

ORIGINAL ARTICLE OPEN ACCESS

# Analytical and Quality Control Validation of a Novel Symmetric Dimethylarginine Assay in Dogs and Cats

Portia Tshidi Mashego | Emma H. Hooijberg 

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, Gauteng, South Africa

**Correspondence:** Emma H. Hooijberg ([emvet1122@gmail.com](mailto:emvet1122@gmail.com))**Received:** 17 March 2025 | **Revised:** 3 May 2025 | **Accepted:** 13 June 2025**Funding:** This study was supported by AgriSETA.**Keywords:** creatinine | dispersion | method validation | quality control validation | SDMA

## ABSTRACT

**Background:** An immunoturbidometric assay for symmetric dimethylarginine (SDMA) measurement on automated chemistry analyzers has recently become available.

**Objective:** To perform analytical validation of the EUROlyser SDMA assay in dogs and cats.

**Methods:** Method validation experiments were performed using stored canine and feline serum. Quality control validation was performed according to Westgard. Performance goals were derived from SDMA biological variation data for both species.

**Results:** Imprecision ranged from 3.7%–7.8% (dogs) to 6.0%–11.8% (cats) with a dispersion of  $\pm 35\%$  for dogs and  $\pm 44\%$  for cats. The assay showed linearity (up to 85  $\mu\text{g}/\text{dL}$  [dogs], 75  $\mu\text{g}/\text{dL}$  [cats]); the preliminary LoQ was 9.5 and 6.9  $\mu\text{g}/\text{dL}$ , respectively. Recovery was 19.7% and 6.5%, respectively. Severe hemolysis resulted in a significant bias in both species. The EUROlyser method showed a significant negative proportional and constant bias in dogs and a significant positive proportional and negative constant bias in cats, compared to the comparative method. In dogs, the mean bias ( $-19.5\%$ ) and the bias at clinical decision limits exceeded the desirable bias; in cats, the mean bias ( $-4.0\%$ ) and the bias at clinical decision limits was  $< 8\%$ . Because of high imprecision, the  $\text{TE}_A$  that could be controlled for was 35%, with a 1–2.5 s rule using patient pools as quality control material.

**Conclusions:** The novel SDMA assay showed acceptable analytical performance, but high dispersion has consequences for the interpretation of results at reference limits and serial measurements. Method-specific reference intervals and decision limits should be generated for both species, particularly for dogs, given the significant bias vs. the comparative method.

## 1 | Introduction

Symmetric dimethylarginine (SDMA) is a marker of glomerular filtration rate (GFR) which has become widely used as a renal biomarker in dogs and cats. The relationship between SDMA, GFR, and serum creatinine in cats and dogs has been well documented [1–7]. More recent publications have focused on the diagnostic utility of SDMA (and creatinine) in detecting kidney disease (chronic kidney disease (CKD), acute kidney injury (AKI) and nephroliths) in cats and dogs [8–13]. The International Renal Interest Society (IRIS) has incorporated SDMA concentrations into its CKD staging guidelines [14].

Symmetric dimethylarginine was first measured in dogs and cats using liquid chromatography-mass spectrometry (LC–MS) [4, 6, 15]. Although LC–MS is accurate and precise, the method is time-consuming, not readily available, and costly to perform [16]. To facilitate the use of SDMA in clinical settings, IDEXX Laboratories developed an enzyme immunoassay (IDEXX EIA) for use in their reference laboratories and a point-of-care (IDEXX POC) assay [17, 18]. Another reference laboratory assay and a POC assay are now available from a second source (EUROlyser Diagnostica GmbH, [www.eurolyser.com](http://www.eurolyser.com)). The EUROlyser reference laboratory SDMA assay is a homogenous immunoturbidometric assay (hereafter referred to as EUROlyser ITA) developed

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2025 The Author(s). *Veterinary Clinical Pathology* published by Wiley Periodicals LLC on behalf of American Society for Veterinary Clinical Pathology.

for SDMA measurement in dogs and cats, which can be used on various automated chemistry analyzers [19]. Information on the nature and species origin of the antibody used in the kit is not available. The manufacturer reports a linear range of 0–100 µg/dL with a limit of quantification of 0.7 µg/dL; imprecision is reported to range from 4.4% to 6.5%, with a good correlation with LC–MS and the IDEXX EIA, with a mean bias < 5.2% [19]. The EUROLyser ITA was recently shown in an independent study to have an inter-assay imprecision of 3.8%–6.5% with a mean bias of –6.7% compared to the IDEXX EIA for canine serum [20]. Apart from this information, to our knowledge, there are no publications concerning the analytical performance of the EUROLyser ITA method in dogs or cats.

The aim of this study was to validate the EUROLyser ITA for measurement of SDMA in cats and dogs. Specific objectives were firstly to evaluate assay imprecision and bias through a range of experiments, including linearity, short- and long-term imprecision, spike and recovery, interference, limits of blank, detection, and quantification; and secondly to perform a method comparison study with the IDEXX EIA as the reference method. The gold standard SDMA LC–MS method was not available in South Africa, and so a field method (the IDEXX EIA) was used as the reference method in the comparison study. The third objective was to perform quality control (QC) validation and formulate a QC strategy for the EUROLyser ITA SDMA assay. Lastly, the relationship between EUROLyser ITA SDMA and serum creatinine was explored. Creatinine is well established as a marker of GFR, and the association between creatinine and SDMA, using other assays, has been extensively documented. For example, in cats a moderate correlation, based either on calculation of  $r$  or  $r^2$ , ranging from  $r = 0.41$  to  $0.84$  and  $r^2$  of  $0.73$  has been reported [1, 2, 4, 10]. Reported correlations in dogs range from  $r = 0.55$  to  $0.95$  and  $r^2$  of  $0.33$  [6, 7, 11, 13]. We did not aim to determine reference intervals or evaluate the diagnostic utility of the EUROLyser assay, but we included the comparison with creatinine to gain some preliminary information about the diagnostic performance of this assay.

For cats, the SDMA method validation data was evaluated using published biological variation (BV) based performance goals: desirable imprecision ( $I_D$ ) 10%, desirable bias ( $B_D$ ) 8%, and desirable total error ( $TE_D$ ) 24% [21]. For dogs, BV-based goals were calculated from a canine BV study with SDMA measured with the IDEXX EIA method, where intra-individual variation was 16% and inter-individual variation 22%, resulting in  $I_D$  8%,  $B_D$  7%, and  $TE_D$  20% [22, 23]. We hypothesized that, based on information from the manufacturer and a previous publication, analytical performance would meet these performance goals [19, 20].

## 2 | Material and Methods

### 2.1 | Study Setting and Samples

This prospective study was performed using canine and feline serum samples stored in the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria. Informed consent was obtained for the use of the stored samples, and the study was approved by the University of Pretoria Animal Ethics

and Research Committee (REC 080-23). The EUROLyser ITA assay was run on the Roche Cobas Integra 400 Plus wet chemistry analyzer (Roche Products [Pty] Ltd., Basel, Switzerland) in the Clinical Pathology Laboratory. Samples for method comparison were analyzed at IDEXX Laboratories (Pty) Ltd., Kyalami, South Africa.

Left-over stored serum samples, banked for up to 60 months at  $-20^{\circ}\text{C}$ , from healthy and unhealthy canine and feline patients of the Veterinary Academic Hospital, were used. Samples were chosen according to their creatinine concentration, to obtain the estimated concentration of SDMA required for the various experiments. Samples used for analytical validation contained at least 0.5 mL of serum each and were visually inspected for hemolysis, lipemia and icterus. Only clear and slightly hemolytic (pink-tinged), icteric (faint yellow) and lipemic (slightly hazy) samples were accepted. Moderately to severely lipemic (hazy to milky), hemolyzed (light to dark red) and icteric (yellow to orange) samples and samples with small volumes were excluded. For method comparison, samples had to have a minimum volume of 1 mL. The EUROLyser SDMA reagent kit was set up and calibrated on the Roche Cobas Integra analyzer according to the manufacturer's instructions. Serum samples or pools were left to thaw and reach room temperature, then mixed well using a vortex and centrifuged to ensure there were no fibrin or cryoprotein precipitates. Two types of samples were used as quality control materials (QCM): EUROLyser SDMA QCM (levels 1 & 2) and serum canine patient sample pools (low and high).

