IDEXX Catalyst SDMA Test for in-house measurement of SDMA concentration in serum from dogs and cats

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Introduction

SDMA is a methylated form of arginine, that is found in intracellular proteins in all nucleated cells of vertebrates and is excreted through the kidneys. SDMA correlates well with glomerular filtration rate (GFR) in people,[1] dogs,[2,3] and cats.[4–6] SDMA is more sensitive than creatinine (CREA) and increases earlier. Whereas creatinine doesn't increase above the reference interval until up to 75% of kidney function is lost, studies have demonstrated that SDMA increases when there is on average a 40% decrease and as little as 25% decrease in glomerular filtration rate (GFR).[2,3,5] SDMA increases with both acute or active kidney injury and chronic kidney disease, allowing veterinarians to intervene earlier for more successful outcomes. Furthermore, SDMA, unlike CREA, is not impacted by lean body mass.[6,7] For these reasons, the IDEXX SDMA® Test quickly becomes an essential parameter on all routine chemistry profiles.

The Catalyst® SDMA Test is a new immunoassay system from IDEXX that is designed to measure SDMA concentrations in serum or lithium heparin plasma samples from dogs and cats without the need to dilute the sample. It is designed to produce prompt, reliable and accurate test results in the veterinary clinic on either the IDEXX Catalyst One® or IDEXX Catalyst Dx® chemistry analyzers. It has the same reference interval (0–14 µg/dL), interpretive guidelines, and reportable range (0–100 µg/dL) as the reference laboratory IDEXX SDMA Test.

The four objectives of this study were to evaluate the:

- Performance of the new test by a method comparison to a reference method (method comparison).
- Precision of the assay using control fluids (precision).
- Analytical specificity (analogue specificity).
- Impact of hemolysis, lipemia, and icterus on the reported concentration (interfering substances).

Materials and methods

Data was collated in Microsoft Office Excel® 2016* before being exported to JMP® 13.0.0† for statistical analysis, including the Method Comparison add-in.‡

Method comparison

Residual serum samples were collected from 107 dogs and 113 cats, including a mixture of healthy animal and clinical patients. All samples were analyzed once using the Catalyst SDMA Test and twice with a liquid chromatography-mass spectrometry (LC-MS) analytical method.§ The average of the LC-MS results was taken as the reference method and compared to the Catalyst SDMA Test result.

Passing–Bablok linear regression analysis was completed for each species. Correlation coefficients were interpreted as follows: $r = 0.90–1.0$, defined very high correlation; 0.70–0.89, high correlation; 0.50–0.69, moderate correlation; 0.30–0.49, low correlation; and 0–0.29, little, if any, correlation.[8]

The regression analysis was also used to look for statistical evidence of systematic error (constant and/or proportional bias). Ninety-five percent confidence intervals (CI) for the y-intercept that did not include the value zero were considered evidence of constant bias. Ninety-five percent CI for the slope that did not include the value 1.0 were considered evidence of proportional bias.

The results from each method, to the nearest whole number, were classified per the following thresholds: ≤14 µg/dL (within reference interval); 15–19 µg/dL; ≥20 µg/dL. The classifications were then compared in a contingency table for each species.

Precision

Precision was assessed per Clinical and Laboratory Standards Institute (CLSI) EP5-A method guidelines.[9] Two levels of control fluid (fluid A and fluid B), were assayed on a Catalyst Dx Chemistry Analyzer. For each species, there were 4 replicates run each morning and each afternoon for 10 days, giving a total of 80 replicates per species. Total precision was calculated per CLSI EP5-A method guidelines.

*Microsoft Office Excel® 2016, Microsoft Corporation, Redmond, Washington, USA.

†JMP® 13.0.0, SAS Institute Inc. Cary, North Carolina, USA.

‡Method Comparison Add-in, SAS Institute Inc. Cary, North Carolina, USA.

§The LC-MS method was described in an abstract at AACC 2015. The LC separation was achieved using XBridge™ reversed-phase (RP) C18 column and an ion-pairing agent. The API 4000™ triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) was operated in multiple reaction monitoring (MRM) mode with positive electrospray interface. The MRM transition for SDMA was observed at m/z 203.2 ➔ 172.1.[10]

Analogue specificity

Cross-reactivity testing was completed by spiking three different control materials with physiologically relevant concentrations of arginine, asymmetric dimethylarginine (ADMA), or monomethyl-L-arginine (MMA). PBS (phosphate buffer solution) was used as a control to compensate for volume change with the additive ("control").

For each control fluid, there were four aliquots (spiked with ADMA, spiked with MMA, spiked with arginine, and "control"), giving a total of 12 aliquots that were each analyzed six times with the Catalyst SDMA Test and a mean was calculated.

