
Peer reviewed version

Link to published version (if available):
10.1177/1098612X15622676

Link to publication record in Explore Bristol Research
PDF-document

University of Bristol - Explore Bristol Research
General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
http://www.bristol.ac.uk/pure/about/ebr-terms
Evaluation of a Hand-Held Point-of-Care Analyser for Measurement of Creatinine in Cats

JA Reeve1,2 S Warman1,2, D Lewis4, N Watson1,2 and K Papasouliotis1,3

Corresponding author: Jenny A Reeve BVSc, MRCVS
jenny.reeve@bristol.ac.uk, phone: 0117 928 9420, fax: 0117 928 9628
Langford Veterinary Services, Small Animal Hospital, Langford House, Langford, BS40 5DU, England, UK

1 School of Veterinary Sciences, University of Bristol, Langford House, Langford, BS40 5DU, England, UK
2 Small Animal Hospital, Langford Veterinary Services, Langford House, Langford, BS40 5DU, England, UK
3 Diagnostic Laboratories, Langford Veterinary Services, Langford House, Langford, BS40 5DU, England, UK
4 Vets Now Hospital, 123-145 North Street, Glasgow, G3 7DA, Scotland, UK

Abstract

Objectives: To evaluate whether a hand-held creatinine analyser (StatSensor® Xpress; SSXp), available for human patients, can be used to reliably measure creatinine in cats.
Methods: Analytical performance was evaluated by determining within-run and between-run coefficient of variation (CV, %), total error observed (TEobs, %) and sigma metrics. 50 client-owned cats presenting for investigation of clinical disease had creatinine measured simultaneously, using SSXp (whole blood and plasma) and a reference instrument (Konelab, serum); 48 paired samples were included in the study. Creatinine correlation between methodologies (SSXp vs Konelab) and sample types (SSXpwhole blood vs SSXplasma) was assessed by Spearman’s correlation coefficient and agreement was determined using Bland-Altman difference plots. Each creatinine value was assigned an IRIS stage (1-4); correlation and agreement between Konelab and SSXp IRIS stages was evaluated.

Results: Within-run CV (4.23-8.85%), between-run CV (8.95-11.72%), TEobs (22.15-34.92%) and sigma metrics (≤3) did not meet desired analytical requirements. Correlation between sample types was high (SSXpwhole blood vs SSXplasma; r = 0.89) and between instruments was high (SSXpwhole blood VS Konelabserum; r = 0.85) to very high (SSXplasma vs Konelabserum; r = 0.91). Konelab and SSXp whole blood IRIS scores exhibited high correlation (r = 0.76). PCV did not significantly affect SSXp determination of creatinine. Bland-Altman difference plots identified a positive bias for the SSXp (7.13μmol/l SSXp whole blood; 20.23μmol/l SSXplasma) compared with the Konelab. Outliers (1/48 whole blood; 2/48 plasma) occurred exclusively at very high creatinine concentrations. The SSXp failed to identify 2/21 azotaemic cats.

Conclusions and relevance: Analytical performance of the SSXp in feline patients is not considered acceptable. The SSXp exhibited high to very-high correlation compared to reference methodology but the two instruments cannot be used interchangeably. Improvements in SSXp analytical performance are needed before its use can be recommended in feline clinical practice.
**Introduction**

Chronic kidney disease (CKD) is highly prevalent within the feline population, affecting up to 20% of clinic-attending cats, with increasing incidence with age.\(^1,2\) The disease is invariably progressive and remains an important cause of morbidity and mortality, particularly within the geriatric population.\(^3,4,5\) Recent literature has explored the incidence of occult, hospital acquired, acute kidney injury (HA-AKI) and identified a prevalence within the feline population of up to 21.2%.\(^6\) Despite a trend towards evaluating more sensitive serum and urine biomarkers of renal disease, creatinine remains an important diagnostic marker.\(^7,8\)

Point-of-care (PoC) creatinine testing has found utility in human medicine in both screening programmes for the prevalence of CKD and early identification of kidney injury in at-risk patients.\(^9,10,11\) PoC analyzers typically have small sample requirements and a rapid turnaround time, providing real-time clinical information.\(^11-15\)

