Application Note

OES™ Quantification of cTnI in Whole Serum

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Introduction

Acute myocardial infarction (AMI) affects approximately 1.1 million people in the United States, with a 30% mortality rate. A specific protein marker found in cardiac muscle, cardiac troponin I (cTnI), has shown its superior and essential role for early diagnosis of acute myocardial infarction, as well as for post-infarction risk assessment. The amount of cTnI in blood initially rises within 2-3 hours after the onset of chest discomfort and remains elevated for 7-14 days. Troponin levels are positively correlated with the extent of heart muscle damage. A rapid and high-sensitivity cTnI assay allows detection of even trivial myocardial damage, and therefore provides physicians with the necessary information to administer critical care at an earlier time.

LamdaGen has developed a highly sensitive cTnI assay. The assay is described as an Optical Enhancement System™ (OES™)-based immunoassay – also known as a plasmonic ELISA.

This high-sensitivity cTnI plasmonic ELISA utilizes sandwich immunoassay-coupled Localized Surface Plasmon Resonance (LSPR) technology to precisely quantitate the concentration of cTnI in human whole blood, plasma and serum specimens. The enhanced sensitivity of the OES plasmonic ELISA is achieved through an enzyme-catalyzed precipitation reaction occurring on the LSPR sensor surface following specific intermolecular binding. Subsequent deposition of these precipitates on the LSPR surface leads to pronounced changes in sensor color and absorption spectra in a dose-dependent manner. The analyte quantitation is highly reproducible. Signal development typically takes three to ten minutes. The color change is permanent, and the sensors can be dried and archived for future reference.

Materials and Methods

<u>LamdaGen's Plasmonic ELISA</u>

LamdaGen's proprietary localized surface plasmon resonance (LSPR) biosensor chips were first functionalized with a thiol-based self-assembled monolayer (SAM). The capture antibodies, mouse monoclonal anti-cTnI antibodies (clone 801; HyTest, Finland) were then immobilized on the sensor surface following standard EDC/NHS activation. After a thorough wash and blocking process, the LSPR sensors were treated with HyTest's troponin I-T-C Complex at the desired final concentration by diluting a stock solution with fetal bovine serum (FBS)-containing LamdaGen's analyte buffer. 10ng/ml BSA in FBS-containing analyte buffer was included as a negative control. After incubation, the detection antibodies, biotinylated mouse monoclonal anti-cTnI antibodies (clone 19C7, HyTest, Finland) and streptavidin-alkaline phosphatase conjugates were added sequentially following the standard ELISA approach.

OES™ Reaction (signal amplification)

After washing the sensors twice with Tris buffer (20mM Tris pH7.5, 150mM NaCl), NBT/BCIP was added to each spot of the LSPR biosensor. After 3 mins, the reaction was stopped by rinsing the sensor three times with deionized water.

Plasmons were measured before and after the OES reaction (Fig. 1) for each active site (sensor spot) using a LamdaGen LightPath S4 instrument.

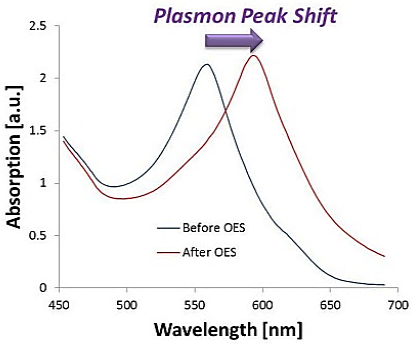

Figure 1. Plasmons are measured before (blue trace) and after (red trace) the OES reaction.

Results and Conclusions

Figure 2 shows the results for the detection of cTnI spiked into 87% whole serum using LamdaGen's plasmonic ELISA. The limit of detection defined as the lowest concentration of analyte that yields a reliable plasmon response, was 0.64pg/ml. The ultra-sensitivity of LamdaGen's OES-based cTnI test is attributed to its LSPR technology platform.

The proprietary method described is easily adapted for the detection of many other clinical biomarkers for early and rapid diagnosis.

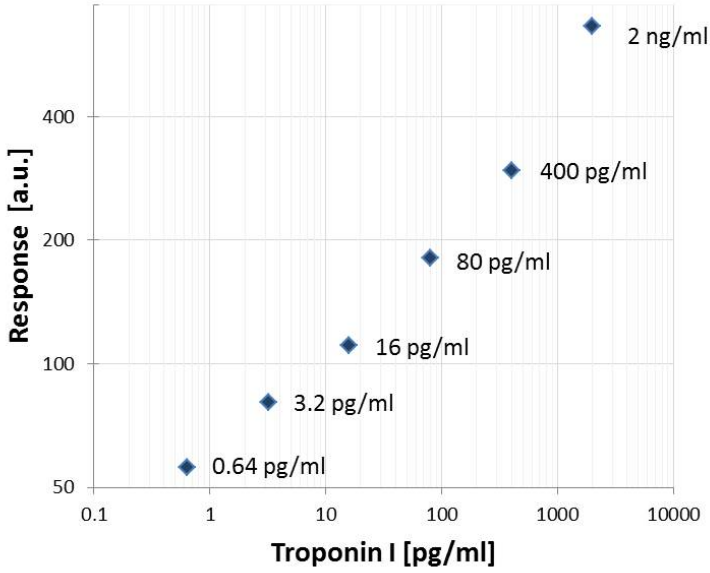

Figure 2. Highly sensitive quantitation of cTnI in 87% FBS with LamdaGen's plasmonic ELISA.

The response is expressed as the plasmon changes in LSPR absorbance spectra with respect to the negative control sample monitored in the spectral range between 450nm and 670nm. Dose response curve was obtained by spiking cTnI into 87% fetal bovine serum. Each data point represents the average of five independent measurements.

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