

# EX VIVO SENSITIVITY AND ROBUSTNESS OF A NOVEL HOME-SCREENING TEST FOR FELINE HEMATURIA

Khenifar E, del Castillo JRE, Gara-Boivin C  
Université de Montréal, St-Hyacinthe, Canada

**Key finding:** The lower limit of repeatable detection is 100 RBC/ $\mu$ L  $\leftrightarrow$  12 [8-16] RBC/HPF. The color remains stable at least for 48 hours.

## Introduction


**Background:** Hematuria is a frequent sign in feline urinary tract diseases<sup>1</sup>. Detecting hematuria is a challenge for cat owners. A novel hematuria-screening test for use at home on the litter has just completed its development.

## Objectives and hypothesis

- Hypothesis** The test can consistently detect clinically relevant microscopic hematuria
- Objectives**
- determine the hematuria lower limit of detection in vitro
  - verify its robustness in altered urine composition

## Materials and methods

### In vitro:

Fig. 1: Test tube	Dilution rank	Concentration (RBC/ $\mu$ L)	Fig. 2: Urine placed on 4 random granules
	0 = stock	8540000	
	1	640500	
	2	32025	
	3	1600	
	4	800	
	5	400	
	6	200	
	7	100	
	8	50	
	9	25	
	10	13	
	11	6	
	12	3	
	13 = blank	0	

**Fig. 3: Chromogenic intensity scale**





0	
1+	
2+	
3+	

Table 1: Quadruplicate testing of 50  $\mu$ L of feline RBC-fortified pooled feline urine

- Granules chromogenic score readings performed at 3 and 30 minutes and at 24 and 48 hours after exposure to fortified urine
- In parallel, centrifugation of the urine samples and additional blood detections (dipstick of the supernatant and microscopy of the sediment)
- Protocol repeated on 4 pools of urine with different specific gravity (SG): 1.026, 1.037, 1.047 and 1.053 g/mL

### Ex vivo:

- Greco-latin square (3 squares of 4 periods per 4 sequences)
- 6 male and 6 female cats
- 4 fluid therapies given awake (6 hours at 10 mL $\cdot$ kg<sup>-1</sup> $\cdot$ h<sup>-1</sup>), causing transient polyuria with differential effects on urinary composition:
  - A : NH<sub>4</sub>Cl
  - B : NH<sub>4</sub>Cl + NaCl 5%
  - C : NaHCO<sub>3</sub>
  - D : NaHCO<sub>3</sub> + NaCl 5%
- Spontaneous micturition collected on polypropylene beads litter, tested for pH, SG and proteinuria, and then fortified with RBC as in Fig. 2, and color changes of granules scored as in Fig. 3.
- Data analysis : generalized linear mixed model for ordinal repeated measures

## Results

### In vitro:

- Microhematuria recorded at concentrations  $\leq$  1965 RBC/ $\mu$ L in this experiment
- Lower limit of detection: 100 RBC/ $\mu$ L  $\leftrightarrow$  12 [8-16] RBC/High Power Field (x400) at the microscopic examination of the sediment from 5 mL of urine

