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Comparison of 2 point-of-care analyzers and the Eurolyser assay with an IDEXX reference laboratory method for measurement of symmetric dimethylarginine in dogs

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OBJECTIVE
To determine the concordance of 2 point-of-care (POC) analyzers and 2 reference laboratories (RLs) for serum symmetric dimethylarginine (SDMA) analysis in dogs. We hypothesized that the Vcheck V200 POC, IDEXX Catalyst POC, and Eurolyser assays would have an acceptable agreement with the IDEXX RL SDMA results.

METHODS
This was a prospective study conducted between August 2019 and March 2023. Blood collected from dogs treated at a referral hospital underwent SDMA analysis by 2 POC analyzers (IDEXX Catalyst and Vcheck V200) and 2 RL methods (Eurolyser and IDEXX). Dogs with suspected or known renal disease were preferentially included later in the study.

RESULTS
75 samples were included in the final analysis. There was a difference in SDMA results obtained from Eurolyser assays but not IDEXX Catalyst POC and Vcheck V200 POC assay compared to IDEXX RL results. When applied to the International Renal Interest Society chronic kidney disease staging classification, there was almost perfect agreement between Eurolyser and Vcheck V200 POC SDMA compared to IDEXX RL SDMA.

CONCLUSIONS
While there was a strong to excellent correlation between assays, the results obtained via each assay demonstrated that there may be significant bias and analytical variation affecting the results. However, this may have minimal effect when applied clinically.

CLINICAL RELEVANCE
Analyzer and method-specific reference intervals should be established for SDMA analysis. There is preliminary evidence to support the use of Eurolyser and Vcheck V200 POC SDMA assays in the staging of canine chronic kidney disease.

Keywords: SDMA, catalyst, Vcheck, eurolyser, IDEXX

Symmetric dimethylarginine (SDMA) analysis has become widely utilized in canine veterinary practice for assessment of renal function and is often routinely analyzed during disease investigation, wellness monitoring, and screening.

Symmetric dimethylarginine is produced by all nucleated cells continually and is excreted primarily by the kidneys, without apparent tubular reabsorption or significant metabolism, meeting many of the criteria of an ideal renal function biomarker.[1] Symmetric dimethylarginine has high specificity for renal dysfunction in dogs, although patient characteristics such as age and breed may affect the applied reference intervals, and some other disease states may be associated with elevated SDMA, notably lymphoid malignancy.[2–4]

Symmetric dimethylarginine may be a more sensitive biomarker for early-stage renal disease than serum creatinine (sCr) when assessed by population-based reference intervals.[1,4] Symmetric dimethylarginine appears to be less affected by body and muscle

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mass than sCr.[5] One study[4] of dogs with rapidly progressive chronic kidney disease (CKD) due to hereditary nephropathy demonstrated elevation of SDMA before elevation of sCr using prespecified cutoff values. Another study[6] of dogs with naturally occurring CKD suggested that SDMA may increase above the reference interval several months before elevation in sCr. In this study, all dogs had an elevation of SDMA above the reference interval before the development of elevated sCr by a mean of 9.8 months. However, another study[7] of client-owned dogs with CKD found that SDMA and sCr had similar diagnostic performance for the detection of decreased GFR. Sensitivity and specificity were similar for SDMA and sCr in this study using prespecified cutoffs. Of note is that most dogs incorrectly categorized by one analyte in this study were correctly classified by the other, highlighting the benefit of assessing multiple biochemical parameters, combined with clinical correlation, in the assessment of renal disease.

Since 2015, the assessment of SDMA has been incorporated into the International Renal Interest Society (IRIS) staging of CKD guidelines.[8] It is recommended that patients are staged by measurement of both SDMA and sCr at 2 or more separate time points in which they are fasted, hydrated, and stable.[8] If SDMA persistently suggests a higher stage than sCr, it is recommended to assign patients to that higher stage. It is important that clinicians consider individual patient factors unrelated to renal function that may influence the SDMA and sCr. Patients are substaged by assessment of blood pressure and proteinuria. The current IRIS guidelines, at the time of writing, note that the recommendations made regarding SDMA are based on published literature utilizing SDMA as measured by IDEXX proprietary technology. The IRIS guidelines utilize a cutoff of 18 µg/dL for the diagnosis of stage 1 CKD, in line with a study of dogs with naturally occurring kidney disease that found this cutoff would improve specificity without compromising the sensitivity of SDMA for detecting decreased GFR.[9] However, the guidelines also note that persistently elevated SDMA above 14 µg/dL may be used to diagnose early CKD, in line with the reference interval provided for most breeds of dogs greater than 12 months of age. A recent study[10] suggested a higher reference interval for older dogs as assessed by 2 methods of measurement, including the IDEXX ELISA. This study population consisted of older nonazotemic dogs but defined renal azotemia as a creatinine > 161 µmol/L and urine specific gravity < 1.030. While a proportion of the dogs included (50/120) had inadequately concentrated urine (urine specific gravity < 1.030), meaning that early CKD could not be entirely excluded for a proportion of the reference interval study population, it would still seem that both age and assay-specific reference intervals should be applied.

Liquid chromatography-mass spectrometry is considered the gold standard for canine SDMA analysis. This methodology is not widely available, is often costly, and is therefore inconvenient for routine veterinary analysis.[11] To the author's knowledge, there currently are limited studies[10,12–14] evaluating the performance of non-IDEXX laboratory proprietary SDMA methodologies, and, unlike cats, independent comparative analytical performance analysis of IDEXX Catalyst point-of-care (POC) and IDEXX reference laboratory (RL) for canine SDMA is lacking.

The objective of this study was to determine if canine serum SDMA results obtained by the IDEXX Catalyst POC, Bionote Vcheck V200 POC, and Eurolyser RL analyses are comparable with IDEXX RL SDMA. Our hypothesis was that each of these analyzers would have an acceptable agreement with the IDEXX RL SDMA results.

Methods

This prospective study was performed between August 2019 and March 2023. Initially, patients were included irrespective of health status. However, due to Vcheck V200 POC SDMA results frequently being below the reportable range (< 10 µg/dL) from February 2021, dogs with known or suspected renal disease were preferentially included. Ethics approval was granted by the Queensland Government Department of Agriculture and Fisheries Animal Ethics Committee Reference No. CA2019/07/1299.

To be eligible for inclusion, blood testing was to be performed at the recommendation of the treating clinician. Dogs were not excluded based on age, sex, body condition, or illness. Informed owner consent was obtained. Dogs could be included on more than one occasion.

