A RAPIDLY RECONFIGURABLE, UNIVERSAL POINT-OF-CARE TEST PLATFORM

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ABSTRACT

This paper reports the development of a new rapidly reconfigurable, universal Point-of-Care Test (POCT) system based on Siloam's Optimiser microfluidic platform technology. The key benefit of this system is the ability to develop/validate assays using conventional lab-automation tools and rapidly adopting new assays for POCT use. A wide-dynamic range beta-HCG (total) 15 minute assay with operating range of 5 IU/L to 68,500 IU/L and a high-sensitive cardiac Troponin-I (HS cTnI) 20 minute assay with detection limit of 0.04 ng/mL are demonstrated as case studies for the Universal POCT system.

KEYWORDS: point-of-care, microfluidics, immunoassay, high-sensitivity

INTRODUCTION

We developed an open source POCT system, named TROVA™, based on our Optimiser™ microfluidic technology [1], as shown in figure 1. Unlike all other POCT systems that are specifically designed for a given assay (or a small group of assays), any functional microplate based assay can be adapted to the open-source POCT system. One key component of this system is the microfluidic test cartridge incorporating Optimiser microfluidic reaction cells and storage reservoirs. The POCT cartridge uses the same Optimiser reaction cell and assays developed on Optimiser microplate format can be directly transferred to POCT test cartridge. This is a key benefit for the Optimiser technology based POCT system. We have demonstrated that assays can be developed on the Optimiser microplate and the exact same performance can be reproduced with the POCT test cartridge. This allows rapidly developing and validating assays on Optimiser microplates using conventional microplate automation tools, and then migrating the assay to the POCT test cartridge.

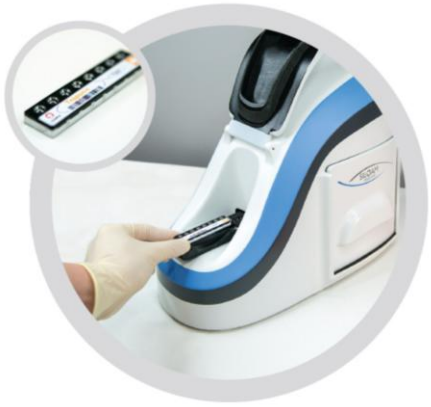

Figure 1: Photograph of POCT system

The POCT system uses 2 key components – a microfluidic test cartridge and a modular analyzer system [2]. The microfluidic test cartridge contains five Optimiser microfluidic reaction cells and three storage reservoirs, as shown in figure 2. The test cartridge is assembled with four components: an injection molded strip with microfluidic reaction cells and reservoirs, a patterned tape to seal the bottom of the strip, an absorbent pad and a basket to hold the absorbent pad. The strip is injection molded from clear polystyrene with micro-machined hardened steel molds. Test-specific assay reagents (antibodies/controls) are either pre-coated or stored in dry form on the cartridge. The design allows the test cartridge to be configured for multiple types of tests: for instance up to 4 analytes and control (cardiac biomarker panel) or for 2 analytes across a 5-log dynamic range (pregnancy/fertility panel). Again, the microfluidic reaction cell in the POCT test cartridge is exactly identical to the microfluidic reaction cell on the Optimiser 96-well microplate. The identical reaction cell ensures that the same performance can be achieved with the POCT system as that in Optimiser microfluidic platform.

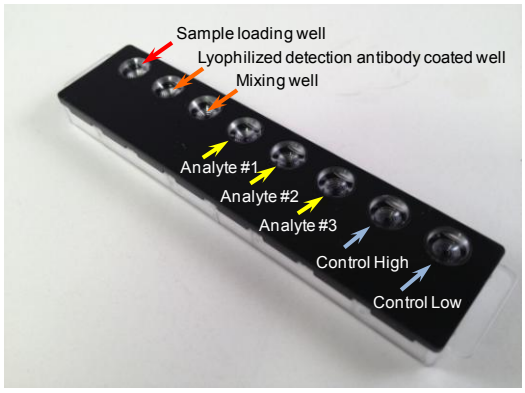

Figure 2: Photograph of Microfluidic test cartridge.

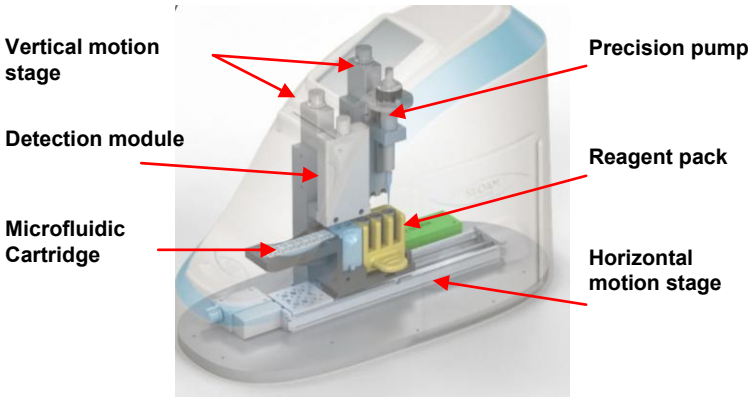

Figure 3: POCT system configuration

As illustrated in figure 3, the analyzer contains a precision liquid-handler, high-sensitivity chemiluminescence detection module and linear stages for cartridge positioning. There is an assay buffer pack in the analyzer which consists

