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ORIGINAL ARTICLE

Clinical application of the StatSensor and StatSensor Xpress point-of-care creatinine measurement devices in dogs

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Abstract

Background: Creatinine is a universally important blood parameter used to detect and monitor acute and chronic kidney disease. Reliable measurements at the bedside remain a challenge in human and veterinary medicine. Despite its potential, a trustworthy point-of-care creatinine biosensor has yet to be established.

Objectives: We aimed to determine the precision and accuracy of the StatSensor (SS) and StatSensor Xpress (SSX) handheld creatinine measurement devices in dogs.

Methods: Paired creatinine samples from dogs with normal (creatinine ≤159 μmol/L), moderate (159–354 μmol/L), and marked (>354 μmol/L) azotemia were compared with a commercial enzymatic analyzer. Within-day precision and linearity studies were performed prior to method comparison studies. Method comparison was evaluated using Bland–Altman, concordance correlation coefficient, Deming, and Passing-Bablok regression analysis.

Results: Seventy-eight dogs were enrolled in the study, including 28 (35%), 25 (32%), and 26 (33%) with normal, moderate, and marked azotemia. Total error surpassed recommendations for all devices, and linearity deviated from identity for the SS1 and SS2. The concordance correlation coefficients of the SS1, SS2, SSXI, and SSX2, were 0.69, 0.59, 0.82, and 0.44, respectively. Bland–Altman analyses showed a high variation in the differences, and relationships showed high heteroskedasticity with negative systemic bias among high creatinine concentrations.

Conclusions: Neither the SS and SSX are considered acceptable for clinical applications in dogs. Further research is indicated for the development of a reliable, cost-effective, point-of-care creatinine analyzer to improve the rapid detection and monitoring human and veterinary patients.

KEYWORDS
analyzer, azotemia, biosensing techniques, kidney, uremia

1 | INTRODUCTION

Creatinine is a universally important blood parameter that has clinical implications for the detection and monitoring of acute and chronic kidney disease. As creatine phosphate is broken down at a constant rate within muscle tissues, it is released into the circulation as a non-protein bound molecule and freely filtered by the glomerulus.[1] Although creatinine is secreted by the renal tubules to a small

degree in male dogs, it does not undergo reabsorption, and extrarenal elimination is considered negligible.[1,2] In veterinary medicine, blood creatinine concentrations have been used in the evaluation of prerenal, renal, and postrenal azotemia, in addition to providing a clinical estimate of glomerular filtration rate.[1] Creatinine concentrations are often trended in-hospital to quantitatively assess the effectiveness of various therapies in the management of acute kidney injury; the long-term management of chronic kidney disease is often similarly dependent on these trends. Its routine measurement in hospitalized patients also facilitates the detection of relative elevations from baseline, which may have serious implications, particularly in critically ill patient populations.[3] Early detection of these changes provide the optimal groundwork for intervention and improvement in patient outcomes.[4]

Despite its frequency of measurement and potentially grave clinical implications, reliable measurement of creatinine at the bedside has remained a challenge in both human and veterinary medicine.[5] Even among veterinary laboratories, substantial variability has been identified, highlighting its intrinsic difficulties as a biomarker.[6,7] The Jaffé method, where picric acid reacts with creatinine to form a chromogen with a specific wavelength absorption range, has contributed to the widespread rapid measurement of creatinine.[5] However, it is nonspecific for creatinine, temperature, and pH sensitive, often requires a large volume of serum, and may overestimate creatinine concentrations compared with other analytical techniques.[5] While enzymatic laboratory clinical analysis has been reported to be one of the most accurate detection methods and least susceptible to interferences, these machines are not always readily accessible, have variable turnovers times, and are associated with considerable costs.[8] High-performance liquid chromatography, often considered the gold standard for creatinine measurement, is similarly limited by technical and economic constraints.[9] The use of a handheld point-of-care (PoC) creatinine analyzer would facilitate both rapid screening during outpatient visits and serial in-hospital monitoring for various conditions, such as acute kidney injury. Despite its potential, a trustworthy PoC creatinine biosensor has yet to be established in either human or veterinary medicine.[5]

The objective of this study was to determine the precision and accuracy of two commercially available handheld creatinine measurement devices in dogs with normal, moderate, and severe azotemia of various etiologies.

2 | MATERIALS AND METHODS

2.1 | NOVA StatSensor and StatSensor Xpress

The StatSensor (SS) and the StatSensor Xpress (SSX) (Nova Biomedical Corporation, Waltham, USA) are small handheld battery-powered devices that analyze creatinine concentrations on whole blood delivered by capillary action into multilayer enzyme reagent strips termed biosensors. The enzymatic conversion of creatinine to hydrogen peroxide is facilitated by creatinine amidohydrolase, creatine amidohydrolase, and sarcosine oxidase, with subsequent amperometric detection of hydrogen peroxide. The SS and SSX reportable ranges are 27 to 1056 μmol/L as stated by the manufacturer. The required sample is 0.0012 mL of anti-coagulated whole blood for both machines. Two models of each, the SS (SS1 and SS2) and the SSX (SSX1 and SSX2), were used in the study.