A PoC creatinine analyzer is an attractive tool in feline clinical practice, given the high incidence of CKD in this species and their relative risk of kidney injury. Various publications have assessed the use of PoC creatinine analyzers in human medicine and a single abstract reports their use in dogs.\(^16\) To the authors’ knowledge, such devices have not previously been evaluated in cats. We evaluated a hand-held analyser that is available for use in human patients; the ‘StatSensor® Xpress-i™ Creatinine Hospital Meter’ (SSXp; Nova Biomedical Corporation, Waltham, MA, USA). The aims of this study were (1) to perform imprecision studies using the SSXp to determine whether device performance was acceptable for the measurement of creatinine in cats and (2) to compare the results obtained from clinical samples using the SSXp to those generated from a commercial high-throughput wet
chemistry analyzer (Konelab PRIME 60, Thermo Scientific, Finland). Ethical approval was granted for the study by the University of Bristol Ethics Committee.

Materials and Methods

Clinical cases and samples

Privately owned cats presented to The Feline Centre at Langford between January 2013 and January 2014, for investigation of clinical disease, were eligible for inclusion. Blood (1.5-3ml, jugular venepuncture) was collected into an EDTA tube (TEKLAB, 0.5 ml) for routine haematology and a non-anticoagulant (plain) tube (BD Vacutainer, 1-2.5 ml) for serum biochemical analysis, which included creatinine measurement using the Konelab analyser (Konelabserum). The remaining blood was used for immediate creatinine measurement by the SSXp (1.2µl whole blood [SSXpwhole blood]) and then to fill a microhaematocrit tube (NRIS microhaematocrit tubes, Soda Lime Glass Na-Heparinized [80 iu/ml], Vitrex Medical) to enable centrifugation for determination of packed cell volume (PCV) and measurement of creatinine in the supernatant plasma using the SSXp (SSXpplasm).

Icteric, haemolysed or lipaemic samples were excluded from analysis due to known interferences of bilirubin, haemoglobin and lipaemia with the creatinine methodology employed by the Konelab, and the lack of information regarding the effect of these interferences on the SSXp methodology.17

For the imprecision studies, a pooled serum sample was created using excess serum from clinical samples, stored in frozen aliquots; this sample was first analyzed in duplicate using the Konelab to confirm that the creatinine concentration was within the analytical range of the SSXp.

StatSensor Xpress
The SSXp is a small hand-held battery powered device that utilizes amperometric methodology, detecting \( \text{H}_2\text{O}_2 \) elimination from an immobilized three-enzyme membrane. Single-use Multi-Well™ technology reagent test strips are inserted into the device. Delivery of the clinical sample fills the test strip by capillary action. An audio sound confirms sample receipt and the creatinine concentration (\( \mu \text{mol/l or mg/dl} \)) is displayed after 30 seconds. The reported measurable creatinine range is 27-1056 \( \mu \text{mol/l} \) (0.31-11.95mg/dl); results outwith this are reported as 'Hi' or 'Lo'.

Three quality control solutions (StatSensor Creat Control C1, C2, C3; Nova Biomedical [QCM]) were provided which were run daily and each time a new canister of strips was opened.\(^\text{18}\) The purpose of this was to evaluate device performance prior to sample analysis. In the event of QCM results outside the provided reference interval, algorithms for device calibration were available from the manufacturer.

**Konelab**

Creatinine concentration was measured in serum samples using an enzymatic colorimetric methodology. Calibration procedure and sample analysis were performed according to the manufacturer's instructions.\(^\text{17}\) Control material (Biostat Diagnostic Systems) with low, normal and high values was included in every run of Konelab samples. Methodology accuracy was assessed by continuous bimonthly participation in an external quality assurance programme (RIQAS; Randox). The measuring range is 10-2500\( \mu \text{mol/l} \) (0.11-28mg/dl), extending to 10,000\( \mu \text{mol/l} \) (113mg/dl) following dilution. Imprecision is \( \leq 4.5\% \) total CV; within-run and between-day CV range from 0.4-1.4\% and 0.3-0.5\%, respectively. This laboratory's established reference interval for feline serum creatinine was 133-175\( \mu \text{mol/l} \) (0.37-1.98mg/dl).
Imprecision

Imprecision is the lack of repeatability of a result, during sequential analysis of a sample and is expressed as coefficient of variation (CV; %). Imprecision was assessed by within- and between-run repeatability using the three QCM and the serum pool.

