Sensitive detection of cTnI in whole blood on MagArray biosensors

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MagArray

"Enabling Better Outcomes through Better Diagnostics"

Abstract

Background: Separation of plasma or serum from the whole blood is often essential for the detection of protein biomarkers on various platforms. Here we report the direct detection of cTnI in whole blood on MagArray platform with no extra steps of processing the whole blood samples. We attribute this unique capability to the fundamental detection mechanism of MagArray platform, by which magnetic signals are generated and detected using magnetic nanotags. In contrast to systems based on optical signals, magnetic signals are not affected by the common optical interference in complex matrices. The detection of cTnI in whole blood samples demonstrates MagArray's biosensors are well suited for complex biological matrices such as whole blood samples.

Objective: A feasibility study of direct detection of protein biomarkers in whole blood without the extra steps of sample processing, to demonstrate a wash-free cTnI assay.

Methods: Antibody pairs for cTnI assay were screened and selected on MagArray platform, and the assay was first developed using purified cTnI in buffers. The assay is then used for the detection of purified cTnI added to whole blood. The assay consists of sequential additions of sample and nanotag solution to the reaction well with no washing. The whole assay time is 12 minutes. Standard curves of cTnI in both pure buffer and whole blood were established and compared. Protein interference from hemoglobin, albumin, and IgG spiked into whole blood was also investigated.

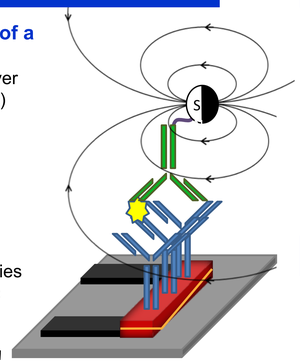
Results: The detection sensitivity of cTnI in whole blood without any sample processing on MagArray platform is close to 10pg/ml. As a comparison, the sensitivity of cTnI detection is approaching 1pg/ml in pure buffer. We assume this is due to the higher viscosity of whole blood samples that slow down the binding rates of analyte and detection antibody. Also, the assay is found to be relatively insensitive of interference from IgG and albumin. High concentration of hemoglobin (40%wt) led to lower signals which again were likely caused by the higher viscosity of the hemoglobin spiked samples.

Conclusions: MagArray platform provides a unique opportunity of detecting proteins in whole blood. Since no extra step is required to process whole blood samples, the complexity of the assay format is greatly reduced. The detection of magnetic signals, rather than optical signals, is a key benefit of the MagArray platform for protein detection in complex biological matrices.

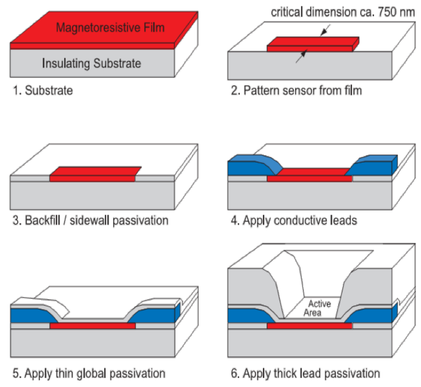
Introduction

1. Principle of Operation: Case of a sandwich immunoassay

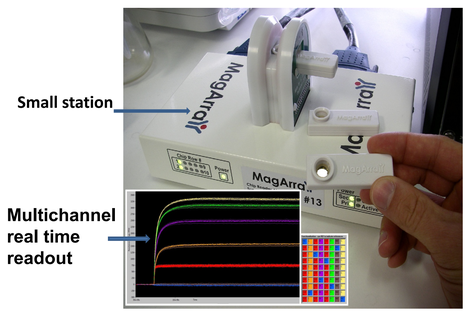
1) Spot a unique capture antibody over each sensor (80 sensors per chip)
2) Incubate with Fluid of Interest
   - Blood
   - Plasma
   - Serum
   - Urine
   - Saliva
   - Milk
3) Add biotinylated detection antibodies
4) Add streptavidin labeled magnetic nanoparticle tags
5) Detect magnetic signal - but only from surface-bound nanoparticles!



