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# Rapid, high sensitivity, point-of-care test for cardiac troponin based on optomagnetic biosensor

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ABSTRACT

*Background:* We present a prototype handheld device based on a newly developed optomagnetic technology for the sensitive detection of cardiac troponin I (cTnI) in a finger-prick blood sample with a turnaround time of 5 min.
*Methods:* The test was completed in a compact plastic disposable with on-board dry reagents and superparamagnetic nanoparticles. In our one-step assay, all reaction processes were precisely controlled using electromagnets positioned above and below the disposable. Nanoparticle labels (500 nm) bound to the sensor surface via a sandwich immunoassay were detected using the optical technique of frustrated total internal reflection.
*Results:* A calibration function measured in plasma demonstrates a limit of detection (mean of blank plus 3-fold the standard deviation) of 0.03 ng/mL cTnI. A linear regression analysis of the region 0.03–6.5 ng/mL yields a slope of $37 \pm 4$, and a linear correlation coefficient of $R^2 = 0.98$. The measuring range could be extended substantially to 100 ng/mL by simultaneously imaging a second spot with a lower antibody concentration.
*Conclusions:* The combination of magnetic particles and their fine actuation with electromagnets permits the rapid and sensitive detection of cTnI. Because of the potential high analytical performance and ease-of-use of the test, it is well suited for demanding point-of-care diagnostic applications.


## 1. Introduction

The development of technologies that deliver high performance in a format suitable for the point-of-care is a difficult challenge. Not only must the analytical requirements such as high sensitivity and precision be fulfilled but the test must be fast, integrated for ease-of-use, robust, and low cost and preferably be in a portable or handheld form [1,2]. Magnetic nanoparticles are often used to increase the speed and sensitivity of analytical quantifications by being a carrier for reagent or analyte concentration [3,4]. They can also be employed as particulate labels in immunoassays and nucleic acid testing [5–8]. The ability to finely manipulate magnetic nanoparticles with a magnetic field [5,6,9,10] makes them suitable for enhancing the speed and for the integration of various assay processes in a high performance test for the point-of-care. We have recently reported an optomagnetic technology that combines the precise control of the motion of magnetic particles with electromagnets and their sensitive detection on a surface using f-TIR imaging [11]. The optomagnetic biosensor platform offers speed, sensitivity and a high degree of analyte multiplexing. The technology can potentially be used in conjunction with any label-based affinity assay for the detection of a wide range of analytes including proteins, small molecules and nucleic acids. Its compact, robust form along with the use of a low cost, mass-manufacturable plastic disposable is very advantageous for the POC environment.

An example of a high performance assay that is desirable to have in a POC format, is the testing for cardiac markers in the diagnosis of acute coronary syndrome due to the time critical nature of the disease [12]. Cardiac troponin I and T (cTnI, cTnT) are markers highly specific for myocardial damage. They can be used alone or in combination with other cardiac markers (e.g. CK-MB, myoglobin) to positively diagnose an acute myocardial infarction [13]. As the recommended clinical cutoff for troponin is the 99th percentile (0.01 ng/mL concentration range) [14], assays for this marker must be highly sensitive and precise. There is a variety of detection technologies utilized in POC devices, including immunochromatographic assays (employing for example, Au nanoparticles or fluorescent molecules/

*Abbreviations:* f-TIR, frustrated total internal reflection; POC, point-of-care; cTnI, cTnT, cardiac troponin I and T; MES, 2-(N-morpholino)ethanesulfonic acid; EDC, N-3-dimethylaminopropyl-N-ethylcarbodimide hydrochloride.

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particles as labels) [15–17], and enzyme-linked immunosorbant assays (with electrochemical or optical detection) [18]. However, very few can offer sensitive and precise troponin testing in a handheld or portable format. The majority of POC tests for cardiac markers are desktop devices that require either long turnaround times (>10 min), large sample volumes (>100 μL) or sample pre-treatment, all of which significantly decrease the ease-of-use of the device [19]. Of the existing POC handheld technologies, even fewer are able to rapidly deliver a sensitive and precise troponin result from a finger-prick blood volume [20,21].

We have developed a sensitive, 5-minute prototype POC test for cTnI using the optomagnetic biosensor. The test is a one-step sandwich immunoassay performed in a stationary liquid in which all assay processes are integrated by the use of magnetic forces acting on magnetic nanoparticle labels (Fig. 1). In the first phase of the assay, nanoparticles highly loaded with antibody move through the solution for effective troponin molecule capture. Subsequently actuating magnets are engaged to move and transport the particles with high speed to the sensor surface for binding. Thereafter, a sequence of finely tuned magnetic pulses is applied to facilitate optimal binding and mixing of the nanoparticles containing cTnI molecules at the antibody-functionalized surface. After the particles react with the sensor surface, free and non-specifically bound particles are rapidly removed with a magnetic wash by applying a magnetic field oriented away from the detection surface. Seamless integration of the assay steps facilitate the design of a simple, single-chamber cartridge, in which dry reagents, including magnetic particles are deposited. The absence of a fluidic wash or fluidic handling enables the use of very small assay volumes, on the order of 1 μL, which easily accommodates finger-prick blood samples. The results described provide a proof-of-principle of the analytical performance achievable using the opto-magnetic technology and offer a basis for further optimizations necessary to achieve the same challenging precision requirements demanded of laboratory cTnI tests.