2.2 | Vitros 4600 chemistry analyzer

The Vitros 4600 Chemistry Analyzer (VCA) (Ortho Clinical Diagnostics, Inc, Rochester, USA) served as the reference standard for method comparison. Creatinine was analyzed on heparinized plasma samples using the Vitros Crea Slide method; creatinine is hydrolyzed to creatine and subsequently converted to sarcosine and urea by creatine aminohydrolase. Sarcosine oxidase oxidizes sarcosine to glycine, formaldehyde, and hydrogen peroxide. A leuco dye undergoes peroxidase-catalyzed oxidation producing a colored product resulting in a change in reflection density proportional to the concentration of creatinine. The VCA reportable range is 4 to 1238 μmol/L, as stated by the manufacturer. The required sample is 0.006 mL of serum or heparinized plasma. All measurements were carried out in duplicate, and the mean was calculated. The Vitros 4600 Chemistry Analyzer was routinely calibrated using Ortho Clinical calibrators, and a daily quality control protocol was adhered to throughout the duration of the study. Following calibration, a quality control sample was performed in duplicate with comparison to provided means. A mean was established following 20 quality control samples and it was ensured that the mean was within the limits provided by Ortho Clinical. Quality control was set to flag values outside of two standard deviations of the mean. Trends in quality control evaluations were evaluated monthly. The within laboratory coefficient of variation of the VCA was 1.0%-4.8%. The reference laboratory is also a part of a Veterinary Laboratory Association Quality Assurance Program, which provides information on how values compare to other customers using the same machine.

2.3 | Within-day precision

Within-day precision was evaluated using three different concentrations of quality controls on the SS and the SSX. Each quality control solution was serially tested 10 times. Analytical CV was compared with the American Society for Veterinary Clinical Pathology (ASVCP) guidelines with a reported range of 3.65%-10.95% for the recommended analytical CV.[10]

2.4 | Total error

Observed total error (TE_{obs}) was calculated from quality control data as the sum of the CV and the bias as determined by the following formula: TE_{obs} (%) = 2×CV+bias (%).[10] Bias was calculated by the following

formula: Bias (%) = (target mean − measured mean)/target mean × 100. Observed total error (TE_{obs}) was compared with to the ASVCP guidelines with a recommended allowable total error (TE_a) of 20%.[10]

2.5 | Linearity

Linearity of the SS and the SSX was assessed via a Nova Creatinine Linearity Kit (Nova Biomedical Corporation, Waltham, USA) over the range of 84–862 µmol/L by averaging quadruplicate measurements of five linearity concentrations and comparing the average to the expected value for each concentration. Linearity was further assessed via simple linear regression, visual inspection of the scatterplot, and evaluation of the slope and 95% CI.

2.6 | Method comparison

Dogs presented to the Mathew J. Ryan Veterinary Hospital, University of Pennsylvania, were prospectively enrolled in the study based on in-hospital PoC testing, blood work accompanying dogs for referral or by routine testing in the clinical pathology laboratory. One milliliter of whole blood was collected from client-owned dogs following owner consent. Blood samples were obtained either by direct venipuncture of the cephalic, lateral saphenous, or jugular vein or drawn from an indwelling intravenous catheter after removal of a 6 mL pre-sample. Following collection, 0.0012 mL of anti-coagulated whole blood was used for SS measurements and 0.0012 mL for SSX measurements. The remainder of the sample was centrifuged to obtain heparinized plasma samples, which were then analyzed using the VCA. Daily quality control testing was performed on the creatinine monitors and the laboratory VCA. All plasma samples were classified as lipemic, icteric, or hemolytic, if applicable, with evaluations of both the physical appearance and a numeric value assigned by the VCA. Samples were subsequently classified as either normal (creatinine concentration ≤159 µmol/L), moderate (159–354 µmol/L), or marked (>354 µmol/L) azotemia on the VCA. Data collection was continued until a minimum of 25 samples were collected for each group. Following data collection, samples were subsequently classified according to IRIS staging of chronic kidney disease (CKD) for further analysis.[11] Dogs were permitted to be enrolled in the study a second time following owner consent if a minimum of 48 hours had elapsed between sample collection.

2.7 | Statistical analysis

A commercial statistical analysis program (Stata/IC 16.1; StataCorp LLC) was used for statistical analysis. The normality of data was assessed using the Shapiro–Wilk test. Descriptive data were presented as the median and range as not all data sets were normally distributed. Fisher's exact test was used to compare all proportions. Continuous variables were compared between groups using the two-sample independent *t*-test for normally distributed variables and the Wilcoxon rank-sum test for data that were not normally distributed. The coefficient of variation (% CV) from the mean and standard deviation from replicate measurements was calculated for evaluation of within-day precision.

The average measurements of each SS1, SS2, SSX1, and SSX2 analyzer were plotted on a graph against the assigned creatinine concentration of the five linearity solutions. Lin's concordance correlation coefficient (CCC) was assessed to evaluate equivalence between the measures. Consistent with other correlation statistics, the CCC varies from −1 to 1. A CCC of 1 corresponds to a perfect agreement between the pair of measures compared. In a similar fashion, a value of −1 represents perfect inverse agreement. The value of 0 is associated with no agreement.[12] Simple linear regression was also performed, and the slope and 95% confidence interval (CI) were reported.

The agreement between methods was evaluated using Bland–Altman analysis to calculate both the average difference with 95% CI and the limits of agreement (average difference +/− 1.960 standard deviation) between the results of the SS1, SS2, SSX1, and SSX2 with the VCA as the reference standard. Since the Bland–Altman analysis does not specify if an agreement is sufficient, a clinical goal was specified at an acceptable average difference of 26.4 µmol/L, where the standard deviation of the difference did not exceed the average difference.[13] This acceptable average difference was derived from the IRIS grading of acute kidney injury, where the detection of an absolute increase in creatinine concentration of 26.4 µmol/L in a 48-hour period is associated with an acute kidney injury.[14] The frequency of outliers defined as points outside the 95% CI of the difference was recorded and deemed acceptable if the percent of outliers from the total number of observations did not exceed 5%. Additionally, Deming and Passing-Bablok regression were performed to further characterize agreement. Two-sample independent t-tests were used to compare Lin's concordance coefficients between devices.

