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Comparison of a full-spectrum multi-analyte clinical analyser with six reference instruments using canine and feline blood samples

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ABSTRACT: In this study, we report the characterisation of a novel centrifugation and spectrum-integrated veterinary clinical analyser, the AmiShield^{TM}, which has been developed for the multiplex measurement of biochemical, electrolyte and immunoassay parameters in a point-of-care testing environment. The aims of this study were to evaluate the analytical performance of the AmiShield^{TM} and to compare it with six reference instruments using clinical blood samples. Two hundred and four canine and 120 feline blood samples collected from veterinary teaching hospitals were analysed in parallel using the AmiShield and appropriate reference instruments. All results were evaluated separately for canine and feline specimens. The instrument's analytical performance was evaluated initially for short- and long-term precision, bias, and observed total error using quality control material. This was followed by comparison of clinical specimens on the AmiShield analyser in parallel with the Vitros and Hitachi for biochemical parameters, VetScan and SNAPshot for total bile acids, and VetLyte and Biolyte for electrolytes. Overall, the AmiShield analyser's performance met the standards of the American Society for Veterinary Clinical Pathology for total allowable error for most analytes, and can be considered suitable for use in veterinary clinical practices. Using canine samples, excellent correlation coefficients ($r \geqq 0.92$) were identified for 14 analytes of various categories including glucose, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, amylase, blood urea nitrogen, creatinine, phosphorus, Na^+, K^+, Cl^- and total bile acid, while good correlations ($0.91 \geqq r \geqq 0.80$) were recorded for albumin ($r = 0.91$). Bland-Altman difference plots also showed agreement (greater than 95% within Limits of Agreement) for glucose, total protein, albumin, alanine aminotransferase, alkaline phosphatase, total bilirubin, amylase, blood urea nitrogen, creatinine, Na^+, K^+, Cl^- and total bile acid between AmiShield and the reference instruments. However, aspartate aminotransferase and phosphorus exhibited higher outliers, implying potential problems associated with matrix interferences such as lipemic samples, which warrant further study. This study demonstrates that the AmiShield compares favourably with standard reference instruments, and the new device generated data of high quality for most analytes in clinical canine and feline samples. The capability of reliably measuring multi-category analytes in one device using minute amounts (170 µl) of whole blood and short turn-around times (< 15 min) underlines the high potential of the device as a good alternative in-house diagnostic application.

Keywords: point-of-care; multi-analytes; whole blood

List of abbreviations

ALB = albumin, **ALP** = alkaline phosphatase, **ALT** = alanine aminotransferase, **AMY** = amylase, **AST** = aspartate aminotransferase, **BUN** = blood urea nitrogen, **CRE** = creatinine, **GLU** = glucose, **LOA** = limits of agreement (± 2 SD from the mean difference), **PHOS** = phosphorus, **TBA** = total bile acid, **TBIL** = total bilirubin, **TE_{obs}** = observed total error, **TP** = total protein

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In-house veterinary clinical analysers undoubtedly play an important role in in situ diagnosis in the modern veterinary practice. They provide veterinarians with valuable diagnostic information regarding disease progression, therapeutic response, and can also help in identifying underlying diseases in an apparently healthy animal. The information usually comes from measurements of various analytes using different methodologies and protocols. However, most available devices can only aim to offer one or a limited number of specific diagnostic categories of data, such as biochemistry, blood gas, electrolytes, or immunoassays. For instance, Hitachi (Hitachi 717 Chemistry Analyser; Hitachi, Tokyo, Japan) and Vitros (Johnson and Johnson Vitros 350 Chemistry System; Ortho Clinical Diagnostics, Johnson and Johnson, Melbourne, Australia) devices exhibit superior accuracy in the determination of biochemistry markers. Biolyte (Biolyte 2000 Electrolyte Analyser; BioCare Corporation, Taoyuan, Taiwan) and VetLyte (VetLyte Blood Electrolyte Analyser; IDEXX Laboratories, Westbrook, Maine, USA) are popular tools for measuring electrolytes while VetScan (Abaxis VetScan VS2 veterinary blood chemistry analyser; Abaxis, Union City, USA) and SNAPshot (SNAPshot Dx Analyser; IDEXX Laboratories, Westbrook, Maine, USA) analysers are good for total bile acid (TBA) analysis. However, it would be costly and inconvenient for a veterinary clinic to set-up and maintain multiple analysers for various categories of routine analytes. In addition, the technical requirements associated with multiple machineries such as larger blood sample volumes (up to 1–2 ml), pre-centrifugation of the sample and pre-loading of the diluents, have, taken together, further complicated the situation. A good clinical system for individual veterinary clinics should aim to provide a point-of-care testing environment which must produce reliable results regardless of the user's skill, as well as a shortened turnaround time and simplified sample handling steps (Broughton and Buckley 1987).

