies

Chemtrak® H-1 was tested on the three in-house biochemistry analysers, for 13 plasma biochemical analytes (albumin, alkaline phosphatase [ALP], alanine aminotransferase [ALT], total bilirubin, calcium, chloride [not available for Abaxis], creatinine, glucose, phosphate, potassium, sodium, total protein, and urea) on 7 or 10 occasions ('runs') on one single day to assess within-day precision. The number of runs varied since electrolytes were not tested on the IDEXX machine on three occasions due to human error.

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, results were obtained from Chemtrak® H-1 for all 13 analytes, each tested once daily, on 11 to 15 days over a one month period, to assess between-day precision. The number of runs varied due to insufficient supply of test 'slides' on individual days, exceeding the total number of tests provided.

Chemtrak® H-3 was used to assess a different range of concentrations for the same analytes on these same three analysers once daily, on 8 to 11 days over the same one month period. Again, the number of runs varied due to insufficient supply of test 'slides' on individual days, exceeding the total provided number of tests or, rarely, instrument failure.

Analyses were not repeated within-day using Chemtrak-3, since materials for only a limited number of tests were provided by each manufacturer. Between-day imprecision was considered to provide the most useful information when considering variation in serial collections from patients in which specific analytes were being frequently monitored as, in these situations, samples are usually collected from patients on different days. Between-day imprecision is generally greater than within-day imprecision.[6]

Quality control analyses using the same batch of Chemtrak® H-1 and Chemtrak® H-3 were also run at the commercial laboratory on the same 15 days (between-day) over the same one month period.

Laboratory methods

The assay methods used by each instrument for each analyte are shown in Table 1.

The Cobas-Integra 400 analyser at a NATA accredited commercial laboratory (Gribbles Veterinary Pathology, Rhodes, Sydney, Australia) underwent assay calibration (with concurrent verification) weekly to monthly (depending on the individual analyte) with calibrators provided by the manufacturers and traceable to the International Federation of Clinical Chemistry (IFCC) standards. QCMs were stored at 4 to 8˚ C according to manufacturer's instructions at both test sites. Heska and Abaxis analysers were less than one year old and installed to the manufacturers' specifications by the distributing agents. Although 10 years old, the IDEXX analysers had been serviced by the manufacturer three months prior to the assessment period. Formal assessments commenced after training by the distributing agent and after a familiarization period of at least 10 runs on each analyser.

Statistical analyses

Assessments in relation to biological variation

Feline and canine biological variation (i.e. CV_I) data for cats and dogs from prior studies [5, 7-9] of the analytes assessed in this study are presented in Table 2, and results for each analyte were categorized as minimum quality precision ($CV_A \leq 0.75 \times CV_I$,) desirable quality precision ($CV_A \leq 0.5 \times CV_I$,) or optimum quality precision ($CV_A \leq 0.25 \times CV_I$). [10-11]

Means (with associated 95% CI), standard deviations and CV_As for each analyte for each in-house analyser were calculated from within-day and between-day results. CV_As within each quality standard were determined for each analyte on each analyser for Chemtrak-1 (within-day and between-day) and Chemtrak-3 (between-day only) for both species.

Comparisons to commercial laboratory analyser

CV_As for each analyte on each analyser (for both QCMs) were compared to the CV_A for that analyte on the commercial analyser with a bootstrap technique implemented using R (http://www.r-project.org/) adapted from previously published code [12] to allow two-sided interpretation. The following criteria were used to interpret the statistical results:

- p-value of < 0.025 = significant difference whereby $CV_{A\ \text{in-house}} > CV_{A\ \text{commercial}}$
- p-value of > 0.975 = significant difference whereby $CV_{A\ \text{commercial}} > CV_{A\ \text{in-house}}$
- p-value between 0.025 and 0.975 = no significant difference in CV_As

Results

For within-day Chemtrak-1 results (Table 3), the IDEXX analyser, 11/13 analytes attained the minimum precision standard for cats (exceptions were albumin and chloride); and 12/13 analytes for dogs (except calcium). On the Heska analyser, 10/13 analytes reached the minimum standard for cats (except calcium, chloride and creatinine); and 12/13 analytes for dogs (except calcium). On the Abaxis analyser, 7/12 attained the minimum performance standard for cats (except ALP, calcium, creatinine, phosphate and sodium); and 9/12 for dogs (except calcium, creatinine and potassium).

For between-day Chemtrak-1 results (Table 4), on the commercial laboratory analyser, 10/13 analytes attained the minimum performance standard for both cats and dogs (the exceptions

Table 2 Cutpoints for minimum, desirable and optimal quality requirements for precision for biochemistry analytes based on biologic variation in cats and dogs