2. Fabrication of Chips – Simplified



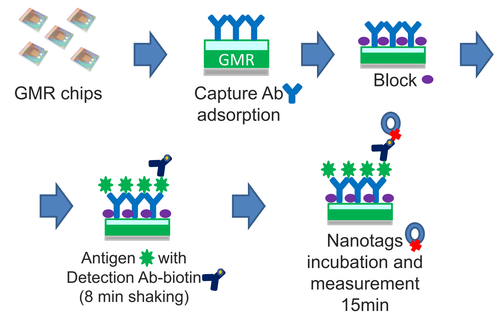
3. A Miniaturized Test Station



Methods

Sample measurements: detection antibodies were mixed with samples and the mixtures were added to the reaction wells on the chips and shaken for 8 min. Magnetic particles were then added to the chips for signal generation. The measurements were run for 15min in order to study the signal-time relationship. The whole assay involved no rinsing procedures and took less than 15 min in optimized assays.

Assay Format

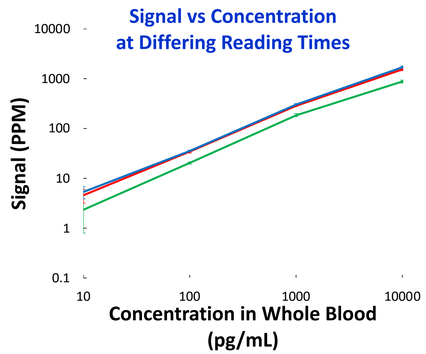


Results

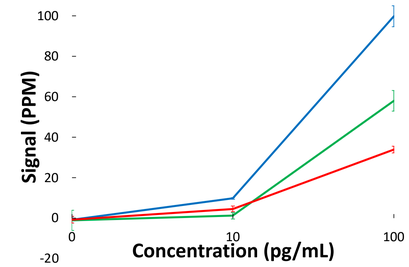
Sample measurements:

- detection antibodies were mixed with samples
- 8 min shaking
- magnetic nanotags directly added with no washing
- signal generation and recording

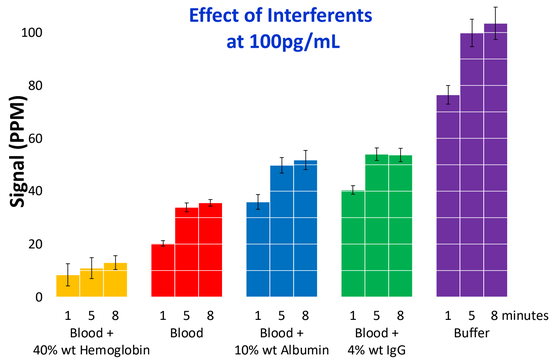
To determine the optimal measurement time, signals at different times were recorded and compared as shown here.



Effect of media on the assay performance: buffer, serum and whole blood: sensitivity of cTnI detection in different media is compared and shown here. For a 9 min assay, the sensitivity is between 10-100pg/ml for serum and whole blood, while the sensitivity is between 1-10pg/ml in PBS (with BSA). Since we are mostly interested in the assays using whole blood, we focused on the whole blood sample in interference studies.



Interference studies: hemoglobin, hematocrit, and antibodies we then studied the effects of common interferents in whole blood and compared with assays in buffer. Shown here are the signals of 100pg/ml cTnI in whole blood spiked with high concentration of different interferents. The same assays in pure buffer and pure blood are included for comparison. There was great effect from 40% hemoglobin, but only very little effect from 4% IgG or 10% albumin.



Conclusions

A wash-free assay for detecting cTnI in whole blood is developed on MagArray platform

Fast assay: 9-15 min. total assay time
Sensitivity 10-100pg/ml in whole blood
Convenience: quantifies cTnI in whole blood with no washing

MagArray biosensor is a simple, rapid platform for measuring protein biomarkers in complex biological matrices