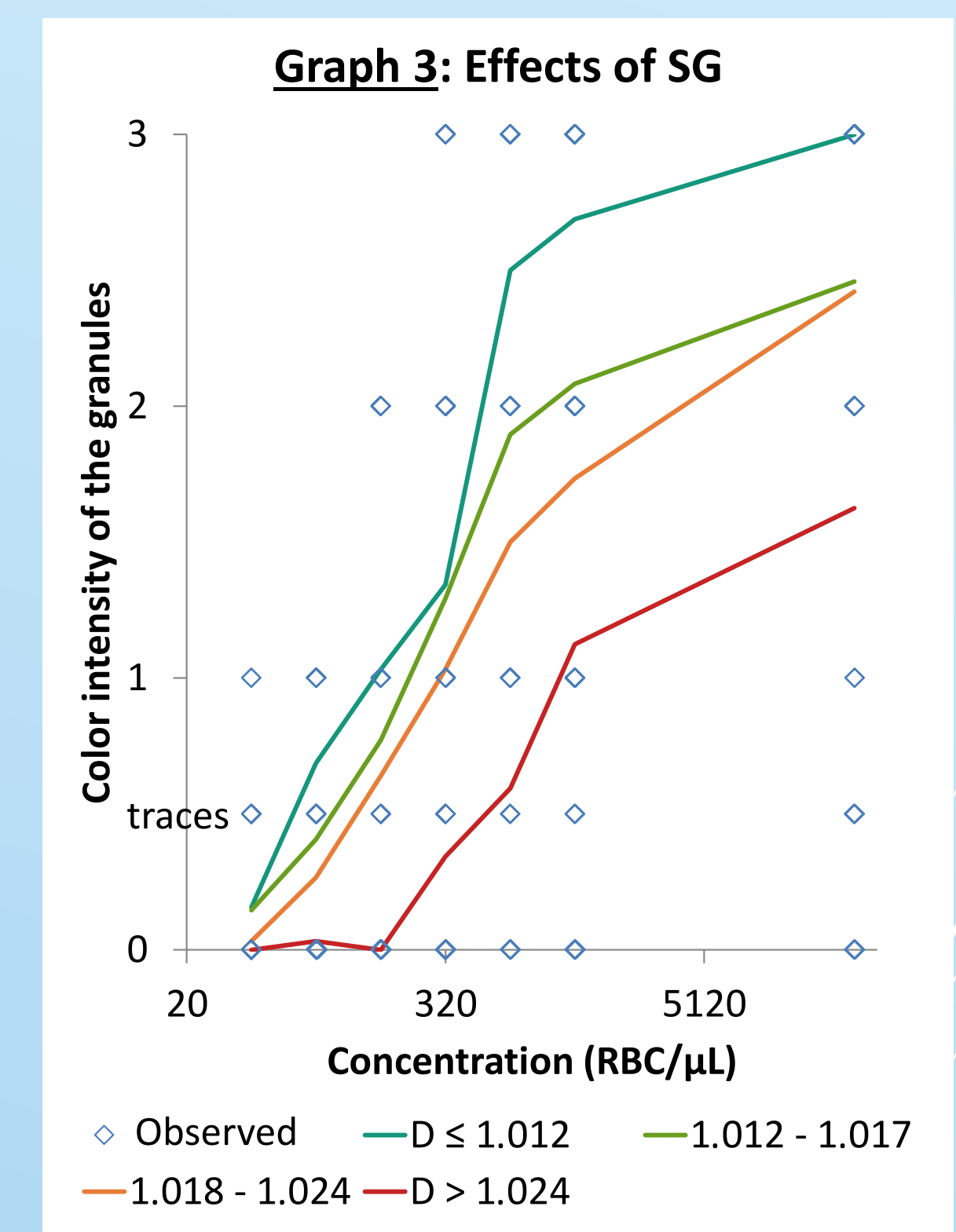
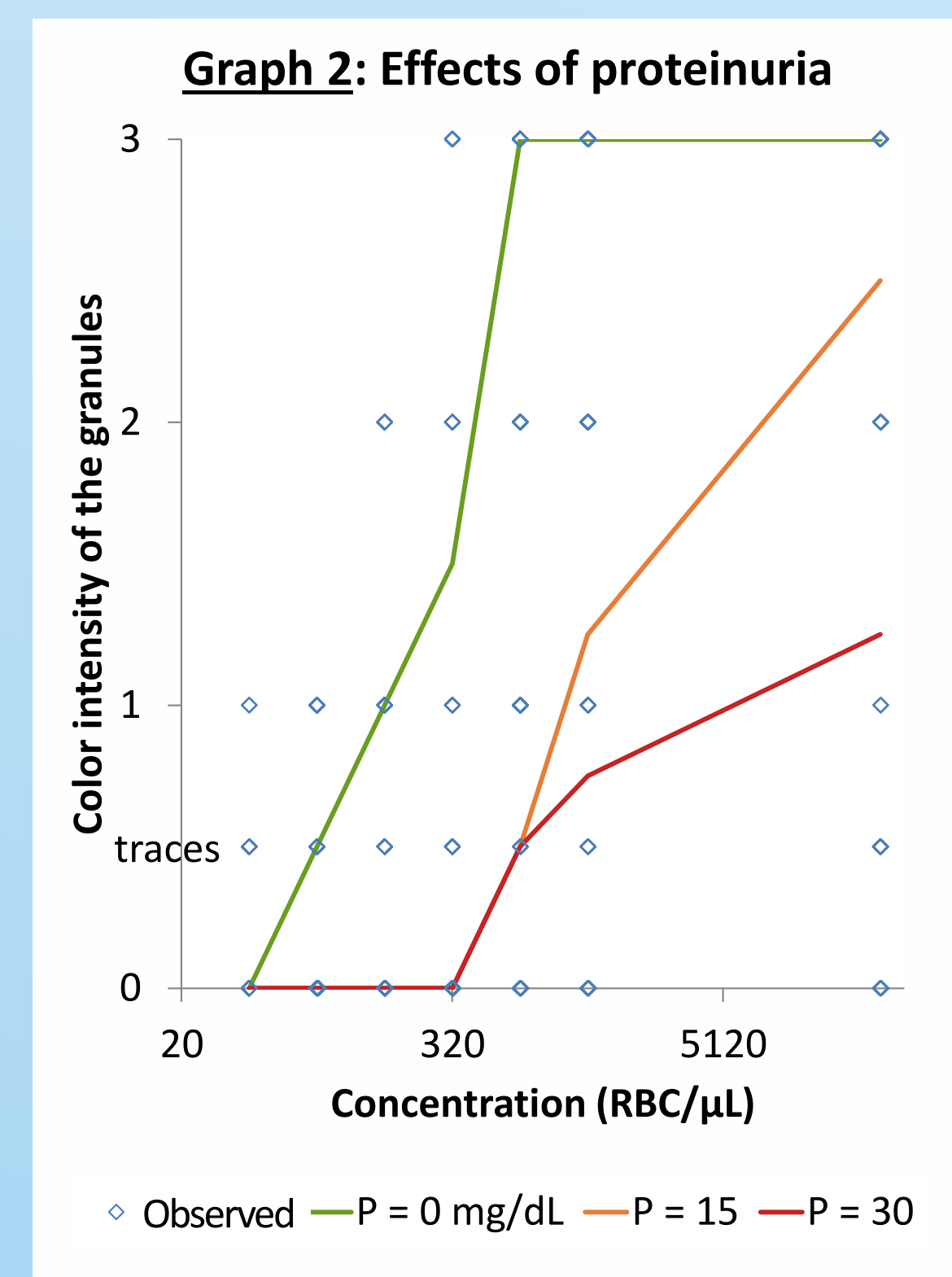
