Simultaneous detection of cardiac troponin T forms in the diagnosis of myocardial infarction

Alvar Rosenqvist, M.Sc. (Tech.) Selma Salonen and Ph.D. Satu Lahtinen
Department of Life Technologies, University of Turku

BIOTECHNOLOGY (tech.)

UNIVERSITY OF TURKU

Introduction

Cardiac troponin T and I (cTnT and cTnI) are commonly used biomarkers in myocardial infarction diagnostics as they play a role in the regulation of muscle contraction. As a result of cell damage, troponins are released into the bloodstream, where they begin to break down into smaller fragments over time (fig. 1). Current cTnT tests recognize most of these fragments but can't tell, if the troponin T is fragmented or not.

The troponin concentration can be elevated due to other diseases or health conditions, which makes it more difficult to diagnose a myocardial infarction. cTnT released in a myocardial infarction is first in an intact or slightly fragmented form, which is why the identification of different forms of cTnT would improve the diagnostic usability of the test.[1]

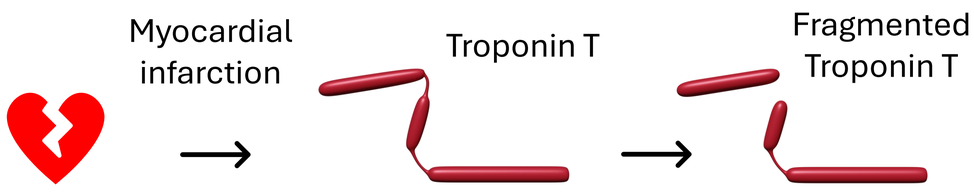

Figure 1. The release and fragmentation of cardiac troponin T.

Materials and Methods

The simultaneous detection of different forms of cTnT was implemented by using two antibodies conjugated to different labels. One of them recognized only the long form of cTnT and the other also the fragmented forms. The capture antibody applied to the well was able to bind most of the common forms of troponin T (fig. 2).

Upconverting nanoparticles (UCNP) were used as labels. The emission light they produce is at a shorter wavelength than the excitation light, which enables a low background signal level, because the autofluorescence is at a longer wavelength than the measured excitation light. The emission light produced by UCNPs can be altered by using different lanthanoids.[2] This enabled the utilization of UCNP labels as colored markers in a multiplex assay.

The signal was measured with microplate reader at 470 and 550 nm wavelengths respectively for the emission spectrum of the labels. 980 nm laser was used for the excitation and the signal was calculated from average of nine points.

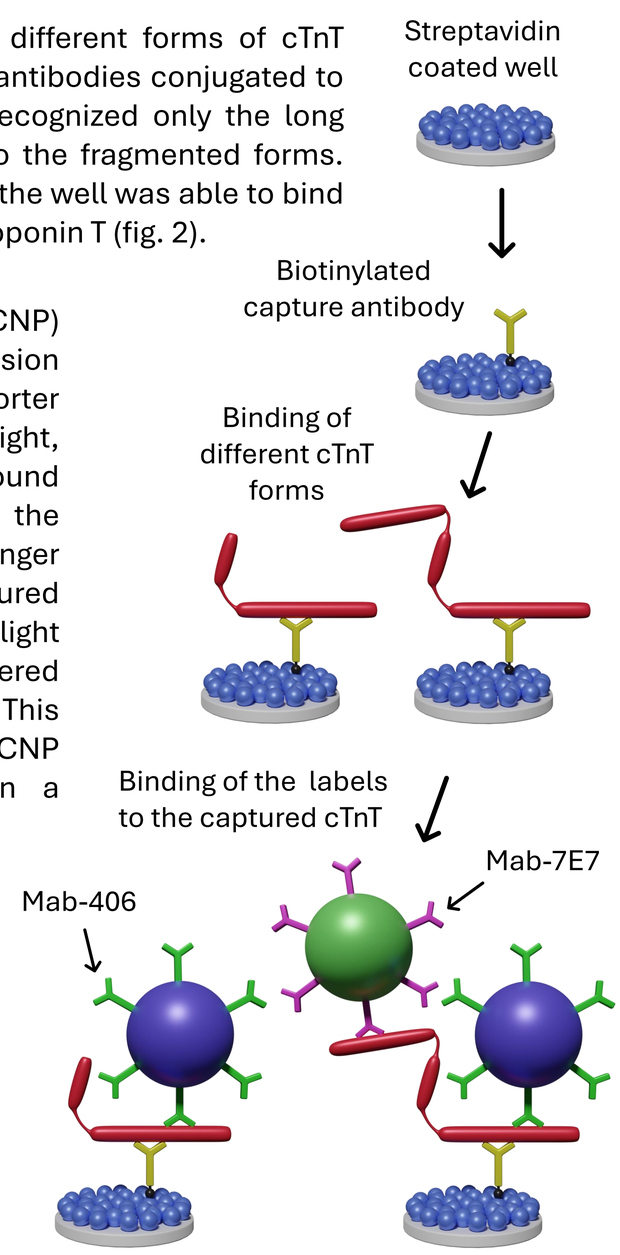

Figure 2. The structure of immunoassay.