### 2.2 | Method Validation Experiments

For the repeatability (short-term/intra-assay imprecision) study, two levels (low and high) of pooled samples were prepared for each species. Symmetric dimethylarginine was measured on each pool 20 times within a single analytical run (i.e., on 1 day) [24]. Twenty aliquots were prepared from the left-over pooled samples and stored at  $-20^{\circ}\text{C}$  for the reproducibility (long-term/inter-assay imprecision) study. These samples were measured once daily for 20 days, after thawing and mixing [24].

For the linearity study, samples with creatinine concentrations ranging from 800 to 1500 µmol/L were combined to make 1 mL of a pooled sample with a high SDMA concentration for each species. Five levels of dilutions were prepared using 0.9% NaCl (level 1 blank) as a diluent and measured in triplicate [24]. The canine pooled sample concentration was 85.1 µg/dL with dilution targets of 63.9, 42.6, 21.3 µg/dL, and a blank. The feline pooled sample concentration was 75.1 µg/dL with dilution targets of 56.3, 37.5, 18.8 µg/dL, and a blank.

For the limit of blank (LoB), limit of detection (LoD) and limit of quantification (LoQ) studies, a 1 mL pooled sample for each species with very low SDMA concentration and physiological saline blank were used; SDMA was measured five times within a single run on the pool and blank for each species. The left-over pooled sample was aliquoted into four tubes and stored at  $-20^{\circ}\text{C}$ . This process was repeated for the next 4 days [24].

For the spike and recovery study, a pooled sample with a targeted SDMA concentration of about 10 µg/dL was made for each

species. Six 200  $\mu\text{L}$  aliquots were prepared from each pool. The first three aliquots were spiked with three different volumes of high calibrator (Level 6, SDMA 100  $\mu\text{g}/\text{dL}$ ) and the last three aliquots were spiked with three identical volumes of saline; SDMA was measured in duplicate [24].

To evaluate the effect of hemolysis, leftover patient serum tubes for each species containing a total of 4 mL of blood clot were used to make up 1.5 mL of hemolysate. This was achieved by freeze-thawing and centrifuging the serum clot tubes 3–4 times over 7 days to lyse the cells [25]. The samples were centrifuged at 4000 rpm for 8 min to produce the supernatant hemolysate. Hemoglobin concentration was determined using the ADVIA 2120i hematology analyzer (Siemens Healthineers, South Africa) and hemolysate was further diluted with distilled water to achieve a hemoglobin concentration close to 100 g/L. This stock solution was then sequentially diluted using equal volumes of distilled water to result in solutions with five concentrations of hemoglobin. Serum samples without hemolysis, icterus, or lipemia were retrieved, thawed, and mixed properly with a vortex and then pooled for each species. Symmetric dimethylarginine concentrations in the pools were determined; pools were then divided into six aliquots of 135  $\mu\text{L}$  of serum, and 15  $\mu\text{L}$  of each concentration of hemolysate solution was added to each of five different tubes to create different degrees of hemolysis. Distilled water was added to a sixth tube. Samples were measured in triplicate.

An Intra-lipid 20% fat emulsion (Fresenius Kabi AB, Sweden) was used to make up a lipid-containing solution to simulate lipemia. To achieve this, a stock solution was prepared by diluting 24  $\mu\text{L}$  of the Intra-lipid fat emulsion with 788  $\mu\text{L}$  of 0.9% NaCl to achieve a triglyceride concentration close to 30 mmol/L (as measured on the Roche Cobas Integra 400 Plus). Distilled water was used to make a 2-fold sequential dilution series of five levels. Pooled serum samples were created for each species, and SDMA concentration was measured; six aliquots of 180  $\mu\text{L}$  were prepared. 60  $\mu\text{L}$  of lipid stock solution from the 5 lipid tubes were each pipetted into five aliquots to create different degrees of lipemia; 60  $\mu\text{L}$  of distilled water was added to the sixth tube. Samples were measured in triplicate.

For the method comparison study, 50 samples from each species, with a minimum volume of 1 mL were selected. Creatinine concentrations were used to guide selection so that samples contained expected SDMA concentrations spanning the measuring range. 0.5 mL of each 1 mL sample was aliquoted into two identically labeled tubes. The resulting 200 samples (50  $\times$  2 aliquots per species) were kept at  $-20^\circ\text{C}$  until the day of analysis when one batch of feline and canine samples was transported in a temperature-controlled container via courier to the IDEXX Laboratory in Johannesburg (about 1 h transit time). Once samples arrived at IDEXX both laboratories commenced SDMA analysis. Samples were measured only once due to limited resources.

Creatinine measurements were available for all samples used in the method comparison and were compared to the SDMA results. Creatinine was measured on the Roche Cobas Integra 400 Plus using the modified Jaffe method.

## 2.3 | QC Validation

Both commercial EUROlyser SDMA QCM (2 levels) and stored aliquoted serum canine patient sample pools (low and high) were used as daily QCM to monitor assay performance during the study.

## 2.4 | Statistical Analysis

After sample analysis, all results for each study were transferred to Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using MedCalc (MedCalc Statistical Software version 22.016; MedCalc Software Ltd., Ostend, Belgium; <https://www.medcalc.org>; 2023). A  $p$  value of  $<0.05$  was considered statistically significant.

Using a Microsoft Excel spreadsheet, imprecision was determined by calculating the CV (%) as follows:

$$\text{CV}(\%) = \frac{\text{SD}}{\text{mean}} \times 100$$

Dispersion was calculated using the intra-individual variation ( $\text{CV}_I$ ) from canine and feline biological variation studies, and the inter-assay imprecision ( $\text{CV}_A$ ) as [20, 21, 23]:

$$\text{Dispersion} = \pm 1.96 \times \sqrt{(\text{CV}_A^2 + \text{CV}_I^2)}$$

Linearity data was evaluated by calculating the mean of the triplicate measurements. Simple linear regression was performed, with the measured means plotted on the  $y$  axis and target values on the  $x$  axis. The regression graph was visually inspected for linearity and the slope and intercept of the regression line, with their 95% confidence intervals, were derived from the regression equation. For LoB and LoD and LoQ, the SD and CV were calculated from the repeated measurements of the blank and the low pool samples: [26]

$$\text{LoB} = \text{mean of blank} + (1.645 \times \text{SD of blank})$$

$$\text{LoD} = \text{LoB} + (1.645 \times \text{SD low concentration pool})$$

$$\text{LoQ} = \text{LoB} + (10 \times \text{SD low concentration pool})$$

To calculate recovery, means of paired spiked and paired diluted samples were calculated. The SDMA concentration difference between the dilution and the addition of paired samples, % recovery for each sample, average of recoveries, and percentage proportional error were calculated.

$$\% \text{Recovery} = \frac{(\text{SDMA addition sample} - \text{SDMA dilution sample})}{\text{amount SDMA added}} \times 100$$

$$\% \text{Proportional error} = 100 - \% \text{Average recovery}$$

For the hemoglobin and lipemia interference study, the mean of the duplicates was calculated for each species and interference. The percentage bias of each sample was calculated in each species.

$$\text{Bias}(\%) = \frac{\text{Measured value} - \text{Target value (sixth tube)}}{\text{Target (sixth tube)}} \times 100$$

For the method comparison study, first a scatter plot was constructed, with results of the EUROLyser ITA plotted on the *y* axis against the IDEXX EIA on the *x* axis, for each species. Linear regression was performed, and residuals were calculated. Any data point with a residual outside of 2 SDs (of the residuals) was discarded as an outlier. Passing-Bablok regression analysis was performed, and Bland–Altman difference plots were constructed [24, 27]. The regression equation was used to determine whether statistically significant constant or proportional bias was present. The regression equation was also used to determine whether clinically significant bias was present at the IDEXX EIA upper reference limit (14 µg/dL) and at different IRIS clinical decision limits [14]. Regarding the Bland–Altman plots, the percentage difference between the two methods was plotted on the *y* axis, and the mean of both methods on the *x* axis. The 95% limits of agreement, as well as lines indicating the TE<sub>D</sub> (20% for dogs, 24% for cats), were also included in the Bland–Altman plots.

The association between creatinine and SDMA was investigated using Spearman's correlation analysis, and scatter plots were created.