Blood samples were collected into EDTA (BD Vacutainer K3EDTA; 2 mL) and serum clot activator (Vacuette CAT Serum Clot Activator; 2 or 4 mL) tubes for hematology and biochemistry analyses, respectively. Blood was allowed to clot within the clot activator tube for approximately 15 minutes before being centrifuged at 1,500 rpm for 5 to 10 minutes, and the serum was harvested. The serum was separated into 3 aliquots (> 0.5 mL in each of 3 red top tubes; Vacuette CAT Serum Clot Activator; 2 mL) for (1) in-clinic POC SDMA analysis via IDEXX Catalyst One (IDEXX Laboratories); (2) referral to IDEXX RL (IDEXX Laboratories) for creatinine and SDMA analysis (Beckman Coulter AU680 and DxC 700 AU [2019 to September 2022] and Beckman Coulter AU5800 [from September 2022 onward]); (3) referral to QML Vetnostics RL for full biochemistry analysis (Cobas 8000; Roche), total T4 analysis (Immulite 2000; Siemens), and SDMA analysis via both Vcheck V200 POC (Bionote Inc) and Eurolyser (Cobas 8000; Roche) methods. The EDTA blood samples were concurrently submitted to QML Vetnostics RL for hematologic analysis (XN-V; Sysmex). Samples sent to RLs were refrigerated (4 °C) until they were collected via routine courier service and transported chilled on ice bricks the same day as collection. Reference laboratory SDMA analyses (IDEXX and Eurolyser) were performed on the same day as the sample submission. Point-of-care analyses (IDEXX Catalyst and Vcheck V200) were performed the same day as sample collection or on separated serum aliquots that had been stored at 4 °C or −20 °C

for a maximum of 7 days or 3 months, respectively, based on previous studies[4,13] evaluating the stability of canine SDMA. Point-of-care analyses were performed as per manufacturer instructions.[15,16]

The manufacturer provided information for each of these SDMA methods as follows: Eurolyser SDMA is an immunoturbidimetric assay with a measurement range of 0 to 100 µg/dL.[17,18] Vcheck V200 POC SDMA is an immunofluorescent assay with a measurement range of 10 to 100 µg/dL.[19] The IDEXX Catalyst POC and IDEXX RL SDMA assays are immunoassays, each with a reportable range of 0 to 100 µg/dL.[16] Analyzer-specific imprecision determined by between run precision from pooled canine serum, together with the corresponding mean SDMA concentrations in brackets, were as follows: Eurolyser SDMA had an analytical precision (CV_A) of 6.53% [11 µg/dL] and 3.8% [31 µg/dL] and Vcheck V200 POC SDMA had a CV_A of 10.96% [13.0 µg/dL] and 10.26% [53 µg/dL] (in laboratory-recorded data: Dr. Brett Stone, QML Vetnostics Laboratory, September 2024). IDEXX RL imprecision was determined from quality control material, and corresponding mean SDMA concentrations in brackets were a CV_A of 9% [10 µg/dL)] and 4.0% [50 µg/dL] (personal communication via email, September 1 2024; IDEXX Laboratories). Analyzer-specific generated imprecision data were not available for the IDEXX Catalyst POC, with manufacturer-reported CV_A at differing SDMA concentrations of 6.2% (15.5 µg/dL) and 5.6% (36 µg/dL) for the IDEXX Catalyst SDMA.[16,20] Two-level (within reference interval and elevated SDMA concentrations) quality control material was run once or twice daily for Eurolyser and IDEXX RL SDMA, respectively. Vcheck V200 calibration was performed monthly as per manufacturer instructions. IDEXX Catalyst cleaning and quality control was performed monthly as per clinic operating procedures and manufacturer recommendations. For the IDEXX RL method, 3 instruments were utilized at 1 RL over the study period whereby SDMA analysis was performed interchangeably using either AU680 or DxC 700 AU (Beckman Coulter) analyzers from 2019 to September 2022 with the AU5800 (Beckman Coulter) analyzer solely used from September 2022 onward. Clinical equivalence was demonstrated between the AU5800 and DxC 700 AU and between the AU680 and DxC 700 AU (personal communication via email, October 11, 2024; IDEXX Laboratories).

Data analysis

Data were analyzed for normal distribution using the Kolmogorov-Smirnov normality test and evaluated visually for normality and symmetry with histograms. Data were then analyzed via simple linear and Passing-Bablok regression methods, paired-sample sign test statistics, and difference plots (Bland-Altman) using Excel (Microsoft Excel for Microsoft 365, version 2405), Analyse-it for Microsoft Excel (version 6.01.1), and SPSS (IBM SPSS Statistics 29.0.1.0) using the IDEXX RL SDMA as the reference method as per American Society for Veterinary Clinical Pathology (ASVCP) guidelines.[21] Symmetric dimethylarginine concentrations outlined for IRIS staging of CKD were used for error grid analysis and weighted κ statistics.[22] To avoid potential discordant results associated with sample interferences between methods, any samples that reported analyzer semi-quantitative hemolysis and/or icterus indices of ≥ 2+ and/or triglyceride concentrations of ≥ 5.0 mmol/L via biochemistry analysis (Cobas 8000; Roche) were excluded. Bland-Altman difference plots assessed both the 95% limits of agreement of the observed differences (LoA_o) as well as 95% limits of agreement based on combined inherent imprecision (LoA_{CII}) using the analytic variation of each method being compared as follows[23]:

$$LoA_o = mean_{Diff} \pm 1.96\ SD_{Diff}.$$

$$LoA_{CII} = 1.96 \times \sqrt{(CV^2_{A\ method\ 2} + CV^2_{A\ IDEXX\ RL}}.$$

To capture the highest potential level of agreement, the largest CV_A available for each method (IDEXX RL, 9%; IDEXX Catalyst POC, 6.2%; Vcheck V200 POC, 10.96%; and Eurolyser, 6.53%) was utilized for LoA_{CII} calculations resulting in LoA_{CII} values of 21%, 28%, and 22% for IDEXX Catalyst POC versus IDEXX RL, Vcheck V200 POC versus IDEXX RL, and Eurolyser versus IDEXX RL, respectively. Total allowable error (TE_A) can be directly substituted for LoA_{CII} percentage so that the limits of agreement based on TE_A (LoA_{TEa}) can be interpreted identically to LoA_{CII}.[23] Methods were considered comparable if ≤ 5% of Bland-Altman plot differences were outside the LoA_{TEa}. Analytical performance and method comparison results were also compared to desirable canine SDMA analytical performance specifications of CV_A of 7%, bias of 6%, and total error of 17.6% based on biological variation.[24]