Percentage change was calculated as:

$$\%\ \text{change} = 100 \times \frac{\text{Mean "spiked" SDMA concentration} - \text{mean "control" SDMA conentration}}{\text{Mean "control" SDMA concentration}}$$

Percentage change was calculated as:

$$\%\ \text{cross-reactivity} = 100 \times \frac{\text{Mean "spiked" SDMA concentration} - \text{mean "control" SDMA conentration}}{\text{Additive concentration}}$$

The mean "spiked" SDMA concentration for each aliquot was compared with the mean "control" SDMA concentration to look for a statistically significant difference (Tukey-Kramer HSD; $P<0.05$).

Interfering substances

Interference caused by the presence of hemoglobin, lipids, or bilirubin was assessed per CLSI EP7-A2 method guidelines.[11] Canine serum samples, which were visibly clear of interferents, were collected and pooled. Aliquots of the pooled sample were then prepared and spiked with varying concentrations of the substances shown in table 1. Each aliquot was run in duplicate on eight Catalyst One chemistry analyzers in a random order.

Table 1. Preparation of aliquots of the pooled sample to assess common interfering substances.

Interference	Spiking material	Number of levels of interfering substance
Hemolysis	Lysed canine red blood cells to produce hemoglobin	7
Lipemia	Intralipid 20%	5
Icterus	Ditaurobilirubin (DTB; a synthetic bilirubin derivative)	7

An all-pairs Tukey-Kramer HSD test ($P<0.05$) was performed with the mean SDMA concentration of each aliquot to look for statistically significant differences.

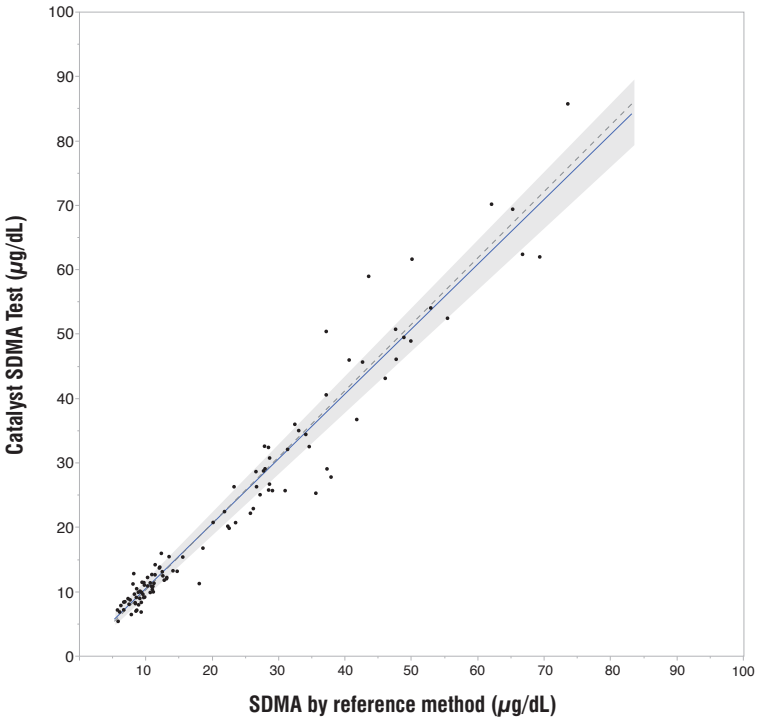
Results

Method comparison

The regression plots are shown in figures 1A (canine) and 1B (feline). The results are summarized in tables 2 and 3. The Catalyst SDMA Test showed excellent correlation to the reference method with no evidence of bias for either species. For the classification of results, there was strong agreement between the two methods.

Figure 1. Passing-Bablok regression.
Regression line shown in blue (with 95% CI); unity shown as gray-dashed line.