IRIS limit agreements between the SS1, SS2, SSX1, and SSX2 and reference analyzer were assessed using the weighted kappa statistic and percent agreement. The interpretation of the kappa values was based on previous recommendations.[15] For all comparisons, $P < 0.05$ was considered statistically significant.

3 | RESULTS

A total of 71 dogs were prospectively enrolled in the study from April 2018 to September 2020. Eight dogs were included in the study twice, resulting in a total of 79 paired samples. Thirty-nine samples were each analyzed on the SS1 and SS2, 38 samples were analyzed on the SSX1, and 42 samples were analyzed on the SSX2. The median time between samples was 10.5 days (range: 2 days, 39 days). The median age of all dogs was 8 years (range: 4 months, 15 years). The median age of dogs in the normal, moderate, and marked azotemia groups was 5 years (range: 1 year, 14 years), 11.5 years (range: 4 months, 15 years), and 6 years (range: 1 year, 15 years).

Non-azotemic dogs were significantly younger than dogs with moderate azotemia (P = 0.002), though no other age-related differences were identified between groups. Thirty (44%) dogs were spayed females, 28 (41%) dogs were castrated males, 7 (10%) dogs were intact males, and 3 (4%) dogs were intact females. Intact male dogs were overrepresented among the marked azotemia group ($P = 0.002$). Twenty-nine (41%) dogs were mixed breed dogs, 4 (6%) dogs were Chihuahuas, 3 (4%) dogs were Maltese, and 2 (3%) dogs were of the following breeds: Shetland Sheepdog, Mastiff, Great Dane, German shepherd, Cavalier King Charles spaniel, and beagle. Three (4%) dogs did not have their breed classified.

Twenty-eight (35%) samples were classified as normal (creatinine concentration ≤159 μmol/L), 25 (32%) samples were classified as moderate (159–354 μmol/L), and 26 (33%) samples were classified as marked (>354 μmol/L) azotemia on the VCA. Mild and moderate hemolysis were noted in 21 (27%) and 2 (3%) samples, respectively. Mild and marked icterus were noted in 3 (4%) and 1 (1%) samples, respectively. Mild and moderate lipemia was noted in 13 (16%) and 2 (3%) samples, respectively. Non-azotemic samples were significantly more likely to have mild lipemia than samples with moderate or marked azotemia ($P < 0.001$). No other differences pertaining to sample quality were identified between groups.

Within-day precision studies for quality control material on the SS1, SS2, SSX12, and SSX2 analyzers are presented in Table 1. Mean creatinine concentrations for low and medium quality control concentrations were within the manufacturer's expected ranges for all analyzers. Mean creatinine concentrations for high-quality control concentrations were within the manufacturer's expected ranges for the SS1 but were above the upper end of the manufacturer's expected range for the SS2, SSX1, and SSX2. Additionally, TE_{obs} was outside the recommended TE_a for the SS1 (high control), SS2 (low control), SSX1 (low and medium control), and SSX2 (low, medium, and high control). Analytical CV was within the recommended range provided by ASVCP guidelines for all analyzers among all control groups, though not all CV_a targets were met.

Measurements of 5 linearity reagents on the SS1, SS2, SSX12, and SSX2 analyzers, and corresponding correlation coefficients are presented in Table 2. All analyzers showed acceptable concordance, and results were linear; however, the slope statistically deviated from identity for both the SS1 and SS2, and a negative bias was identified.

Three dogs had creatinine concentrations outside the SS and SSX reportable range (>1056 μmol/L) on the reference analyzer and were subsequently excluded from the method comparison. Of note, all samples with creatinine concentrations above the reportable range were detected on the SS and SSX, though a significant negative bias was appreciated. The creatinine concentrations of the excluded dogs were 1088 μmol/L (SS2 466 μmol/L, SSX2 513 μmol/L), 1167 μmol/L (SS1 644 μmol/L, SSX2 595 μmol/L), and 1645 μmol/L (SS2 872 μmol/L, 1019 μmol/L) on the reference analyzer.

In comparison to the reference measure, Lin's concordance correlation coefficient (rho_c) for the SS1, SS2, SSX1, and SSX2 was 0.69, 0.59, 0.82, and 0.44, respectively. The rho_c of the SSX2 was significantly lower than the SS1 ($P = 0.039$) and SSX1 ($P = 0.001$), while the rho_c of the SS2 was also significantly lower than the SSX1 ($P = 0.017$). Hence in comparison to the reference methodology, the SS1 and SSX1 were superior to the SSX2, and the SSX1 was superior to the SS2. No other differences were found between the devices.

Further analysis using Bland–Altman agreement between two measures (Figures 1 and 2) showed high average differences surpassing 26.4 μmol/L for all devices and a high level of variation in the differences where the standard deviation of the average difference was significantly greater than the identified mean difference (Table 3). The frequency of outliers was 8%, 5%, 8%, and 5% for the SS1, SS2, SSX1, and SSX2, respectively. All relationships showed a high level of heteroscedasticity with all outliers in among the highest creatinine values, consistent with a negative systemic bias at these concentrations.

