Multiplex Measurement of Rat Kidney Cytokines in Serum and Urine using the Fluorokine® MAP Assay

Jane Schmidt, Ph.D., Michael Rynning, and Richard Fuerstenberg | R&D Systems, Inc., 614 McKinley Pl. NE, Minneapolis, MN, 55413

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www.RnDSystems.com

ABSTRACT

Drug toxicity can result in major organ damage to the kidneys. The traditional methods for monitoring drug-induced nephrotoxicity in toxicology studies include measurement of serum creatinine and blood urea nitrogen levels, but in recent years, more sensitive kidney biomarkers have emerged. The PCK rat is a recently identified rodent model of polycystic kidney disease that can be used as a model for general kidney disease or damage. We measured TIM-1/KIM-1/HAVCR, FABP1/L-FABP, Lipocalin-2/NGAL, Cystatin C, and Osteopontin in serum and urine from normal (Sprague Dawley®) and PCK rats using the Fluorokine® MAP Rat Kidney Toxicity Panel and observed a significant increase of TIM-1/KIM-1/HAVCR and Lipocalin-2/NGAL in PCK rat serum compared to normal rat serum. We also observed statistically significant differences for Lipocalin-2/NGAL and Cystatin C in PCK urine when compared to normal urine.

Fluorokine Multianalyte Profiling kits are bead-based multiplex immunoassays designed for use with the Luminex® 100™, Luminex 200™, or Bio-Rad® Bio-Plex® dual laser, flow-based sorting and detection analyzers. They offer the ability to quantify multiple analytes simultaneously with less user time, less sample volume, and a lower cost per result compared to conventional ELISAs. Five biomarkers can be measured in a user-defined multiplex using the Rat Kidney Toxicity Panel. The assay is fully validated for accurate, precise, and reproducible results in rat serum, plasma, and urine.

INTRODUCTION

The kidney plays a major role in the metabolism and excretion of drugs, making it vulnerable to various drug-induced injuries. During drug development, nephrotoxicity in animal models is a major factor in the failure of many prospective compounds. Traditional methods of monitoring drug-induced nephrotoxicity in preclinical toxicology studies measure serum creatinine (SCr) and blood urea nitrogen (BUN) levels. However, these markers of renal function lack the sensitivity and specificity to adequately detect nephrotoxicity before significant kidney damage has occurred.[1-3] In recent years, FABP1/L-FABP, Lipocalin-2/NGAL, TIM-1/KIM-1/HAVCR*, Cystatin C, and Osteopontin have been identified as kidney toxicity protein markers that are more sensitive than SCr and BUN.[1,4,5]

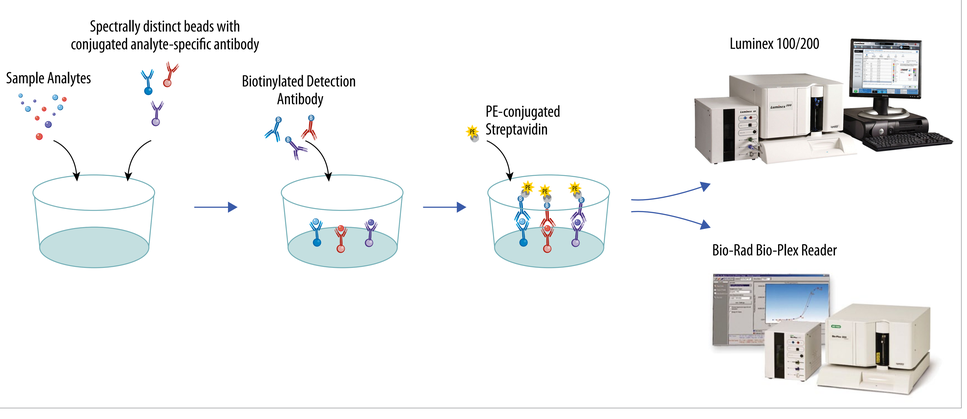
The PCK rat is a recently identified model of polycystic kidney disease that can also be used as a model of general renal disease and kidney damage. Serum and urine samples from PCK rats were analyzed using the Rat Fl uorokine Multianalyte Profiling (MAP) Kidney Toxicity Panel (R&D Systems, Catalog # LRK000). The Rat Fluorokine MAP Kidney Toxicity Panel is a bead-based multiplex immunoassay that can simultaneously assess the levels of FABP1/L-FABP, Lipocalin-2/NGAL, TIM-1/KIM-1/HAVCR, Cystatin C, and Osteopontin in a single sample of serum, plasma, or urine. The profiles of the five biomarkers in serum and urine from PCK rats were compared to those in serum and urine from normal (Sprague Dawley) rats.