The traditional filter-based optical design is usually based on the use of a selected wavelength, thus limiting their application. Each time when a new marker emerges, a new wavelength has to be selected and validated, as was seen with TBA. In contrast, spectrum-based optical detection allows scanning of the full range of wavelengths and has been gradually accepted as a standard method applicable both in research and in *in vitro* diagnostics in industry. A new veterinary clinical analyser, AmiShield™ (ProtectLife International Biomedical Inc., Taoyuan, Taiwan), incorporating micro-spectrum and micro-fluidic technology, is introduced in this study. To the authors' knowledge, there have been no independent performance evaluations or method comparisons with current clinical analysers published with respect to the AmiShield. The capability of measuring more than 10 multi-category analytes including biochemical parameters, electrolytes and hormone markers per run, together with the short analysis time and minimal sample volume requirements, has made this analyser an attractive alternative in a veterinary clinic setting and warrants further investigation.

The aims of this study were to (1) evaluate AmiShield's analytical performance relative to precision using quality control materials and to describe the coefficient of variation, bias, observed total error relative to current guidelines and (2) to compare canine and feline clinical specimens on the AmiShield analyser in parallel with the Vitros and Hitachi analysers for biochemical parameters, VetScan and SNAPshot for total bile acids and VetLyte and Biolyte for electrolytes.

MATERIAL AND METHODS

Blood sampling from animals. Clinically healthy or diseased samples (174 canine and 120 feline) were obtained after screening of cases admitted to National Taiwan University Veterinary Hospital and 30 healthy or diseased canine samples were obtained from National Chung Hsing University Veterinary Medicine Teaching Hospital in the period October 2013–December 2014.

Approximately 1–1.5 ml of whole blood from dogs and cats were collected using lithium heparin tubes. The heparinised whole blood and/or plasma samples were used when appropriate. Samples with obvious haemolysis were excluded. Moderate lipemic samples were still used for testing. The whole blood sample was used for the measurement of glucose (GLU), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), amylase (AMY), blood urea nitrogen (BUN), creatinine (CRE) and phosphorus (PHOS). The plasma was obtained by centrifugation at 1096 × *g* for 5 min and used for the measurement of Na^+, K^+, Cl^-,

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and total bile acid (TBA). Every clinical specimen was tested within 5 h after collection on the different instruments. All results were evaluated separately for canine and feline specimens.

Instruments and detection methods. The reference instruments included the Vitros (Johnson and Johnson Vitros 350 Chemistry System; Ortho Clinical Diagnostics, Johnson and Johnson, Melbourne, Australia) and Hitachi (Hitachi 717 Chemistry Analyser; Hitachi, Tokyo, Japan) devices for biochemical parameters; VetScan (Abaxis VetScan VS2 veterinary blood chemistry analyser; Abaxis, Union City, USA) and SNAPshot (SNAPshot Dx Analyser; IDEXX Laboratories, Westbrook, Maine, USA) for TBA; and VetLyte (VetLyte Blood Electrolyte Analyser; IDEXX Laboratories, Westbrook, Maine, USA) and Biolyte (Biolyte 2000 Electrolyte Analyser; BioCare Corporation, Taoyuan, Taiwan) for electrolytes. Specifically, GLU, TP, ALB, AST, ALT, ALP, TBIL, AMY, BUN, CRE, PHOS, Na^+, K^+, Cl^- and TBA were measured using both AmiShield™ (ProtectLife International Biomedical Inc., Taoyuan, Taiwan) and Vitros at National Taiwan University Veterinary Hospital, or using both AmiShield and Hitachi at National Chung Hsing University Veterinary Medicine Teaching Hospital. In this study, Vitros and Hitachi served as the reference instruments for biochemistry. For electrolytes, VetLyte and Biolyte served as reference instruments. For TBA, VetScan, SNAPshot, and Hitachi were all used as references. The methodologies for all employed reference instruments are summarised in Tables 1 and 2. The AmiShield and the reference instruments ran the samples in parallel within the timeframe of 5 h. Measurements of quality control samples were performed every day on all analysers before testing.