Within-run repeatability was determined by measuring creatinine in the same sample 20 times sequentially (QCM and feline serum pool).

Between-run repeatability was determined from the values generated over a 20 day period (QCM) and by measuring creatinine in duplicate daily for five consecutive days (feline serum pool).19

Sample type and method comparisons

On each day of testing, SSXp whole blood was analysed immediately following sample collection and SSXp plasma within 15-30 minutes afterwards. Plain tubes were transported to the Diagnostic Laboratories where Konelab serum was run within 45-60 minutes post blood collection.

Data collection and statistical analysis

Data regarding the date of sample acquisition, cats’ signalment, disease, creatinine results and PCV were recorded in a commercially available spreadsheet (Microsoft Excel, USA). All statistical analyses were performed using the 2D graphing and statistics software Prism® 4 GraphPad (GraphPad Software Inc., USA).

Imprecision, total error observed (TEobs) and sigma (σ) metrics

Within- and between-run imprecision were determined by calculating the CV (%):

\[ CV = 100 \times \frac{\text{standard deviation (SD)}}{\text{mean}} \]
Total error observed (TE_{obs} \%,) is the sum of random error (imprecision) and systematic error, with the former represented by CV and the latter represented by bias (inaccuracy of the generated result compared to the true value). TE_{obs} for creatinine was determined by the following formula:\textsuperscript{19}

\[
TE_{obs}(\%) = 2 \times CV + \text{bias}(\%)
\]

Bias was calculated using the QCM according to the following formula:

\[
\text{Bias}(\%) = (\text{target - measured})/\text{target}
\]

Where 'target' is the mean creatinine value reported by the manufacturer and 'measured' is the mean creatinine value measured by the SSXp over the 20 day period.\textsuperscript{18}

Since three different QCM (C1, C2, C3) were used daily, bias was determined from each of the three controls.

TE_{obs} was assessed in two ways: (1) TE_{obs}(\%) values for each of the QCM [TE_{obs-C1}, TE_{obs-C2}, TE_{obs-C3}] were calculated using the specific between-run CV and bias for each QCM as determined above; (2) feline-specific TE_{obs}(\%) was calculated using the between-run CV for the feline serum pool and each one of the three QCM bias as determined above. Since bias was determined for C1, C2 and C3, feline-specific TE_{obs-C1}(\%), TE_{obs-C2}(\%) and TE_{obs-C3}(\%) were calculated for comparison. SSXp methodology was considered acceptable if TE_{obs} < total allowable error (TE_{A}). Since published TE_{A} values vary throughout the literature, these were taken from the guidelines of the American Society for Veterinary Clinical Pathology (ASVCP) (TE_{A}: 20\%) and published studies (TE_{A}: 17\%).\textsuperscript{19,20}

Sigma metric values (\(\sigma\)), a universally-accepted objective measure of analytical methodologies, were calculated according to the following formula:\textsuperscript{19,20,21}

\[
\sigma = (\text{TE}_{A}(\%) - \text{bias}(\%))/\text{CV}
\]

TE_{A} values were taken from published studies, bias was taken as the bias for the QCM C1 and CV as the between-run CV for the feline pool.\textsuperscript{19,20} As the TE_{A} values differed between the two sources (17\% vs 20\%), \(\sigma\) was calculated for both to generate one for the low (\(\sigma_{\text{low}}\)) and one for the higher (\(\sigma_{\text{high}}\))
Interpretation of $\sigma$ values was performed as follows: $\leq 3$: poor; $>3$: marginal; $>4$: good; $>5$: excellent; $>6$: world-class.\textsuperscript{22,23}