Results

The signals dropped approximately 30% when both UCNP-labels were used compared to a single label assay (fig. 3).

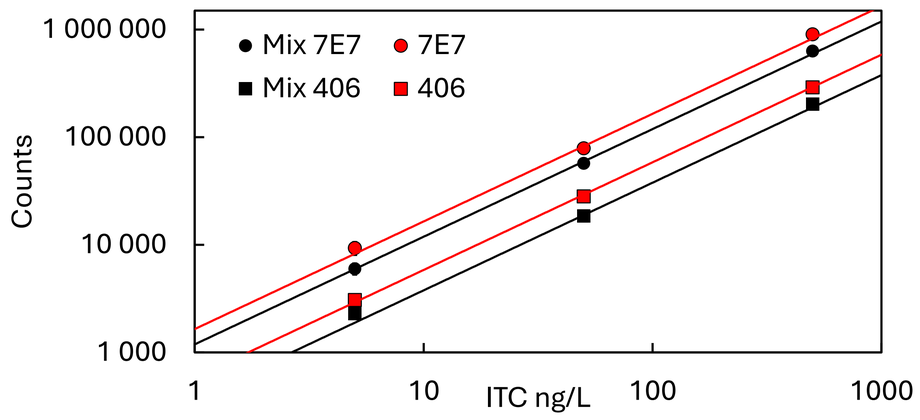

Figure 3. Comparison of signal generation in single label and multi label assays. The antibody 406 recognizes most cTnT forms and the 7E7 only the long form. Black lines (Mix) portray the signal when both labels were present in the well. Red lines portray the signal when only one of the labels was used.

There were some problems when the assay performance was tested on plasma samples collected from healthy individuals (fig. 4). One of the samples had extremely high measured concentration of long troponin T even though the measured total troponin T was similar to other samples. Because of this the calculated limit of detection was 23,91 ng/L for the long cTnT and 4,75 ng/L for the total troponin T.

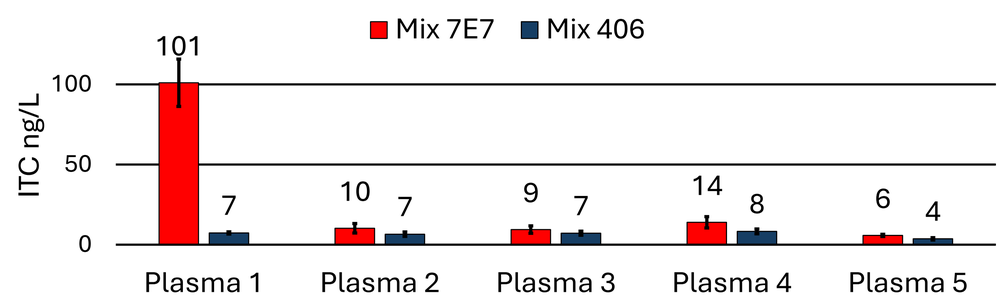

Figure 4. The assay performance on plasma samples. The blood was collected from five healthy individuals. Twelve replicates were used.

The nonspecific signal was present both in multiplexed assays and single label assays.

Conclusions

The use of two UCNP -labels seems like a promising way to detect and measure the concentrations of different cTnT forms in a multiplexed assay, but there are still issues with high nonspecific signals in the plasma samples.

The quality of conjugation and coating of the UCNP labels plays a crucial role in the assay performance and could be studied more in the future.

References

[1]Katrukha, I. A. & Katrukha, A. G. (2021) Myocardial Injury and the Release of Troponins I and T in the Blood of Patients. Clin Chem 67:124–130.
[2]Kale, V., Päkkilä, H., Vainio, J., Ahomaa, A., Sirkka, N., Lyytikäinen, A., … Soukka, T. (2016) Spectrally and Spatially Multiplexed Serological Array-in-Well Assay Utilizing Two-Color Upconversion Luminescence Imaging. Anal Chem 88:4470–4477.