Analyte	Within cat CV (CV_I)	Minimum quality (0.75 * CV_I)	Desirable quality (0.5 * CV_I)	Optimum quality (0.25 * CV_I)	Analyte	Within dog CV (CV_I)	Minimum quality (0.75 * CV_I)	Desirable quality (0.5 * CV_I)	Optimum quality (0.25 * CV_I)
Albumin	3.00%	2.25%	1.50%	0.75%	Albumin	5.80%	4.35%	2.90%	1.45%
ALP	12.45%	9.33%	6.22%	3.11%	ALP	13.40%	10.05%	6.70%	3.35%
ALT	17.94%	13.45%	8.97%	4.48%	ALT	19.50%	14.63%	9.75%	4.88%
Calcium	2.34%	1.75%	1.17%	0.58%	Calcium	1.20%	0.90%	0.60%	0.30%
Chloride	1.17%	0.88%	0.59%	0.29%	Chloride**	2.96%	2.22%	1.48%	0.74%
Creatinine	5.97%	4.48%	2.99%	1.49%	Creatinine	6.60%	4.95%	3.30%	1.65%
Glucose	7.49%	5.62%	3.75%	1.87%	Glucose	10.70%	8.03%	5.35%	2.68%
Phosphorus	8.49%	6.37%	4.25%	2.12%	Phosphorus	12.70%	9.53%	6.35%	3.18%
Potassium	3.63%	2.72%	1.81%	0.91%	Potassium*	3.30%	2.48%	1.65%	0.83%
Sodium	0.86%	0.65%	0.43%	0.22%	Sodium**	2.77%	2.08%	1.39%	0.69%
Total Bilirubin	87.46%	65.59%	43.73%	21.86%	Total Bilirubin	27.50%	20.63%	13.75%	6.88%
Total Protein	8.31%	6.23%	4.15%	2.08%	Total Protein	5.30%	3.98%	2.65%	1.33%
Urea	10.43%	7.82%	5.22%	2.61%	Urea	13.10%	9.83%	6.55%	3.28%

CV_I: intra-individual coefficient of variation
Feline biologic variation results from Baral et al., 2013
Canine biologic variation results from Ruaux et al., 2012 unless otherwise stated
* Jensen et al., 1993
** Schweindenwein et al., 2012

for cats were chloride, creatinine and sodium; and for dogs were calcium, chloride and creatinine). On the Abaxis analyser, 9/12 analytes attained this standard for both cats and dogs (exceptions for cats were creatinine, potassium and sodium; and for dogs were calcium, creatinine and potassium). On the IDEXX analyser 9/13 analytes attained the minimum performance standard for cats (except albumin, chloride, potassium, sodium) and 10/13 analytes for dogs (except calcium, chloride and potassium). On the Heska analyser, 8/13 analytes reached this standard for cats (except albumin, calcium, chloride, potassium and sodium) and 9/13 for dogs (except calcium, chloride, potassium, sodium).

For between-day Chemtrak-3 results (Table 4), 12/13 analytes determined by the IDEXX analyser attained the minimum performance standard for cats (except albumin) and all analytes attained this standard for dogs; 10/12 of analytes determined by the Abaxis analyser attained this standard for cats (except calcium and sodium) and 11/12 reached this standard for dogs (except calcium); 10/13 analytes determined by the Heska analyser, reached this standard for cats (except chloride, potassium and sodium) and 9/13 reached this standard for dogs (except calcium, chloride, potassium, sodium); 9/13 of analytes determined by the commercial laboratory analyser, attained this standard for cats (except calcium, chloride, creatinine and potassium) and 10/13 for dogs (except calcium, chloride, creatinine and sodium).

For Chemtrak-1, the commercial analyser had the lowest observed CV_As for 6/13 analytes (ALP, bilirubin, glucose, phosphate, potassium and sodium) (Table 5). However the in-house analysers had lower observed CV_As for ALT (Heska), calcium (Abaxis and IDEXX), chloride (IDEXX and Heska), creatinine (IDEXX and Heska), total protein (Abaxis and IDEXX) and urea (IDEXX and Heska).

Table 3 Within-day performance with QCM (Chemtrak 1) on Heska VetScan, IDEXX VetTest and Heska analysers.