**Sample type and method comparisons**

Relationships between creatinine values for each collected set of data (Konelab\textsubscript{serum} vs SSXp\textsubscript{whole blood}, Konelab\textsubscript{serum} vs SSXp\textsubscript{plasma}, SSXp\textsubscript{whole blood} vs SSXp\textsubscript{plasma}) were evaluated for normality using the D’Agostino-Pearson omnibus normality test. Using the same data sets, Spearman’s correlation coefficient was calculated to measure the strength of relationship between (a) methodologies (SSXp vs Konelab), (b) SSXp values obtained from different sample types (whole blood vs plasma) and (c) PCV and SSXp\textsubscript{whole blood} creatinine results. To further explore the clinical applicability of the device, every creatinine value was also assigned an IRIS stage ($<140\mu\text{mol/l} [<1.6\text{mg/dl}] = \text{IRIS stage 1}; 140-250\mu\text{mol/l} [1.6-2.8\text{mg/dl}] = \text{IRIS stage 2}; 251-440\mu\text{mol/l} [2.9-5.0\text{mg/dl}] = \text{IRIS stage 3}; >440\mu\text{mol/l} [>5.0\text{mg/dl}] = \text{IRIS stage 4}$) and correlation of IRIS staging between methodologies (Konelab vs SSXp) was evaluated, with IRIS stage 1 considered non-azotemic and IRIS stages 2-4 considered azotemic.\textsuperscript{24} Correlation coefficients were interpreted as proposed by Zady: 0.9-1 very high correlation, 0.70-0.89 high correlation, 0.50-0.69 moderate correlation, 0.30-0.49 low correlation and $<0.30$ little, if any, correlation.\textsuperscript{25}

Sensitivity and specificity for the SSXp’s ability to determine non-azotemic vs azotemic samples (using a cut-off of 140$\mu\text{mol/l}$, based upon IRIS staging) were calculated using the following formulae:

$$\text{Sensitivity} = \frac{\text{true positive results}}{\text{true positive results} + \text{false negative results}}$$

$$\text{Specificity} = \frac{\text{true negative results}}{\text{true negative results} + \text{false positive results}}$$

Deming regression analysis was used to determine the mathematical relationship and the constant (intercept) and proportional (slope) error of the SSXp relative to the Konelab and SSXp\textsubscript{plasma} relative to the SSXp\textsubscript{whole blood} values. Bland-Altman analysis was used to assess agreement between the two
instruments and between SSXp results obtained from the two different sample types. Agreement was considered good when the 95% limits of agreement (LOA; mean of the differences ±2SD) were narrow and the bias was small.

**Results**

*Clinical cases*

50 samples from 44 cats were analysed for inclusion in the study. Two samples, both from the same cat on different occasions, were visibly icteric, thus excluded from analysis. No sample was visibly haemolysed or lipaemic. Of the 43 cats included in the study, ages ranged from 1 year 1 month to 16 years (median 9 years 11 months). There were 20 male neutered, one male entire and 22 female neutered cats of a variety of breeds; 33 Domestic Shorthair, two Domestic Longhair, two British Shorthair, two Persian and one each of Burmese, Norwegian Forest, Siamese and Somali. Thirty one of the 43 cats presented for a single clinical problem and 12 had multiple clinical problems, encompassing a variety of diseases; endocrine (20), urinary tract (15), respiratory (6), cardiac (6), hepatic (4), neurological (3), nutritional (2), gastro-intestinal (2), neoplastic (1) and haematological (1).

*StatSensor*

No technical problems occurred when using the device. The error code ‘E-4’, indicating insufficient sample, occurred three times during precision analysis and repeatability; repeat samples were successfully measured. No error codes occurred whilst measuring clinical samples.

Manufacturer provided target ranges for C1, C2 and C3 QCM were 44-124µmol/l, 133-239µmol/l and 398-663µmol/l (0.50-1.40mg/dl, 1.50-2.70mg/dl and 4.50-7.50mg/dl), respectively. No QCM failures were identified during the study; every result was within its designated range.
Imprecision, total error observed (TE\textsubscript{obs}) and sigma (\sigma) metrics

The imprecision studies identified SSXp imprecision (CV, \%) within- and between-run of 4.23-8.85% and 8.95-11.72% respectively (Table 1).

