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S-PLEX℠ Troponin I Assay with fg/mL Sensitivity

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1 Abstract

Cardiovascular toxicity is a potential complication of multiple anticancer therapies. Some drugs, such as anthracyclines, have been implicated in irreversible cardiac dysfunction. Cardiac troponin is a specific and sensitive marker of myocardial injury. Serum and plasma concentrations of troponin are often below the limits of detection in apparently healthy individuals. Sensitivity of troponin assays has improved by two orders of magnitude over the last several years, but few assays can detect basal levels of serum troponin in more than 50% of apparently healthy individuals, limiting the utility of the assay for early measurement of low levels of cardiac damage during clinical research.

S-PLEX is a novel ultrasensitive immunoassay platform based on MSD's MULTI-ARRAY® electrochemiluminescence (ECL) technology. An S-PLEX cardiac troponin I assay was developed and analytically characterized for research use. Serum and plasma samples from 24 apparently healthy individuals, 90 individuals with cardiovascular complications, and 25 individuals receiving anthracycline chemotherapy were measured. The assay required 25 µL of sample per measurement and was run on the MESO® SECTOR S 600 and MESO QuickPlex® SQ 120 instruments. The lower limit of detection was determined to be 10 fg/mL and the limits of quantitation ranged from 31 fg/mL to 160,000 fg/mL. The assay was anchored to the NIST reference material SRM 2921. Control samples run at three levels (n=8 per plate, 8 plates, 2 days, 2 operators) had total CVs of 7% to 8% (n=64). Spike recovery and dilution linearity had recoveries between 80% and 120%. Specificity of the assay was demonstrated by analyte depletion using several commercially available anti-troponin specific antibodies. There was no detectable cross-reactivity to skeletal troponin. As expected, troponin I serum or plasma concentrations were high (~10,000 fg/mL to >160,000 fg/mL) for 90 individuals with cardiovascular complications. Troponin I serum concentrations in 25 samples from individuals receiving anthracycline chemotherapy had a median concentration of 306 fg/mL and a range of <1Q to 5,820 fg/mL, comparable to a set of apparently healthy specimens (n=24) with a median concentration of 226 fg/mL and a range of <1Q to 4,330 fg/mL. The S-PLEX troponin I assay was capable of detecting troponin in 44 of the 49 samples from individuals with no known cardiovascular disease.

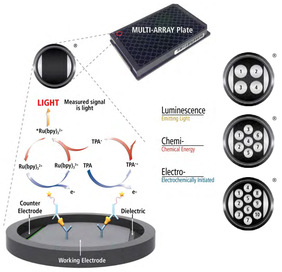
In conclusion, we have developed a highly specific and sensitive cardiac troponin I assay that is capable of accurately measuring the cardiac troponin I protein concentration in samples from individuals with known cardiovascular disease, as well as 90% of the apparently healthy individuals tested. This assay can be used to study low levels of cardiac troponin I in serum and plasma samples and may provide a useful research tool for the detection of cardiac troponin I, a biomarker linked to cardiac damage.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY microplates. We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY technology with significantly higher sensitivity.

Electrochemiluminescence Technology

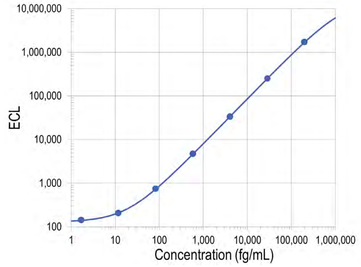
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.



3 Calibrator Anchoring

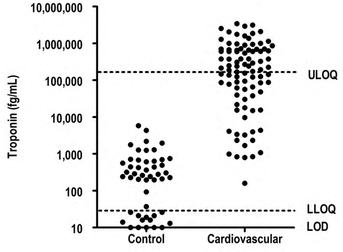
The assay calibrator was anchored to the NIST reference material SRM 2921.

4 Representative Calibration Curve



A representative calibration curve is shown with estimated limit of detection (LOD) typically around 10 fg/mL.

5 Serum/Plasma Concentrations



Approximately 90 serum or plasma samples from troponin positive individuals and from individuals who had been diagnosed with congestive heart failure were obtained from commercial vendors and tested. Troponin-positive samples were diluted 10-fold prior to testing; the remaining samples were tested neat.

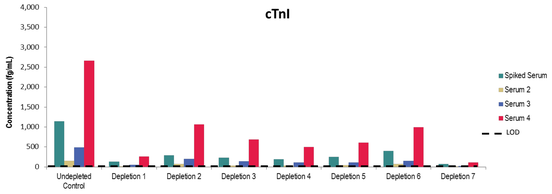
Control samples in this graph are samples from individuals without known cardiovascular disease. Samples were not matched for age, gender, sample type (serum/plasma), or other factors. Five samples with concentrations below the LOD (10 fg/mL) are shown as 10 fg/mL.