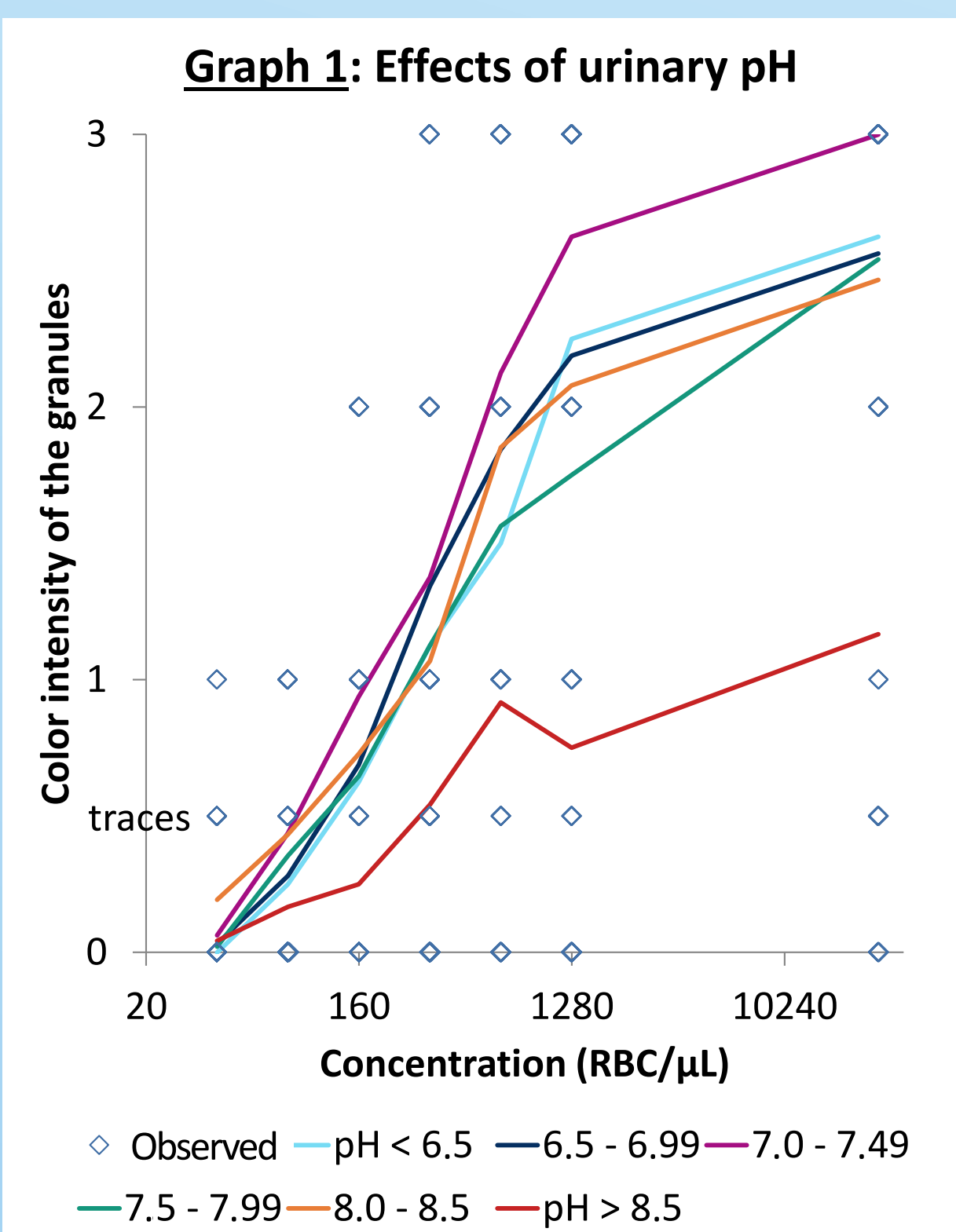