Measurement using the AmiShield veterinary clinical analyser. For measurements of blood biochemistry, 170 µl of whole blood sample were administered into an unpacked AmiShield comprehensive shield panel disc, and measured according to the manufacturer's protocol. For measurements of electrolytes and TBA, 170 µl of serum or plasma sample were pre-diluted using diluents pre-packed inside the disc pouch. Then, 170 µl of diluted sample were administered onto respective assay discs for analysis according to the AmiShield operation manual.

The AmiShield allows plasma separation by centrifugation of the whole blood inside the analyser; necessary diluents were precisely measured out, mixed and distributed to the cuvettes containing reagents. Full spectrum-based responses can give results in 15 min for 11 analytes.

AmiShield precision. The precision of the AmiShield was determined based on the guidelines of the American Society of Veterinary Clinical

Table 1. Methodologies used by the AmiShield, Vitros and Hitachi analysers for biochemical analysis

Analyte	AmiShield	Vitros[a]	Hitachi[a]
Glucose	enzymatic method – Trinder reaction	enzymatic method – Trinder reaction	enzymatic method – Trinder reaction
Total protein	colorimetric method – biuret	colorimetric method – biuret	colorimetric method – biuret
Albumin	colorimetric method – BCG	colorimetric method – BCG	colorimetric method – BCG
Aspartate aminotransferase	enzymatic method	enzymatic method	enzymatic method
Alanine aminotransferase	enzymatic method	enzymatic method	enzymatic method
Alkaline phosphatase	enzymatic method	enzymatic method	enzymatic method
Total bilirubin	colorimetric method – diazo	colorimetric method – diazo	colorimetric method – DCA
Amylase	colorimetric method	colorimetric method	colorimetric method
Blood urea nitrogen	enzymatic method – GLDH	enzymatic method – GLDH	enzymatic method – GLDH
Creatinine	enzymatic method	enzymatic method	Jaffe method
Phosphorus	molybdate method	molybdate method	molybdate method

BCG = bromocresol green, DCA = 2,4-dichloroaniline, GLDH = glutamate dehydrogenase

[a]The Vitros and Hitachi devices use reagents from VITROS Chemistry Products and DiaSys Diagnostic Systems GmbH, respectively

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Table 2. Methodologies used by the AmiShield, VetScan, SANPshot Dx, VetLyte, Hitachi, and Biolyte for total bile acid and electrolyte analysis

Analyte	AmiShield	VetScan	SNAPshot	Hitachi
Total bile acid	cycling enzymatic method	cycling enzymatic method	SNAP Bile Acid	colorimetric endpoint test
Analyte	AmiShield		VetLyte	Biolyte
Na^+	enzymatic method using β-galactosidase	NA	ISE Fluid Pack	ISE
K^+	enzymatic reaction using PK/LDH		ISE Fluid Pack	ISE
Cl^-	thiocyanate method		ISE Fluid Pack	ISE

PK = pyruvate kinase, LDH = lactate dehydrogenase, NA = not applicable, ISE = ion selective electrode

Pathology (ASVCP) laboratory quality assurance document (Harr et al. 2013). For assessment of the level of imprecision, intra-assay (within-run) variations were assessed by running 15 replicates of quality control material (Bio-Rad Laboratories, Irvine, USA) in a single day. The inter-assay (between-run) variations were assessed by running the same quality control material in duplicate on 15 consecutive working days. The quality control material used in the assay gives slightly higher values than the reference intervals. Intra- and inter-assay repeatability was expressed as the coefficient of variation (CV %), which was calculated according to the following equation:

$$CV = SD/mean \times 100$$

where: CV = coefficient of variation (%); SD = standard deviation

The measured bias (%) of AmiShield was calculated by using the measured mean and the quality control material manufacturer's reported mean for the assayed control material, according to the following formula:

$$bias = (mean_{man} - mean_{meas})/mean_{man} \times 100)$$

where: bias = measured bias of AmiShield (%); $mean_{man}$ = quality control material manufacturer's reported mean; $mean_{meas}$ = measured mean

If bias (%) is a negative number, then the absolute number should be used in calculation. Observed total error was calculated according to the following equation:

$$TE_{obs} = 2CV + bias$$

where: TE_{obs} = observed total error (%); CV = coefficient of variation (%); bias = measured bias of AmiShield (%)

Method comparison. Correlations between AmiShield and reference instruments were determined by Deming regression using EP Evaluator Release 6 (Data Innovations LLC, South Burlington, USA). The value of Pearson's correlation coefficient was either considered excellent ($r \geqq 0.92$), good (r = 0.80–0.91), fair (r = 0.59–0.79), or poor ($r < 0.59$), based on previous studies (Papasouliotis et al. 2006; Papasouliotis et al. 2008). Data were also plotted on Bland-Altman difference plots to evaluate the degree of agreement between AmiShield and the reference instruments.