<table>
<thead>
<tr>
<th>CV (%)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>Serum pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean target:</td>
<td>Mean target:</td>
<td>Mean target:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>84µmol/l (0.95mg/dl)</td>
<td>186µmol/l (2.10mg/dl)</td>
<td>530µmol/l (6.00mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Within-run</td>
<td>6.32</td>
<td>8.85</td>
<td>5.07</td>
<td>4.23</td>
</tr>
<tr>
<td>Between-run</td>
<td>11.72</td>
<td>10.13</td>
<td>10.23</td>
<td>8.95</td>
</tr>
</tbody>
</table>

Table 1: Precision values (CV, \%) for creatinine measurement by the SSXp, as determined by within-run and between-run CV for the three levels of QCM and the pooled feline serum sample.

CV, coefficient of variation (\%); QCM, quality control media; C1, C2 and C3, levels 1, 2 and 3 QCM, respectively.

Quality requirements

Average (mean) TE\textsubscript{obs} (between-run) for QCM was 29.36 (range 22.15-34.92) and for the feline serum pool was 25.87 (range 19.78-29.39) (Tables 2 and 3). No within-run QCs fell outside 20% of the mean target value. 5/20 C1, 1/20 C2 and 5/20 C3 between-run QCs fell outside 20%.
Table 2: Calculated bias and $TE_{\text{obs}}$ of the SSXp at all three level QCM (C1, 2 and 3)

$QCM$, quality control media; $TE_{A}$, total allowable error; $TE_{\text{obs}}$, total error observed; $TE_{\text{obs-C1-3}}$, total error observed for each of the three QCM

<table>
<thead>
<tr>
<th>Serum pool</th>
<th>$TE_{\text{obs-C1}}$</th>
<th>$TE_{\text{obs-C2}}$</th>
<th>$TE_{\text{obs-C3}}$</th>
<th>$\text{Sigma}_{\text{Low}}$</th>
<th>$\text{Sigma}_{\text{High}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>29.39</td>
<td>19.78</td>
<td>28.45</td>
<td>0.616</td>
<td>0.951</td>
</tr>
</tbody>
</table>

Table 3: Calculated total error observed and sigma metrics of creatinine measurement of the feline serum pool by the SSXp

$TE_{A}$, total allowable error; $TE_{\text{obs}}$, total error observed; $\text{Sigma}_{\text{Low}}$, sigma metrics calculated for $TE_{A}$ of 17%; $\text{Sigma}_{\text{High}}$, sigma metrics calculated for $TE_{A}$ of 20%

Creatinine

Analysis of the 48 clinical samples generated a wide range of Konelab creatinine values (range: 48-1299µmol/l [0.54-14.69mg/dl], median: 129.5µmol/l [1.46mg/dl]), reflecting all IRIS CKD stages (stage 1: 27 samples, stage 2: 17, stage 3: one, stage 4: three) thus enabling evaluation of SSXp performance across a wide range of creatinine concentrations.

The range of creatinine results generated by the SSXp on clinical samples was 63-682µmol/l (0.71-7.71mg/dl; median 141µmol/l [1.60mg/dl]) for SSXp$_{\text{whole blood}}$ and 73-664µmol/l (0.83-7.51mg/dl; median 170.5µmol/l, [1.93mg/dl]) for SSXp$_{\text{plasma}}$. No ‘Hi’ or ‘Lo’ results were generated.

Comparison studies
Comparing SSXp and Konelab, very high correlation was demonstrated between SSXp\textsubscript{plasma} and Konelab\textsubscript{serum} \((r = 0.91, P<0.0001)\) and high correlation between SSXp\textsubscript{whole blood} and Konelab\textsubscript{serum} \((r = 0.85, P<0.0001)\). LOA were very wide \((474.6 \mu\text{mol/l} [5.37\text{mg/dl}] \text{ and } 433.6\mu\text{mol/l} [4.90\text{mg/dl}]\) respectively). Similarly high correlation was demonstrated between SSXp\textsubscript{whole blood} and SSXp\textsubscript{plasma} \((r = 0.89, P<0.0001, \text{ although again, with relatively wide LOA: } 206.78\mu\text{mol/l} [2.34\text{mg/dl}])\). There was no statistically significant correlation between PCV and SSXp\textsubscript{whole blood} creatinine values \((r = -0.28)\), across the tested range (PCV 18-42%). These findings are shown in Tables 4 and 5.