of washing buffer, dilution buffer and substrate. Those buffers are designed to allow for a full day of operation and can be used for all types of assay. The POCT system requires minimum operation. The buffer reagent will be placed in the system at the beginning of the day. For each test, operator will place the test cartridge into the system, then dispense the sample into the corresponding well of the cartridge. After pressing the start button, the motion stage will pull the cartridge into the system. For a typical procedure, the pipettor will aspirate buffer from reagent pack, reconstitute the lyophilized antibody with the buffer, aspirate sample, mix sample with reconstituted antibody, dispense mixture into the microfluidic detection chamber, load washing buffer, and finally load substrate. Then the light intensity from the detection chamber is measured by detector module. The entire procedure will be done by the system automatically and the result will be presented in display at the end.

WIDE RANGE BETA-HCG TEST WITH POCT SYSTEM

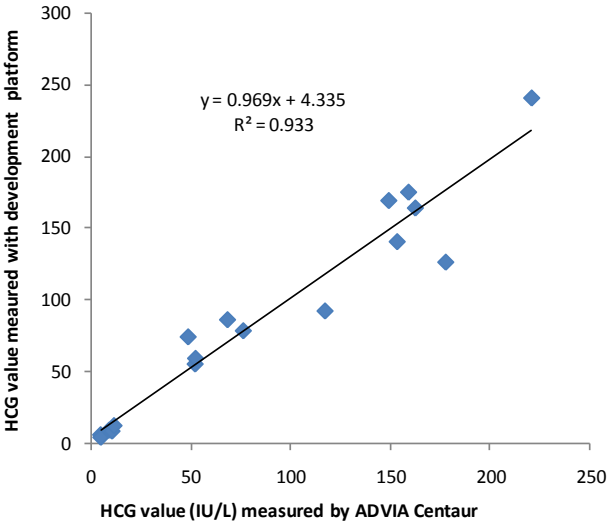

Figure 4: Correlation of measured HCG value in serum samples with on Optimiser microfluidic platform and Siemens ADVIA Centaur system.

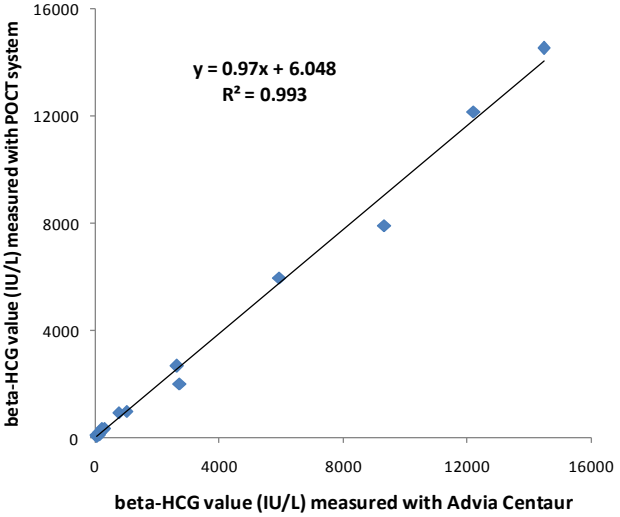

Figure 5: Correlation of measured HCG value in serum samples with POCT system and Siemens ADVIA Centaur system.

A wide dynamic range beta-HCG assay was developed for the POCT system. It has the range from 5 IU/L - 68,500 IU/L and total assay time of 16 min. The assay uses a test cartridge which contains pre-coated beta-HCG capture antibody and dried detection antibody, and on-board auto-dilution to extend dynamic range. Two microfluidic detection cell of the cartridge are pre-coated with capture antibody, blocked and dried. An on-board dilution procedure will be performed automatically in one of the reservoirs. Then the system will dispense undiluted sample & detection antibody mixture into one detection well and diluted mixture into the other detection well. As described above, the assay was first optimized on Optimiser platform and then transferred to POCT system. Figure 4 shows an optimized beta-HCG assay developed on the Optimiser microfluidic plate format and compared against Siemens ADVIA Centaur system. Seventeen patient samples have been measured. Excellent correlation and linearity were achieved (R^2 = 0.99, slope = 0.98). Then the assay was transferred to the POCT system based on the optimized conditions from Optimiser platform. Figure 5 shows results for the same assay on the POCT system with similar performance for plasma (R^2 = 0.99). The POCT system uses a stored calibration curve which explains the slight difference in results. The POCT system is also capable to handle whole blood sample. Eight spiked whole blood samples were measured with the POCT system. The results were compared with results from POCT assay with plasma collected from those spiked blood samples, as shown in figure 6. The results show good correlation (R^2 = 0.997) between them. The HCG value in collected plasma is higher than blood since there is only 55% plasma in blood. So the HCG value measured with whole blood can be reported as HCG value in plasma after multiplying 1.803.