## 2. Materials and methods

### 2.1. Reagents

All buffer materials unless otherwise stated were supplied by Sigma Aldrich Corporation. Superparamagnetic particles functionalized with carboxylic acid groups (MasterBeads 500 nm diameter) were purchased from Ademtech. A number of antibody pairs recognizing various epitopes on cTnI were screened and found to be very effective for the detection of cTnI using the optomagnetic biosensor technology. In this work we focus on results from the pair consisting of the monoclonal antibody A34780359P (BiosPacific Inc.) as the tracer coupled to the particles, and goat polyclonal (Hytest Ltd) as the capture antibody immobilized on the sensor surface. Calibrators were prepared from human troponin ITC complex (Hytest, reference SRM(r)2921) by diluting either in pure human citrate plasma pool from 20 apparently healthy donors or in EDTA whole blood from single healthy donors. Concentration values stated in this work were based on serial dilutions made directly in citrate plasma or EDTA whole blood. In experiments with whole blood, the samples were used within 12 h of collection to avoid effects from degradation.

### 2.2. Optomagnetic biosensor platform

Fig. 2A shows a schematic of the optomagnetic analyzer/reader consisting of the f-TIR detection optics and the actuating electromagnetic coils [11]. These were designed for and incorporated into a handheld format, a fully functional experimental version of which is displayed in Fig. 2B. For the data presented in this report, an open laboratory setup, interfaced with a personal computer, was used in order to collect more detailed data and to have more setup modification flexibility.

The analyzer was used in combination with an injection-molded disposable cartridge containing a reaction chamber for the assay (Fig. 2C). The bottom surface of the reaction chamber was functionalized with capture antibody and served as the sensing surface. Magnetic nanoparticles at the sensor surface were detected using the optical principle of frustrated total internal reflection (f-TIR) [11,22,23]. Systems for f-TIR imaging are well suited for near-patient applications because they take advantage of the superior performance of optical detection (high sensitivity, multiplexing, insensitivity to magnetic fields and chemical interference) while being robust, low cost and readily integrated into a portable format. The signal at time $t$ was calculated for each spot, averaging over an area of approximately $100\times100$ μm, using the formula $\text{Signal}(t)=[R(0)-R(t)]/R(0)]$, where $R(0)$ is the reflected light intensity in the absence of magnetic particles at the sensor surface and $R(t)$ is the reflected light intensity at time $t$ during the assay. The end point signal for an assay was determined from the difference between the signal upon sample introduction into the chamber and signal after the magnetic wash (see section III of Fig. 3A). The current optics in our f-TIR setup enables microarrays consisting of more than 30 distinct spots (~100 μm diameter) to be simultaneously imaged. In Fig. 2D, the f-TIR image of an array consisting of four spots is shown. By printing different capture antibodies on the spots and using a mixture of magnetic particles with the corresponding tracer antibodies, it is possible to multiplex a high number of different analytes.

The actuating electromagnets consist of two magnetic systems, a top coil 2 mm above the sensor surface and a bottom magnet system positioned 1 mm below the sensing surface. The magnetic flux was

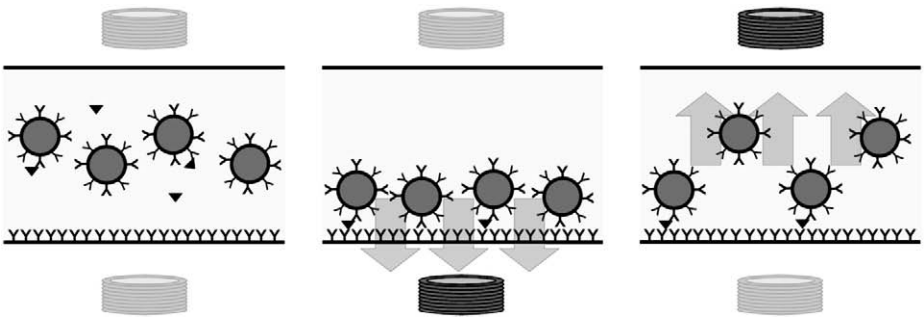

**Fig. 1.** Depiction of the reaction chamber and actuation magnets showing successively the assay processes: analyte binding by antibody-functionalized nanoparticles (top and bottom magnets off), nanoparticle binding to the sensor surface and magnetic removal of free and weakly bound nanoparticles.