While concordance analysis showed very good to excellent agreement between SS and SSX (rho_c = 0.87–0.98), in contrast, the Bland–Altman analysis showed a high level of variance. Bland–Altman, Deming, and Passing-Bablok slopes all approached one.

Using the SS1 (N = 38), 10 (26%) dogs were within IRIS limits for stage II, 8 (21%) for stage III, and 2 (5%) for stage IV CKD. Comparatively, using the reference analyzer, 6 (16%) of these dogs were within IRIS limits for stage II, 9 (24%) for stage III, and 6 (16%) for stage IV CKD. Five of 6 (83%) dogs within IRIS limits for stage IV CKD were misclassified by the SS1 as within IRIS limits for stage III (N = 3) or II (N = 2). Dogs within IRIS limits for stage III CKD dogs were misclassified by the SS1

TABLE 1 Within-day precision study for quality control material on the StatSensor1, StatSensor2, StatSensor Xpress 1, and StatSensor Xpress 2 point-of-care creatinine measurement devices in dogs

	Low control				Medium control				High control			
	SS1	SS2	SSX1	SSX2	SS1	SS2	SSX1	SSX2	SS1	SS2	SSX1	SSX2
Expected mean creatinine (range) (μmol/L)	84 (44–124)				186 (133–239)				376 (283–469)			
Mean creatinine (μmol/L)	93	104	93	93	180	193	211	214	457	470	562	547
Standard deviation	3	6	6	5	4	5	13	10	18	13	28	50
Within-day imprecision (% CV)	3	6	7	5	2	3	6	5	4	3	5	9
Observed total error (% TE_{obs})	17	35	24	22	8	9	26	24	22	17	16	21

Abbreviations: CV, coefficient of variation; SS1, StatSensor 1; SS2, StatSensor 2; SSX1, StatSensor Xpress 1; SSX2, StatSensor Xpress 2.

TABLE 2 Measurements of 5 linearity reagents on the StatSensor1, StatSensor2, StatSensor Xpress1, and StatSensor Xpress 2 point-of-care creatinine measurement devices, and corresponding correlation coefficients

	Assigned Low	Assigned High	Assigned Mean	StatSensor1 Mean	StatSensor2 Mean	StatSensor Xpress1 Mean	StatSensor Xpress2 Mean
Creatinine (μmol/L)							
Concentration 1	44	124	84	94	91	95	97
Concentration 2	133	239	186	180	179	195	197
Concentration 3	283	469	376	329	342	384	387
Concentration 4	398	663	531	470	470	541	545
Concentration 5	663	1061	862	746	752	891	901
Concordance coefficient				0.97	0.97	1.0	1.0
Linear regression slope (95% CI)				0.83 (0.81, 0.85)	0.84 (0.79, 0.89)	1.02 (0.98, 1.06)	1.03 (0.98, 1.07)