For QC validation, the results of both the pooled (low and high) patient QCM and commercial EUROLyser SDMA QCM were recorded on a Microsoft Excel spreadsheet, and the mean, CV, and SD were calculated. An online calculator and normalized OPSpecs Chart ([www.westgard.com](http://www.westgard.com)) were used to evaluate appropriate control rules [28]. Bias was set at 0% for the patient pools. For the commercial QCM, bias was calculated as:

$$\text{Bias (\%)} = \frac{\text{Measured value} - \text{Target value}}{\text{Target value}} \times 100$$

The normalized *x* and *y* values obtained from the online OPSpecs calculator were plotted on the normalized OPSpecs Chart for 90% probability of error detection (Ped), probability of false rejection (Pfr) < 5%, and *n* = 2 (for two control levels) to select the QC rule to be used.

### 3 | Results

The intra- and inter-assay imprecision was lower than *I<sub>D</sub>* (8% dog, 10% cat) apart from the low feline pool inter-assay imprecision, which was slightly high at 11.8% (Table 1). Using inter-assay imprecision, dispersion was calculated to be ±35% for dogs (both low and high pools) and ±43% to 45% (high pool-low pool, mean ±44%) for cats. Based on visual inspection of the linearity regression plots (Figure 1) and the regression equation, there was no deviation from linearity (dog up to 85.1 µg/dL, *r* = 1.00, slope 0.990 [95% CI 0.987–1.010] and intercept 0.234 [95% CI –0.377 to 0.845]; cat up to 75.1 µg/dL, *r* = 1.00, slope 1.010 [95% CI 0.924–1.109] and intercept –0.576 [95% CI –4.832 to 3.680]).

For the LoD and LoQ experiments, the mean, SD, and CV for the low canine pool were 6.1 µg/dL, 0.95 µg/dL, and 15.5%, and for the low feline pool were 6.6 µg/dL, 0.69 µg/dL, and 10.4%, respectively. The LoB was 0.0 µg/dL. For canine serum, the LoD was 1.6 µg/dL and the LoQ was 9.5 µg/dL. For feline serum, the LoD was 1.1 µg/dL and the LoQ was 6.9 µg/dL. For canine serum, the overall recovery was 80.3%, and the proportional error (bias) was 19.7%. One

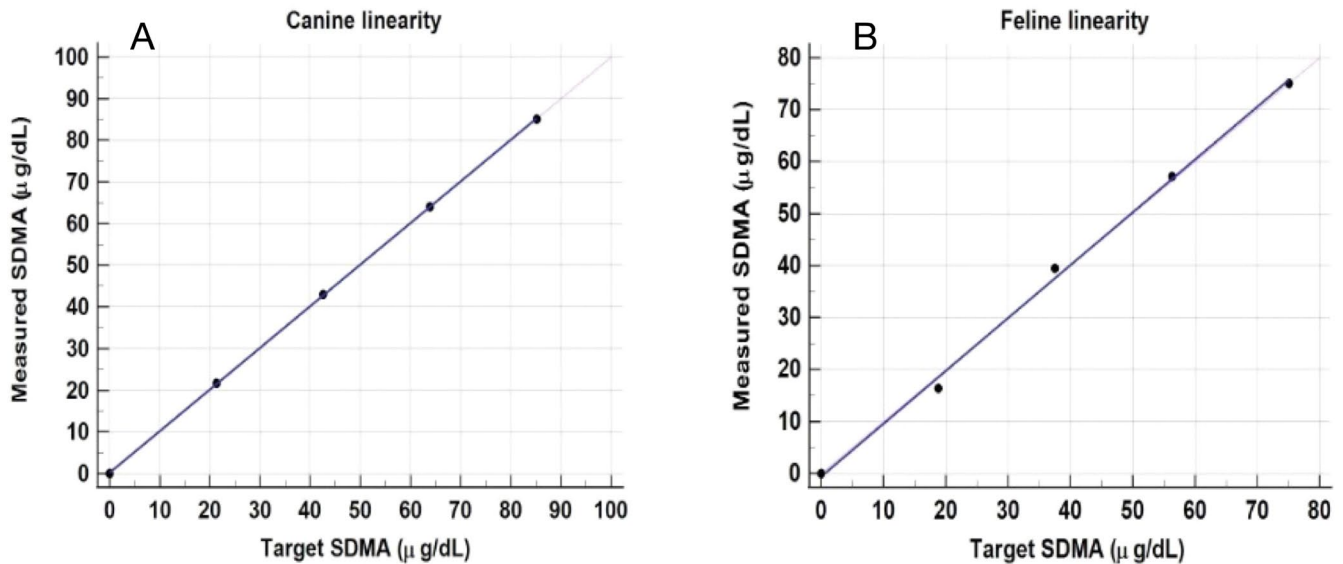
**TABLE 1** | Intra- and inter-assay imprecision study results for the EUROLyser SDMA reference laboratory immunoturbidometric assay using canine and feline serum.

Species	Study type	Pool mean (µg/dL)	SD (µg/dL)	CV (%)
Dog	Intra-assay	9.4	0.5	5.4
		50.5	1.9	3.7
	Inter-assay	10.2	0.8	7.4
Cat	Intra-assay	56.3	4.4	7.8
		10.7	0.6	6.0
	Inter-assay	44.8	1.6	3.7
		13.0	1.5	11.8
		52.8	4.9	9.2

Abbreviations: CV, coefficient of variation; SD, standard deviation; SDMA, symmetric dimethylarginine.

sample had a very poor recovery of 57.1% (with analysis repeated) and the other two had recoveries of 85.0% and 99.0% (average 92.0%). For feline serum, the average recovery was 93.47%, and the proportional error (bias) was 6.53%. The bias in feline serum was less than the *B<sub>D</sub>* of 8%, and the bias in canine serum overall was less than the TE<sub>D</sub> of 20%. A clinically significant bias of variable direction of >*B<sub>D</sub>* ±7% was present in canine serum with severe hemolysis, and a clinically significant positive bias >*B<sub>D</sub>* ±8% was present in feline serum with moderate to severe hemolysis (Table 2). A clinically significant bias of 11.6% was present in canine serum with severe lipemia, whereas there was no significant bias in feline serum with lipemia (Table 3).

In terms of the method comparison, three outliers were identified and paired data removed from the canine dataset, and four paired results were removed as outliers from the feline dataset. With Passing-Bablok regression analysis (Figure 2, Table 4), a strong positive linear correlation between the two methods was seen (*r* = 0.888, *p* < 0.0001 for feline measurements and *r* = 0.981, *p* < 0.0001 for canine measurements). Based on the Cusum test, all data sets were linear (*p* = 1.00 canine, *p* = 0.86 feline). Based on the regression equation, for dogs, the EUROLyser ITA method showed a significant negative constant and proportional bias, and in cats there was a significant negative constant bias and a significant positive proportional bias compared to the IDEXX EIA [27]. In dogs, the Bland–Altman 95% limits of agreement were –46.9% (95% CI –53.9% to –39.8%) to 7.9% (95% CI 0.8%–14.9%). The mean bias was –19.5% (95% CI –23.6% to –15.4%) (Figure 3A). The bias, calculated using the regression equation, at the IDEXX EIA upper reference limit and IRIS clinical decision limits exceeded *B<sub>D</sub>* of ±7% (Table 5). In addition, 21/47 (45%) paired measurements were outside of the TE<sub>D</sub> of 20% (Figure 3A). Applying the regression equation to the IDEXX EIA upper reference limit of 14 µg/dL yields an adjusted preliminary upper reference limit of 10.9 µg/dL for dogs using the EUROLyser ITA method; the effect of the bias on the IRIS cutoffs, using the regression equation, is also shown (Table 5). In cats, the Bland–Altman 95% limits of agreement were –38.9% (95% CI –48.0% to –29.8%) to 7.9% (95% CI 21.8%–40.0%). There was an acceptable mean bias of –4.0% (95% CI –9.3% to 1.3%)



**FIGURE 1** | Canine (A) and feline (B) linearity regression plot showing the linear relationship between target and measured values for the EUROLyser SDMA reference laboratory immunoturbidometric assay.

