Volumetric absorptive microsampling for iohexol and creatinine based GFR determination in cats: Aligning animal welfare with practical feasibility

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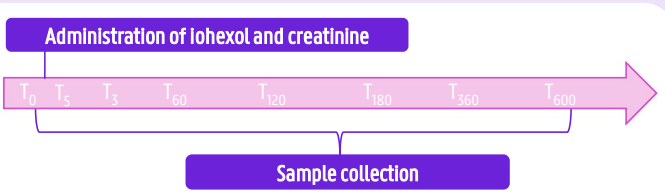
INTRODUCTION AND AIMS

Chronic Kidney Disease (CKD) is not only common in humans, but also in cats, and early detection of CKD is crucial for better prognosis. Currently, the gold standard to assess renal function is measurement of glomerular filtration rate (GFR), allowing early detection of decreased kidney function. To overcome the practical limitations of this procedure, microsampling can be used. Application of volumetric absorptive microsampling (VAMS) in feline nephrology would be of tremendous value, aligning with animal welfare and improving practical feasibility of GFR measurements. Moreover, application of this methodology in human medicine allows for a more practical procedure for GFR measurement, facilitating application in routine clinical care.

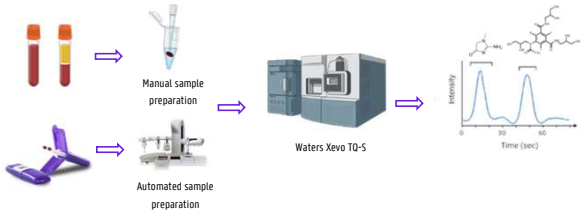
In this context, we developed and validated an LC-MS/MS method to simultaneously measure iohexol and creatinine in plasma, blood and VAMS samples, the latter collected via ear-prick. Furthermore, a clinical study was conducted in which 23 cats were enrolled to collect both conventional venous blood, plasma and VAMS samples, in parallel for GFR measurement. Clinical method validation was performed using the validated methods. Finally, an application study was conducted to evaluate the clinical applicability of ear-prick sampling for iohexol and creatinine based GFR determination in cats.

METHODS

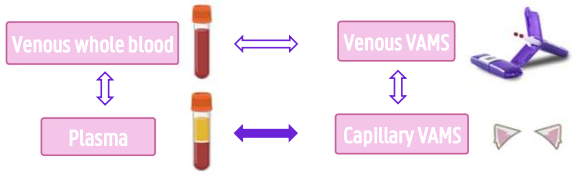
SAMPLING PROTOCOL



SAMPLE ANALYSIS



CLINICAL VALIDATION



APPLICATION

Validation of correction formulas to reliably convert cVAMS results to plasma results

RESULTS

ANALYTICAL VALIDATION

- ✓ Accuracy and precision
- ✓ Matrix effect and recovery
- ✓ Selectivity
- ✓ Stability studies
- ✓ Hematocrit effect
- ✓ Robustness of extraction procedure

Implementation of 3R principle in bio-analytical method validation

Hematocrit-independence

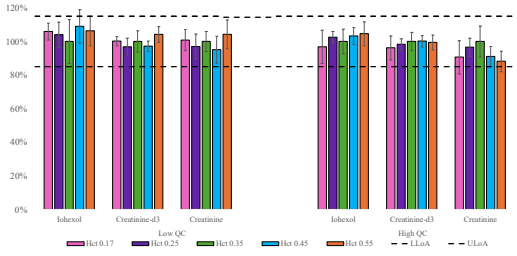


Figure 1 Effect of the hematocrit (hct) on the recovery (RE) from VAMS samples. The RE was determined from VAMS samples (n = 6) with hct values ranging from 17 to 55%, and normalised to the RE at reference hct (35%). No hct-dependence was apparent and the RE was within the pre-set acceptance criterion at all hct levels, for both analytes, demonstrating the **robustness of the automated extraction procedure**.

Accuracy & precision

		Nominal value (µg/mL)	Accuracy (bias)	Repeatability (CV)	Total imprecision (CV)
Iohexol	LLOQ	2	1.4%	8.0%	8.0%
	QCL	5	-2.9%	9.4%	9.4%
	QCM	50	-5.4%	0.9%	7.8%
	QCH	300	-0.5%	4.2%	7.2%
	QCL cat	5	1.0%	5.5%	7.5%
	QCH cat	300	2.5%	5.3%	7.2%
Creatinine-d3	LLOQ	2	-2.2%	8.3%	8.3%
	QCL	5	-3.3%	9.6%	9.6%
	QCM	50	-2.6%	2.5%	3.0%
	QCH	300	0.9%	2.8%	6.6%
	QCL cat	5	1.1%	5.5%	8.0%
	QCH cat	300	2.8%	4.3%	6.2%

Table 1 Accuracy (bias), repeatability (CV) and total imprecision (CV) data at the different QC levels for iohexol and creatinine-d3 in VAMS samples. QCL and QCH prepared in cat blood are indicated in pink.

CLINICAL VALIDATION

Large cVAMS *vs.* plasma differences

- Blood *vs.* plasma
- Use of VAMS sampling technique
- Capillary *vs.* venous

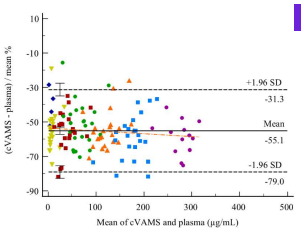


Figure 2 Bland-Altman comparison of cVAMS and plasma concentrations for iohexol

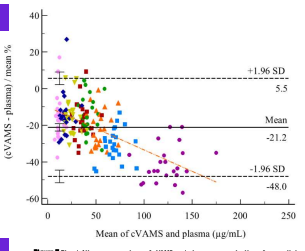


Figure 3 Bland-Altman comparison of cVAMS and plasma concentrations for creatinine

Reliable conversion of cVAMS results to plasma results!

Iohexol: hematocrit-based correction

$$x\,(plasma) = \frac{y\,(cVAMS)}{(1 - HT)} * 0.88$$

Creatinine: time-dependent Blood/Plasma ratio correction

$$x\,(plasma) = \frac{y\,(cVAMS\,Tx)}{\left(\frac{B}{P}Tx\right)} * 0.92$$

APPLICATION

Excellent agreement!

- Iohexol: 94% within 20% difference
- Creatinine: 96% within 20% difference

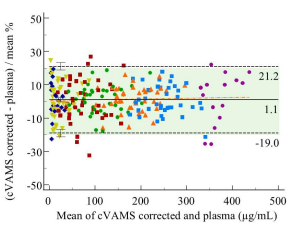


Figure 4 Bland-Altman comparison of corrected cVAMS and plasma concentrations for iohexol

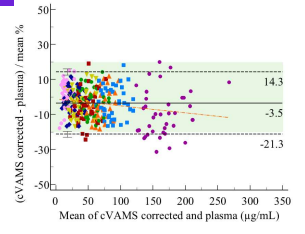


Figure 5 Bland-Altman comparison of corrected cVAMS and plasma concentrations for creatinine

CONCLUSION

Our method fulfilled all pre-set validation acceptance criteria. We applied our established correction formulas derived from the clinical validation data successfully resulting in **excellent agreement** for both iohexol and creatinine between **capillary VAMS and plasma concentrations**. Consequently, we demonstrated that **ear-prick sampling using VAMS is a suitable alternative** to conventional venous sampling to determine iohexol and creatinine for GFR determination in cats.