SSXp\textsubscript{whole blood} determination of azotemia (compared to Konelab\textsubscript{serum}) as based on IRIS staging (creatinine ≥140\mu\text{mol/l} [≥1.6\text{mg/dl}]) had sensitivity of 90\% and specificity of 82\%.

<table>
<thead>
<tr>
<th>Study</th>
<th>Correlation Coefficient</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Konelab\textsubscript{serum} vs SSXp\textsubscript{whole blood} ((n=48))</td>
<td>0.85</td>
<td>0.73 – 0.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>b) Konelab\textsubscript{serum} vs SSXp\textsubscript{plasma} ((n=48))</td>
<td>0.91</td>
<td>0.85 – 0.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>c) SSXp\textsubscript{whole blood} vs SSXp\textsubscript{plasma} ((n=48))</td>
<td>0.89</td>
<td>0.82 – 0.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>d) PCV vs SSXp\textsubscript{whole blood} ((n=46))</td>
<td>-0.28</td>
<td>-0.53 – 0.02</td>
<td>0.0609*</td>
</tr>
<tr>
<td>e) Konelab\textsubscript{serum} vs SSXp\textsubscript{whole blood} (IRIS stages) ((n=48))</td>
<td>0.76</td>
<td>0.61–0.86</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4. Comparison studies, as expressed by Spearman’s correlation coefficient and 95\% confidence intervals for creatinine measurement between different methodologies (Konelab vs SSXp) \((a, b)\), creatinine measurement between different matrices (SSXp\textsubscript{whole blood} vs SSXp\textsubscript{plasma}) \((c)\), creatinine measurement vs PCV \((d)\) and IRIS stages between different methodologies (Konelab vs SSXp) \((e)\)

\(r\), Spearman’s correlation coefficient; CI, confidence interval; *, not statistically significant

Constant error, proportional error and bias for the SSXp relative to the Konelab and SSXp\textsubscript{plasma} relative to the SSXp\textsubscript{whole blood} values are presented in Table 5 and Figures 1(a-d) and 2(a,b).
<table>
<thead>
<tr>
<th></th>
<th>Deming regression</th>
<th>Bland-Altman</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>95% CI</td>
</tr>
<tr>
<td>Konelab&lt;sub&gt;serum&lt;/sub&gt;</td>
<td>0.56±</td>
<td>0.47–0.65</td>
</tr>
<tr>
<td>vs SSX&lt;sub&gt;whole&lt;/sub&gt;</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konelab&lt;sub&gt;serum&lt;/sub&gt;</td>
<td>0.48±</td>
<td>0.40–0.57</td>
</tr>
<tr>
<td>vs SSX&lt;sub&gt;plasma&lt;/sub&gt;</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>SSX&lt;sub&gt;whole&lt;/sub&gt;</td>
<td>0.80±</td>
<td>0.70–0.91</td>
</tr>
<tr>
<td>blood</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>vs SSX&lt;sub&gt;plasma&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Deming regression analysis results, depicting constant (intercept) and proportional (slope) error, with 95% confidence intervals. Bland-Altman data used to determine agreement between methodologies.

CI, confidence interval; LOA, limits of agreement.

Figure 1; Deming-regression plots between Konelab and (a) SSX<sub>whole</sub> blood and (b) SSX<sub>plasma</sub>. Bland-Altman difference plots between Konelab and (c) SSX<sub>whole</sub> blood and (d) SSX<sub>plasma</sub>.

[Insert figure 1a]
[Insert figure 1b]
[Insert figure 1c]
[Insert figure 1d]
Discussion

To the authors’ knowledge, this is the first study in which analytical and clinical performance of a hand-held PoC creatinine analyser has been evaluated for use in cats.