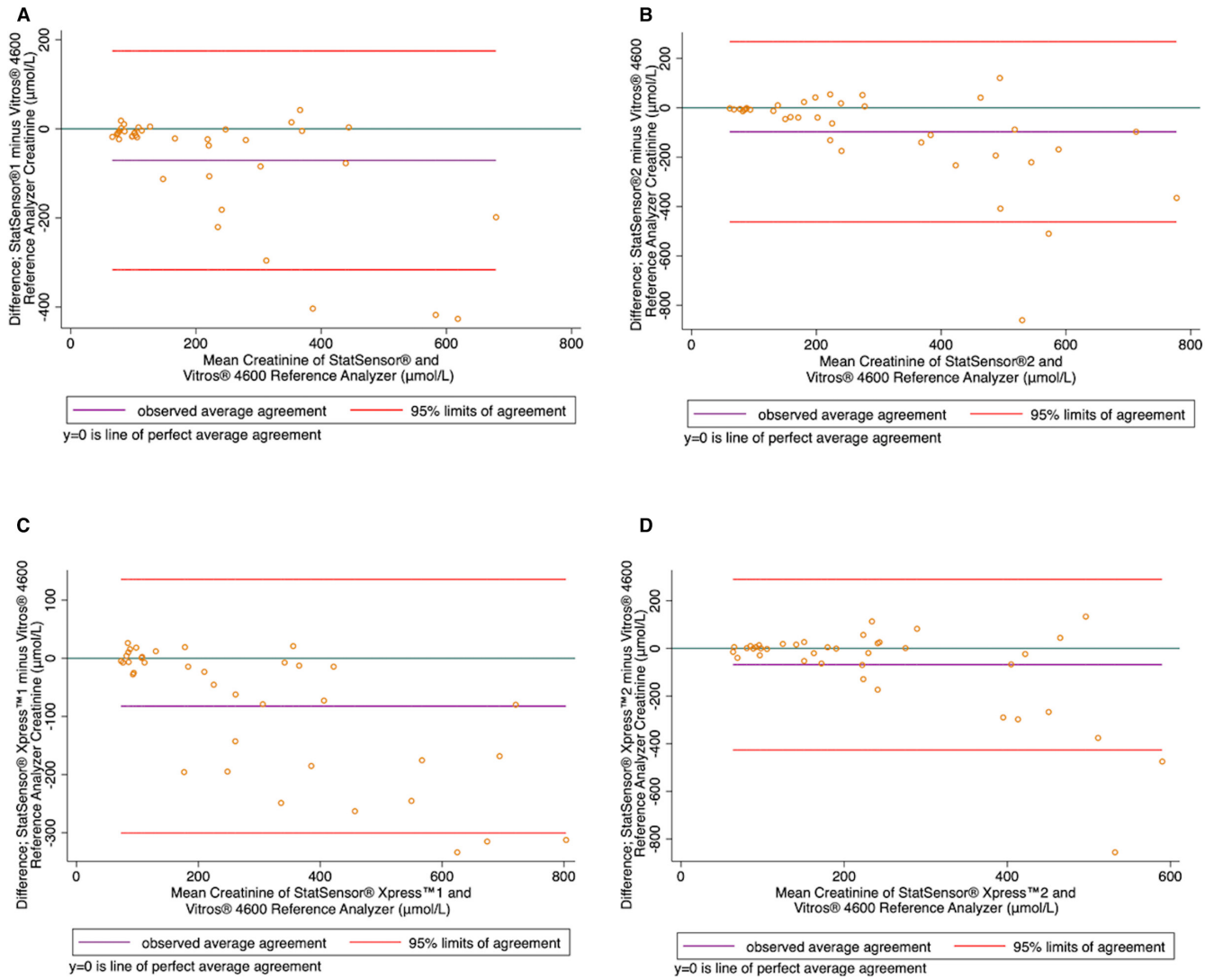

FIGURE 1 Bland–Altman plots of method comparisons for the (A) StatSensor 1, (B) StatSensor 2, (C) StatSensor Xpress 1, and (D) StatSensor Xpress 2, with the Vitros 4600 Chemistry Analyzer as reference standard in dogs

in 4 samples, 3 of which were within IRIS limits for stage II and 1 within limits for stage IV. Weighted kappa analysis identified 74% agreement in IRIS limit classification for the SS1 (kappa 0.62, $P < 0.001$).

Using the SS2 (N = 37), 12 (32%) dogs were within IRIS limits for stage II, 10 (27%) for stage III, and 6 (16%) for stage IV CKD. Comparatively, using the reference analyzer, 11 (30%) of these dogs

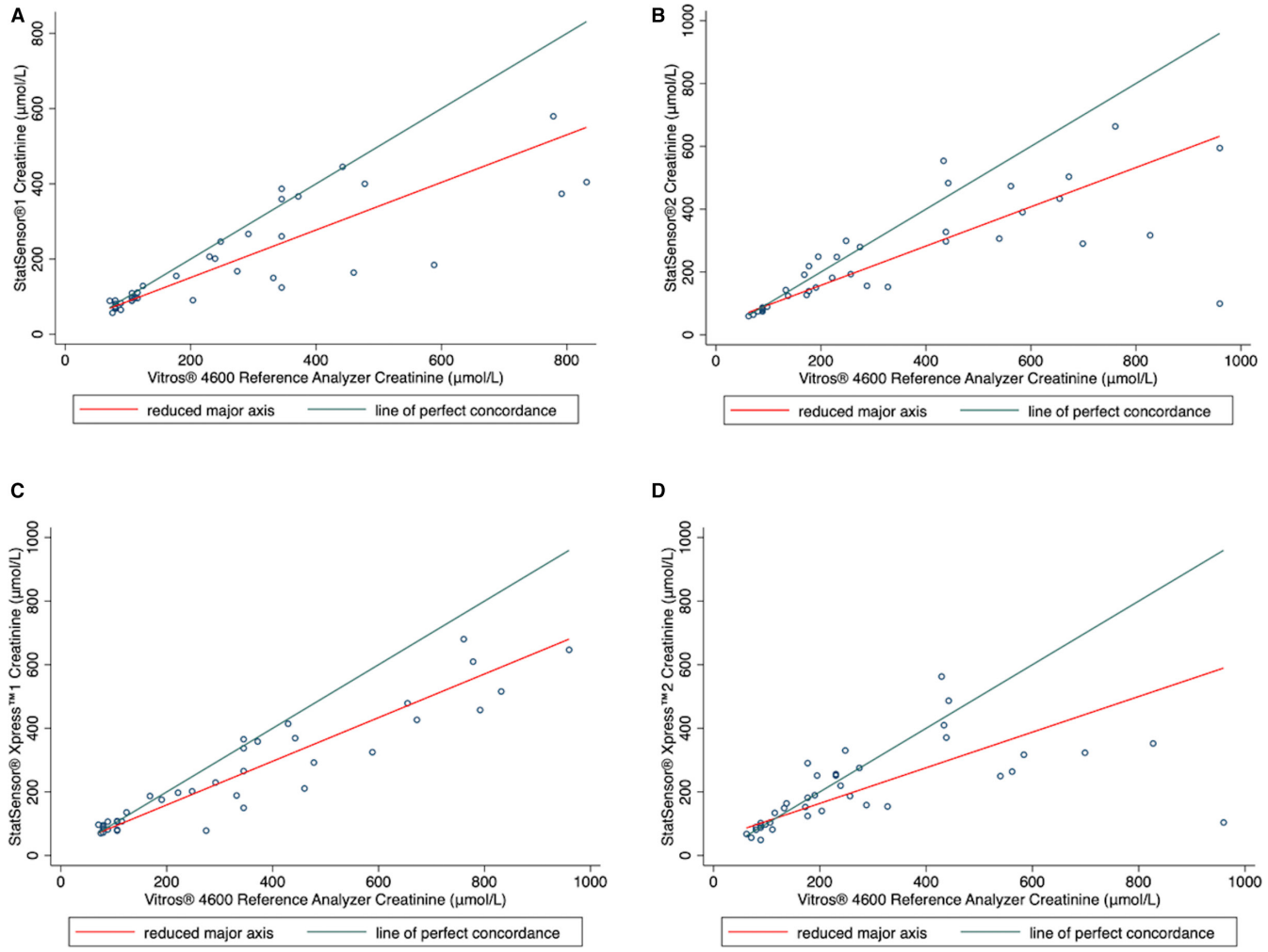

FIGURE 2 Graphic representations of the concordance correlation coefficient for method comparisons of the (A) StatSensor 1, (B) StatSensor 2, (C) StatSensor Xpress 1, and (D) StatSensor Xpress 2, with the Vitros 4600 Chemistry Analyzer as reference standard in dogs