Analyte (units)	Analyzer	Mean	95% CI (lower limit, upper limit)	SD	CV_A (%)	Cat	Dog
	Abaxis	68.70	68.11, 69.29	0.82	**1.19**	D	O
Albumin (g/L)	IDEXX	38.40	37.71, 39.09	0.97	2.53	-	D
	Heska	51.10	50.69, 51.51	0.57	**1.12**	D	O
	Abaxis	46.40	43.14, 49.66	4.55	9.81	-	M
Alkaline Phosphatase (ALP)(U/L)	**IDEXX**	65.10	64.12, 66.08	1.37	**2.10**	O	O
	Heska	49.50	48.89, 50.11	0.85	**1.72**	O	O
	Abaxis	30.90	29.30, 32.50	2.23	**7.22**	D	D
Alanine Aminotransferase (ALT) (U/L)	**IDEXX**	37.20	35.49, 38.91	2.39	**6.42**	D	D
	Heska	32.70	31.94, 33.46	1.06	**3.24**	O	O
	Abaxis	14.90	14.49, 15.31	0.57	**3.83**	O	O
Total Bilirubin (umol/L)	**IDEXX**	13.60	13.23, 13.97	0.52	**3.82**	O	O
	Heska	16.20	15.75, 16.65	0.63	**3.89**	O	O
	Abaxis	1.66	1.63, 1.68	0.04	2.41	-	-
Calcium (mmol/L)	IDEXX	1.71	1.70, 1.72	0.02	1.17	D	-
	Heska	1.65	1.64, 1.67	0.02	1.21	M	-
Chloride (mmol/L)	IDEXX*	111.29	110.41, 112.17	0.95	0.85	M	D
	Heska	116.10	114.54, 117.66	2.18	1.88	-	M
	Abaxis	69.90	65.96, 73.84	5.51	7.88	-	-
Creatinine (Umol/L)	**IDEXX**	69.70	68.94, 70.46	1.06	**1.52**	D	O
	Heska	60.00	58.69, 61.31	1.83	3.05	M	D
	Abaxis	3.52	3.47, 3.57	0.06	**1.70**	O	O
Glucose (mmol/L)	**IDEXX**	3.50	3.42, 3.57	0.11	**3.14**	D	D
	Heska	3.21	3.17, 3.25	0.06	**1.87**	O	O
	Abaxis	0.93	0.89, 0.97	0.06	6.45	-	M
Phosphate (mmol/L)	**IDEXX**	0.82	0.81, 0.83	0.02	**2.44**	D	O
	Heska	0.88	0.87, 0.89	0.01	**1.14**	O	O
	Abaxis	2.71	2.66, 2.76	0.07	2.58	M	_
Potassium (mmol/L)	IDEXX*	2.66	2.61, 2.71	0.05	1.88	M	D
	Heska	2.44	2.40, 2.48	0.05	2.05	M	M
	Abaxis	146.80	144.87, 148.73	2.70	1.84	-	M
Sodium (mmol/L)	**IDEXX***	156.57	156.08, 157.07	0.53	**0.34**	D	O
	Heska	146.00	144.89, 147.51	2.11	1.45	-	M
	Abaxis	71.20	70.39, 72.01	1.14	**1.60**	O	D
Total Protein (g/L)	**IDEXX**	66.60	65.76, 67.44	1.17	**1.76**	O	D
	Heska	69.80	68.86, 70.74	1.32	**1.89**	O	D
	Abaxis	5.32	5.23, 5.41	0.12	**2.26**	O	O
Urea (mmol/L)	**IDEXX**	5.16	5.12, 5.20	0.05	**0.97**	O	O
	Heska	5.49	5.45, 5.53	0.06	**1.09**	O	O

Ten tests were performed per analyte-analyzer combination except those marked with '*' where 7 tests were performed. The columns 'Cat' and 'Dog' indicate whether the analyte reached the minimum (M), desirable (D), or optimum (O) quality precision standard for that species; '-' indicates that the minimum standard was not reached. CV_As reaching desirable or optimum quality standards for both dogs and cats are in bold.

For Chemtrak-1, the Heska analyser had a significantly lower CV_A (p=0.99) than the commercial laboratory analyser for ALT and the CV_A was not significantly different (p-values between 0.025 and 0.975) for a further six analytes (ALP, ALT, chloride, creatinine, glucose, total protein, and urea). The IDEXX analyser had a lower CV_A approaching significance (p=0.96) for calcium and the CV_A was not significantly different for a further 5 analytes (ALP, calcium, chloride, creatinine, total protein and urea). The CV_As did not differ significantly between the Abaxis analyser and the commercial laboratory analyser for 4/12 analytes (albumin, calcium, total protein and urea). For Chemtrak-3, the commercial analyser had the lowest observed CV_A for only 3/13 analytes (ALP, bilirubin, and phosphate). For Chemtrak-3, for 22/38 in-house-analyte combinations, CV_As did not differ significantly from the CV_As from the commercial laboratory analyser. CV_As from the Heska analyser did not

Table 4 Between-day performance on QCM (Chemtrak-1 and Chemtrak-3) by the Abaxis VetScan, IDEXX Vet Test and Heska analysers with comparisons of analytical coefficients of variation (CV_As) to those from a commercial laboratory Cobas-Integra analyser (Gribbles) by a bootstrap technique. The columns Cat' and 'Dog' indicate whether the analyte reached the minimum (M), desirable (D), or optimum (O) quality precision standard for that species; '-' indicates that the minimum standard was not reached. CV_As reaching desirable quality standards for both cats and dogs are in bold. ** indicates a p-value<0.025, meaning that the CV_A of the commercial laboratory analyser was significantly lower than that of the in-house analyser. A single * asterisk indicates a p-value between 0.025 and 0.05 which provides some evidence that the CV_A is lower. †† indicates a p-value>0.975 meaning that the CV_A of the in-house analyser was significantly lower than that of the commercial analyser. A single † indicates a p-value between 0.95 and 0.975, which provides some evidence that the CV_A is lower.

