Evaluation of the MSD Multiplex Electrochemiluminescent Immunoassay for the Determination of Cardiac Toxicity Biomarkers in Rat Serum

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Introduction
ICH guidelines require that preclinical safety pharmacology studies to assess the risk of cardiotoxicity be conducted before beginning any clinical development program.

Investigation of cardiac toxicity in general toxicology studies is not covered by specific guidelines, with the following exception: the investigation of cardiac toxicity in the conduct of pre-clinical and clinical studies for the development of new drugs for the treatment of diabetes.

Cardiovascular complications are common among diabetic patients, and some recently introduced therapies have been shown to exacerbate these complications. The endpoints to be examined include the biomarkers of cardiac toxicity, troponin I (cTnI) and troponin T (cTnT), and are clearly indicated in a Guidance document issued by the FDA in 2008.

The purpose of this study was to evaluate the Cardiac Injury Panel 2 Assay Kit, a multiplex 96-well electrochemiluminescent immunoassay from MSD (Meso Scale Discovery), for the determination of cTnI, cTnT, and fatty acid binding protein 3 (FABP3) in rat serum. These biomarkers can also be measured using the MSD Muscle Injury Panel 1 Assay Kit, which includes the muscle injury biomarkers MyoG and skeletal muscle troponin I (sTnI).

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.

Conclusions
It was determined that the Cardiac Injury Panel 2 kit is suitable for the determination of cTnI, cTnT, and FABP3 in rat serum samples for support of biomarker analysis.

Characteristics of “Ideal” Toxicity Biomarkers
- Specific: High tissue/serum ratio
- Not expressed in non-target tissues, even under pathologic circumstances
- Differentiates toxicity type (e.g., acute, chronic, necrosis, hypertrophy)
- Sensitive: Low (and measurable) baseline relative to change induced by toxicity
- Marker of “early,” reversible toxicity
- Immediate release with injury
- Predictive: Sufficiently long and characterized half-life in blood
- Release proportionate to extent of injury
- Gives indication of reversibility
- Robust: Rapid, simple, accurate, and inexpensive detection in all relevant species
- Bridge preclinical and clinical
- Noninvasive/accessible

Fatty acid binding protein 3 (FABP3) is found in high concentration in myocytes (several-fold greater than in skeletal muscle). It is rapidly released into circulation with cell injury after myocardial ischemia and necrosis. FABP3 is expressed primarily in the heart and skeletal muscle, but is also present in the brain, liver, and small intestine.

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>Parallelism</th>
<th>Specificity</th>
<th>Stability</th>
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</thead>
<tbody>
<tr>
<td>cTnI</td>
<td>0.016 – 23.4</td>
<td>99.3 – 114.5</td>
<td>1.1 – 5.0</td>
<td>102.9 – 110.0</td>
<td>Demonstrated for dilutions through 128-fold</td>
<td>Demonstrated for dilutions through 16-fold</td>
<td>No cross-reactivity observed with cTnT or FABP3 Antibodies</td>
<td>Met acceptance criteria for short-term, long-term, and freeze/thaw stability</td>
</tr>
<tr>
<td>cTnT</td>
<td>0.032 – 47.0</td>
<td>99.4 – 111.4</td>
<td>4.3 – 14.9</td>
<td>91.7 – 106.4</td>
<td>Demonstrated for dilutions through 128-fold</td>
<td>Demonstrated for dilutions through 8-fold</td>
<td>No cross-reactivity observed with cTnI or FABP3 Antibodies</td>
<td>Met acceptance criteria for short-term, long-term, and freeze/thaw stability</td>
</tr>
<tr>
<td>FABP3</td>
<td>0.066 – 95.2</td>
<td>102.2 – 123.7 (ULOQ)</td>
<td>1.0 – 18.3</td>
<td>94.5 – 164.5</td>
<td>Demonstrated for dilutions from 4-fold to 128-fold</td>
<td>Demonstrated for dilutions from 2-fold to 16-fold</td>
<td>No cross-reactivity observed with cTnI or cTnT Antibodies</td>
<td>Met acceptance criteria for short-term, long-term, and freeze/thaw stability</td>
</tr>
</tbody>
</table>

References
O’Brien, PJ. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. Toxicology 2008 Mar 20; 245(3):206-18.
MSD Multi-Spot Assay System Cardiac Injury Panel 2 (rat) Assay Kit 17379-v4-2010Aug.