were within IRIS limits for stage II, 8 (22%) for stage III, and 10 (27%) for stage IV CKD. Five of 10 (50%) dogs within IRIS limits for stage IV CKD were misclassified by the SS2 as within IRIS limits for stage III, while 1 dog within limits for stage IV CKD was misclassified by the SS2 as having a normal creatinine concentration. Dogs within IRIS limits for stage III CKD were misclassified by the SS2 in 6 samples, 4 of which were classified as within IRIS limits for stage II and 2 as within limits for stage IV. Weighted kappa analysis identified 59% agreement in IRIS limit classification for the SS2 (kappa 0.46, $P < 0.001$).

Using the SSX1 (N = 38), 8 (21%) dogs were within IRIS limits for stage II, 9 (24%) for stage III, and 7 (18%) for stage IV CKD. Comparatively, using the reference analyzer, 5 (13%) of these dogs were within IRIS limits for stage II, 10 (26%) for stage III, and 10 (26%) for stage IV CKD. Four of 10 (40%) dogs within IRIS limits for stage IV CKD were misclassified by the SSX1 as within IRIS limits for either stage III (N = 3) or II (N = 1). Dogs within IRIS limits for stage III CKD were misclassified by the SSX1 in 5 samples, 3 of which were classified as within IRIS limits for stage II, 1 as within limits for stage IV, and one as having a normal creatinine concentration. Weighted kappa analysis identified 74% agreement in IRIS limit classification for the SSX1 (kappa 0.64, $P < 0.001$).

Using the SSX2 (N = 39), 14 (36%) dogs were within IRIS limits for stage II, 11 (28%) for stage III, and 2 (5%) for stage IV CKD. Comparatively, using the reference analyzer, 13 (33%) of these dogs were within IRIS limits for stage II, 8 (21%) for stage III, and 6 (15%) for stage IV CKD. All 6 (100%) dogs within IRS limits for stage IV CKD were misclassified by the SSX2 as within IRIS limits for either stage III (N = 4), II (N = 1), or as having a normal creatinine concentration (N = 1). Dogs within IRIS limits for stage III CKD were misclassified by the SSX2 in five samples, three of which were classified as within IRIS limits for stage II and two for stage IV. Weighted kappa analysis identified 59% agreement in IRIS limit classification for the SSX2 (kappa 0.43, $P < 0.001$).

Statistical analysis was repeated following the exclusion of samples with any degree of hemolysis, lipemia, or icterus (N = 35) that may have interfered with creatinine analysis (Table 4). Concordance was lower, variation in the differences remained high, and a high level of heteroscedasticity persisted with a negative systemic bias at high creatinine concentrations for the SS1, SS2, SSX1, and SSX2

TABLE 3 Pearson's correlation coefficient, slope, concordance correlation coefficient, average difference, standard deviation, 95% limits of agreement, Deming slope, and Passing-Bablok intercept and slope for method comparisons of the StatSensor1, StatSensor 2, StatSensor Xpress 1, and StatSensor Xpress 2, with the Vitros 4600 Chemistry Analyzer as reference standard and a comparison between point-of-care creatinine measurement devices in all dogs

Analyzer	r[a]	Slope	r_c[b]	Avg Diff	SD	95% LOA	Deming Slope (95% CI)	PB Intercept (95% CI)	PB Slope (95% CI)
Reference standard: Vitros 4600 Chemistry Analyzer									
StatSensor 1	0.83	0.63	0.69	−70.78	125.26	−316.28, 174.71	0.58 (0.38, 0.78)	11.34 (−10.39, 30.76)	0.80 (0.63, 0.95)
StatSensor 2	0.72	0.63	0.59	−97.33	186.05	−461.99, 267.32	0.53 (0.27, 0.78)	21.93 (5.54, 38.49)	0.68 (0.59, 0.83)
StatSensor Xpress 1	0.94	0.69	0.82	−82.35	111.34	−300.57, 135.87	0.67 (0.57, 0.77)	27.33 (8.10, 46.08)	0.71 (0.60, 0.82)
StatSensor Xpress 2	0.55	0.56	0.44	−68.15	182.81	−426.44, 290.14	0.38 (−0.00, 0.77)	18.40 (−11.41, 54.63)	0.81 (0.47, 1.06)
Reference standard: StatSensor®1									
StatSensor Xpress 1	0.94	1.03	0.94	5.57	51.47	−95.31, 106.45	1.03 (0.87, 1.19)	4.00 (−18.93, 22.86)	1.03 (0.88, 1.27)
StatSensor Xpress 2	0.95	1.17	0.87	22.88	19.56	−15.47, 61.22	1.17 (0.82, 1.53)	0.86 (−149.16, 47.87)	1.18 (0.83, 2.48)
Reference standard: StatSensor 2									
StatSensor Xpress™1	0.98	1.02	0.98	10.48	42.96	−73.71, 94.68	1.02 (0.88, 1.16)	7.91 (−43.64, 68.46)	1.04 (0.71, 1.20)
StatSensor Xpress 2	0.92	0.85	0.90	−4.69	55.47	−113.41, 104.04	0.84 (0.62, 1.06)	8.14 (−8.76, 21.86)	0.99 (0.88, 1.12)

Abbreviations: Avg Diff, average difference; LOA, limits of agreement; PB, Passing-Bablok; SD, standard deviation.