Analyte (units)	Analyser	Chemtrak 1 n	Mean	SD	CV_A%	Cat	Dog	P value#	Chemtrak 3 n	Mean	SD	CV_A%	Cat	Dog	P value#
	Abaxis	14	68.36	1.01	**1.50**	D	D	0.92	11	45.55	0.93	2.1	M	D	0.06
Albumin	IDEXX	15	38.60	1.68	4.40	-	D	<0.01**	11	24.45	1.37	5.6	-	M	<0.01**
(g/L)	**Heska**	13	51.46	1.51	2.90	-	D	0.01**	9	33.33	0.5	**1.5**	D	D	0.72
	Cob-Int	30	45.10	0.8	1.80	-	D		28	30.04	0.51	1.7	M	D	
	Abaxis	14	45.07	3.27	7.30	M	M	<0.01**	11	486.8	8.34	**1.7**	O	O	0.92
	IDEXX	15	65.80	2.62	**4.00**	D	D	0.20	11	332.0	18.8	**5.7**	D	D	<0.01**
ALP (U/L)	**Heska**	13	50.00	2.61	**5.20**	D	D	0.07	10	357.4	19.0	**5.3**	D	D	0.03*
	Cob-Int	31	45.55	1.67	**3.70**	D	D		29	339.8	8.31	**2.4**	O	O	
	Abaxis	14	32.43	2.82	**8.70**	D	D	<0.01**	11	213.0	4.74	**2.2**	D	O	<0.01**
	IDEXX	15	35.73	2.6	**7.30**	D	D	<0.01**	11	219.2	4.36	**2.0**	D	O	<0.01**
ALT (U/L)	**Heska**	13	32.77	0.6	**1.80**	D	O	0.99††	11	215.0	12.0	**5.6**	D	D	<0.01**
	Cob-Int	29	29.28	0.88	**3.00**	D	O		27	232.4	1.55	**0.7**	O	O	
Total	**Abaxis**	14	13.57	1.91	14.10	O	M	<0.01**	11	102.8	6.4	**6.2**	O	O	0.02**
Bilirubin	**IDEXX**	14	10.79	1.85	17.10	O	M	<0.01**	9	92.22	8.29	**9.0**	O	D	<0.01**
(umol/L)	**Heska**	13	13.54	1.56	**11.50**	O	D	0.03*	11	98.82	5.78	**5.8**	O	O	0.02**
	Cob-Int	30	15.43	0.86	**5.60**	O	D		28	123.6	4.64	**3.8**	O	O	
	Abaxis	14	1.61	0.02	1.50	M	-	0.66	11	2.96	0.05	1.8	-	-	0.50
Calcium	IDEXX	15	1.70	0.02	1.20	M	-	0.96†	10	2.88	0.02	0.7	D	M	0.99††
(mmol/L)	Heska	13	1.63	0.04	2.60	-	-	0.02**	9	3.21	0.05	1.4	M	-	0.76
	Cob-Int	35	1.61	0.03	1.70	M	-		33	3.11	0.06	1.8	-	-	
Chloride	IDEXX	14	111.7	3.95	3.50	-	-	0.89	9	95.22	0.67	0.7	M	D	>0.99††
(mmol/L)	Heska	11	113.0	5.63	5.00	-	-	0.689	9	96.11	2.98	3.1	-	-	0.94
	Cob-Int	90	111.2	6.48	5.80	-	-		86	96.63	6.44	6.7	-	-	
	Abaxis	14	68.79	10.5	15.30	-	-	0.01**	11	565.3	21.8	3.9	M	M	0.80
Creatinine	IDEXX	15	68.47	2.95	4.30	M	M	0.85	11	668.3	23.9	3.6	M	M	0.92
(Umol/L)	**Heska**	13	65.31	2.9	4.40	M	M	0.70	10	518.7	9.42	**1.8**	D	D	0.94
	Cob-Int	37	66.51	5.08	7.60	-	-		33	517.8	27.7	5.4	-	-	
	Abaxis	14	3.60	0.1	**2.90**	D	D	<0.01**	11	18.88	0.18	**1.0**	O	O	0.572
Glucose	**IDEXX**	15	3.47	0.14	4.00	D	M	<0.01**	11	16.49	4.87	**2.2**	D	O	<0.01**
(mmol/L)	**Heska**	13	3.19	0.05	**1.50**	O	O	0.31	11	17.45	0.16	**0.9**	O	O	0.69
	Cob-Int	22	3.22	0.04	**1.30**	O	O		28	18.6	0.19	**1.0**	O	O	
	Abaxis	14	1.10	0.06	5.80	M	D	<0.01**	11	2.87	0.07	**2.3**	D	O	<0.01**
Phosphate	**IDEXX**	13	0.88	0.03	**3.30**	D	D	0.02**	8	2.24	0.05	**2.4**	D	O	0.02**
(mmol/L)	**Heska**	13	0.94	0.04	**3.80**	D	D	<0.01**	9	2.70	0.08	**3.0**	D	O	<0.01**
	Cob-Int	29	0.84	0.02	**2.00**	O	O		27	2.39	0.03	**1.2**	O	O	
	Abaxis	14	2.71	0.15	5.40	-	-	<0.01**	11	6.08	0.13	2.2	M	M	<0.01**
Potassium	**IDEXX**	14	2.71	0.23	8.50	-	-	<0.01**	9	6.46	0.05	**0.8**	O	O	0.56
(mmol/L)	Heska	11	2.39	0.12	5.10	-	-	<0.01**	9	5.66	0.19	3.3	-	-	<0.01**
	Cob-Int	87	2.60	0.02	**0.60**	O	O		81	6.09	0.05	**0.9**	O	D	
	Abaxis	14	147.6	2.84	1.90	-	M	<0.01**	11	125.7	1.95	1.6	-	M	0.01**
Sodium	IDEXX	14	157.2	2.23	1.40	-	M	<0.01**	9	127.1	0.78	0.6	M	O	0.62
(mmol/L)	Heska	11	145.1	6.32	4.40	-	-	<0.01**	9	122.0	3.57	2.9	-	-	<0.01**
	Cob-Int	88	142.9	1.01	0.70	-	D		82	117.3	0.89	0.8	-	D	
Total	**Abaxis**	14	71.64	1.15	**1.60**	O	D	0.77	11	39.45	0.82	**2.1**	D	D	0.70
Protein	**IDEXX**	15	65.87	0.99	**1.50**	O	D	0.91	11	39.36	0.92	**2.3**	D	D	0.55
(g/L)	**Heska**	13	67.77	1.54	**2.30**	D	D	0.17	10	40.6	1.26	**3.1**	D	D	0.16
	Cob-Int	29	72.79	1.37	**1.90**	O	D		27	39.93	0.96	**2.4**	D	D	
	Abaxis	14	5.44	0.19	**3.40**	D	D	0.33	11	23.62	0.41	**1.8**	O	O	0.90
Urea	**IDEXX**	15	5.27	0.14	**2.60**	O	O	0.81	11	18.93	0.29	**1.5**	O	O	0.93
(mmol/L)	**Heska**	13	5.53	0.14	**2.60**	O	O	0.67	10	23.7	0.47	**2.0**	O	O	0.78
	Cob-Int	21	5.43	0.17	**3.00**	D	D		27	24.42	0.58	**2.4**	O	O	