A. Canine: n = 107; correlation (r) = 0.98; slope = 0.98; intercept = 0.44 μg/dL



B. Feline: n = 113; correlation (r) = 0.94; slope = 1.00; intercept = -0.95 μg/dL

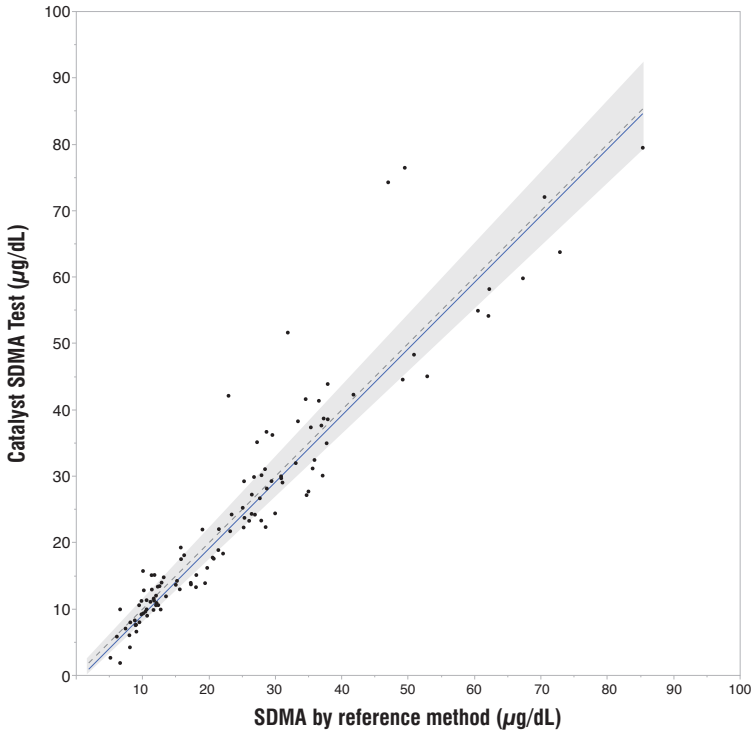


Table 2. Regression analysis by species.

	Canine	Feline
n	107	113
Correlation (*r*)	0.98	0.94
Mean difference (standard deviation) µg/dL	-0.28 (3.84)	-0.02 (5.69)
Intercept (95% CI) µg/dL	0.44 (-0.54 to 0.99)	-0.95 (-2.18 to 0.03)
Slope (95% CI)	0.98 (0.92 to 1.02)	1.00 (0.94 to 1.07)

Table 3. Contingency table by species.

A. Canine: n = 107; overall concordance = 95%

		Mean SDMA concentration from reference method		
		≤14 µg/dL	15–19 µg/dL	≥20 µg/dL
Catalyst SDMA Test result	≤14 µg/dL	49%	3%	0%
	15–19 µg/dL	2%	2%	0%
	≥20 µg/dL	0%	0%	44%

For the discordant samples, the median absolute difference was 2 µg/dL.

B. Feline: n = 113; overall concordance = 85%

		Mean SDMA concentration from reference method		
		≤14 µg/dL	15–19 µg/dL	≥20 µg/dL
Catalyst SDMA Test result	≤14 µg/dL	31%	6%	0%
	15–19 µg/dL	4%	4%	4%
	≥20 µg/dL	0%	1%	50%

For the discordant samples, the median absolute difference was 3 µg/dL.

Precision

The results of the precision analysis are shown in table 4. The new method shows a total coefficient of variation (CV) of <10%. This is consistent with the high-throughput immunoassay for SDMA used at IDEXX Reference Laboratories.[¶]

Table 4. Summary of results from the precision study.

Control fluid	Species	Replicates	Mean SDMA concentration (µg/dL)	Standard deviation (µg/dL)	CV%
Fluid A	Canine	80	15.50	0.96	6.2
	Feline	80	18.39	1.25	6.8
Fluid B	Canine	80	36.01	2.02	5.6
	Feline	80	44.99	2.54	5.6

Analogue specificity

The results of the specificity analysis are shown in table 5. There was no statistically significant change seen with any of the spiked aliquots.

Table 5. Summary of the analogue specificity study. Additive concentration after spiking: MMA 50 µg/dL; ADMA 50 µg/dL; arginine 2,500 µg/dL.

Control material	SDMA concentration in "control" (µg/dL)	Additive	% Change	% Cross-reactivity
1	13.90	MMA	0.91	0.25
		ADMA	6.76	1.88
		Arginine	2.82	0.02
2	28.41	MMA	-6.83	-5.90
		ADMA	-0.02	-0.02
		Arginine	1.36	0.02
3	43.14	MMA	1.92	1.02
		ADMA	0.36	0.19
		Arginine	-0.46	0.00

[¶]Abstract at AACC 2015 showed total precision was ≤10%.[12]

Interfering substances

The results of the interfering substances study are shown in table 6. The common interfering substances examined here had no statistically significant impact on the reported SDMA concentrations.

Table 6. Impact of interfering substances.

Hemolysis		Lipemia		Icterus	
Hemoglobin concentration (mg/dL)	Catalyst SDMA Test average concentration (µg/dL)	Intralipid concentration (mg/dL)	Catalyst SDMA Test average concentration (µg/dL)	DTB concentration (mg/dL)	Catalyst SDMA Test average concentration (µg/dL)
Not spiked	11.02	Not spiked	12.84	Not spiked	13.77
31.25	11.95	250	12.88	1	13.50
62.5	12.09	500	13.02	3	13.29
125	11.41	750	12.95	10	13.54
250	12.03	1,000	13.28	20	13.25
375	12.63			30	13.61
500	11.53			40	12.99

Conclusions

The new Catalyst® SDMA Test demonstrates excellent correlation with the reference method. It provides veterinarians an accurate, precise, reliable, and convenient option to diagnose and monitor dogs and cats with kidney disease using the in-house Catalyst Dx® and Catalyst One® chemistry analyzers.

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