Acceptability was determined using Deming regression analysis for the presence of systemic error (proportional or constant bias), a method requiring 0 and 1 to lie within the 95% CI of the intercept and slope, respectively. In addition, a good agreement in the Bland-Altman difference plot was regarded as a percentage of non-outliers of the limits of agreement (LOA) of greater than 95%; conversely, a percentage of non-outliers of less than 95% was regarded as poor agreement (Jensen and Bantz 1993; Gary et al. 1999; Jensen Kjelgaard-Hansen 2006; Bland and Altman 2010; Flatland et al. 2014). The percentage of non-outliers of the LOA was defined as the percentage of data points not outside the LOA in the Bland-Altman difference plots.

RESULTS

Precision of AmiShield

Eleven analytes, including GLU, TP, ALB, AST, ALT, ALP, TBIL, AMY, BUN, CRE and PHOS, were evaluated by AmiShield for precision; the results are summarised in Table 3. Overall, the precision of the AmiShield fulfilled all ASVCP requirements and it can be considered as suitable for use in most

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Table 3. The precision values (coefficient of variation (CV), %), bias and observed total error (TE) of the AmiShield Veterinary Clinical Analyser (ASVCP) using control material. Imprecision, bias and observed total error was calculated by using quality control material

Analyte	Between-run precision (CV, %)	Within-run precision (CV, %)	Bias (%)	TE_{obs} (%)	ASVCP allowable TE_a (%) value
Glucose	3.4	4.3	0.3	8.9	20
Total protein	7.3	2.9	0.1	6.0	10
Albumin	4.4	3.4	0.6	7.4	15
Aspartate aminotransferase	8.3	2.9	1.4	7.2	30
Alanine aminotransferase	8.4	6.9	0.6	14.5	25
Alkaline phosphatase	5.1	6.2	2.4	14.9	25
Total bilirubin	6.8	2.7	0.7	6.1	30
Amylase	13.7	11.2	0.3	22.8	25
Blood urea nitrogen	7.0	4.1	1.0	9.1	12
Creatinine	8.0	6.5	2.6	15.6	20
Phosphorus	5.0	2.7	0.9	6.3	15

TE_a = allowable total error, TE_{obs} = observed total error, TE_{obs} (%) = 2 CV (%) + bias (%), TP = total protein

veterinary clinical practices. The within-run variation ranged from 2.7 to 11.2% while between-run coefficients of variation ranged from 3.4 to 13.7%. The observed total error of each biochemical analyte was lower than the value of the ASVCP allowable total error, which indicates that AmiShield is suited for use in veterinary clinical practices (Table 3).

Biochemistry parameters

The correlation between AmiShield and Vitros (the reference instruments in National Taiwan University Veterinary Hospital) on 11 biochemical analytes using canine or feline samples are shown in Figures S1 and S2 (in electronic supplementary ma-

Table 4. Correlation coefficients, regression data, and difference plot data for canine samples measured using the AmiShield and Vitros

Canine analyte	*n*	Deming's regression					Bland-Altman difference plots			LOANO
		r	y-intercept	95% CI of intercept	slope	95% CI of slope	mean difference	LOA	outliers (*n*)	
Glucose	172	0.98	4.04	0.30–7.79‡	0.95	0.92–0.99§	–0.9	–21.9–20.1	8/172	95.35
Total protein	173	0.98	0.42	0.22–0.63‡	0.93	0.91–0.96§	–0.04	–0.6–0.5	3/173	98.27
Albumin	167	0.91	0.29	0.11–0.47‡	0.91	0.85–0.97§	0.02	–0.3–0.4	1/167	99.40
Aspartate aminotransferase	142	0.99	–1.37	–5.60–2.87	0.98	0.96–0.99§	–4.37	–49.2–40.5	9/142	93.66*
Alanine aminotransferase	174	0.99	6.53	–2.20–15.25	0.96	0.94–0.97§	–6.57	–108.0–94.9	2/174	98.85
Alkaline phosphatase	174	0.99	25.02	11.2–38.9‡	0.89	0.87–0.90§	–31.57	–258.0–194.9	1/174	99.43
Total bilirubin	115	0.99	0.2	0.06–0.35‡	0.99	0.96–1.01	0.17	–1.2–1.5	4/115	96.52
Amylase	104	0.97	55.94	102–209‡	0.86	0.82–0.90§	–5.15	–330.6–320.3	3/104	97.12
Blood urea nitrogen	167	0.98	–0.56	–2.01–0.89	1.01	0.98–1.04	–0.23	–14.0–13.6	8/167	95.21
Creatinine	147	0.98	–0.03	–0.14–0.08	1.02	0.99–1.05	0.01	–1.0–1.0	5/147	96.60
Phosphorus	152	0.97	0.35	0.08–0.62‡	0.94	0.90–0.98§	–0.05	–1.7–1.6	8/152	94.74*