differ significantly compared to the commercial laboratory analyser for 8/13 analytes (albumin, ALP, calcium, chloride, creatinine, glucose, total protein, and urea). CV_As from the Abaxis analyser did not differ significantly compared to the commercial laboratory analyser for 7/12 analytes (albumin, ALP, calcium, creatinine, glucose, total protein and urea) and the IDEXX analyser had either significantly lower, or not significantly different CV_As, compared to the commercial analyser for 7/13 analytes (calcium, chloride, creatinine, potassium, sodium, total protein and urea), with calcium ($p=0.99$) and chloride ($p>0.99$) having significantly lower CV_As.

CV_As for the in-house analysers were either less than ($p>0.975$) or not significantly different (p between 0.025 and 0.975) from CV_As for the commercial laboratory analyser for 18/38 of Chemtrak-1 assessments and 22/38 of Chemtrak-3 assessments and therefore 40/76 of all assessments. When combining Chemtrak-1 and Chemtrak-3 assessments, the Heska analyser had significantly lower or not significantly different CV_As to the commercial laboratory analyser for 16/26 of analyses; the IDEXX equipment had 13/26 and the Abaxis instrument, 11/24. A single extreme result was obtained for glucose from the IDEXX analyser, where the result was approximately ten times less than the other analysers' results with Chemtrak-3; the CV_A was 2.25% and 29.5% when this result was excluded and included, respectively.

Discussion

The most important finding of this study is that seven analytes (ALP, ALT, glucose, phosphorus, total bilirubin, total protein and urea) met desirable quality precision standards for Chemtrak-3 and minimum quality standards for Chemtrak-1 on all analysers, meaning that clinicians can be confident that results determined for these analytes are repeatable and that variation in results due to imprecision is small relative to variation in biological status. However, 15-31% of analyte determinations did not reach minimum quality precision standards pooled across both QCMs for cats and dogs.

Since commercial laboratory biochemical results are usually considered the 'gold standard' by veterinarians, it was surprising that fewer analytes reached the minimum quality precision standard (as determined by biological variation) for the commercial analyser compared to two of the in-house analysers (see Table 5). Given the results in relation to biological variation, it was not surprising that the precision of more than half of all analyte determinations across all in-house analysers for both Chemtrak-1 and Chemtrak-3 was not significantly different to or better than, that determined by the commercial laboratory analyser. The IDEXX analyser had a lowest CV_A (of all four analysers) for more assessments with similar results from the commercial laboratory analyser.

In the seminal work concerning biological variation,[13] Cotlove reported that the lowest biologic variation occurred for analytes where there is strict homeostatic regulation of the stability of the composition and volume of extracellular and intravascular fluids (such as sodium, potassium, chloride and albumin) as well as total calcium which has complex regulatory mechanisms. In general, those analytes that did not reach the described quality standards in this study were those that Cotlove identified as having lowest biologic variation, and included the electrolytes (sodium, potassium, and chloride), calcium and albumin. In addition, the desirable quality standard (for both species) for creatinine, an analyte not considered to be under strict homeostatic control,[13] was only reached by the Heska analyser

Table 5 Summary of number of results reaching at least minimum[1], at least desirable[2] and optimum[3] quality precision standards based on biologic variation in cats and dogs for each analyser.