GENERAL METHODS

SAMPLES

Serum and urine samples from normal (Sprague Dawley) rats (n=20) and PCK rats (n=10) were purchased from Bioreclamation, LLC.

ASSAY PROCEDURE



RESULTS

TABLE 1

					Serum		Urine	
Analyte	**Standard Curve Ranges**	**Average Sensitivity (pg/mL)**	**Average Intra-assay Precision**	**Average Inter-assay Precision**	**Average Recovery**	**Average Linearity**	**Average Recovery**	**Average Linearity**
TIM-1/KIM-1/HAVCR	14-10,170 pg/mL	1.5	5.2%	6.8%	97%	91%	94%	91%
FABP1/L-FABP	3,409-2,485,500 pg/mL	72	7.0%	10.8%	102%	92%	96%	95%
Lipocalin-2/NGAL	8-5,900 pg/mL	2.6	6.5%	8.1%	104%	99%	90%	96%
Cystatin C	26-19,000 pg/mL	2.4	7.3%	11.0%	102%	85%	96%	106%
Osteopontin	13-9,200 pg/mL	0.6	4.8%	7.9%	104%	102%	99%	96%

Analytical Performance of the Rat Fluorokine MAP Kidney Toxicity Panel. The Rat Fluorokine MAP Kidney Toxicity Panel was rigorously tested in-house to ensure optimal assay performance for all analytes and sample types.

Sensitivity – The Minimum Detectable Dose was determined by adding two standard deviations to the MFI of twenty zero standard replicates and calculating the corresponding concentration.

Intra-assay Precision - Three samples of known concentration were tested twenty times on one plate to assess precision within an assay.

Inter-assay Precision – Three samples of known concentration were tested in thirty-six separate assays to assess precision between assays.

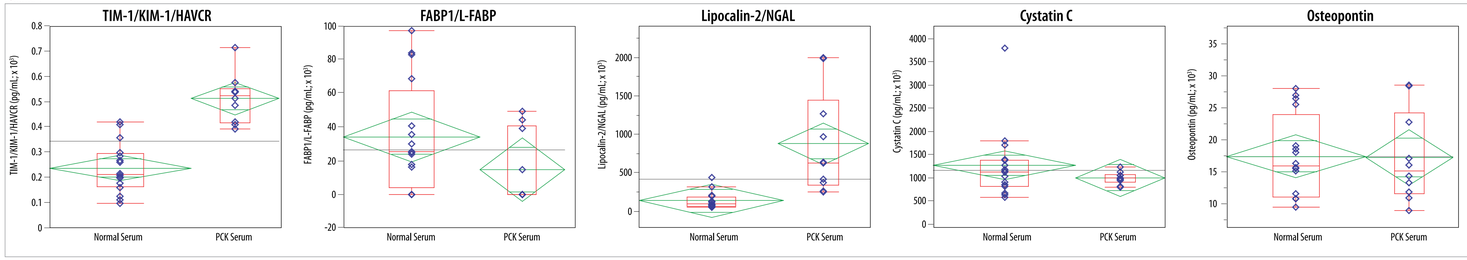
Recovery and Linearity – To assess the accuracy of the assays, serum and urine samples were spiked with recombinant proteins at three levels and evaluated for recovery. The samples were also serially diluted to evaluate assay linearity.

TABLE 2

	TIM-1/KIM-1/HAVCR		FABP1/L-FABP		Lipocalin-2/NGAL		Cystatin C		Osteopontin	
	Normal Serum	**PCK Serum**	**Normal Serum**	**PCK Serum**	**Normal Serum**	**PCK Serum**	**Normal Serum**	**PCK Serum**	**Normal Serum**	**PCK Serum**
Average (pg/mL)	236	512	45,343	36,850	135,413	877,800	1,265,125	995,200	17,431	17,228
Range (pg/mL)	98 - 420	390 - 712	ND - 96,400	ND - 49,200	51,600 - 434,000	256,000 - 2,000,000	580,000 - 3,800,000	790,000 - 1,240,000	9,460 - 28,000	8,940 - 28,600

Concentrations of Kidney Toxicity Biomarkers Measured in Normal and PCK Rat Serum using the Rat Fluorokine MAP Kidney Toxicity Panel. ND = Not Detectable.