LOA = limits of agreement (± 2 SD from the mean difference), LOANO = percentage of non-outliers of the limits of agreement

*Results with poor agreements, non-outliers < 95%

‡Confidence interval for intercept does not include the value zero, indicating statistical evidence of constant bias

§Confidence interval for slope does not include the value 1.0, indicating statistical evidence of proportional bias

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Table 5. Correlation coefficients, regression data, and difference plot data for feline samples measured using the AmiShield and Vitros

Feline analyte	*n*	Deming's regression					Bland-Altman difference plots			LOANO
		r	y-intercept	95% CI of intercept	slope	95% CI of slope	mean difference	LOA	outliers (*n*)	
Glucose	88	0.98	–1.01	–6.94–4.92	1	0.96–1.04	–0.93	–27.4–25.5	4/88	95.45
Total protein	103	0.96	0.04	–0.36–0.44	0.98	0.93–1.03	–0.12	–0.8–0.5	7/103	93.20*
Albumin	62	0.84	–0.05	–0.45–0.36	0.98	0.84–1.12	–0.11	–0.5–0.2	2/62	96.77
Aspartate aminotransferase	100	0.98	4.38	–1.98–10.7	1	0.96–1.04	4.58	–45.0–54.1	5/100	95.00
Alanine aminotransferase	103	0.99	5.59	–2.60–13.8	0.99	0.97–1.02	4.08	–67.8–76.0	4/103	96.12
Alkaline phosphatase	100	0.99	1.68	–3.90–7.26	1.08	1.04–1.12§	8.83	–39.5–57.1	3/100	97.00
Total bilirubin	52	0.99	0.34	0.14–0.54‡	0.91	0.87–0.96§	0.17	–1.4–1.7	2/52	96.15
Amylase	19	0.94	226	–53.4–505	0.85	0.70–0.99§	–42.04	–646.4–562.3	2/19	89.47*
Blood urea nitrogen	120	0.99	0.78	–1.24–2.79	1.01	0.98–1.03	1.01	–14.3–16.3	11/120	90.83*
Creatinine	114	0.97	–0.15	–0.29–0.01‡	1	0.96–1.04	–0.15	–1.1–0.8	3/114	97.37
Phosphorus	86	0.96	–0.14	–0.61–0.32	1.01	0.95–1.08	–0.06	–1.9–1.8	4/86	95.35

LOA = limits of agreement (± 2 SD from the mean difference), LOANO = percentage of non-outliers of the limits of agreement

*Results with poor agreements, non-outliers < 95%

‡Confidence interval for intercept does not include the value zero, indicating statistical evidence of constant bias

§Confidence interval for slope does not include the value 1.0, indicating statistical evidence of proportional bias

terial (ESM); for the supplementary material see the electronic version) and Table 4, and Table 5. Using Deming's regression (Figure S1 in ESM and Table 4) and Bland-Altman difference plots (Figure S2A in ESM and Table 4) to evaluate the comparative results of the 174 canine samples from National Taiwan University Veterinary Hospital, identical correlation factor values were obtained from the two different statistical analysis methods. Excellent coefficient of correlation ($r \geqq 0.92$) were calculated for 10 analytes of various categories, namely, GLU (0.98), TP (0.98), AST (0.99), ALT (0.99), ALP (0.99), TBIL (0.99), AMY (0.97), BUN (0.98), CRE (0.98) and PHOS (0.97), while a good correlation ($0.91 \geqq r \geqq 0.80$) was calculated for ALB ($r = 0.91$). The data range, the slope and intercept, the correlation coefficient and the sample size (n) are also shown in Table 4. Slopes of between 0.86 and 1.02 were obtained (Table 4). Bland-Altman difference plots also showed good agreement (percentage of non-outliers of LOA over 95%) for most biochemical analytes between AmiShield and Vitros (Table 4). The percentages of non-outliers in the LOA for canine AST and PHOS were 93.7% and 94.7%, respectively.

