Evaluation of Multiplex Electrochemiluminescent Immunoassays for Quantitative Determination of Kidney Injury Biomarkers in Rat Urine

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Introduction

Traditional clinical markers of kidney injury, such as BUN and serum creatinine, lack sufficient sensitivity for early detection of injury and are not specific for the site of injury; thus, they have limited clinical utility. Site-specific nephrotoxicity biomarkers can be used to detect early stage renal injury and to localize that injury to specific sites within the kidney.

The purpose of this study was to evaluate two 96-well multiplex electrochemiluminescent immunoassays from MSD (Meso Scale Discovery) which quantitatively measure the levels of biomarkers of kidney toxicity in rats. The Kidney Injury Panel 1 Assay measures lipocalin-2 (NGAL), osteopontin, albumin, and TIM-1/KIM-1/HAVCR, and the Acute Kidney Injury (AKI) panel measures the levels of alpha GST, GSTYb1 and RPA-1 in rat urine as indicators of renal damage.

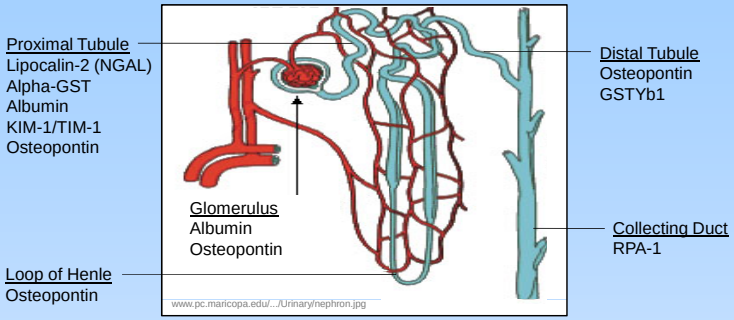
Conclusions

It was determined that, with certain limitations, the multiplex electrochemiluminescent immunoassays were suitable for analysis of kidney toxicity biomarkers in rat urine. Multiplex detection of the biomarkers in urine affords early and site specific detection of renal injury in rats.

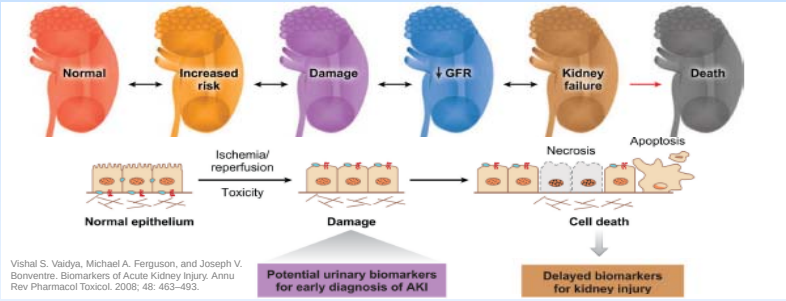
Methods

Evaluation of these methods included assessment of assay range, precision and accuracy, lower and upper limits of quantification, specificity, spike recovery, dilutional linearity, parallelism, and stability.

Sites of Kidney Injury Biomarkers in Rats



Kidney Injury Continuum



Validated Kidney Injury Biomarkers

Lipocalin-2 (NGAL): up-regulation observed in rat proximal tubule cells after ischemia-reperfusion injury; raised plasma levels found to be strongly correlated with decreased renal function in certain patients with renal damage

Osteopontin: constitutively expressed by the distal tubular epithelium in rodent and human kidney; up-regulation by proximal tubular epithelial cells in a number of rodent models of renal injury

Albumin: an increased level in urine is an indicator of albuminuria, which can result from kidney damage

TIM-1/KIM-1/HAVCR (kidney injury molecule-1): expressed in renal proximal tubule epithelial cells; expression is markedly up-regulated in the proximal tubule in acute kidney injury of certain types

Alpha GST and GSTYb1: found in the proximal and distal renal tubules, respectively; neither is released in a healthy animal, so detection is both a sensitive and site specific indicator of kidney injury

Renal Papillary Antigen 1 (RPA-1): first urinary biomarker associated with injury to the luminal epithelial cells of the collecting duct; shown to be an early biomarker of renal papillary necrosis (RPN);

Results

Biomarker	Range (ng/mL)*	Inter-assay Accuracy (% of Nominal)	Inter-assay Precision [CV (%)]	Recovery (% of Nominal)	Linearity	Parallelism	Specificity
Lipocalin-2	0.25 – 181	101.3 – 119.1	5.6 – 11.4	96.9 – 112.7	Not demonstrated beyond 2-fold dilution	Not demonstrated beyond 2-fold dilution	Cross-reactivity observed with OPN and TIM-1 Antibodies
Osteopontin	0.066 – 48.2	88.4 – 109.3	6.2 – 18.7	48.1 – 52.4	Demonstrated for dilutions from 16- to 128-fold	Not Assessed (Samples not available)	No cross-reactivity observed
Albumin	64.2 – 46800	90.6 – 104.7	4.8 – 15.5	75.4 – 125.1	Demonstrated for dilutions up to 128-fold	Demonstrated for dilutions from 2- to 16-fold	No cross-reactivity observed
KIM-1/TIM-1	0.01 – 2.46	81.2 – 115.0	5.7 – 20.8	85.1 – 101.8	Demonstrated for dilutions up to 128-fold	Demonstrated for dilutions from 2- to 128-fold	No cross-reactivity observed
Alpha GST	6.0 – 1470	89.0 – 110.4	6.7 – 19.7	61.6 – 90.3	Demonstrated for dilutions up to 128-fold	Demonstrated for dilutions up to 32-fold	No cross-reactivity observed
GSTYb1	1.9 – 470	94.3 – 112.0	5.2 – 9.9	144.6 – 170.9	Demonstrated for dilutions from 4- to 128-fold	Demonstrated for dilutions from 2- to 64-fold	No cross-reactivity observed
RPA-1	3.6 – 880 *Units are U/L	95.5 – 110.9	6.3 – 24.4	111.3 – 125.4	Demonstrated for dilutions up to 64-fold	Demonstrated for dilutions up to 128-fold	No cross-reactivity observed

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MSD Multi-Spot Assay System Argutus AKI Test (rat) Assay Kit 17742-v8-2010Sep.

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