Results

This prospective study included 81 canine blood samples. Five samples were excluded due to lipemia, icterus, and/or hemolysis, and a further sample was excluded due to IDEXX RL analysis not being performed. The remaining 75 samples were obtained from 61 dogs, 30 males (23 neutered and 7 intact) and 31 females (28 spayed and 3 intact), including 21 mixed-breed dogs and 28 varieties of purebred dogs ranging in age from 0.25 to 14 years. Symmetric dimethylarginine results and descriptive statistics obtained via the 2 POC and 2 RL methods are provided **(Supplementary Table S1)**. The Kolmogorov-Smirnov normality test and histogram evaluation demonstrated that all data sets were not normal and not symmetrical, and left skewed even after Log transformation. A paired-sample sign test was thus performed to evaluate for significant difference, with significance set as $P < .05$. IDEXX RL SDMA concentrations ranged from 3 to 90 µg/dL, and the correlation coefficient (r) within the simple linear regression was < 0.975 for both of the POC methods, Passing-Bablok regression analysis was also performed (graphs not shown). A summary of results from simple linear and Passing-Bablok

regression methods, paired-sample sign test statistics, and Bland-Altman difference plots data analysis for SDMA results obtained via each of the IDEXX Catalyst POC, Vcheck V200 POC, and Eurolyser methods against the IDEXX RL SDMA results are provided **(Table 1)**. The Vcheck V200 POC analyzer reported SDMA concentrations to 1 decimal place, and the results were rounded to the nearest whole number. The Vcheck V200 POC analyzer had the lowest reportable range limit of 10 µg/dL with all SDMA results lower than this reported as < 10 µg/dL.

For each of the Bland-Altman difference plots, the number of data points exceeding the LoA_o and LoA_{TEa} was visually determined.[23] Interassay bias was determined as the difference between the mean results of each analyzer expressed as both units and percentages as reported previously for feline SDMA comparisons.[12]

Weighted κ and error grid analyses were used to quantify the agreement between each of the method comparisons based on SDMA concentrations used for IRIS staging of canine CKD as stage 1 (SDMA, < 18 µg/dL), stage 2 (18 to 35 µg/dL), stage 3 (36 to 54 µg/dL), and stage 4 (> 54 µg/dL).[8] Tabulated interassay CKD stage agreement concurrently allowed for stratification of each set of results into performance zones for error grid analysis as follows: zone A, no effect on clinical action (both SDMA results within the same IRIS stage); zone B, altered clinical action with little or no effect on clinical outcome (SDMA results differing by 1 IRIS stage); zone C, altered clinical action likely to affect clinical outcome (SDMA results differing by 2 IRIS stages); and zone D, altered clinical action which could have significant clinical risk (SDMA results differing by 3 IRIS stages). Zone definitions used were based on those

Table 1—Results of simple linear and Passing-Bablok regression methods, paired-sample sign test statistics, and Bland-Altman difference plots comparing symmetric dimethylarginine (SDMA) results from IDEXX Catalyst point-of-care (POC), Vcheck V200 POC, and Eurolyser with IDEXX reference laboratory (RL) SDMA results.

SMDA results

IDEXX Catalyst POC versus IDEXX RL (n = 68)				
Simple linear regression and paired-sample sign test statistics	*r* (95% CI)	Slope (95% CI)	Intercept (µg/dL) (95% CI)	Sign test 2-tailed *P* value (*Z* score)
	0.95 (0.88 to 1.03)	0.76 (0.70 to 0.82)	2.10 (0.75 to 3.46)	.053 (−1.94)
Passing-Bablok regression	Slope (95% CI)	Intercept (µg/dL) (95% CI)		
	0.78 (0.67 to 0.87)	2.06 (0.82 to 3.25)		
Bland-Altman difference plot	Mean difference (bias) (µg/dL) (95% CI)	Bias (%)	LoA_o y-intercepts	
	−2.16 (−3.29 to −1.04)	to 8.58	6.95 to 11.28	
Vcheck V200 POC versus IDEXX RL (n = 42)				
Simple linear regression and paired-sample sign test statistics	*r* (95% CI)	Slope (95% CI)	Intercept (µg/dL) (95% CI)	Sign test 2-tailed *P* value (*Z* score)
	0.93 (0.81 to 1.05)	0.87 (0.76 to 0.98)	1.18 (−1.91 to 4.26)	.349 (−.937)
Passing-Bablok regression	Slope (95% CI)	Intercept (µg/dL) (95% CI)		
	0.83 (0.66 to 0.97)	2.32 (−1.00 to 5.44)		
Bland-Altman difference plot	Mean difference (bias) (µg/dL) (95% CI)	Percent bias	LoA_o y-intercepts	
	−1.84 (−0.16 to −3.53)	−5.76	8.75 to 12.43	
Eurolyser versus IDEXX RL (n = 75)				
Simple linear regression and paired-sample sign test statistics	*r* (95% CI)	Slope (95% CI)	Intercept (µg/dL) (95% CI)	Sign test 2-tailed *P* value (*Z* score)
	0.99 (0.95 to 1.03)	0.9 (0.86 to 0.93)	0.53 (−0.27 to 1.33)	< .001 (−3.653)
Passing-Bablok regression	Slope (95% CI)	Intercept (µg/dL) (95% CI)		
	0.91 (0.86 to 0.95)	0.17 (−0.34 to 0.90)		
Bland-Altman difference plot	Mean difference (bias) (µg/dL) (95% CI)	Percent bias	LoA_o y-intercepts	
	−1.31 (−0.74 to −1.87)	−6.73	3.53 to −6.14	

LoA_o = 95% Limits of agreement of the observed differences.