Similar correlation coefficient results were also observed using 120 feline samples as shown in Figure S2B in ESM and Table 5. Using 120 feline samples, excellent coefficients of correlation ($r \geqq 0.92$) were calculated for 10 of the 11 analytes of various categories, namely, GLU (0.98), TP (0.96), AST (0.98), ALT (0.99), ALP (0.99), TBIL (0.99), AMY (0.94), BUN (0.99), CRE (0.97) and PHOS (0.96), while a good correlation ($0.91 \geqq r \geqq 0.80$) was calculated for ALB ($r = 0.84$). The data range, the slope and intercept, the correlation coefficient and the sample size (n) are all shown in Table 5. Slopes of between 0.85 and 1.01 were obtained (Table 5). In both canine and feline samples, in 10 of the 11 analytes, the AmiShield and Vitros measured very similar values. Bland-Altman difference plots also showed good agreement (percentage of non-outliers of LOA over 95%) for most biochemical analytes between the AmiShield and Vitros (Table 5). Only three analytes (TP: 93.2%; AMY: 89.5%; and BUN: 90.83%) showed a percentage of non-outliers of LOA of less than 95%.

The correlation coefficient of the Hitachi at the National Chung Hsing University Veterinary Medicine Teaching Hospital with the AmiShield was also calculated. Thirty canine samples were analysed at the National Chung Hsing University Veterinary Medicine Teaching Hospital using the Hitachi and AmiShield within 5 h (Figure S1B in

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ESM). Similar correlation coefficients were calculated (data not shown). Excellent correlation coefficients ($r \geqq 0.92$) were calculated for seven analytes of various categories, namely, GLU (0.99), AST (0.98), ALT (0.98), ALP (0.97), TBIL (0.97), AMY (0.92), and CRE (0.99), while good correlations ($0.91 \geqq r \geqq 0.80$) were calculated for TP (0.89), BUN (0.90), ALB (0.84), and PHOS (0.89).

TBA

One hundred canine samples were analysed for TBA values using AmiShield and VetScan on the same day. An excellent correlation coefficient result ($r = 0.99$) was achieved (Figure S3A in ESM). Similar correlations were also found in comparison with the SNAPshot ($r = 0.87$) and Hitachi ($r = 0.95$). Comparatively, a larger bias was found between AmiShield, SNAPshot and Hitachi, respectively (Figures S4B and S4C in ESM), and a minimal level of outliers was found between AmiShield and reference instruments (Figure S4 in ESM).

Electrolytes

A total of 97 canine samples were analysed for Na^+, K^+, and Cl^- values using AmiShield and VetScan on the same day. An excellent correlation coefficient result ($r = 0.92$) was achieved for K^+, while good correlations were found for Na^+ (0.90) and Cl^- (0.90) (data not shown). Similar correlations were also found in comparison with the VetLyte (Figures S3B, S3C, and S3D in ESM) and BioLyte (data not shown). A minimal level of outliers was found between AmiShield and VetScan (Figure S5A in ESM), VetLyte (Figure S5B in ESM) and Biolyte (Figure S5C in ESM).

DISCUSSION

The demand for an instrument that is capable of measuring multi-analytes with short turnaround times, small sample volumes and reliable outcomes has lent impetus to the development of miniaturised and inexpensive analytical devices (Tsai and Doong 2004; Tsai and Doong 2005). The miniaturised devices currently available can be divided into wet or dry chemistry-based methods. Wet chemistry-based devices such as the Abaxis VetScan, exhibit superior accuracy in the determination of a panel of selected multiple biochemistry markers in one run. Dry chemistry-based devices, such as the IDEXX VetTest/VetLyte, Arkray Spotchem EZ/EL and FUJI DRI-CHEM, allow flexibility in choosing variable numbers of analytes for measurement. The AmiShield Multiple analyser described in this study incorporates all of these advantages together in one device. It can measure a panel of selected multiple analytes in 15 min, while at the same time also providing flexibility: up to three parameters of various categories can be tested simultaneously. This feature allows any combination of test items to be custom-made to fit diagnostic needs including emerging new markers.