Imprecision guidelines are provided by Westgard, the governing standard for analytical quality requirements, with desired within-run and between-run imprecision for serum creatinine of 2.98% and 3.96% respectively; indicating inadequate precision of the SSXp (within-run and between-run CV of 4.23-8.85% and 8.95-11.72% respectively), compared to laboratory standard.27 A recent study evaluating the SSXp in human patients (whole blood) reported comparable findings, with mean within-run imprecision of 2.3-5.9% and mean between-run imprecision (using three level QCM) of 4.2-9.0%; therefore similarly failing to meet desired specifications.11 CV values for pooled feline serum (our study) and whole blood (previous human studies) were lower than for QCM.11 This is unexpected; the uniform matrix of the latter would be expected to yield a more consistent result compared with the less homogenous matrix of whole blood, plasma or serum.11

Average TEobs (QCM: mean 29.36%, range 22.15-34.92%; feline serum pool: mean 25.87%, range 19.78-29.39%) also failed to meet desired TEA (17, 20%).18,19,20 Possible explanations for this include machine, reagent strip, operator or environmental error; further work would be needed to identify which factors are most influential. Sigma metrics for the SSXp was consistent with very poor
performance ($\sigma \leq 3$) at both creatinine TE thresholds. A recent study evaluating the SSXp in human patients neither reported TE$_{obs}$ nor sigma metric values, so a direct comparison cannot be made.\textsuperscript{11} Whilst creatinine is reported to be among those biochemical analytes having the greatest imprecision, bench-top veterinary devices perform considerably more precisely; a recent study evaluating analytical performance of a dry chemistry analyser reported TE$_{obs}$ and $\sigma$ for feline creatinine of 10 and 5.7 respectively.\textsuperscript{28,29}

The population of cats in our study had a serum creatinine range, as determined by Konelab, of 48-1299µmol/l (0.54-14.69mg/dl). Despite being reported to measure creatinine over a range of 27-1056µmol/l (0.31-11.95mg/dl), the SSXp range for the same population was from 63-682µmol/l (0.71-7.71mg/dl) and 73-664µmol/l (0.83-7.51mg/dl) for SSXp$_{whole~blood}$ and SSXp$_{plasma}$ respectively, indicating a failure of the SSXp to accurately quantify higher creatinine values. Further studies evaluating greater numbers of markedly azotemic samples are required to further elucidate the SSXp performance at high creatinine concentrations.

Although SSXp is intended for creatinine measurement in whole blood, we also measured creatinine in plasma as the similar matrix of plasma and serum rendered comparison between SSXp and reference methodology more representative. Indeed, SSXp$_{plasma}$ exhibited greater correlation with Konelab$_{serum}$ ($r = 0.91, P<0.0001$) than did SSXp$_{whole~blood}$ with Konelab$_{serum}$ ($r = 0.85, P<0.0001$).

