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 and troponin T, 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 Myl3 and skeletal muscle troponin I (sTnI).

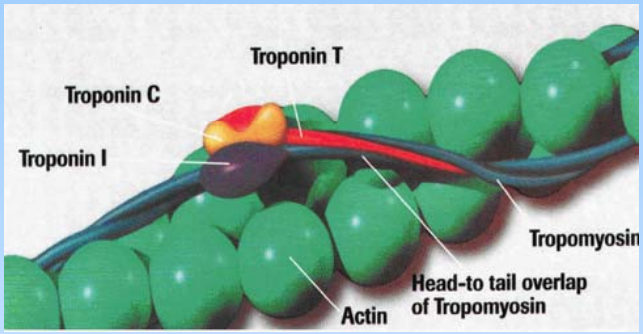
Methods

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.

Diagram of Cardiac Troponin Complex



Chaikhouni A, M.D.; Al-Zaim H, M.D. Troponin I Levels After Coronary Bypass Operations In Aleppo, syria .Heart Views 2007 Mar – May; 8(1).

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	

Modified from the Expert Working Gropup on Biomarkers of Drug-Induced Cardiac Toxicity report to the Nonclinical Studies Subcommittee of the Advisory Committee for Pharmaceutical Sciences (http://www.fda.gov/ohrms/dockets/ac/01/slides/3763s1 00 HOLT/sld005/htm).

Cardiac Injury Panel 2 Biomarkers

Cardiac troponin subunits are specific, sensitive, and robust markers of drug-induced myocardial cell injury in humans, as well as in common laboratory species. Upon muscle damage, troponin I (cTnI) and troponin T (cTnT) are released into the blood. Differences in sequence between cardiac and skeletal troponins allow for the distinction between cardiac and skeletal muscle toxicity.

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.

References

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O'Brien, PJ. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. Toxicity 2008 Mar 20; 245(3):206-18.

Walker, DB. Serum chemical biomarkers of cardiac injury for nonclinical safety testing. Toxicol. Pathol. 2006 Jan; 34(1): 94 – 104.

Wallace, K., *et.al.* Serum troponins as biomarkers of drug-induced cardiac toxicity. Toxicol. Pathol. 2004 Jan; 32(1): 106 – 121.

MSD Multi-Spot Assay System Cardiac Injury Panel 2 (rat) Assay Kit 17379-v4-2010Aug.

MSD Multi-Spot Assay System Muscle Injury Panel 1 (rat) Assay Qualified Kit 17805-v3-2010Jul.

Results

Biomarker	Range (ng/mL)	Inter-assay Accuracy (% of Nominal)	Inter-assay Precision [CV (%)]	Recovery (% of Nominal)	Linearity	Parallelism	Specificity	Stability
cTnI	0.016 – 23.4	99.3 – 114.5	1.1 – 5.0	102.9 – 110.0	Demonstrated for dilutions through 128-fold	Demonstrated for dilutions through 16-fold	No cross-reactivity observed with cTnT or FABP3 Antibodies	Met acceptance criteria for short-term, long-term, and freeze/thaw stability
cTnT	0.032 – 47.0	99.4 – 111.4	4.3 – 14.9	91.7 – 106.4	Demonstrated for dilutions through 128-fold	Demonstrated for dilutions through 8-fold	No cross-reactivity observed with cTnI or FABP3 Antibodies	Met acceptance criteria for short-term, long-term, and freeze/thaw stability
FABP3	0.066 – 95.2	102.2 – 123.7 (ULOQ)	1.0 – 18.3	94.5 – 164.5	Demonstrated for dilutions from 4-fold through 128-fold	Demonstrated for dilutions from 2-fold to 16-fold	No cross-reactivity observed with cTnI or cTnT Antibodies	Met acceptance criteria for short-term, long-term, and freeze/thaw stability

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