**TABLE 2** | Hemolysis interference study results.

	Degree of hemolysis	Hemoglobin (g/L)	Mean SDMA (µg/dL)	Bias %
Canine	Severe	10.5	7.4	-15.2
	Severe	5.3	9.6	9.9
	Moderate	2.6	8.7	0.3
	Mild	1.3	9.2	5.3
	Slight	0.7	8.1	-6.8
	None	0	8.7 (target)	0
Feline	Severe	10.9	14.8	29.8
	Severe	5.5	13.89	21.5
	Moderate	2.7	13.2	15.4
	Mild	1.4	11.4	-0.1
	Slight	0.7	11.8	3.7
	None	0	11.4 (target)	0

Note: The table depicts the bias for the EUROLyser SDMA reference laboratory immunoturbidometric assay with different degrees of hemolysis. Abbreviation: SDMA, symmetric dimethylarginine.

(Figure 3B) with Bland–Altman plot analysis, and the absolute bias at different clinical decision limits was within  $\pm 8\%$  (Table 5). There were 9/46 (20%) paired measurements outside of the  $TE_D$  of 24% (Figure 3B). Applying the regression equation to the IDEXX EIA upper reference limit of 14 µg/dL yields an adjusted preliminary upper reference limit of 13.1 µg/dL for cats using the ITA method, and the effect of the bias on the IRIS cut-offs, using the regression equation, is also shown (Table 5). The dispersion range (using  $\pm 35\%$  for dogs,  $\pm 44\%$  for cats) for each clinical decision limit was also calculated (Table 5).

There was a significant positive correlation between SDMA and creatinine in both dogs ( $r=0.865$ ,  $p<0.0001$ ) and cats ( $r=0.794$ ,  $p<0.0001$ ). For dogs, 30% (14/47) of paired results were below

the upper reference limit for both creatinine and SDMA (adjusted), 57% (27/47) of paired results were above the reference interval for both, there were 2% (1/47) of results with a raised creatinine but normal SDMA and 11% (5/47) of results with a raised SDMA but normal creatinine (Figure 4A). For cats, 26% (12/46) of paired results were below the upper reference limits for both creatinine and SDMA (adjusted), 46% (21/46) of paired results were above the reference interval for both, there were 20% (9/46) of results with a raised creatinine but normal SDMA and 7% (3/46) of results with a raised SDMA but normal creatinine (Figure 4B).

The CVs for the low and high canine patient pool QCM were 7.4% and 8.0% (bias was set at zero). The CVs and biases for the

TABLE 3 | Lipemia interference study results.

	Degree of lipemia	Triglyceride (mmol/L)	Mean SDMA (µg/dL)	Bias %
Canine	Marked	7.36	9.3	11.6
	Marked	3.63	8.5	1.1
	Moderate	1.84	8.6	2.6
	Moderate	0.92	8.1	-3.2
	Slight	0.46	8.5	1.9
	None	0	8.4 (target)	0
Feline	Marked	7.36	10.1	5.76
	Marked	3.63	9.2	-4.1
	Moderate	1.84	9.0	-5.8
	Moderate	0.92	9.0	-6.0
	Slight	0.46	9.3	-2.6
	None	0	9.6 (target)	0

Note: The table below depicts the bias for the EUROLyser SDMA reference laboratory immunoturbidometric assay with different degrees of lipemia. Abbreviation: SDMA, symmetric dimethylarginine.

low and high commercial QCM were 12.9% and 2.0%, and 6.6% and 1.5%, respectively. The normalized *x*- and *y*-coordinates on the Normalized OPSpecs chart represent imprecision and bias, respectively. These coordinates, the candidate QC rules, and the Ped and Pfr for each using the 90% AQA Normalized OPSpec Chart with *n*=2 are shown in Table 6. Because of the high imprecision, no rules could be selected at a TE<sub>D</sub> of 20%, and so QC validation was also performed for a total allowable error (TE<sub>A</sub>) of 30% and 35% (Table 6). No rules could be selected for a TE<sub>A</sub> of 30%, whereas the 1–2.5 *s* rule was suitable using a TE<sub>A</sub> of 35% with canine patient pools.

## 4 | Discussion

EUROLyser ITA SDMA assay imprecision and bias were evaluated through a range of experiments, and the assay met the predetermined performance goals in most experiments for both species but showed a clinically significant bias compared to the reference method for dogs. There was high dispersion in both species. Although the assay mostly met the BV-derived *I*<sub>D</sub>, the degree of imprecision did not allow for QC validation using the BV-derived TE<sub>D</sub> of 20%, and performance could only be controlled for a TE<sub>A</sub> of 35%.

Intra- and inter-assay imprecision were mostly acceptable and were below the *I*<sub>D</sub> in both species except for the inter-assay CV of the low feline pool, which was slightly high, at 11.8%. The higher CV findings in the cat versus the dog could be related to the sample matrix. Imprecision (3.7%–11.8%) was higher than has been reported for the original LC–MS method (1.3%–3.7%) and possibly with a novel LC–MS/MS method (imprecision only reported as “<10%”) [6, 15]. In terms of immunoassays, the IDEXX EIA method had a good inter-assay imprecision (7.7% and 2.3%) for low canine pool and high feline pool, respectively in an early study, but a high imprecision of 13.5% was reported for pooled feline serum in a 2021 study [17, 18]. In this same study, the inter-assay imprecision using the IDEXX POC SDMA assay was 8.7%–11.6% for feline plasma and unacceptably high for feline serum (13.5%) [18]. Another POC SDMA assay, the Bionote Vcheck V200, was reported to have an inter-assay CV of 10.3%–11.0%, and the EUROLyser ITA an inter-assay CV of 3.8%–6.5% with canine serum [20]. In the present study, the EUROLyser ITA assay therefore has an inter-assay imprecision that is between that of the IDEXX EIA and POC assays. Why the imprecision for the EUROLyser ITA here is higher than reported previously is not known, as that study did not include any details of the imprecision experiments [20]. A high inter-assay imprecision leads to high dispersion (together with high intra-individual variation), which has implications for the interpretation of

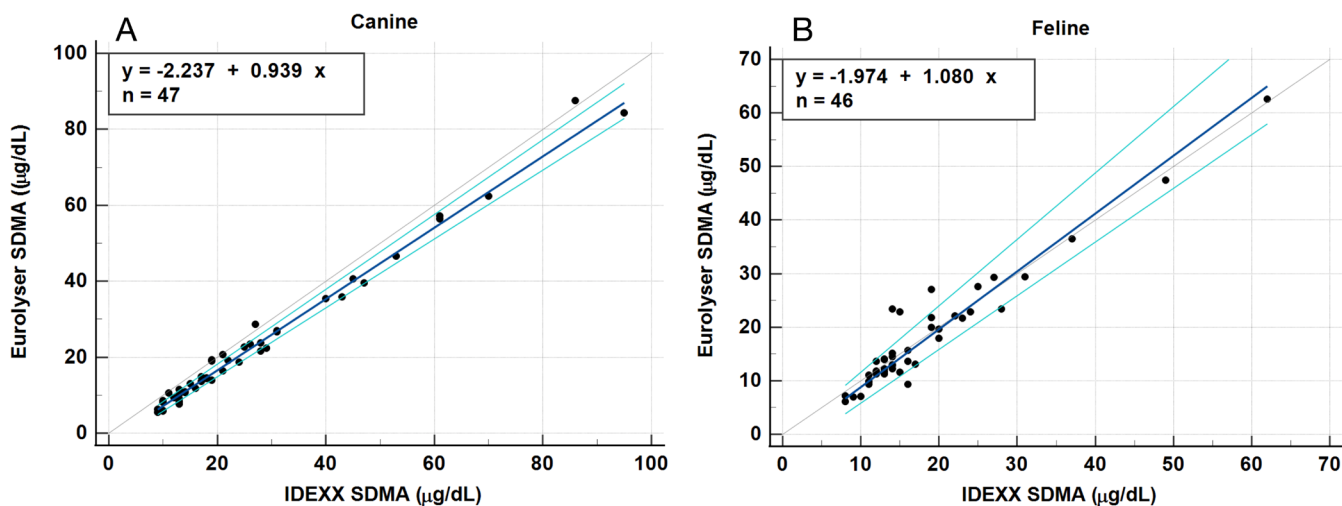
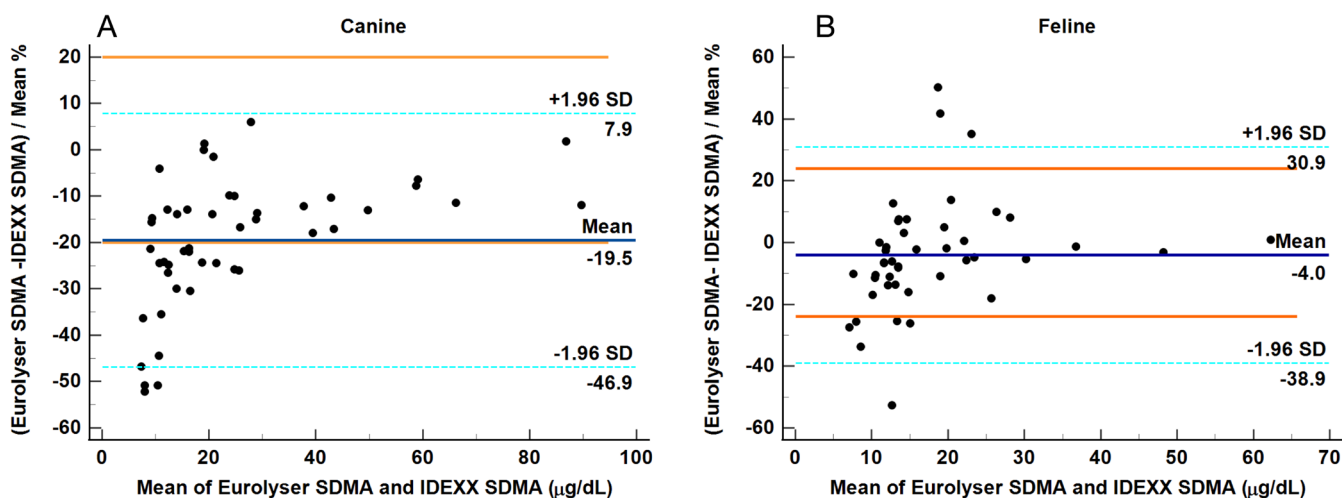


