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.

Results

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

References
 MSD Multi-Spot Assay System Argus A Kit/Assay Kit 17742-vb-2010Sp
 MSD Multi-Spot Assay System Kidney Injury Panel 1 (rat) Assay Qualified Kit 17747-vb-2010Sp

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