Dilution Rank	0 = stock	1	2	3	4	5	6	7	13 = blank
Concentration (RBC/ $\mu$ L)	8.5 $\cdot$ 10 <sup>6</sup>	0.6 $\cdot$ 10 <sup>6</sup>	32 $\cdot$ 10 <sup>3</sup>	1.6 $\cdot$ 10 <sup>3</sup>	800	400	200	100	0
Microscopic observation (RBC/HPF)	>50	>50	>50	>50	>50	34-50	17-27	10-21	0
Granules score 3 min	3+	3+	3+	3+	2+	2+	1+	1+	0
Granules score 30 min	3+	3+	3+	2+	2+	1+	1+	1+	0
Granules score 24h	3+	3+	3+	2+	2+	1+	1+	1+	0
Granules score 48h	3+	3+	3+	2+	2+	1+	1+	1+	0

Table 2: Positive chromogenic responses of the granules (at 48 hours) for the first dilution ranks with SG=1,026

### Ex vivo:

- The I.V. fluids changed the urinary SG, pH and proteins. The limit of detection increased with:
  - pH (320 RBC/ $\mu$ L with pH $\geq$ 8.5)
  - proteinuria (640 RBC/ $\mu$ L with Prot $\geq$ 15 mg/dL)
 } microhematuria still detected (graph 1 and 2)
- No significant effect (p>0.05) of SG (graph 3) or sex



## Discussion

### Necessity of this test:

- Hematuria is a frequent sign in urinary tract diseases<sup>1</sup>, which are common among cats<sup>2</sup>
- Relevance of the diagnosis of hematuria's origin<sup>3</sup> and relevance of an early diagnosis of urinary tract diseases<sup>4</sup>

### Relevance of this detection limit:

- > 5 RBC/high power field (x400) is considered as pathologic<sup>5</sup>.
- The granules detect as low as 12 RBC/HPF (x400) $\leftrightarrow$ 100 RBC/ $\mu$ L, over the pathological limit and under the lower limit of macrohematuria (2500 RBC/ $\mu$ L)<sup>5</sup>.

- Limits of this study:** *ex vivo* controlled conditions. Currently presenting a test in clinics and households with feline patients at risk of hematuria.

## Conclusion

- In both experiment, hematuria was detected in the range of microhematuria. The product has good potential for clinical use.

## Literature cited

- Dorsch, R., et al. (2014). "Feline lower urinary tract disease in a German cat population. A retrospective analysis of demographic data, causes and clinical signs." *Tierarztl Prax Ausg K Kleintiere Heimtiere* 42(4): 231-239.
- Bartlett, P. C., et al. (2010). "Disease surveillance and referral bias in the veterinary medical database." *Prev Vet Med* 94(3-4): 264-271.
- Forrester, S. D. (2004). "Diagnostic approach to hematuria in dogs and cats." *Vet Clin North Am Small Anim Pract* 34(4): 849-866.
- Grauer, G. F. (2005). "Early detection of renal damage and disease in dogs and cats." *Vet Clin North Am Small Anim Pract* 35(3): 581-596.
- Fundamentals of Veterinary Clinical Pathology Second Edition, 2008

## Acknowledgments

- Project funded in part by MITACS acceleration research grants

## For further information

elodie.khenifar@umontreal.ca