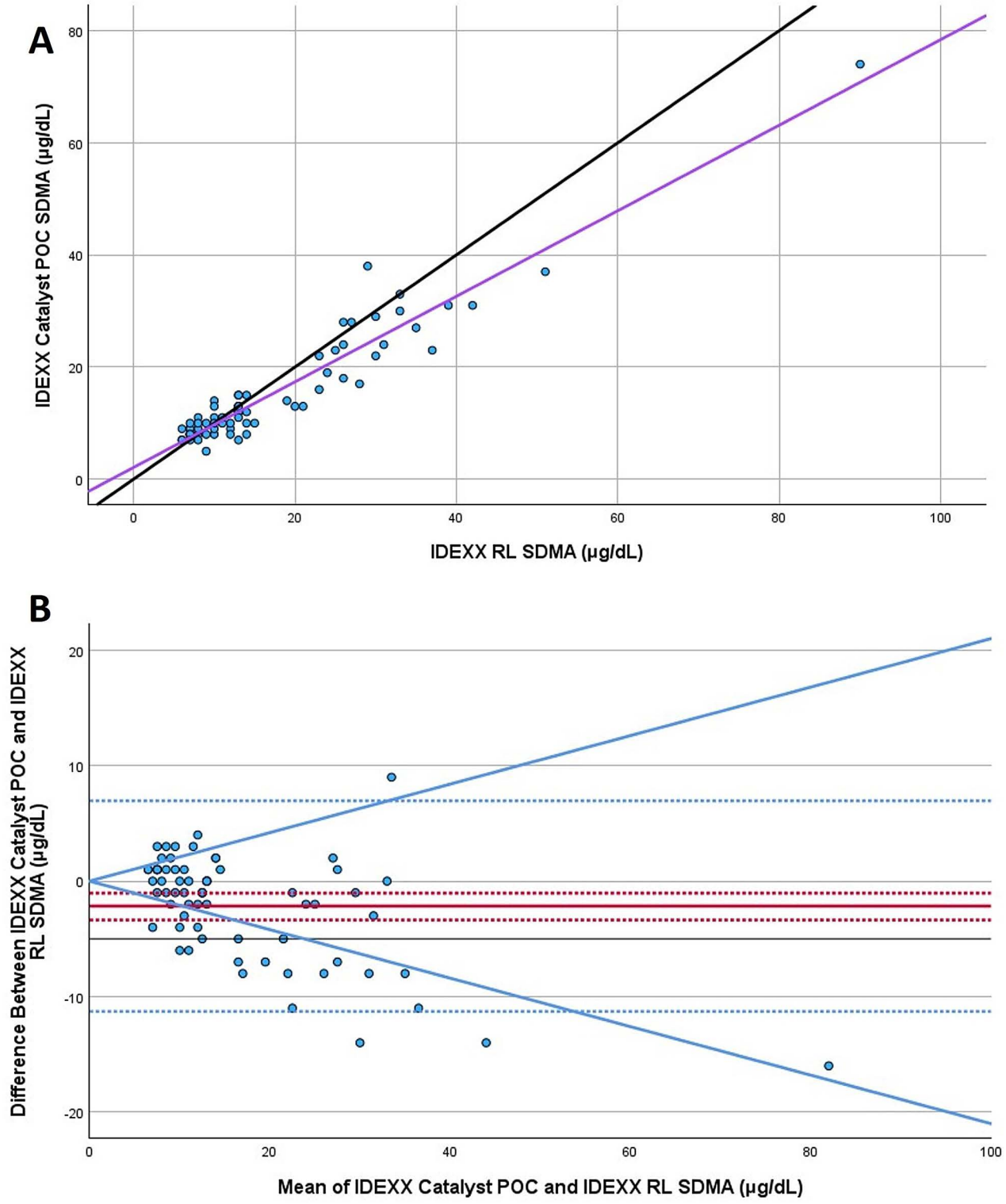

Figure 1—Scatter plot (A) and Bland-Altman difference (B) plot comparing IDEXX Catalyst point-of-care (POC) and IDEXX reference laboratory (RL) symmetric dimethylarginine (SDMA) concentrations. A—Black line, y = x; purple line, line of best fit (y = 0.763x + 2.101). B—Red solid line, mean difference (bias, −2.16); dashed red lines, 95% CI of mean (−1.04 to −3.29); dashed blue lines, limits of agreement of the observed differences (LoA_o: 6.95 to −10.93); solid blue lines, limits of agreement based on total allowable error (LoA_{TEa}: y = ± .21x).

traditionally used to assess the accuracy of blood glucose meters.[25]

Comparison of IDEXX Catalyst POC with IDEXX RL SDMA

Seven samples were excluded from the comparison of IDEXX Catalyst POC and IDEXX RL SDMA results due to 1 "error" result, 1 lost sample for IDEXX Catalyst POC analysis, and 5 samples were excluded due to a change in the IDEXX Catalyst POC method to the use of a slide with incorporated reagent in April 2022. Sixty-eight samples remained for result comparisons (Table 1; **Figure 1**); all of which were performed on the previously available Catalyst SDMA that utilized a cup and separate reagent. Symmetric dimethylarginine results from the IDEXX Catalyst POC and IDEXX RL were positively correlated. The paired-sample sign test supported no difference (P = .053) between IDEXX Catalyst POC and IDEXX RL SDMA results with 38 negative differences, 22 positive differences, and 8 results the same. Passing-Bablok regression analysis demonstrated both a systematic and proportional difference between the 2 methods given that the confidence intervals of the intercept and slope did not contain 0 and 1.0, respectively. The Bland-Altman difference plot demonstrated a mean difference (negative bias) between the IDEXX Catalyst POC and IDEXX RL methods. However, the data points were widely scattered about zero, with 94% of results differing by −11 to 7 µg/dL. The IDEXX Catalyst POC demonstrated an increasing negative proportional bias relative to IDEXX RL results at increasing SDMA concentrations. Four (6%) and 27 (40%) data points exceeded the LoA_o and LoA_{TEa}, respectively.

The stratification of results across the different IRIS CKD stages is provided **(Table 2)** to demonstrate the error grid analysis. The weighted κ result agreement was 0.77, which is interpreted as "substantial agreement" between these 2 methods.[26] Error grid analysis demonstrated that 87% (59/68) of data sets fell within the same IRIS stage (zone A) and 12% (9/73) of data sets differed by 1 IRIS stage (zone B). Zone B data sets differed from 5 to 14 µg/dL.

Comparison of Bionote Vcheck V200 POC with IDEXX RL SDMA

An additional 33 samples were excluded from the comparison of Vcheck V200 POC and IDEXX RL SDMA results; reasons for exclusion included an error Vcheck V200 POC result, 1 lost sample, 28 samples with Vcheck V200 POC SDMA result below the reportable range (< 10.0 µg/dL), and 1 outlier (dog 34). Forty-two samples remained for complete result comparisons (Table 1; **Figure 2**). Symmetric dimethylarginine results from the Vcheck V200 POC and IDEXX RL were positively correlated. The paired-sample sign test supported no difference (P = .349) between Vcheck V200 POC and IDEXX RL SDMA results, with 24 negative differences, 17 positive differences, and 1 result the same. Passing-Bablok regression analysis demonstrated a proportional difference between the 2 methods given that the confidence interval of the slope did not contain 1.0. The Bland-Altman difference plot demonstrated a minimal mean difference (negative bias) between the Vcheck POC and IDEXX RL. However, the data points were widely scattered about zero with 95% of results differing by −12 to 9 µg/dL. Two (5%) and 10 (24%) data points exceeded the LoA_o and LoA_{TEa}, respectively.