[a]Pearson's correlation coefficient.

[b]Concordance correlation coefficient.

TABLE 4 Pearson's correlation coefficient, slope, concordance correlation coefficient, average difference, standard deviation, 95% limits of agreement, Deming slope, and Passing-Bablok intercept and slope for method comparisons of the StatSensor 1, StatSensor 2, StatSensor Xpress 1, and StatSensor Xpress 2 with the Vitros 4600 Chemistry Analyzer as the reference standard and a comparison between point-of-care creatinine measurement devices in dogs, excluding samples with hemolysis, lipemia, or icterus

Analyzer	r[a]	Slope	r_c[b]	Avg Diff	SD	95% LOA	Deming Slope (95% CI)	PB Intercept (95% CI)	PB Slope (95% CI)
Reference standard: Vitros 4600 Chemistry Analyzer									
StatSensor 1	0.76	0.58	0.60	−80.20	151.25	−376.65, 216.25	0.50 (0.24, 0.76)	−5.99 (−26.13, 60.74)	0.93 (0.46, 1.06)
StatSensor 2	0.45	0.56	0.32	−127.96	225.26	−569.46, 313.55	0.34 (−0.13, 0.81)	47.01 (−11.75, 150.18)	0.61 (0.34, 0.86)
StatSensor Xpress 1	0.94	0.76	0.60	−79.94	113.28	−302.00, 142.09	0.58 (0.49, 0.67)	39.67 (11.68, 73.08)	0.65 (0.53, 0.79)
StatSensor Xpress 2	0.38	0.46	0.24	−123.14	236.62	−586.91, 340.64	0.21 (−0.24, 0.66)	78.56 (−34.32, 140.18)	−0.53 (0.26, 1.05)
Reference standard: StatSensor 1									
StatSensor Xpress 1	0.92	1.04	0.92	13.21	−95.60	122.03	1.04 (0.78, 1.31)	16.49 (−29.68, 33.71)	0.94 (0.83, 1.28)
StatSensor Xpress 2	1.00	1.54	0.87	19.16	30.30	−40.23, 78.55	1.54 (0.74, 2.35)		
Reference standard: StatSensor 2									
StatSensor Xpress 1	0.96	0.90	0.95	0.88	47.00	−91.22, 92.99	0.89 (0.20, 1.59)		
StatSensor Xpress 2	0.87	0.81	0.84	−12.16	73.31	−155.84, 131.53	0.79 (0.47, 1.11)	14.47 (−23.56, 83.19)	0.96 (0.63, 1.18)

Abbreviations: Avg Diff, average difference; LOA, limits of agreement; PB, Passing-Bablok; SD, standard deviation. Passing-Bablok regression omitted for insufficient cases.

[a]Pearson's correlation coefficient.

[b]Concordance correlation coefficient.

compared with the reference analyzer. Concordance analysis continued to show very good to excellent agreement between SS and SSX with a persistent high level of variance. IRIS limit classification and weighted kappa analysis continued to show a similar proportion of misclassification among stage III and IV CKD.

4 | DISCUSSION

The objective of this prospective study was to determine the precision and accuracy of two commercially available handheld creatinine measurement devices in dogs with normal, moderate, and severe azotemia of various etiologies. With the exception of a previously published abstract investigating the SSX in dogs hospitalized in an intensive care unit, this serves as the first published validation study of handheld creatinine measurement devices in dogs.[16] Despite mean creatinine concentrations for low and medium quality control concentrations being within the manufacturer's expected ranges, mean creatinine concentrations for the SS2, SSX1, and SSX2 were above the upper end of the expected range for the high-quality control concentrations. Furthermore, TE_{obs} exceeded TE_a for all analyzers, and not all CV_a targets were met. This study identified a significant negative bias of the SS and the SSX in dogs with marked azotemia on the VCA. At normal and moderately elevated creatinine concentrations, the SS and the SSX, served as reliable devices; however, as all analyzers either failed to detect or markedly underestimated marked azotemia (>354 μmol/L) in numerous subjects, the clinical utility of such devices is limited. A significant negative bias was detected among linearity analyses for the SS1 and SS2, which may have contributed to the method comparison findings. However, this does not apply to the SSX1 and SSX2. While concordance analysis showed a variable level of agreement of SS and SSX with the reference measure, the Bland–Altman analysis revealed high values of variance among the measures, bringing into question the precision of these methods. Furthermore, although dogs were excluded from method comparison if their creatinine concentrations were above the SS and SSX manufacturer reportable range (>1056 μmol/L) on the reference analyzer, creatinine concentrations for these dogs were still readable and were significantly lower than the reference analyzer, raising concern for the utility of these devices as independent analyzers.