Analyser			Abaxis	IDEXX	Heska	Cobas-Integra
Within day (Chemtrak-1)	Minimum	Cat	7/12	11/13	10/13	
		Dog	9/12	12/13	11/13	
	Desirable	Cat	6/12	10/13	8/13	
		Dog	6/12	12/13	9/13	
	Optimum	Cat	4/12	4/13	7/13	
		Dog	4/12	6/13	7/13	
Between day (Chemtrak-1)	Minimum	Cat	9/12	9/13	8/13	9/13
		Dog	9/12	10/13	9/13	10/13
	Desirable	Cat	6/12	6/13	7/13	8/13
		Dog	6/12	7/13	8/13	10/13
	Optimum	Cat	2/12	3/13	3/13	5/13
		Dog	0/12	1/13	3/13	5/13
Between day (Chemtrak-3)	Minimum	Cat	10/12	12/13	10/13	9/13
		Dog	11/12	13/13	9/13	10/13
	Desirable	Cat	7/12	9/13	9/13	8/13
		Dog	9/12	10/13	9/13	10/13
	Optimum	Cat	4/12	3/13	3/13	6/13
		Dog	6/12	7/13	4/13	6/13
Between day Pooled Chemtrak-1 and 3	Minimum	Cat	19/24	21/26	18/26	19/26
		Dog	20/24	23/26	18/26	20/26
Between day Pooled Chemtrak-1 and 3 for cats and dogs	Minimum	Cat and dog	39/48	44/52	36/52	38/52

1 at least minimum means $CV_A \leq 0.75 \times CV_I$ ie any of minimum, desirable or optimum quality precision standards;
2 at least desirable means $CV_A \leq 0.5 \times CV_I$ ie either of desirable or optimum quality precision standards
3 $CV_A \leq 0.25 \times CV_I$
CV_A = analytical CV; CV_I = biological CV

when assessing Chemtrak-3. For analytes with low biological variation, the 'noise' due to analytical imprecision is likely to be large relative to biological variability, both within an individual and between individuals.[11, 13] When CV_A approaches CV_I, proportionally more of the observed variation is due to the limitations of the analytical equipment rather than intra-subject (biological) variation.

The current American Society for Veterinary Clinical Pathology (ASVCP) guidelines for biochemistry allowable total error [14] note that some instrument/method performance may not be achievable using biological variation specifications, as is also recognised for human clinical pathology.[11] However, these guidelines also note "Improvements in instrument/method performance in the future may allow improved quality requirements based on biologic variation" [14]. Improvement can only be assessed by measurement and this study represents the initial assessment of precision of small animal analysers by this rigorous standard.

Another difficulty in assessing CV_A arises when results are reported as whole integers (as for numerous analytes in this study). For example, duplicate results for chloride reported as 115mmol/L and 116mmol/L represent analytical variation of 0.87% whereas if the true concentrations were 115.4mmol/L and 115.6mmol/L, the analytical variation would be 0.17%.

Several studies have assessed precision of the same models of the veterinary in-house analysers used in this study.[15-21] However, these precision estimates were neither assessed in relation to biological variation nor statistically compared to a commercial laboratory analyser. In these studies, most analytes had CV_As <5%. Where CV_As were high, most studies noted that the quality control solution had very low concentrations of that analyte[16-18] as was observed in this study. This is consistent with other studies that have shown that precision varies with the concentration being assessed;[22-24] as is the case for bilirubin for which the CV_A can be greater than 20% for concentrations less than 17 umol/L (a concentration similar to Chemtrak-1), and 5 to 6% at concentrations around 100 umol/L (similar to Chemtrak-3) regardless of method used.[22] For many analytes, precision at low concentrations is clinically less relevant since despite analytical variation, results will still be well within reference intervals. Therefore, results from Chemtrak-3 (where concentrations were either above or below the reference interval depending on the analyte) could be considered more clinically relevant. When assessing results for Chemtrak-3 only, more analytes reached the minimum (and desirable) standards and notably, the IDEXX analyser met the minimal standards for dogs for all analytes and, for cats, the only exception was albumin. However, for some analytes, most analysers still failed to reach the minimum standards (particularly those with low biological variation such as calcium, chloride and sodium for cats).

Ideally, QCMs should be 'commutable' with patient plasma or serum; that is, the results from QCMs should be consistent with those from plasma or serum samples from healthy and diseased individuals.[25] Species-specific plasma or serum samples, pooled from multiple individuals, are considered the ideal standard for QCMs instead of commercially available QCMs.[26] However, with pooled plasma samples, analyte concentrations can decline over time, requiring new batches of material to be prepared at regular intervals and the concentrations of analytes within each batch to be re-established to confirm that each batch is comparable.[27] Also analyte concentrations in pooled plasma samples can differ from population normal ranges, pooled plasma may be not be commutable for all analytes being assessed, there can be difficulty recruiting donors with pathologic analyte concentrations, and only limited quantities of pooled plasma may be available. For these reasons, authentic clinical specimen pools are not practical for large multi-constituent surveillance quality assurance studies.[28] Additionally, commercial QCMs are acceptable for precision studies under the American Society for Veterinary Clinical Pathology (ASVCP) guidelines.[29] Therefore, QCMs were chosen as the most practical specimen for determination of precision for the period of this study.