The stratification of results across the different IRIS CKD stages is provided **(Supplementary Table S2)**. The weighted κ result agreement was 0.83, which is interpreted as "almost perfect agreement" between these 2 methods.[26] Error grid analysis demonstrated that 88% (37/42) of data sets fell within the same IRIS stage (zone A) and 12% (5/42) of data sets differed by 1 IRIS stage (zone B).

The 28 samples with a Vcheck SDMA result of < 10 µg/dL were also then included for repeat weighted κ and error grid analyses **(Supplementary Table S3**). Seventy-one percent (20/28) of Vcheck SDMA results < 10 µg/dL had a corresponding IDEXX RL SDMA result ≤ 10 µg/dL and 100% (28/28) of Vcheck SDMA results < 10 µg/dL had a corresponding IDEXX RL SDMA result within the stage I IRIS category (< 18 µg/dL). The weighted κ result agreement was 0.88, which is interpreted as almost perfect agreement between these 2 methods.[26] Error grid analysis demonstrated that 93% (65/70) of data sets fell within the same IRIS stage (zone A), and 7% (5/70) of data sets differed by 1 IRIS stage (zone B). Zone B data sets differed from 4 to 13 µg/dL.

Comparison of Eurolyser RL with IDEXX RL SDMA

A total of 75 samples were available for comparison of Eurolyser RL and IDEXX RL SDMA results (Table 1; **Figure 3**). Symmetric dimethylarginine results from the Eurolyser and IDEXX RL demonstrated excellent correlation (r = 0.99). The paired-sample sign test supported a difference (P = < .001) between Eurolyser and IDEXX RL SDMA results with

Table 2—Agreement between IDEXX Catalyst POC and IDEXX RL SDMA results based on International Renal Interest Society (IRIS) chronic kidney disease staging categories.

	IDEXX Catalyst IRIS stage 1	IDEXX Catalyst IRIS stage 2	IDEXX Catalyst IRIS stage 3	IDEXX Catalyst IRIS stage 4	Total
IDEXX RL IRIS stage 1	44[a]	0	0	0	44
IDEXX RL IRIS stage 2	5[b]	13[a]	1[b]	0	19
IDEXX RL IRIS stage 3	0	3[b]	1[a]	0	4
IDEXX RL IRIS stage 4	0	0	0	1[a]	1
Total	49	16	2	1	68

[a]Zone A error grid analyses. [b]Zone A error grid analyses.

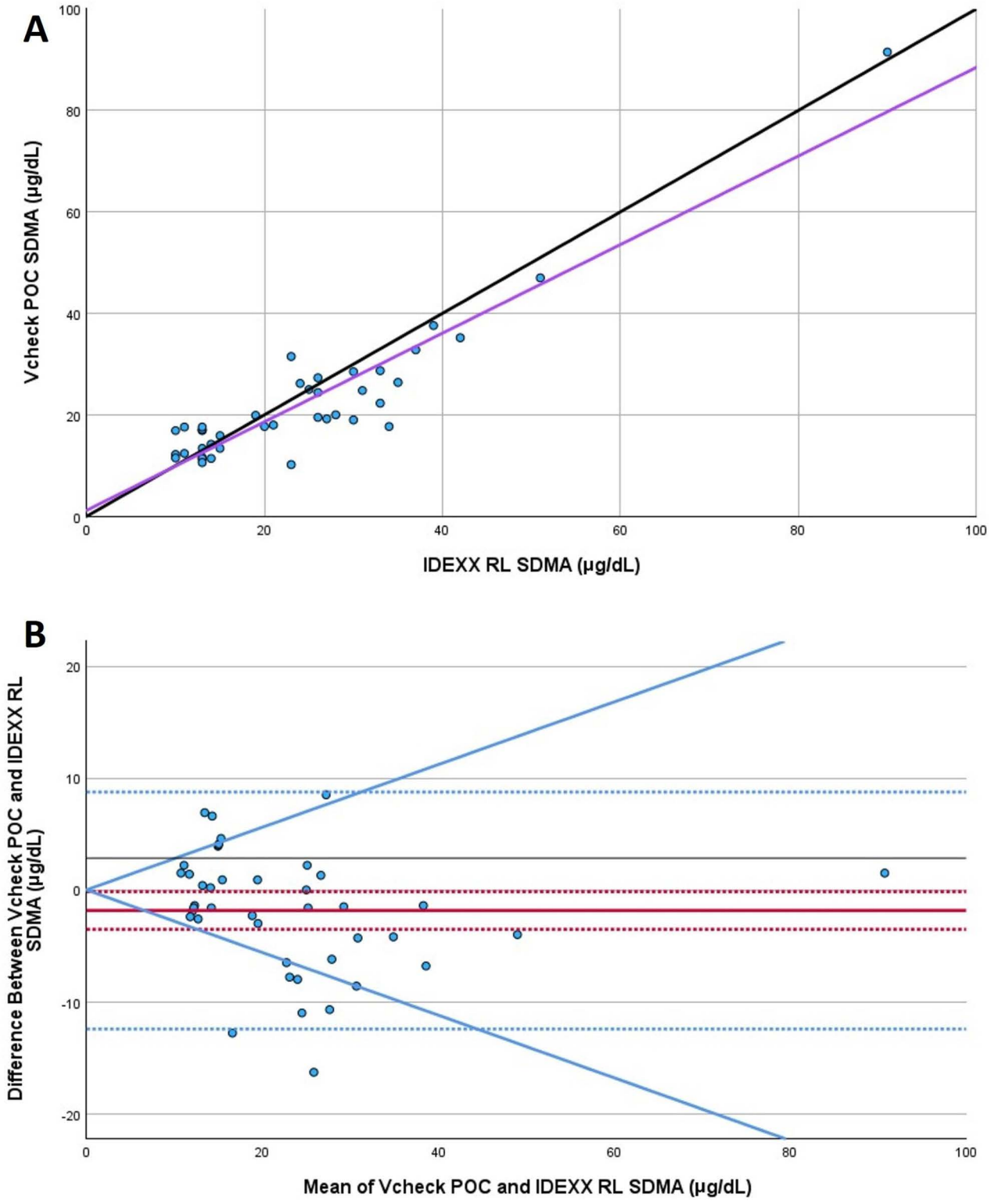

Figure 2—Scatter plot (A) and Bland-Altman difference (B) plot comparing Vcheck V200 POC and IDEXX RL SDMA concentrations. A—Black line, y = x; purple line, line of best fit (y = 0.687x + 5.013). B—Red solid line, mean difference (bias, −1.84); dashed red lines, 95% CI of mean (−0.159 to −3.526); dashed blue lines, LoA_o (8.746 to −12.432); solid blue lines, LoA_{TEa} (y = ± 0.28x).