The Konelab$_{serum}$ vs SSXp$_{plasma}$ and Konelab$_{serum}$ vs SSXp$_{whole~blood}$ regression plots revealed two (1299µmol/l vs 644µmol/l [14.69mg/dl vs 7.29mg/dl], 799µmol/l vs 408µmol/l [9.04mg/dl vs 4.62mg/dl]) and one (1299µmol/l vs 601µmol/l [14.69mg/dl vs 6.80mg/dl]) data points respectively, markedly deviating from the line of best fit. Whilst the trend at lower creatinine values
was for the SSXp (whole blood and plasma) to have a positive bias relative to the Konelab, this
trended towards a considerable negative bias of SSXp compared with Konelab at higher creatinine
values; with increased discordance associated with higher creatinine concentrations as indicated
above. The reason for this switch from a positive to negative bias was not apparent and would require
further investigation. Furthermore, this is of considerable importance when considering clinical
applicability of the device; had a consistent positive or negative bias been identified, this may have
enabled either generation of a device-specific reference range, or, application of a linear algorithm to
SSXp results to yield greater correlation with reference methodology. However, the switch from a
positive to negative bias makes exploring both of these options obsolete. The finding of a negative
bias of the SSXp vs reference methodology at higher creatinine values was also identified in a recent
human study; with demonstration of improved correlation if values >600µmol/l (>6.79mg/dl) were
excluded from analysis (r of 0.99 and 0.97 respectively). The higher correlation in this previous
study can be accounted for by excellent correlation at lower creatinine values, in comparison to the
positive bias exhibited when SSXp is used to evaluate feline creatinine. Similarly, apparently higher
correlation between reference methodology and SSXp evaluation of canine creatinine (r=0.954) in a
previous study can be explained by a narrower range of creatinine values (maximum 610µmol/l
[6.9mg/dl]). Discordance from laboratory methodology with increasing creatinine values has also
been identified in earlier models of the SSXp. It is possible that unmeasured metabolites
associated with azotemia cause interference with the methodology at higher creatinine values. These
deviated data points also appeared as the only outliers on the Bland-Altman difference plots. If these
outliers were excluded from analysis, this considerably narrowed the 95% limits of agreement, from
424.9µmol/l (4.81mg/dl) with outliers, to 150.1µmol/l (1.70mg/dl) without outliers for Konelab Serum
vs SSXp whole blood and from 464.8 µmol/l (5.26mg/dl) with outliers to 114.1µmol/l (1.29mg/dl)
without outliers for Konelab Serum vs SSXp Plasma.
Comparing Konelab$_{\text{serum}}$ vs SSXp$_{\text{whole blood}}$ in 36/48 paired samples the same IRIS stage was established by both instruments. In the remaining 12 paired samples, the SSXp and Konelab IRIS stages differed by a single stage; in 6/12 the SSXp creatinine value would have resulted in a falsely diagnosed azotemia (SSXp stage 2 vs Konelab stage 1) and in 2/12 the SSXp failed to identify an azotemia (SSXp stage 1 vs Konelab stage 2 for both). These findings (36/48 correctly staged, 19/21 azotaemic patients identified) demonstrate reasonable sensitivity (90%) and specificity (82%) of the SSXp for identification of azotemia as based on IRIS staging (creatinine $\geq 140\mu\text{mol/l} \geq 1.6\text{mg/dl}$).

Previous studies have considered variability between whole blood and plasma matrices and thus evaluated the impact of PCV on PoC testing, specifically regarding blood glucose and lactate concentrations.$^{30,31}$ Regarding the former, previous studies have consistently identified an inverse correlation between HCT and blood glucose concentration; proposed mechanisms for this relationship include erythrocytes causing mechanical impedance of plasma diffusion into the reagent strip, blood viscosity affecting fluid permeability in the reagent layer and other matrix-associated complications (e.g. micro-clot formation, protein deposition, fibrin aggregation) or haemolysis.$^{31}$ Conversely, PCV does not appear to influence lactate measurement; with a lack of correlation reported ($r = 0.04; P = 0.77$; PCV range 14-57.5%). Our findings of no significant correlation between PCV and SSXp$_{\text{whole blood}}$ ($r = -0.28$) suggest that within the tested range (18-42%), PCV does not significantly affect SSXp measurement of creatinine. Further testing would be required to evaluate the effect of severe anaemia or erythrocytosis. The previous study evaluating the SSXp in human patients used whole blood but did not evaluate the impact of PCV on creatinine determination.$^{11}$

**Conclusions**
The SSXp was easy-to-use, providing rapid patient-side results. Precision studies identified poor repeatability and reproducibility, not meeting acceptable guidelines for a PoC device. Despite this, high to very-high correlation was exhibited, compared to the reference methodology for creatinine determination. The SSXp correctly classified the IRIS stage in 36/48 of patients, although 2/21 azotemic cats were not identified. Improved device precision and accuracy are required prior to recommending its’ use in feline patients.

Acknowledgements

The authors would like to thank all the clinicians and nursing staff involved in the cases included in the study and Nova Biomedical for providing the StatSensor Xpress and associated materials.

Funding

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

Conflicts of interest

The authors do not have any potential conflicts of interest to declare.

References