Statistical comparisons of CVs have been problematic since the distributions of results can vary between populations [12] as observed in the current study. Parametric approaches have been used, but these methods make assumptions that may not be valid about the underlying distribution of the statistical parameter.[30-32] The bootstrapping (also known as resampling) technique[12] used in this study overcomes these problems. However, this approach only provides information about whether results differ in comparison to those from the commercial analyser but provides no information about the likely magnitude of differences; the results for one analyser may vary more greatly than the commercial analyser yet still be within the biological variations guidelines (as for ALT on the IDEXX and Heska analysers). Further, in-house analyser results may not be statistically different to the commercial

laboratory analyser on occasions where neither the in-house analyser nor the commercial laboratory analyser meets the biological variation standards (as is the case for creatinine when assessed with Chemtrak-1 on the Abaxis analyser).

Some individual analyte inconsistencies were noted; the reason for the very high albumin results for all samples tested on the Abaxis analyser alone is unclear. All in-house analysers, as well as the commercial analyser, determine albumin by the 'bromcresol green' method.[33] The high values from the Abaxis analyser may relate to some other substance in the QCM bound by the bromcresol green dye however this does not occur with plasma samples.[34]

There was also a single aberrant, low reading of glucose with Chemtrak-3 on the IDEXX machine. This glucose result was approximately ten times lower than the other results found for this analyte using Chemtrak-3 and most likely represents random error. In clinical use, unusual results such as this should trigger repeat analysis of the same or a different sample from the patient, and thus, the CV_A without this result was also assessed; this was 2.25% (down from 29.5% when this result was included). Thus this IDEXX equipment mostly gives glucose results with a 2.25% CV_A at a concentration approximately double the high end of the reference interval but occasionally gives spurious incorrect results.

Finally, in summarizing the relative performance of the in-house analysers: the IDEXX analysers (followed by the Abaxis analyser) met the minimum quality standards on more occasions than the other analysers for both QCM's for both dogs and cats (see Table 5). The commercial laboratory analyser performed better for Chemtrak-1 analyses and had more analytes reaching the optimum standards for Chemtrak-1 and Chemtrak-3 for both dogs and cats. Confining analyses to Chemtrak-3, which is likely to be more clinically relevant, the IDEXX analyser had 25/26 of assessments (dog and cat) meeting the minimum standard, the Abaxis analyser had 21/24 of assessments meeting the minimum standard and the Heska and commercial laboratory analysers each had 19/26 of assessments meeting this standard. However, comparisons between analyser models are limited by variation in precision between instruments of the same model. In a recent study of multiple in-house analysers, precision varied between analysers of the same model. [21]

It can be concluded the precision (repeatability) of results from these in-house and commercial laboratory analysers for ALP, ALT, glucose, phosphorus, total bilirubin, total proteins and urea, is generally high, so large differences between repeated results from the same patient are more likely to be due to biological changes rather than analyser variation. The few analytes (such as calcium, creatinine and electrolytes) that do not achieve desirable or minimally acceptable specifications for precision based on biologic variation require special attention including repeated testing, monitoring of trends within the patient over time, and interpretation in conjunction with results of other tests and clinical evaluation in order to correctly interpret these results.

Acknowledgments The authors would like to thank Dr Kathleen Freeman for her expertise, guidance and support as well as Gribbles Pathology (Heska Dri-chem and commercial laboratory), IDEXX Pathology, REM Systems (Vetscan) for providing analysers, and analytes and allowing independent scrutiny of their products.

Funding statement This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest statement The authors have no interest or association with any of the companies represented in this study and declare that there is no conflict of interest.