52 negative differences, 20 positive differences, and 3 results the same. Passing-Bablok regression analysis demonstrated a proportional difference between the 2 methods given that the confidence interval of the slope did not contain 1.0. The Bland-Altman difference plot demonstrated a mean difference

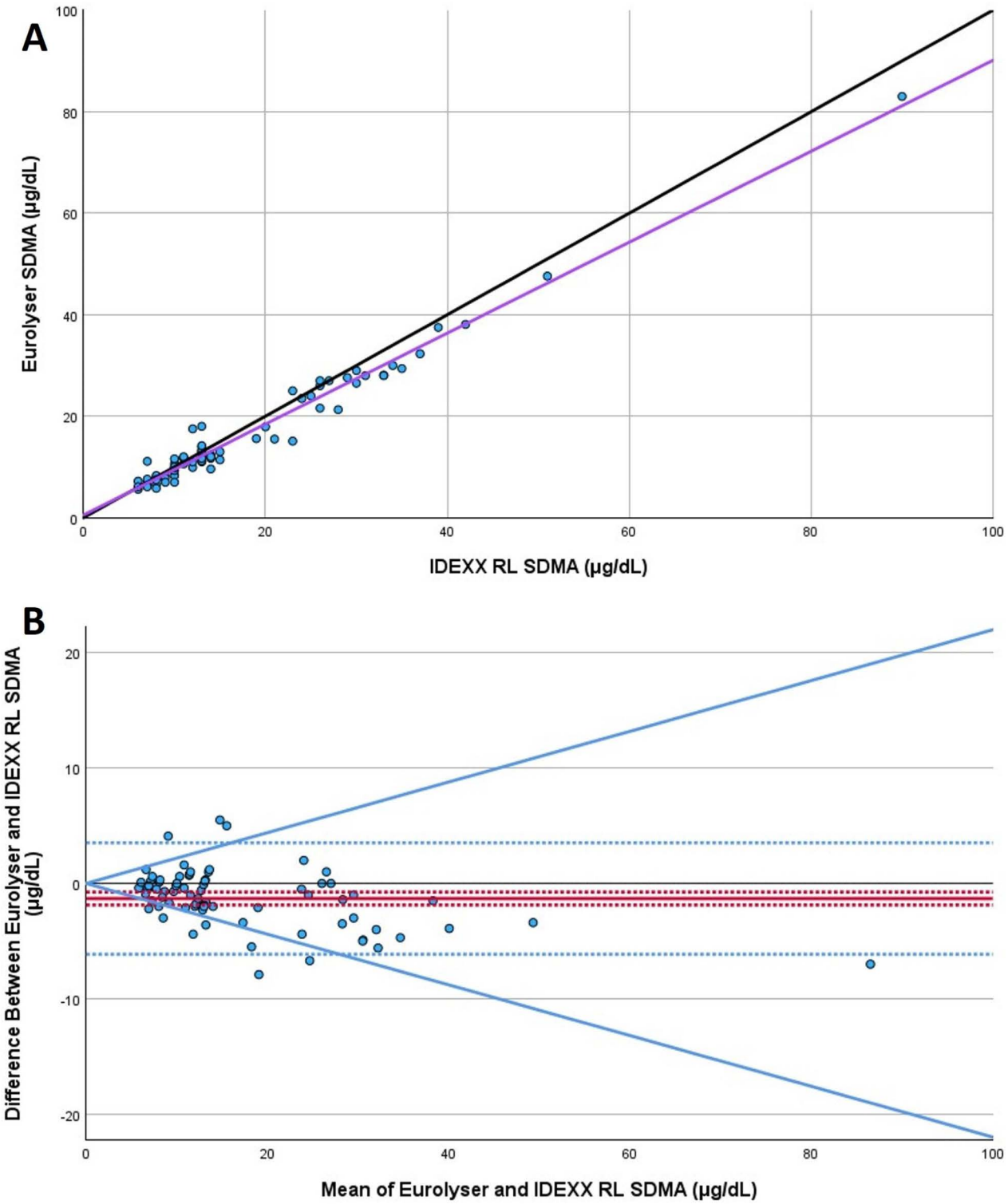

Figure 3—Scatter plot (A) and Bland-Altman difference (B) plot comparing Eurolyser and IDEXX RL SDMA concentrations. A—Black line, y = x; purple line, line of best fit (y = 0.895x + 0.532). B—Red solid line, mean difference (bias, −1.307); dashed red lines, 95% CI of mean (−0.739 to −1.874); dashed blue lines, LoA_o (3.526 to −6.143); solid blue lines, LoA_{TEa} (y = ± 0.22x).

(negative bias) between the Eurolyser and IDEXX RL methods. However, the data points were widely scattered about zero with 95% of results differing by −6 to 4 µg/dL. The Eurolyser demonstrated an increasing negative proportional bias relative to IDEXX RL results at increasing SDMA concentrations. Six (8%) and 11 (15%) data points exceeded the LoA_o and LoA_{TEa}, respectively.

The stratification of results across the different IRIS CKD stages is provided **(Supplementary Table S4)**. The weighted κ result agreement was 0.86, which is interpreted as almost perfect agreement between these 2 methods.[26] Error grid analysis demonstrated that 92% (69/75) of data sets fell within the same IRIS stage (zone A) and 8% (6/75) of data sets differed by 1 IRIS stage (zone B). Zone B data sets differed from 3 to 8 µg/dL.

Discussion

To our knowledge, this is the first study to assess the relative accuracy of the Vcheck V200 POC and Eurolyser methods under field conditions compared to a well-characterized reference method for canine SDMA analysis. It is also the first independent study comparing the performance of the IDEXX Catalyst POC and IDEXX RL for canine SDMA analysis. IDEXX RL SDMA was used as the reference method for result comparison as this method has been utilized for the majority of previously published literature[8] regarding canine SDMA and IRIS staging of CKD recommendations is currently based on this method. While duplicate measurements by each method are desirable, single SDMA analysis is routinely performed under field conditions in the clinical setting and hence duplicate analyses were not performed in this study.