FIGURE 2 | Passing-Bablok regression plots of comparative measurements of 47 canine (A) and 46 feline (B) serum samples with the EUROLyser SDMA reference laboratory immunoturbidometric method plotted on the *y* axis and the IDEXX SDMA reference laboratory method plotted on the *x* axis. The thin gray line is the line of identity (*y*=*x*). The thick dark blue line is the line of best fit. The light blue line represents the 95% CI. Black dots on graphs A and B represent the individual data points around the fitted regression line.

**TABLE 4** | Passing-Bablok regression analysis results for comparison of the EUROLyser SDMA reference laboratory immunoturbidimetric assay to the IDEXX EIA SDMA method.

	Canine	Feline
Regression equation ( $Y = a + bX$ )	$Y = -2.237 + 0.939X$	$Y = -1.974 + 1.080X$
Constant bias		
Intercept $a$	-2.237	-1.974
95% CI	-3.275 to -1.517	-4.195 to -0.790
Proportional bias		
Slope $b$	0.939	1.080
95% CI	0.907 to 0.985	1.003 to 1.240

Abbreviations: CI, confidence interval; SDMA, symmetric dimethylarginine.



**FIGURE 3** | Bland-Altman difference plots, comparing the EUROLyser SDMA reference laboratory immunoturbidimetric method to the IDEXX reference laboratory SDMA method for dogs (A) and cats (B). The dark blue line represents the mean bias %, the blue dashed lines represent the 95% limits of agreement and the orange lines represent the desirable analytical error of  $\pm 20\%$  (dog) or  $\pm 24\%$  (cat).

serial results and the strict use of clinical decision limits, as has been noted for SDMA in dogs and cats previously and is reiterated here [18, 20]. Dispersion for the EUROLyser ITA for dogs has been previously calculated to range from  $\pm 28\%$  to  $30\%$ , and dispersion for cats ranged from  $\pm 43\%$  to  $47\%$  with the IDEXX POC assay and  $\pm 40\%$  to  $47\%$  for the IDEXX EIA assay (here dispersion was  $\pm 35\%$  for dogs and  $\pm 44\%$  for cats) [18, 20]. The effect of dispersion is well illustrated by the overlap in the adjusted clinical decision limits for IRIS staging when dispersion is taken into account; for example, a single SDMA measurement of  $35 \mu\text{g/dL}$  in a dog does not necessarily mean the patient is in Stage 3, it could also be in Stage 4; a single SDMA measurement of  $12 \mu\text{g/dL}$  is not definitely less than the upper reference limit as this result is in the dispersion range for Stage 2 (Table 5).

The EUROLyser ITA SDMA assay was accurate, based on good linearity under dilution, up to concentrations of  $75.1 \mu\text{g/dL}$  in the cat and  $85.1 \mu\text{g/dL}$  in the dog. Studies that have evaluated the reportable range of the EIA have spiked native samples to obtain an SDMA concentration of approximately  $100 \mu\text{g/dL}$ . These include studies in cats and dogs, but also in horses, rabbits and cheetahs [6, 11, 17, 18, 29–31]. We chose to use native

serum samples with a predicted high SDMA based on creatinine concentration, and not spiked samples, as native samples better represent the patient matrix. The lower EUROLyser ITA upper reportable limits, as determined here, compared to the IDEXX EIA, are not expected to have clinical implications, as the IRIS SDMA cutoffs for Stage 4 CKD are much lower ( $38.00 \mu\text{g/dL}$  in cats,  $54.00 \mu\text{g/dL}$  dogs) [14]. The lowest concentration at which SDMA could be reliably quantified for dogs was estimated to be  $9.5 \mu\text{g/dL}$  and for cats,  $6.9 \mu\text{g/dL}$ , which is significantly above the manufacturer's stated LoQ of  $0.7 \mu\text{g/dL}$  (data and calculation not given). Ideally, as the LoQ is based on performance goals, it should be set based on precision profiling of low concentration samples [26]. We did not specifically perform these experiments, but the low pool mean for the canine intra-assay study was very close to the estimated canine LoQ, at  $9.4 \mu\text{g/dL}$  with an acceptable CV of  $5.4\%$ , whereas the CV of  $15.5\%$  at a mean of  $6.1 \mu\text{g/dL}$  was too high (low pool used for the LoD and LoQ experiments), so the true LoQ likely lies between  $6.1$  and  $9.5 \mu\text{g/dL}$ . For cats, the CV at the LoD low pool mean of  $6.6 \mu\text{g/dL}$  was  $10.4\%$  and only just exceeded the  $I_D$  of  $10\%$ , whereas the CV of  $6.0\%$  for the intra-assay study pool with a mean of  $10.7 \mu\text{g/dL}$  was acceptable, so the true LoQ is likely very close to the estimate of  $6.9 \mu\text{g/dL}$ . No numerical value for the IDEXX EIA LoQ was found.

**TABLE 5** | Bias for the EUROlyser SDMA reference laboratory immunoturbidometric assay at IRIS clinical decision limits with adjusted limits and expected range of results (95% probability), based on the Passing-Bablok regression analysis and dispersion. IRIS cutoffs are based on data gathered from the IDEXX EIA.