The precision results (Table 3) demonstrated that AmiShield exhibited analytical short- and long-term precision values that meet the guidelines of the ASVCP. Thus, the analyser is suitable for use in clinical applications. The precision studies revealed coefficients of variation of biochemical analytes to be below 11.2% for within-run, and below 13.7%, for between-run analyses. The observed total error was acceptable according to the guidance of the ASVCP. These data indicate that the AmiShield is suitable for the multiplex determination of these analytes in a point-of-care environment.

For the most commonly measured blood biochemistry parameters (GLU, TP, AST, ALT, ALP, TBIL, AMY, BUN, PHOS and CRE), AmiShield showed an excellent correlation with the Vitros Analyser in canine and feline samples (Figure S1 in ESM, Tables 4 and 5). However, both canine and feline testing results showed that the 95% CI of slopes of several values did not include 1; further, the 95% CI of the intercept did not include 0. This indicates proportional and constant discrepancies between the AmiShield and the reference instruments. Thus, the results of the AmiShield should not be directly interchanged with those of reference instruments. Rather, results should be interpreted using reference intervals established by the AmiShield analyser itself.

Bland-Altman difference plots also revealed a good agreement between the AmiShield and the reference instruments for most analytes based on a percentage of non-outliers of the LOA larger than 95% (Figure S2 in ESM, Tables 4 and 5). Although several outliers were observed, which may potentially complicate clinical decision making, this

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represented a total of only six out of 1687 tests (0.36%) using canine samples (Figure S2A in ESM and Table 4). Affected parameters included one case each for AST (15 versus 63 U/l), ALT (56 versus 110 U/l), BUN (9 versus 31 mg/dl) and PHOS (4.8 versus 16.2 mg/dl). In all cases the level measured by the AmiShield was lower than that of the reference instrument. While the current design precludes the possibility of reanalysing these samples, an operator and/or instrument error cannot be ruled out. One notable parameter of concern is ALB. The Deming regression between the AmiShield and the Vitros (Table 4) demonstrated only a good agreement (r = 0.91 for canine samples, r = 0.84 for feline samples). From the methodological point of view, the bromocresol green-dye binding method utilised by AmiShield had been reported to not be reliable in various bird species. However, a previous study demonstrated good-to-excellent correlation for canine (r = 0.98) and feline (r = 0.90) samples in a wet-reagent chemistry analyser format (Papasouliotis et al. 2006). Since the wet chemistry method tends to have higher dilution ratio that could reduce the matrix effect, further investigation with higher ALB concentrations is needed to validate the impact of the matrix effect on this particular parameter. In the current study, we noted that the Vitros showed slightly better agreements with the AmiShield for more parameters (10 vsersus 7) than the Hitachi analyser (Figure S1 in ESM); this might be explained by the newer Vitros model which utilises a different analytical method for some parameters (Table 1), in addition to the larger sample sizes. Whether or not this is also associated with the locations where the animals were admitted and personnel effects is not clear, but together, these results suggest that the novel point-of-care testing analyser gives accurate and reliable results that are equivalent to most commonly used instruments. Thus, the device has great potential as an application in different clinical settings.

Deming's regression and Bland-Altman difference plots revealed excellent correlations and good agreements for the TBA results (Figures S3A and S4 in ESM). A small bias (3.5 mmol/l) when compared SNAPshot was found. This might be due to differences in methodology: a modified ELISA is used in SNAPshot, whereas a cycling enzymatic method is employed by the AmiShield and VetScan. Although one outlier was identified (102 mmol/l by AmiShield versus 140 mmol/l by VetScan), the value was above the respective reference interval to a similar extent; thus, this disagreement between the AmiShield and VetScan would not affect clinical decision making in this case. In contrast, one outlier resulting in disagreement with SNAPshot (2 mmol/l by AmiShield versus 19 mmol/l by SNAPshot) may be significant enough to affect the clinical decision. Therefore, comparative interpretation between two distinct methodologies should be made with great care. Overall, the AmiShield was demonstrated to have excellent correlations and good agreements with reference instruments (only eight outliers out of 132 samples) in clinical TBA measurement (Figure S4 in ESM).