6 Specificity

Cross-reactivity

The assay recognizes native troponin ITC complex and free native and recombinant troponin I. There is no cross-reactivity to skeletal troponin; 600,000 fg/mL of skeletal troponin gave a signal below the assay LOD.

Analyte Depletion



Depletion experiments were performed with seven antibodies, which include antibodies not used in the S-PLEX cTnI assay.

Depletion was performed on four apparently healthy donor serum samples, one of which was spiked with serum from an individual experiencing a cardiac event. The spiked sample was included to ensure that the assay is able to measure and deplete the most common isoforms of cTnI complex found in both healthy and diseased individuals. Samples were depleted using antibody-labeled magnetic beads. Due to the well known diversity of cTnI complexes found in individuals, two or three different antibodies with diverse epitopes were used per depletion condition shown. Controls for the experiment included untreated sample as well as samples depleted with mouse IgG conjugated magnetic beads. Measured levels of analyte were similar for the undepleted sample and the mouse IgG incubated samples (data not shown). All of the apparently healthy donor samples are measurable above the LOD in each assay. Depletion efficiency was variable for different conditions tested but several conditions showed complete depletion of cTnI from samples. The variability in depletion efficiency is most likely due to the complex mixture of cTnI complex in samples and the various epitopes associated with the antibodies selected for depletion.

These data coupled with acceptable spike recovery and dilution linearity data below indicate that the assay measures the true analyte, not an artifact present in the sample matrix.

7 Spike Recovery & Dilution Linearity

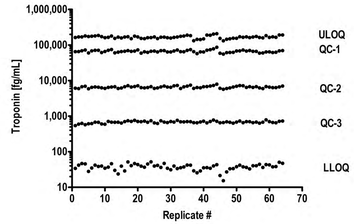
25 serum, 9 EDTA plasma, and 14 heparin plasma samples from apparently healthy donors were spiked with three levels of troponin ITC complex purified from human heart tissue. Average spike recovery values are well within the acceptable range (80-120%). However, some of these samples showed low spike recovery. It is known that some individuals have anti-troponin antibodies (Vylegzhanina 2017, *Clin Chem* 63: 343), which could explain the low spike recovery. Results are shown at left, below.

29 apparently healthy serum samples spiked with cardiac troponin ITC complex were used to determine dilution linearity from 2-, 4-, and 8-fold dilutions. 97% of the samples tested showed acceptable dilution linearity. Results are shown at right, below.

	Serum	EDTA Plasma	Heparin Plasma
Spike Level (fg/mL)	Average % Recovery	Average % Recovery	Average % Recovery
7,678	87	89	83
3,839	85	88	78
548	91	90	80

	Serum
Fold Dilution	Average % Recovery
2	100
4	98
8	101

8 Reproducibility

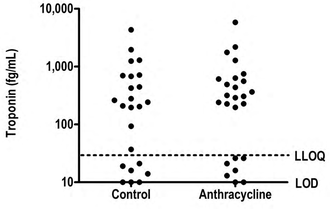


	Troponin (fg/mL)	%CV (n=64)
QC-1 (High)	50,000	7.9
QC-2 (Mid)	6,000	6.7
QC-3 (Low)	700	7.0
ULOQ	160,000	9.1
LLOQ	31	19

Control samples were run by two operators at multiple levels in replicates of eight per plate, on a total of eight 96-well plates over two days. ULOQ (the highest concentration quantifiable) and LLOQ (the lowest concentration quantifiable) had CV <20% and concentration recovery within 80-120%.

The graph and the table show results for three QC samples and the ULOQ and LLOQ samples.

9 Anthracycline-treated Individuals



Cardiovascular toxicity is a potential complication of multiple anticancer therapies. Some drugs, such as anthracyclines, have been implicated in irreversible cardiac dysfunction. Remnant serum samples from 20 breast cancer and 5 lymphoma individuals who had been treated with anthracycline were obtained from commercial sample vendors. Treatment dose and time from treatment to blood draw information was not provided to us. Control samples in this graph are serum samples from presumably healthy individuals without known anthracycline treatment. Samples were not matched for age, gender, or other factors. Samples with concentrations below the LOD (10 fg/mL) are shown as 10 fg/mL.

In this sample set, there was no significant effect of anthracycline treatment on serum troponin concentration.

10 Conclusion

We have developed a highly specific and sensitive cardiac troponin I assay that is capable of accurately measuring the cardiac troponin I protein concentration in samples from individuals with known cardiovascular disease, as well as 90% of the apparently healthy individuals tested. The results from this study do not support anthracycline treatment causing a significant shift in serum troponin concentration; further experiments need to be performed to be conclusive. The data demonstrate that the developed assay can be used to study low levels of cardiac troponin I in serum and plasma samples and may be a useful tool for measuring cardiac troponin I, a biomarker linked to cardiac damage, during clinical research.

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