	Existing decision limit for dogs	Bias and adjusted EUROlyser CDL (regression equation)	Range of results around EUROlyser CDL based on dispersion ( $\pm 35\%$ )	Existing decision limit for cats	Bias and adjusted EUROlyser CDL (regression equation)	Range of results around EUROlyser CDL based on dispersion ( $\pm 44\%$ )
IDEXX EIA URL	14.0 $\mu\text{g/dL}$	-22.1% 10.9 $\mu\text{g/dL}$	7.1–14.7 $\mu\text{g/dL}$	14.0 $\mu\text{g/dL}$	-6.1% 13.1 $\mu\text{g/dL}$	7.3–18.9 $\mu\text{g/dL}$
IRIS Stage 2 lower CDL	18.0 $\mu\text{g/dL}$	-18.5% 14.7 $\mu\text{g/dL}$	9.6–19.8 $\mu\text{g/dL}$	18.0 $\mu\text{g/dL}$	-3.0% 17.5 $\mu\text{g/dL}$	9.8–25.2 $\mu\text{g/dL}$
IRIS Stage 3 lower CDL	35.0 $\mu\text{g/dL}$	-12.5% 30.6 $\mu\text{g/dL}$	20.0–41.2 $\mu\text{g/dL}$	25.0 $\mu\text{g/dL}$	-0.1% 25.0 $\mu\text{g/dL}$	14.0–36.0 $\mu\text{g/dL}$
IRIS Stage 4 lower CDL	54.0 $\mu\text{g/dL}$	-10.2% 48.5 $\mu\text{g/dL}$	31.6–65.4 $\mu\text{g/dL}$	38.0 $\mu\text{g/dL}$	2.8% 39.1 $\mu\text{g/dL}$	39.1–56.3 $\mu\text{g/dL}$

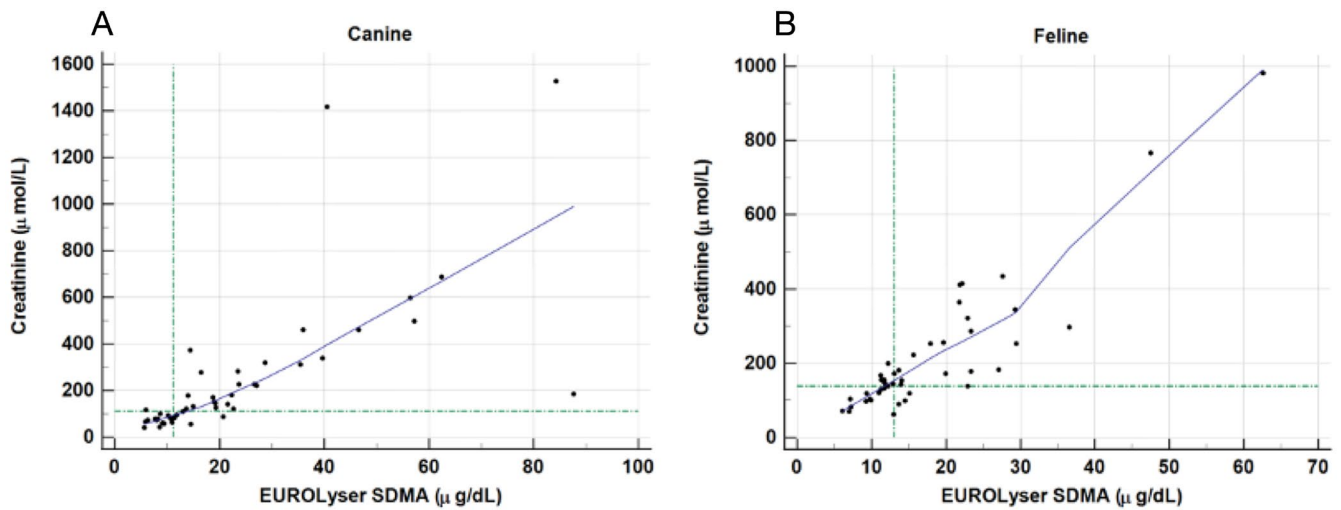
Abbreviations: CDL, clinical decision limit; EIA, enzyme immunoassay; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine; URL, upper reference limit.

Hemolysis can affect the accuracy of analyte measurement in immunoturbidometric assays by chemical, spectrophotometric, or dilutional mechanisms [25]. Symmetric dimethylarginine measurement was affected by severe hemolysis in dogs and by moderate to severe hemolysis in cats, with the EUROlyser ITA. A recent study conducted on canine serum using the LC-MS and the IDEXX EIA methods also showed an effect of increasing hemolysis on SDMA measurement, although another study found no effect on SDMA measured by the IDEXX EIA in canine serum [6, 15]. Studies in rabbits and rats have shown an interference from hemolysis using the IDEXX EIA [30, 32]. Patient samples affected by moderate to severe hemolysis should not be used for SDMA measurement using the EUROlyser ITA, and the effect of hemolysis on SDMA using the IDEXX EIA for dogs and cats should be further clarified.

Lipemia can also affect the accuracy of analyte measurement in immunoturbidometric assays by light scattering from lipoprotein particles present in the sample [33]. In this study, severe lipemia in canine samples caused a positive bias, but there was no significant interference in feline samples. This is in contrast to a study using canine serum samples with an LC-MS and the IDEXX EIA method that found no interference of lipemia, but this could have been related to the limited numbers of moderate to severely lipemic samples present [15]. A marked reduction of SDMA concentration in severely lipemic samples has been noted for rabbits and rats with the IDEXX EIA [30, 32]. There may therefore be species-specific differences in the effect of lipemia using the IDEXX EIA and the EUROlyser ITA assays.

A poor unacceptable recovery of 57.09% was observed in one of the spiked canine samples, which was repeatable. The average recovery of the other two samples was 92.0%. Poor recovery indicates a high proportional systematic error, thought to be due to a sample matrix effect. The reason for the poor recovery in one sample is unknown, as this sample was pooled from several serum samples and did not exhibit any hemolysis, lipemia, or icterus. It should be noted that the unidentified cause of the inaccuracy could be present in other canine patient samples, and SDMA results should always be interpreted together with serum creatinine, urinalysis results, and other clinical findings. The EUROlyser ITA showed an excellent average recovery of 93.47% with a proportional error of 6.53% in feline samples. No other studies have noted poor recovery using other SDMA assays in cats and dogs [2, 3, 6].

The EUROlyser ITA was compared to the IDEXX EIA by single measurements of canine and feline serum samples, with SDMA concentrations spanning the clinically important range. Limitations of this method comparison study include the lack of duplicate measurements for the method comparison experiment and the use of a field method (the IDEXX EIA) rather than the gold standard LC-MS method as the reference method. Several outliers were excluded from statistical analysis, based on evaluation of residuals, because of a large difference between the two paired results, which was presumed to represent a large random error in either one of the paired measurements. A statistically and clinically significant negative bias of -19.5% between the IDEXX EIA and EUROlyser ITA methods was present for canine serum samples, with the difference between almost half



**FIGURE 4** | Scatter plots showing SDMA versus creatinine for dogs (A) and cats (B). The dashed green lines represent the laboratory upper reference limit for creatinine and the adjusted upper reference limit for the EUROLyser SDMA reference laboratory immunoturbidometric method. The dark blue line represents the Local Regression Smoothing trend line with a span of 80%.

of paired measurements greater than the  $TE_D$  of 20%. A lower bias of  $-6.7\%$  with 15% of paired measurements greater than a total allowable error of 22% has previously been reported for the EUROLyser ITA versus the IDEXX EIA for canine serum [20]. The EUROLyser ITA manufacturer reports a negative bias between these two methods of  $-5.2\%$  for dogs [19]. The reason for a bias is most likely related to differences in antibody binding between the two assays, and the differences in the magnitude of the bias between our study and other studies may be due to the different chemistry analyzer platforms used for all the assays, or differences in calibration between the same assay in different locations. The implications of this unacceptably high bias between the two methods in this study are that reference intervals and clinical decision limits for dogs determined using the IDEXX EIA cannot be used for the EUROLyser ITA, and should be determined for each laboratory using the assay. The IRIS SDMA staging cutoffs are based only on the IDEXX EIA method, and an indication of how the bias may affect using SDMA for staging is illustrated in Table 5 [14]. The high proportion of individual differences greater than the  $TE_D$  is in part due to the high dispersion of SDMA with both of these assays, and paired measurements may have resulted in less variation. It should be noted that a bias between two field methods does not mean that one method is better than the second method, only that they give different results.

The manufacturer reports a mean bias between the IDEXX EIA and EUROLyser ITA methods of  $-0.3\%$  in cats, whereas in our study, the mean bias was  $-4.0\%$  [19]. This is less than the  $B_D$  of 8%, although 20% of paired measurements exceeded the TEa of 24%. A high negative mean bias of  $-30.2\%$  was reported for feline serum for SDMA measured using the IDEXX EIA versus the IDEXX POC methods, and the bias for plasma samples was  $-11.5\%$  [18]. In that study, up to 34% of differences were greater than the  $TE_D$ , which was partly due to the imprecision of both assays [18]. Although the mean bias and the bias at the upper reference limit and clinical cutoffs for feline samples were within the  $B_D$  here, it is still recommended that method-specific reference intervals and cutoffs (for IRIS staging, see

Table 5) be determined for cats with the EUROLyser ITA assay. Alternatively, manufacturer adjustments to calibration could improve bias.