References

1.Jensen AL, Kjelgaard-Hansen M. Method comparison in the clinical laboratory. *Vet. Clin. Path.* 2006;35(3):276-286.
2.Harris E. Statistical principles underlying analytic goal-setting in clinical chemistry. *Am. J. Clin. Pathol.* 1979;72(2 Suppl):374-382.
3.Fraser CG, Petersen PH. Desirable standards for laboratory tests if they are to fulfill medical needs. *Clin. Chem.* 1993;39(7):1447-1453.
4.Fraser CG, Hyltoft PP, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. *Ann. Clin. Biochem.* 1997;34:8-12.
5.Baral RM, Dhand NK, Freeman KP, Krockenberger MB, Govendir M. Biological variation and reference change values of feline plasma biochemistry analytes. *J. Feline Med. Surg.* 2014;16(4):317-325.
6.Williams GZ, Young DS, Stein MR, Cotlove E. Biological and analytic components of variation in long-term studies of serum constituents in normal subjects. I. Objectives, subject selection, laboratory procedures, and estimation of analytic deviation. *Clin. Chem.* Dec 1970;16(12):1016-1021.
7.Jensen AL, Pedersen HD, Koch J, Aaes H, Flagstad A. Applicability of the critical difference. *J. Vet. Med. A.* 1993;40(1 10):624-630.
8.Ruaux CG, Carney PC, Suchodolski JS, Steiner JM. Estimates of biological variation in routinely measured biochemical analytes in clinically healthy dogs. *Vet. Clin. Path.* 2012;41(4):541-547.
9.Schwendenwein I, Hooijberg E, Hanus B, Frommlet F, Pagitz M. Variance components and critical differences in dogs and cats. Paper presented at: 14th Conference of the European Society of Veterinary Clinical Pathology2012; Ljubljana, Slovenia.
10. Fraser CG. *Biological variation: from principles to practice*: Amer Assn for Clinical Chemistry; 2001.
11. Petersen PH, Fraser CG, Jorgensen L, et al. Combination of analytical quality specifications based on biological within-and between-subject variation. *Ann. Clin. Biochem.* 2002;39(6):543-550.
12. Amiri S, Zwanzig S. Assessing the coefficient of variations of chemical data using bootstrap method. *J. Chemometr.* 2011;25:295-300.
13. Cotlove E, Harris EK, Williams GZ. Biological and analytic components of variation in long-term studies of serum constituents in normal subjects. III. Physiological and medical implications. *Clin. Chem.* Dec 1970;16(12):1028-1032.
14. Harr KE, Flatland B, Nabity M, Freeman KP. ASVCP guidelines: allowable total error guidelines for biochemistry. *Vet. Clin. Path.* 2013;42(4):424-436.
15. Mischke R, Schossier N, Wirth W. Trockenchemische Blutanalysen bei Hund und Katze mit dem VETTEST 8008 im statistischen Vergleich mit naßchemischen Methoden [Blood testing with dry reagents (VetTest 8008) in dogs and cats in comparison with standard methods]. *Kleintierpraxis.* 1992;37:183-199.
16. Little C, Gettinby G, Downs J, Marshall C. Consistency of results from the Vettest 8008 clinical biochemistry analyser. *Vet. Rec.* 1992;130:419-424.
17. Tschudi PR. Evaluation des Trockenchemie-Analysengerätes VetTest 8008 [Evaluation of the dry chemical analysis system VetTest 8008]. *Schweiz. Arch. Tierheilkd.* 1995;137:381-385.
18. Klein A, Mischke R. Klinische Prufung des naßchemischen Analysegerätes VetScan beim Hund [Clinical evaluation of the wet chemical analyser, VetScan, in dogs]. *Prakt. Tierarzt.* 1998;79:1100-1111.
19. Sutton A, Dawson H, Hoff B, Grift E, Shoukri M. Analyte comparisons between 2 clinical chemistry analyzers. *Can. Vet. J.* 1999;40:255-260.
20. Trumel C, Diquélou A, Germain C, Palanché F, Braun JP. Comparison of measurements of canine plasma creatinine, glucose, proteins, urea, alanine aminotransferase, and alkaline phosphatase obtained with Spotchem SP 4430 and Vitros 250 analyzers. *Res. Vet. Sci.* 2005;79(3):183-189.
21. Rishniw M, Pion PD, Maher T. The Quality of Veterinary In-clinic and Reference Laboratory Biochemistry Testing. *Vet. Clin. Path.* 2012;41(1):92-109.
22. Ross J, Fraser M. Analytical clinical chemistry precision. State of the art for fourteen analytes. *Am. J. Clin. Pathol.* 1977;68(1 Suppl):130-141.
23. Ross J, Fraser M, Moore T. Analytic clinical laboratory precision--state of the art for thirty-one analysis. *Am. J. Clin. Pathol.* 1980;74(4 Suppl):521-530.
24. Ross JW, Lawson NS. Analytic goals, concentration relationships, and the state of the art for clinical laboratory precision. *Arch. Pathol. Lab. Med.* 1995;119(6):495-513.
25. Vesper HW, Miller WG, Myers GL. Reference materials and commutability. *Clin Biochem Rev.* 2007;28(4):139.
26. Fraser CG, Peake MJ, Cresswell MA. Problems associated with clinical chemistry quality control materials. *Crit. Rev. Clin. Lab. Sci.* 1980;12(1):59-86.
27. Kjelgaard-Hansen M, Jensen AL, Kristensen AT. Internal quality control of a turbidimetric immunoassay for canine serum C-reactive protein based on pooled patient samples. *Vet. Clin. Path.* 2004;33(3):139-144.
28. Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. *Clin. Chim. Acta.* 2003;327(1):25-37.
29. Flatland B, Freeman KP, Friedrichs KR, et al. ASVCP quality assurance guidelines: control of general analytical factors in veterinary laboratories. *Vet. Clin. Path.* 2010;39(3):264-277.
30. Gupta RC, Ma S. Testing the equality of coefficients of variation in k normal populations. *Commun. Stat-Theor. M.* 1996;25(1):115-132.
31. Nairy KS, Rao KA. Tests of coefficients of variation of normal population. *Commun. Stat-Simul. C.* 2003;32(3):641-661.
32. Pang WK, Leung PK, Huang WK, Liu W. On interval estimation of the coefficient of variation for the three-parameter Weibull, Lognormal and Gamma distribution: a simulation-based approach. *Eur J Oper Res.* 2005;164(2):367-377.
33. Doumas BT, Ard Watson W, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta.* 1971;31(1):87-96.
34. Baral RM, Morton JM, Dhand NK, Krockenberger MB, Govendir M. Bias of feline plasma biochemistry results between three in-house analyzers and a commercial laboratory analyzer: Results cannot be directly compared. *J. Feline Med. Surg.* Accepted.