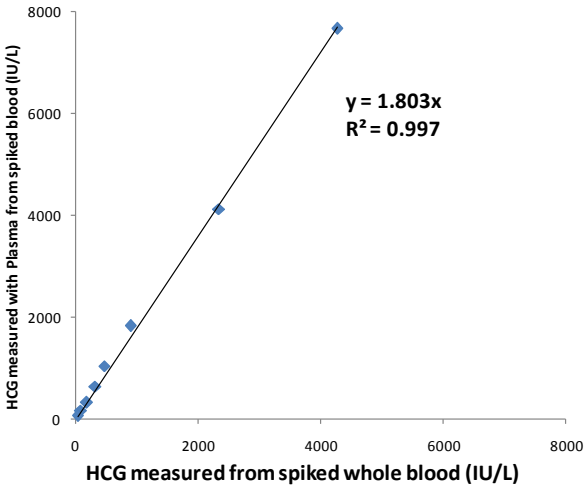

Figure 6: Correlation of measured HCG value in spiked whole blood and the plasma collected from whole blood with POCT system.

HIGH SENSITIVE C-TNI TEST WITH POCT SYSTEM

A high-sensitivity c-TnI assay was also developed using the repeat-load approach to boost sensitivity which was optimized using microplate format on lab-automation [3]. It has detection limit at 0.04 ng/mL and total assay time at 20 min. The test cartridge contains one microfluidic detection cell with pre-coated capture antibody and one reservoir with dried detection antibody. During the assay procedure, the system will repeat dispense 2 µL of sample in to the detection well for 10 times with 1 minute interval. The assay was first optimized on Optimiser platform and then transferred to POCT system. Figure 7 shows an optimized HS c-TnI assay developed on the Optimiser microfluidic plate format and compared against Siemens ADVIA Centaur system. Twelve patient samples have been measured. Excellent correlation and linearity were achieved (R^2 = 0.96). The assay protocol was then migrated to POCT with nearly identical performance and strong correlation to Siemens VISTA Dimension (R^2 = 0.97), as shown in figure 8.

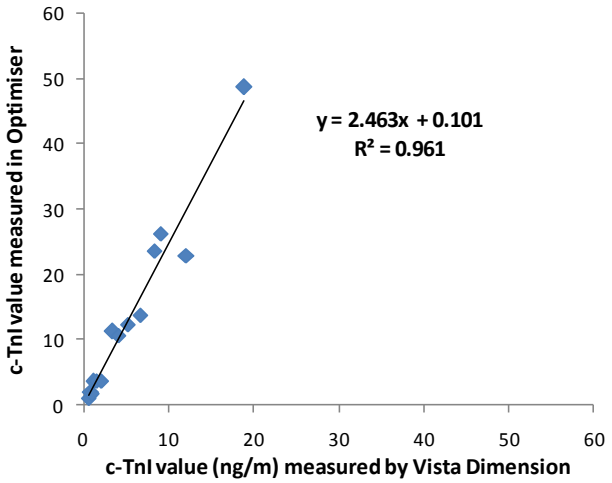

Figure 7: Correlation of measured c-TnI value on development platform (Optimiser microplate) and Vista Dimension system.

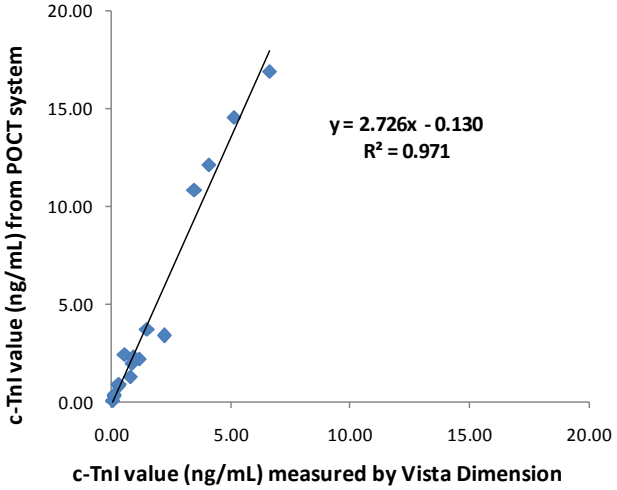

Figure 8: Correlation of measured c-TnI value in plasma samples with POCT system and Vista Dimension system.

CONCLUSION AND DISCUSSION

We have previously demonstrated over 70 assays on the Optimiser platform and it makes the TROVA POCT platform as a Universal Solution for POC test development. Furthermore, the capability of using a common platform allows for new POCT assays to be developed in <3 months with zero transfer risk as opposed to the 12-18 month of time for conventional POCT methods. These advantages are made possible by the core microfluidic technology and offer a new paradigm for POCT assay development.

ACKNOWLEDGEMENT

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