There was a significant positive correlation between serum SDMA and creatinine for both dogs ( $r=0.865$ ) and cats ( $r=0.794$ ). For dogs, for 87% of samples, both SDMA and creatinine were either both below or above their respective upper reference limits, whereas there were some samples (11%) with a raised SDMA but normal creatinine and only a single sample with a raised creatinine but normal SDMA. For cats, 72% of samples had both results either above or below the reference interval, 7% of samples had a raised SDMA but normal creatinine, but there were nine samples (20%) with a raised creatinine but normal SDMA. For discrepant pairs, the results were all close to the reference limit, and discordance is in part due to the high dispersion of SDMA. The 9% of canine samples and 7% of feline samples with an elevated SDMA in the absence of azotemia may reflect the earlier rise in SDMA compared to creatinine or may represent decreased GFR in animals with poor muscle mass [3–5, 7]. There was one canine and nine feline samples with high creatinine and normal SDMA concentrations. Despite earlier studies finding that SDMA was more sensitive than creatinine for detecting decreases in GFR, later studies found that both analytes were similarly sensitive [5, 9, 12]. These results further indicate that creatinine and SDMA should be used together for the detection of early decreases in GFR.

We aimed to perform QC validation and formulate a QC strategy for the novel SDMA assay, but found that the use of statistical control rules was only possible with a  $TE_A$  of 35%, and not with the BV-based  $TE_D$  of 20%. The daily performance of the EUROLyser ITA on board the Cobas Integra 400 Plus analyzer was monitored using both the manufacturer's QCM and patient pool samples interchangeably once a day for the duration of the study. Imprecision ranged from 6.6% to 12.9%, similar to that found in the inter-assay imprecision experiment. This imprecision was too high for the selection of simple control rules using a  $TE_D$  of 20%, as this BV-derived performance goal with a high

**TABLE 6** | Normalized  $x$  and  $y$  axis coordinates, selected QC rule, and associated Ped and Pfr for the EUROLyser SDMA reference laboratory immunoturbidometric assay.

	Canine patient sample pool		Commercial QCM	
	Low	High	Low	High
CV (mean)	7.4% (10.2 µg/dL)	8.0% (56.3 µg/dL)	12.9% (14.3 µg/dL)	6.6% (49.2 µg/dL)
Bias (target)	0% (none)	0% (none)	2.0% (14.0 µg/dL)	1.5% (50 µg/dL)
Using TE <sub>D</sub> = 20%				
$x$ axis coordinate	37	40	65	33
$y$ axis coordinate	0	0	10	8
QC rule	No rule	No rule	No rule	No rule
Ped	N/A	N/A	N/A	N/A
Pfr	N/A	N/A	N/A	N/A
Using TE <sub>A</sub> = 30%				
$x$ axis	25	26	42	22
$y$ axis	0	0	7	5
QC rule	No rule	No rule	No rule	No rule
Ped	N/A	N/A	N/A	N/A
Pfr	N/A	N/A	N/A	N/A
Using TE <sub>A</sub> = 35%				
$x$ axis	21	22	53	27
$y$ axis	0	0	8	6
QC rule	1–2.5 s	1–2.5 s	No rule	1–3 s
Ped	90	90	Not applicable	90
Pfr	0.03	0.03	Not applicable	0.00

Note: QCM: Either a patient pool or commercial QCM. Results are shown for TE<sub>D</sub> and two levels of TE<sub>A</sub>.

Abbreviations: Ped, probability of error detection; Pfr, probability of false rejection; QC, quality control; QCM, QC material; TE<sub>A</sub>, total allowable error; TE<sub>D</sub>, total desirable error.

Ped was too strict for actual assay performance. In this situation, alternative options may include using a multi-rule, additional levels of QCM, decreasing the Ped, or increasing the TE<sub>A</sub> [28, 34]. The disadvantage of using additional QCM is the high cost. Even though utilizing a multi-rule would keep the Pfr low while maintaining Ped, these procedures are more complicated and time-consuming than single-rule procedures. In our study, QC validation was further attempted by relaxing the TE<sub>D</sub> to TE<sub>A</sub> of 30% and 35%. At TE<sub>A</sub> of 35%, the 1–2.5 s rule was suitable for use with both levels of patient pool QCM, and the 1–3 s rule for the commercial QCM high level, but no rule was applicable for the commercial QCM low level. We did not adjust the TE<sub>A</sub> any higher, as that degree of error would potentially have a large impact on clinical decision-making. The process of determining the total allowable error for QC validation based on method performance and QC specifications is termed the “reverse approach” [28, 34]. These findings show that although individual components of analytical error (bias and imprecision) may meet biological variation goals during assay validation, making the

assay suitable for clinical use, this does not mean that assay performance is stable enough for a statistical QC strategy using a BV-derived TE<sub>D</sub> [28]. Further monitoring of QCM results and regular QC validation should be performed to obtain a more robust “reverse” TE<sub>A</sub>. This TE<sub>A</sub> of 35%, rather than the TE<sub>D</sub> of 20%, needs to be taken into account when interpreting test results. Given the high dispersion, and that the TE that can be controlled for is high, duplicate analysis of patient samples for SDMA should be considered.

This study did not aim to validate or establish reference intervals for SDMA with the novel assay. Ideally, the reference individuals included in a reference interval study for renal function biomarkers like creatinine or SDMA should have their normal renal function confirmed by direct measurement of GFR, which is a logistically complex, expensive, and time-consuming process involving clinicians and the laboratory as a collective [13]. This was not within the scope of our study. The fact that this was a retrospective study using stored samples without reference

to clinical records could also be considered a limitation. Still, since all experiments involved in assay validation focus on determining analytical bias and imprecision, and not diagnostic specificity and sensitivity, this is not of high relevance. Stability of SDMA measured with the IDEXX EIA has been shown for serum stored for 10 months at  $-24^{\circ}\text{C}$  and 24 months at  $-80^{\circ}\text{C}$ , but longer periods have not been investigated [15]. Although our experiments focused on SDMA concentrations at the time of measurement, not at the time of collection, degradation of proteins during storage could possibly have introduced some variation to these results.

This method validation study comprehensively investigated the analytical performance of the EUROLyser ITA for SDMA measurement in feline and canine serum. The assay exhibited an imprecision similar to other SDMA assays, which results in high dispersion and has implications for the interpretation of both single and serial results. The method comparison study showed a bias compared to the IDEXX EIA method in both canine and feline samples, but most notably in canine samples, indicating the need for assay-specific reference intervals and an adjustment of the IRIS clinical decision limits. Canine serum pools were suitable for use as QCM to monitor assay performance, with a  $\text{TE}_A$  of 35%. The adoption of this automated EUROLyser ITA assay will increase the availability of SDMA measurements for small animal practice.

---

## Acknowledgments

This work was supported by an AgriSETA bursary from the Department of Higher Education and Training, South Africa. We would like to thank Dr. Emma Strage from the Swedish University of Agricultural Sciences and Dr. A Russell Moore from Colorado State University for their constructive feedback and suggestions for improvement during their evaluation of the MSc dissertation from which this manuscript is derived.

## Conflicts of Interest

The authors declare no conflicts of interest.