For the three electrolytes tested, the AmiShield in general showed good correlations (r > 0.8) and was in good agreement with the VetScan and VetLyte (Figures S3 and S5 in ESM). Two Na^+ measurements (191 versus 131, and 183 versus 141 mmol/l) in moderately icteric samples were observed to deviate from the Biolyte. Therefore, possible matrix interference cannot be ruled out. In addition, the bias of Na^+ (–12.2–11.6 mmol/l) and K^+ (–0.6 to 0.6 mmol/l) was significantly larger than those reported when wet-reagent analysers (Na^+ 1.7–0.2; K^+ 0.4–0.3; Papasouliotis et al. 2008) and point-of-care testing portable analysers (Na^+ 4.5; K^+ 0.2; West et al. 2014) were used. A previous study indicated that when the photometric measurement range (2.5A) is exceeded, then results may be unpredictably low or even negative (Hubl et al. 1994). Thus, one possible reason for the larger bias could be the high triglyceride (greater than 150 mg/dl, lipemic) concentration which may introduce turbidity and affect enzymatic quantitation.

In conclusion, this comparative study demonstrates the great potential of using AmiShield for clinical canine and feline samples. A wide range of analytes can be measured using a single instrument and the generated results show good-to-excellent correlation with commonly trusted reference instruments such as the Vitros, Hitachi, VetScan, SNAPshot, VetLyte, and Biolyte. The reagent disc contains 11 reaction wells and multiple internal quality control wells, thus allowing multiplex assays in a single run without compromising accuracy and reproducibility. Since the reagent mixture is predistributed and freeze-dried on the microfluidic disc, measurement of analytes becomes greatly simplified and only 170 µl of whole blood is required.

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In addition, the fully automated design supports the veterinarian with one-touch and walk-away operation and effectively reduces human error introduced during centrifugation and serum pipetting. One major advantage of this kind of disc analyser is the small sample size; this is beneficial not only for companion animals such as dogs and cats, but is also particularly important for smaller patients such as rodents and other exotic animals. However, problems associated with matrix interference such as lipemic samples that result in higher numbers of outliers require future study. Proportional and constant error was observed for most analytes, which indicates that the values measured by the AmiShield cannot be used interchangeably with those generated by reference instruments and should be interpreted using reference intervals established by the AmiShield analyser itself.

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REFERENCES

Bland JM, Altman DG (2010): Statistical methods for assessing agreement between two methods of clinical measurement. International Journal of Nursing Studies 47, 931–936.

Broughton PM, Buckley BM (1987): Performance requirements of tests performed nearer the patient. Scandinavian Journal of Clinical and Laboratory Investigation 47, 99–104.

Flatland B, Friedrichs KR, Klenner S (2014): Differentiating between analytical and diagnostic performance evaluation with a focus on the method comparison study and identification of bias. Veterinary Clinical Pathology 43, 475–486.

Gary TE, Pratt MC, Cusick PK (1999): Determination of agreement between laboratory instruments. Contemporary Topics in Laboratory Animal Science 38, 56–59.

Harr KE, Flatland B, Nabity M, Freeman KP (2013): ASVCP guidelines: allowable total error guidelines for biochemistry. Veterinary Clinical Pathology 42, 424–436.

Hubl W, Wejbora R, Shafti-Keramat I, Haider A, Hajdusich P, Bayer PM (1994): Enzymatic determination of sodium, potassium, and chloride in abnormal (hemolyzed, icteric, lipemic, paraproteinemic, or uremic) serum samples compared with indirect determination with ion-selective electrodes. Clinical Chemistry 40, 1528–1531.

Jensen AL, Kjelgaard-Hansen M (2006): Method comparison in the clinical laboratory. Veterinary Clinical Pathology 35, 276–286.

Papasouliotis K, Dodkin S, Murphy K, Woloszczuk G, Fleming M (2006): Analysis of canine and feline blood samples using the Kuadro in-house wet-reagent chemistry analyser. Journal of Small Animal Practice 47, 190–195.

Papasouliotis K, Dodkin S, Murphy KF, Sladen A (2008): Comparison of measurements of 18 analytes in canine and feline blood samples using the in-practice Falcor 350 and the reference KoneLab 30i analysers. Journal of Small Animal Practice 49, 494–501.

Tsai HC, Doong RA (2004): Simultaneous determination of renal clinical analytes in serum using hydrolase- and oxidase-encapsulated optical array biosensors. Analytical Biochemistry 334, 183–192.

Tsai HC, Doong RA (2005): Simultaneous determination of pH, urea, acetylcholine and heavy metals using array-based enzymatic optical biosensor. Biosensors and Bioelectronics 20, 1796–1804.

West E, Bardell D, Senior JM (2014): Comparison of the EPOC and i-STAT analysers for canine blood gas and electrolyte analysis. Journal of Small Animal Practice 55, 139–144.

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