FIGURE 1



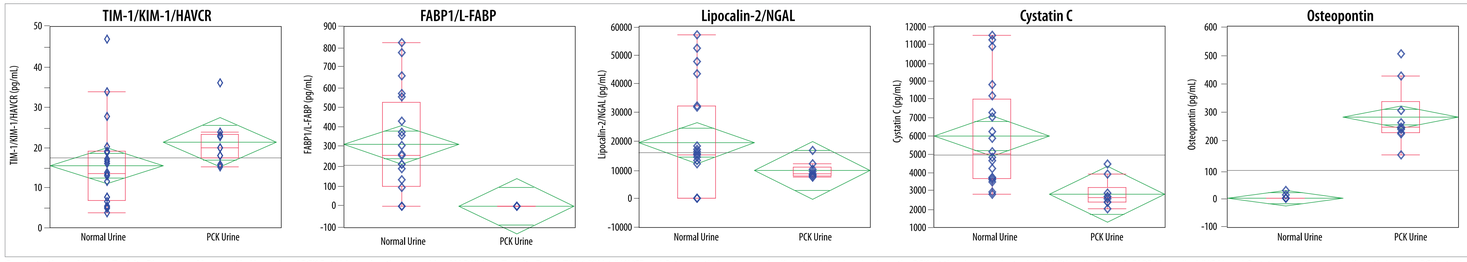
Concentrations of Kidney Toxicity Biomarkers Measured in Normal and PCK Rat Serum using the Fluorokine MAP Kidney Toxicity Panel. TIM-1/KIM-1/HAVCR and Lipocalin-2/NGAL levels were significantly higher in serum from the PCK rat than in normal rat serum. Additionally, FABP1/L-FABP levels were significantly lower in PCK rat serum compared to normal rat serum.

TABLE 3

	TIM-1/KIM-1/HAVCR		FABP1/L-FABP		Lipocalin-2/NGAL		Cystatin C		Osteopontin	
	Normal Urine	**PCK Urine**	**Normal Urine**	**PCK Urine**	**Normal Urine**	**PCK Urine**	**Normal Urine**	**PCK Urine**	**Normal Urine**	**PCK Urine**
Average (pg/mL)	15.56	21.32	309	ND	27,793	9,759	6,012	2,846	2.0	286
Range (pg/mL)	4.00 - 46.95	15.2 - 36.2	ND - 820	ND	ND - 57,170	7,374 - 17,012	2,809 - 11,491	2,011 - 4,454	ND - 26.6	152 - 506

Concentrations of Kidney Toxicity Biomarkers Measured in Normal and PCK Rat Urine using the Rat Fluorokine MAP Kidney Toxicity Panel. ND = Not Detectable.

FIGURE 2



Concentrations of Kidney Toxicity Biomarkers Measured in Normal and PCK Rat Urine using the Fluorokine MAP Kidney Toxicity Panel. TIM-1/KIM-1/HAVCR and Osteopontin levels were significantly higher in urine from the PCK rat than in normal rat urine. Additionally, FABP1/L-FABP, Lipocalin-2/NGAL and Cystatin C levels were significantly lower in PCK rat urine compared to normal rat urine.

CONCLUSIONS

- Significant increases were observed in the levels of TIM-1/KIM-1/HAVCR and Lipocalin-2/NGAL in serum from the PCK rat compared to normal rat serum. A significant decrease was observed in the level of FABP1/L-FABP in serum from the PCK rat compared to normal rat serum.
- Significant increases were observed in the levels of TIM-1/KIM-1/HAVCR and Osteopontin in urine from the PCK rat compared to normal rat urine. Significant decreases were observed in the levels of FABP1/L-FABP, Lipocalin-2/NGAL and Cystatin C in urine from the PCK rat compared to normal rat urine.
- The Rat Fluorokine MAP Kidney Toxicity Panel is a fully validated bead-based multiplex assay that generates accurate, precise, and reproducible results.
- The Rat Fluorokine MAP Kidney Toxicity Panel is an excellent tool for drug toxicology studies because it can simultaneously assess the levels of FABP1/L-FABP, Lipocalin-2/NGAL, TIM-1/KIM-1/HAVCR, Cystatin C, and Osteopontin in a single serum, plasma, or urine sample.
- The Rat Fluorokine MAP Kidney Toxicity Base Kit can be combined with any combination of the five analyte-specific bead sets for greater flexibility in experimental design.

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