There was a strong positive correlation ($r > 0.90$) between both POC analyzers and the Eurolyser method with IDEXX RLSDMA results. However, correlation does not equate to agreement between methods. The Vcheck V200 POC, IDEXX Catalyst POC, and Eurolyser methods all demonstrated a minimal average negative total bias of approximately 1 to 2 µg/dL compared to IDEXX RL SDMA analysis. However, this minimal mean difference (bias) is misleading as the LoA_o intercepts (Bland-Altman difference plots) in each of these comparisons demonstrate that there were often large individual differences in SDMA results compared to the reference method. Ninety-five percent of results differed with the IDEXX RL SDMA results within approximately ± 8, ± 11, and ± 5 µg/dL for the IDEXX Catalyst POC, Vcheck V200 POC, and Eurolyser methods, respectively. The observed percent bias for the IDEXX Catalyst POC and Eurolyser methods but not the Vcheck V200 POC method compared to IDEXX RL results exceeded the bias of 6% based on biological variation.[24] Our observed negative bias between IDEXX Catalyst POC and IDEXX RL SDMA is consistent with the findings of previous studies.[12,13] Overall, there was a difference ($P \leq .05$) in SDMA results obtained with the Eurolyser method but not the IDEXX Catalyst POC and Vcheck V200 POC methods compared to IDEXX RL results. In the case of the IDEXX Catalyst POC, the result was marginal ($P = .053$), and for both POC analyzers, this was an unexpected finding as visual assessment of the IDEXX Catalyst POC and Vcheck V200 POC versus IDEXX RL SDMA results, regression analyses, and difference plots did not appear superior to the Eurolyser method comparison. We propose that this may reflect the low total number of data sets, particularly for the Vcheck V200 POC SDMA where results of < 10 µg/dL were excluded. Similarly, the lower total number of data sets with the Vcheck V200 POC analyzer may potentially affect the lower percent bias observed using this analyzer. Maximum CV_A values for both POC and the IDEXX RL SDMA methods were all above the desirable CV_A of 7.0% based on biological variation.[24] The observed negative bias and wide LoA_o intercepts for each of IDEXX Catalyst POC, Vcheck V200 POC, and Eurolyser analyses indicate that there may be significant bias and analytical variation affecting the results. This is further supported by the large proportion of data sets (40% IDEXX Catalyst POC, 24% Vcheck V200 POC, and 15% Eurolyser), which exceeded the TE_A (LoA_{TEa}) as determined by LoA_{CII}. As > 5% of data sets exceed the TE_A in each instance, for each of the method comparisons undertaken in this study, there is more difference between the methods than can be explained by the method analytical variation and these differences could impact clinical decision making.[23] Therefore, canine SDMA results from each of these analyzers are not comparable with those obtained via the IDEXX RL. This also suggests that the SDMA reference intervals of all the assays evaluated within this study are not interchangeable with the IDEXX RL reference interval and that assay/method-specific reference intervals are needed. This is in keeping with other studies.[12,13] It would also be recommended to use the same method for serial SDMA analyses.

There are no ASVCP Quality Assurance and Laboratory Standards Committee consensus-based clinical recommendations for total error for SDMA, but desirable total error goals based on biological variation data are available.[24,27] Interestingly, the total error goal of 17.6% based on biological variation is lower than any of the TE_A goals as determined by LoA_{CII} in this study. Therefore, this desirable total error goal of 17.6% based on biological variation may not be appropriate or achievable if the biological variation of SDMA differs in unhealthy compared to healthy animals (eg, potentially in those dogs with SDMA results ≥ 18 µg/dL) and/or if CV_A differs from that achieved within the biological variation studies on healthy animals.

Visual assessment (Supplementary Table S1) of data sets indicates that the SDMA results generated by each POC method and the Eurolyser method largely approximate the corresponding IDEXX RL SDMA result. A more direct assessment was made of the potential clinical impact resulting from observed differences in canine SDMA assays based on the well-established canine IRIS CKD staging and treatment guidelines.[8] The agreement of each of the IDEXX Catalyst POC, Vcheck V200 POC, and Eurolyser SDMA results was quantified against their corresponding IDEXX RL SDMA result, based on the IRIS CKD stages, via Cohen weighted κ and error grid analysis. For the Vcheck V200 POC, SDMA results of < 10 µg/dL were able to be included in the Cohen weighted κ and error grid analyses given that these results could be classified as IRIS CKD stage 1 (SDMA < 18 µg/dL).

The Cohen weighted κ analysis provides a quantitative measure of the magnitude of agreement

between each of the compared methods beyond that which would be expected by chance alone, assuming the categories are ordered (as is the case for IRIS CKD stages) and assigning less weight to agreement as categories are further apart.[22] There was almost perfect agreement of SDMA results for both the Vcheck V200 POC and Eurolyser assays, while the IDEXX Catalyst POC demonstrated a lower substantial agreement with IDEXX RL SDMA results for IRIS CKD stage classification.

Error grid analysis (eg, Parkes error grid) has been previously used to assess the clinical accuracy of blood glucose meters whereby the clinical accuracy of a blood glucose value is expressed with respect to clinical relevance regarding potential effect on treatment decision and clinical outcome.[25] Traditionally, error grid results are displayed graphically, separated into 5 sequential risk zones ranging from A (clinically accurate measurements, no effect on clinical action) to E (altered clinical action which could have dangerous consequences).[25] We adapted this methodology using "traffic-light" color coding and the tabulated agreement results to depict the proportion of paired results within the same IRIS stage and the proportion of paired results belonging to different IRIS stages, including how far apart in stages each pair was, for each of the method comparisons. There was an excellent agreement for both the Eurolyser assay (93%) and Vcheck V200 POC assay (92%) with IDEXX RL SDMA for the IRIS CKD stage allocation, while the agreement was slightly weaker with the IDEXX Catalyst POC with 88% of paired results falling within the same IRIS stage as IDEXX RL SDMA results. None of the 3 analyzers produced any results that differed from IDEXX RL SDMA results by more than 1 sequential IRIS stage. Therefore, any of the results causing discordant IRIS CKD stage allocation compared to IDEXX RL SDMA may alter clinical action but are likely to have little or no expected effect on clinical patient outcome.

Dispersion represents a range of possible results based on a single analytical result and is calculated as follows:

$$\text{Dispersion} = \pm 1.96 \times \sqrt{(CV_A^2 + CV_I^2)},$$

where CV_I represents the intraindividual variation of 14% derived from canine SDMA biological variation studies.[24] Based on the "best-case" scenario using the lowest analyzer SDMA CV_A values included in this study for the Eurolyser of 6.53% and 3.8%, dispersion of Eurolyser SDMA results would be ± 30% and ± 28% at SDMA concentrations of 11 and 31 µg/dL, respectively. This means that there is a 95% probability that a single measured Eurolyser SDMA result of 11 µg/dL actually represents a range of possible results from 8.0 to 14.0 µg/dL and a single measured Eurolyser SDMA result of 31 µg/dL actually represents a range of possible results from 22 to 40 µg/dL. Given that error grid zone B data sets differed between 3 and 14 µg/dL, this discrepant IRIS stage classification between methods may entirely, or at least in part, simply reflect the dispersion associated with one or both of those results, particularly when they approximate the strict IRIS stage cutoffs. It should be noted that this degree of best-case scenario dispersion would not meet the SDMA TE_A performance goals of ± 2 to ± 3 µg/dL based on expert opinion for acceptable feline SDMA analytical variation.[28] It is reasonable to presume similar clinician expectations for canine SDMA analytical performance.