Multiple studies in the human medical field have investigated the clinical utility of PoC creatinine devices in various settings, including renal and hemodialysis clinics, in-hospital intensive care and oncology patients, and those undergoing potentially renal-toxic therapy (eg, anti-HIV drug Tenofovir).[17–21] While certain studies suggest that various devices may have acceptable to good results for specific patient populations, their applications continue to be associated with moderate inaccuracies, and a reliable creatinine biosensor with good analytical performance and a fast turnaround of results has yet to be validated in human medicine.[5,22]

Similar investigations in the veterinary medical field are scarce. An abstract investigating the use of the SSX creatinine analyzer in dogs referred to a university teaching hospital reported a Pearson's correlation coefficient of 0.954 with a positive bias (mean +20.09, 95% limits of agreement: −53.30 to 93.46 μmol/L) associated with the use of the PoC analyzer compared with a standard clinical laboratory.[16] The authors concluded that the PoC analyzer showed a good correlation with laboratory values, though separate reference intervals were indicated and further research was recommended.[16] The negative bias identified among our study was of opposing direction and markedly greater magnitude. While the proportion of dogs with moderate and marked azotemia was not reported in the abstract, a low number of dogs with severely elevated creatinine values might have contributed to the difference in these findings as the negative bias identified in our study was found to be largely driven by dogs with reference creatinine concentrations >700–800 μmol/L.

The SSX creatinine analyzer was also recently evaluated in a population of cats presented to a private referral hospital for evaluation of clinical disease.[23] Results of this study found a high correlation for both creatinine concentrations and International Renal Interest Society (IRIS) scores between the PoC analyzer and the reference analyzer, although a relatively small positive bias was associated with the SSX creatinine analyzer. A total of three outliers were noted at very high creatinine concentrations (ie, >600 μmol/L), where values were markedly underestimated by the PoC analyzer, as seen in the current study. Of note, 92% of samples in the feline study were ≤440 μmol/L (ie, IRIS CKD stage 1 or 2). Despite the high correlation, the analytical performance of the analyzer was found to be unacceptable, and its clinical use was not recommended. Similar to the canine abstract, a low number of cats with marked azotemia may have precluded the detection of a negative bias among the PoC analyzer in this study. Contrary to these veterinary reports identifying a positive bias among PoC analyzers, multiple human studies have found a negative bias at high creatinine concentrations, similar to the results of our study.[17–20,24]

There are several potential interferences known to influence creatinine detection, which may contribute to the overall difficulty in establishing an accurate PoC creatinine analyzer. High urea concentrations and other reactive substances among azotemic patients have the potential ability to interfere with the detection of creatinine among certain biosensors.[18] Urea is an intermediate product of the enzymatic conversion of creatinine to hydrogen peroxide that occurs within the multilayer enzyme reagent strips of the SS and SSX devices. Consequently, as urea concentrations are markedly elevated, they may inhibit creatine hydrolysis to sarcosine and urea; subsequently, this may decrease creatinine concentrations on PoC analyzers. However, in-vitro evaluation of the influence of high urea concentrations on the SS device has been found to increase rather than decrease creatinine measurements by 25%-30%.[18] Other substances such as bilirubin, lipids, glucose, and ascorbate may also play a potential role in creatinine interference.[22,25] Exclusion of samples with hemolysis, lipemia, or icterus did not result in improved agreement between the SS, SSX, and reference analyzer in this study. Additionally, certain PoC creatinine analyzers may be affected by the administration of exogenous compounds such as acetaminophen,

creatine, and hydroxyurea which falsely elevate creatinine or dopamine, and dobutamine which may cause a false decrease.[18,25,26] Further research and advancement in PoC technology are required to elucidate what role certain molecules play in the interference of creatinine measurement in dogs with PoC analyzers, particularly those with marked azotemia.

This clinical investigation is limited by its relatively low number of dogs with marked azotemia >700–800 µmol/L, despite controlling for the inclusion of at least 25 dogs with a reference creatinine value >354 µmol/L. Though the reportable range of the SS and SSX is 27 to 1056 µmol/L per the manufacturer, precision and linearity analyses were limited to a range of expected mean concentrations of 84–376 µmol/L and 84–862 µmol/L, respectively. This limits the reliability of method comparison data and clinical applicability for high creatinine concentrations. While the inclusion of patients with varying degrees of hemolysis, icterus, and lipemia may have impacted the PoC detection of creatinine, it reflects an accurate representation of samples from dogs in-hospital with varying degrees of azotemia and remains a relevant variable to overcome with future developments. Furthermore, concurrent medications and fluid additives were not documented at the time of study enrollment and may have resulted in creatinine interference. Linearity testing was limited to the analysis of varying concentrations of quality controls and did not include the use of pooled canine samples, nor was an evaluation of biological variation performed in this study. In the face of their significant negative bias among higher creatinine values and resultant limited clinical utility, further evaluation of analytical performance was not performed. As a result, the authors caution the interpretation of the analytical performance of the devices presented in this study.

The results of this method comparison study have illustrated that the SS and SSX PoC creatinine measurement devices are not considered acceptable for clinical application in dogs. Despite relative reliability when a patient is non-azotemic or has only mild to moderate azotemia, the use of either of these devices may provide false assurance with a normal creatinine measurement despite the presence of severe, life-threatening azotemia. Furthermore, its application in-hospital has the potential to suggest clinical resolution of azotemia despite either unchanged or significant underlying progression of the disease. Further research and technological advancements are indicated for the development of a trustworthy, cost-effective, PoC creatinine analyzer to improve the rapid detection and monitoring of both human and veterinary patients with acute and chronic kidney disease.

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DISCLOSURE

The authors received financial support from Nova Biomedical Corporation for completion of the study, though they have no additional affiliation with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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