## References

1. R. Jepson, H. Syme, C. Vallance, R. E. Jepson, H. M. Syme, and J. Elliott, "Plasma Asymmetric Dimethylarginine, Symmetric Dimethylarginine, L-Arginine, and Nitrite/Nitrate Concentrations in Cats With Chronic Kidney Disease and Hypertension," *Journal of Veterinary Internal Medicine* 22 (2008): 317–324.
2. J. Braff, E. Obare, M. Yerramilli, J. Elliott, and M. Yerramilli, "Relationship Between Serum Symmetric Dimethylarginine Concentration and Glomerular Filtration Rate in Cats," *Journal of Veterinary Internal Medicine* 28 (2014): 1699–1701.
3. J. Hall, M. Yerramilli, E. Obare, J. A. Hall, and D. E. Jewell, "Comparison of Serum Concentrations of Symmetric Dimethylarginine and Creatinine as Kidney Function Biomarkers in Cats With Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 28 (2014): 1676–1683.
4. J. Hall, M. Yerramilli, E. Obare, J. A. Hall, S. Yu, and D. E. Jewell, "Comparison of Serum Concentrations of Symmetric Dimethylarginine and Creatinine as Kidney Function Biomarkers in Healthy Geriatric Cats Fed Reduced Protein Foods Enriched With Fish Oil, L-Carnitine, and Medium-Chain Triglycerides," *Veterinary Journal* 202 (2014): 588–596.
5. J. Hall, M. Yerramilli, E. Obare, J. A. Hall, L. D. Melendez, and D. E. Jewell, "Relationship Between Lean Body Mass and Serum Renal Biomarkers in Healthy Dogs," *Journal of Veterinary Internal Medicine* 29 (2015): 808–814.
6. M. Nabity, G. Lees, M. Boggess, et al., "Symmetric Dimethylarginine Assay Validation, Stability, and Evaluation as a Marker for the Early Detection of Chronic Kidney Disease in Dogs," *Journal of Veterinary Internal Medicine* 29 (2015): 1036–1044.
7. J. Hall, M. Yerramilli, E. Obare, J. A. Hall, K. Almes, and D. E. Jewell, "Serum Concentrations of Symmetric Dimethylarginine and Creatinine in Dogs With Naturally Occurring Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 30 (2016): 794–802.
8. J. Hall, M. Yerramilli, E. Obare, J. A. Hall, J. Li, and D. E. Jewell, "Serum Concentrations of Symmetric Dimethylarginine and Creatinine in Cats With Kidney Stones," *PLoS One* 12 (2017): e0174854.
9. M. Brans, S. Daminet, F. Mortier, L. Duchateau, H. P. Lefebvre, and D. Paepe, "Plasma Symmetric Dimethylarginine and Creatinine Concentrations and Glomerular Filtration Rate in Cats With Normal and Decreased Renal Function," *Journal of Veterinary Internal Medicine* 35 (2021): 303–311.
10. S. Loane, T. Williams, and K. McCallum, "Evaluation of Symmetric Dimethylarginine in Cats With Acute Kidney Injury and Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 36 (2022): 1669–1676.
11. D. Dahlem, R. Neiger, A. Schweighauser, et al., "Plasma Symmetric Dimethylarginine Concentration in Dogs With Acute Kidney Injury and Chronic Kidney Disease," *Journal of Veterinary Internal Medicine* 31 (2017): 799–804.
12. L. Pelander, J. Häggström, A. Larsson, et al., "Comparison of the Diagnostic Value of Symmetric Dimethylarginine, Cystatin C, and Creatinine for Detection of Decreased Glomerular Filtration Rate in Dogs," *Journal of Veterinary Internal Medicine* 33 (2019): 630–639.
13. M. McKenna, L. Pelligand, J. Elliott, D. Cotter, and R. Jepson, "Relationship Between Serum Iohexol Clearance, Serum SDMA Concentration, and Serum Creatinine Concentration in Non-Azotemic Dogs," *Journal of Veterinary Internal Medicine* 34 (2020): 186–194.
14. International Renal Interest Society, "IRIS Staging of CKD," (2023), [https://static1.squarespace.com/static/666b9ecb4064a156963b4162/t/66a6dbc90ca6986e1b5c06bd/172211273243/2\\_IRIS\\_Staging\\_of\\_CKD\\_2023.pdf](https://static1.squarespace.com/static/666b9ecb4064a156963b4162/t/66a6dbc90ca6986e1b5c06bd/172211273243/2_IRIS_Staging_of_CKD_2023.pdf).
15. S. Marynissen, G. Junius, E. Van den Steen, et al., "Serum Symmetric Dimethylarginine in Older Dogs: Reference Interval and Comparison of a Gold Standard Method With the ELISA," *Journal of Veterinary Internal Medicine* 38 (2024): 960–970.
16. D. Polzin, "Chronic Kidney Disease in Small Animals," *Veterinary Clinics of North America. Small Animal Practice* 41 (2011): 15–30.
17. R. Ernst, J. Ogeer, D. McCrann, et al., "Comparative Performance of IDEXX SDMA Test and the DLD SDMA ELISA for the Measurement of SDMA in Canine and Feline Serum," *PLoS One* 13 (2018): e0205030.
18. R. Baral, K. Freeman, and B. Flatland, "Comparison of Serum and Plasma SDMA Measured With Point-Of-Care and Reference Laboratory Analysers: Implications for Interpretation of SDMA in Cats," *Journal of Feline Medicine and Surgery* 23 (2021): 906–920.
19. EUROLyser, "SDMA Reagent Kit Information Sheet," accessed 9 March, 2025, [https://www.eurolyser.com/data/Eurolyser/SDMA\\_Reagent\\_Kit\\_info\\_sheet.pdf](https://www.eurolyser.com/data/Eurolyser/SDMA_Reagent_Kit_info_sheet.pdf).
20. C. Halman, N. Courtman, and B. Stone, "Comparison of 2 Point-Of-Care Analyzers and the Eurolyser Assay With an IDEXX Reference Laboratory Method for Measurement of Symmetric Dimethylarginine in Dogs," *American Journal of Veterinary Research* 86 (2025): ajvr.24.07.0204.

21. R. Baral, K. Freeman, and B. Flatland, "Analytical Quality Performance Goals for Symmetric Dimethylarginine in Cats," *Veterinary Clinical Pathology* 50 (2021): 57–61.
22. B. Flatland, M. Camus, and R. Baral, "Analytical Quality Goals—A Review," *Veterinary Clinical Pathology* 47 (2018): 527–538.
23. A. Hillaert, D. Liu, S. Daminet, et al., "Serum Symmetric Dimethylarginine Shows a Relatively Consistent Long-Term Concentration in Healthy Dogs With a Significant Effect of Increased Body Fat Percentage," *PLoS One* 16 (2021): e0247049.
24. J. E. Arnold, M. S. Camus, K. P. Freeman, et al., "ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (Version 3.0)," *Veterinary Clinical Pathology* 48 (2019): 542–618.
25. F. Marques-Garcia, "Methods for Hemolysis Interference Study in Laboratory Medicine—A Critical Review," *eJIFCC* 31 (2020): 85–97.
26. Clinical and Laboratory Standards Institute (CLSI), *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition* (CLSI, 2012).
27. A. L. Jensen and M. Kjelgaard-Hansen, "Method Comparison in the Clinical Laboratory," *Veterinary Clinical Pathology* 35 (2006): 276–286.
28. K. Freeman, S. Klenner-Gastreich, and J. Korchia, "The Importance of Quality Control Validation and Relationships With Total Error Quality Goals and Bias in the Interpretation of Laboratory Results," *Veterinary Clinical Pathology* 53, no. Suppl 1 (2024): 65–74.
29. H. Schott, L. Gallant, M. Coyne, et al., "Symmetric Dimethylarginine and Creatinine Concentrations in Serum of Healthy Draft Horses," *Journal of Veterinary Internal Medicine* 35 (2021): 1147–1154.
30. M. Strong-Townsend, N. Fabian, G. Skinner, et al., "Assay Validation and Determination of the Reference Interval for Symmetric Dimethylarginine in Healthy Rabbits," *Journal of Exotic Pet Medicine* 49 (2024): 12–17.
31. L. Waugh, S. Lyon, G. A. Cole, et al., "Retrospective Analysis and Validation of Serum Symmetric Dimethylarginine (SDMA) Concentrations in Cheetahs (*Acinonyx jubatus*)," *Journal of Zoo and Wildlife Medicine* 49 (2018): 623–631.
32. D. Hamlin, A. Schultze, M. Coyne, et al., "Evaluation of Renal Biomarkers, Including Symmetric Dimethylarginine, Following Gentamicin-Induced Proximal Tubular Injury in the Rat," *Kidney360* 3 (2022): 341–356.
33. C. Fernández Prendes, M. Castro Castro, L. Sánchez Navarro, L. Rapún Mas, C. Morales Indiano, and T. Arrobas Velilla, "Handling of Lipemic Samples in the Clinical Laboratory," *Advances in Laboratory Medicine* 4 (2023): 5–27.
34. J. Korchia and K. Freeman, "Total Observed Error, Total Allowable Error, and QC Rules for Canine Serum and Urine Cortisol Achievable With the Immulite 2000 Xpi Cortisol Immunoassay," *Journal of Veterinary Diagnostic Investigation* 34 (2022): 246–257.