

Fluorescent Immunoassays for Cardiac Troponin

Subjects: Cardiac & Cardiovascular Systems

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Troponin is a complex of three proteins that regulate the contraction of skeletal and cardiac muscles. The measurement of cardiac troponin levels in patient's blood has become the “gold standard” indicator of myocardial infarction (MI) (“heart attack”). The measurements are conducted using an immunoassay that specifically and selectively detects troponin through antibody-protein binding and emits through a fluorescent output signal for quantification.

Keywords: cardiac troponin ; myocardial infarction ; immunoassays ; fluorescence ; biosensors ; cardiovascular diseases

1. Overview

Cardiovascular diseases (CVDs) are considered one of the major causes of human death globally. Myocardial infarction (MI), characterized by a diminished flow of blood to the heart, presents the highest rate of morbidity and mortality among all other cardiovascular diseases. These fatal effects have triggered the need for early diagnosis of appropriate biomarkers so that countermeasures can be taken. Cardiac troponin, the central key element of muscle regulation and contraction, is the most specific biomarker for cardiac injury and is considered the “gold standard”. Due to its high specificity, the measurement of cardiac troponin levels has become the predominant indicator of MI. Various forms of diagnostic methods have been developed so far, including chemiluminescence, fluorescence immunoassay, enzyme-linked immunosorbent assay, surface plasmon resonance, electrical detection, and colorimetric protein assays. However, fluorescence-based immunoassays are considered fast, accurate and most sensitive of all in the determination of cardiac troponin levels post MI.

2. Cardiac Troponin—A Biomarker for Myocardial Infarction

Troponin is a complex of three proteins (**Table 1**) that regulate the contraction of skeletal and cardiac muscles; (i) Troponin I (TnI) inhibits the ATPase activity and binds to actin, (ii) Troponin C (TnC) binds to calcium ions and induce conformational changes in TnI, and (iii) Troponin T (TnT) attaches to tropomyosin forming Tn-tropomyosin complex [5,6]. The cardiac forms of TnI and TnT are significantly different from those of the skeletal muscles. In the event of MI, cTnI and cTnT are released from the injured heart muscles into the bloodstream and their concentration remains high over several days, even when no other symptoms of MI are present [7]. The normal concentration of cTnI is 1–2 ng/mL or less; after the onset of MI, the concentration of cTnI increases to about 50 ng/mL within 3–6 h reaching as high as 500 ng/mL [4]. Precise quantification of cTn levels in a patient's blood following ischemia/chest pain may indicate whether the patient had a myocardial infarction or not [8].

Table 1. Cardiac Troponin (cTn) subunits/role [9,10].

cTn Subunits	Molecular Weight and Size	Functions
cTnI	24 kDa, 210-residue long protein	Actomyosin ATPase inhibitory subunit
cTnT	36 kDa, 288-residue long protein	Tropomyosin binding subunit
cTnC	18 kDa, a 161-residue long protein with two globular domains	Ca ²⁺ binding subunit and an anchoring protein of Tn complex

4. Immunoassays for cTnI Detection

There are different strategies used for the development of immunoassays, however, “sandwich” immunoassay is one of the most reported assay designs for detection of cTnI in the last few years. This assay design is based on capturing the targeted antigen between a “capture” antibody and a “detecting” antibody where each binds at a different and distant

epitope. The capturing antibody is usually immobilized on a surface, which can be a microplate well, a paper surface (cellulose or nitrocellulose), a nanoparticle, or an electrode. The immobilization process provides a handle to separate the targeted antigen from the other components of the sample, which minimizes interference. The detection antibody is then introduced to bind to the antigen through its exposed molecular surface (epitope). The detecting antibody is associated with the signaling mechanism and signal amplification of the detection process. Different signaling mechanisms were reported and these include optical (colorimetric, fluorescence, emission), electrochemical, and magnetic signals, in addition to radioactivity and surface plasmon resonance (SPR) [4,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27]. Signal amplification, however, is the process by which each antigen-binding (detection) event is translated into a large number of “signaling” molecules or probes to produce a read-out signal.

5. Conclusions

Fluorescent-based sandwich immunoassays are effective and sensitive for quantification of cTnI over several platforms. The heterogeneous matrix in these assays provides a platform for minimizing interference by other biological molecules in the samples, yet it may increase the complexity and length of the assay. On the other hand, the increase in sensitivity and the decrease in limit of detection (LOD) were accompanied for most cases with a linear dynamic range (LDR) which falls below the relevant clinical concentrations of cTnI in patients' blood. This brings another challenge to the ability to apply these assays in point-of-care (PoC) testing or resource-scarce settings, as it requires sample preparations and dilutions. Moreover, most of the reported assays require several steps of solution addition and washing, which increases the time needed to complete the assays.^[1]

The assays with direct fluorescent labels provide a quick detection assay; these are not very sensitive but their narrow LDRs fall within the clinical concentrations. The LOD decreases when a package of fluorescent labels is applied, yet the LDR generally falls into the sub-nano concentrations. On the other hand, the enzyme-linked assays are the most familiar assays and they generally provide an LDR within the needed range but these require time-control to avoid overestimation and false positives. Thus, the development of a quick and sensitive immunoassay for cTnI remains the future goal for many groups and research activities. There is a need to develop assays that are sensitive, specific and work at a wide dynamic range that covers the possible concentration of cTnI in patients' blood with possible MI (5–500 ng/mL). It is important that the assay works with direct blood samples without the need for sample processing and can be applied in PoC settings. The latter requires that the assay can be completed within a short time (minutes rather than hours) and with minimal use of lab equipment.

References

1. Remya Radha; Syeda Shahzadi; Mohammad Al-Sayah; Fluorescent Immunoassays for Detection and Quantification of Cardiac Troponin I: A Short Review. *Molecules* **2021**, *26*, 4812, [10.3390/molecules26164812](https://doi.org/10.3390/molecules26164812).

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