The majority of results in this study fall within IRIS stage 1 and IRIS stage 2 classifications. Ideally, more SDMA results within the higher IRIS stage classifications would have also been included to further assess both the simple linear regression (line of best fit) and error grid analyses over a broader distribution of SDMA concentrations.

One limitation of this study is that while commercial laboratory SDMA analyses were performed on the same day as sample collection/submission, in some instances POC analyses were performed on stored serum samples. The IDEXX Catalyst POC now, at the time of writing, utilizes a slide with incorporated reagent, which is stored frozen, and is recommended for use with serum or heparinized plasma (or whole blood in a specific lithium heparin separator). In our study, the IDEXX Catalyst POC was run utilizing the previously available assay, which included a sample cup and separate reagent kit, which were stored refrigerated. The findings of this study may not be applicable to the currently commercially available single-slide assay with incorporated reagents. For the IDEXX Catalyst POC, most samples were not frozen, but toward the end of the study, samples were frozen for batch analysis due to a lack of calibrator materials for the POC analyzer in the primary clinic where the study was conducted. While these temperatures and storage times are generally considered to fulfill requirements for appropriate SDMA sample handling, an updated IDEXX Catalyst One operator's guide recommends against using frozen samples.[29] Freezing of sera may have therefore potentially impacted IDEXX Catalyst POC results; however, the previous IDEXX Catalyst POC guidelines did not exclude frozen sera from the appropriate sample type. Canine SDMA has been previously measured from sera frozen for longer than 1 year and has shown to be stable for at least 2 years and after 3 freeze-thaw cycles.[4,13] Symmetric dimethylarginine is stable in refrigerated serum for up to 7 days when analyzed in a commercial laboratory while there is conflicting information regarding the stability of SDMA analyzed on POC analyzers using serum refrigerated for up to 7 days.[13,30] Therefore, refrigerated storage of sera for up to 7 days before analysis on either of the POC analyzers may also have impacted both Vcheck V200 POC and IDEXX Catalyst POC results. Ideally, all SDMA analyses would have been performed by each of the methods at the same time frame postsample collection and on sera stored at the same temperatures. However, this was not always possible because of POC equipment malfunctions and/or a lack of reagents or calibrator material. Proprietary adjustments to the IDEXX RL SDMA analyzer mentioned in a separate Australian SDMA

study[12] occurred before our study and therefore should not have affected our results. Future studies should also consider the comparison of all SDMA analyses with the liquid chromatography-mass spectrometry gold standard method.

A significant limitation of this study was the use of 3 separate analyzers for SDMA analysis by the IDEXX commercial laboratory (IDEXX Laboratories) over the course of our study, particularly given that this was the reference method (IDEXX RL) to which results from the other analyzers were then compared. The interchangeable use of 2 analyzers over a large duration of our study period (Beckman Coulter AU680 and DxC 700 AU analyzers from 2019 to September 2022) hindered the identification of which analyzer was specifically used to generate the majority of the IDEXX RL SDMA results. Therefore, data for each of these analyzers could not be individually compared further. While it certainly is not ideal to combine results from multiple methods/analyzyers, in-laboratory validation studies demonstrated each of these analyzers to have clinically equivalent performance (personal communication via email, October 11, 2024; IDEXX Laboratories). Commercial laboratories will undertake analyzer and/or analytical method changes from time to time, adhering to quality assurance standards requirements such as those outlined within ASVCP guidelines.[21] While the use of different RL analyzers over the course of the study is a significant limitation and may potentially reduce the robustness of our findings, the results presented here would reflect the differences in SDMA results expected by clinicians interchangeably using the POC or commercial laboratory methods evaluated over the study period. We therefore feel that this remains a valid study conducted under real-world field conditions.

Monthly Vcheck V200 calibration and IDEXX Catalyst cleaning and quality control each performed as per manufacturer recommendations does not guarantee daily acceptable performance by either of these POC analyzers. Future studies involving POC equipment should ensure that more frequent quality control analysis with or without external quality assessment (proficiency testing) program participation is undertaken. Daily monitoring of in-built instrument quality control functions and a minimum of weekly analysis of at least 1 level of external quality control material is recommended for POC analyzers by ASVCP.[31]

This study demonstrated negative bias, wide LoA_o intercepts, and > 5% of data sets exceeding the TE_A for each of IDEXX Catalyst POC, Vcheck V200 POC, and Eurolyser analyses, indicating that canine SDMA results from each of these analyzers are not comparable with those obtained via the IDEXX RL. Furthermore, this suggests that the reference intervals between all the assays compared here are not directly interchangeable with the IDEXX RL reference intervals and that assay/method-specific reference intervals are needed. This is in keeping with other studies.[12,13] This highlights the need to use the same method/analyzer for serial SDMA monitoring. It is important to note that documenting that 2 methods are not interchangeable does not prove that one of the methods is inferior to another.[23] Additionally, the results obtained from each of the SDMA method comparisons performed in this study clearly highlight that the use of simple linear regression and correlation coefficients alone may be inadequate for method comparison studies and result in misleading data interpretation. All comparison of methods studies should follow current ASVCP guidelines.[21]

Discordant IRIS CKD stage allocations by the assays evaluated here would at least, in part, simply reflect the dispersion associated with one or both of those results and is expected to have little or no effect on clinical patient outcome. This offers preliminary supportive evidence for the inclusion of Eurolyser RL SDMA and Vcheck V200 POC SDMA results along with the IDEXX proprietary SDMA analyses for staging of canine CKD.

Further independent studies assessing the comparative performance of different POC and RL SDMA methodologies would be encouraged to determine the CV_A and dispersion of SDMA results for each assay, preferably using patient sera at SDMA concentrations approximating the upper limit of the reference interval and IRIS stage cutoffs. Where possible, comparative studies should utilize gold-standard liquid chromatography-mass spectrometry SDMA analysis as the reference method. Veterinary clinicians should also be acutely aware that even if operating at the optimal performance (eg, based on CV_A values presented in this study) both RL and POC SDMA methods may have a degree of dispersion that would cloud the definitive interpretation of a single SDMA result with respect to diagnosis, staging, or assessment of progression of CKD. Ongoing studies assessing the clinical utility of SDMA over sCr measurements are recommended, including further longitudinal studies with serial intraindividual assessment.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.