SINGLE-STEP, MULTI-PARAMETER MONITORING OF LIVER FUNCTION ON A PORTABLE CENTRIFUGAL ANALYZER

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ABSTRACT

This paper describes the first use of a compact, integrated and automated centrifugal microfluidic platform equipped with paired emitter detector diodes (PEDD) for carrying out a 5-parameter enzymatic liver assay panel with colorimetric end-point and kinetic detection (Fig. 1). Starting with single-step pipetting of finger prick blood, this "Lab-on-a-Disc" (LoaD) system [1, 2] controls multi-stage sample preparation and flow distribution into six outer reaction chambers by an array of dissolvable film (DF) valves [3]. The measurements on albumin (ALB), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and total (TBIL) bilirubin show good quantitative correlation with standard benchtop and hospital laboratory results.

KEYWORDS: Liver assay panel, portable, centrifugal analyzer, integrated microfluidic platform

INTRODUCTION

In resource-poor settings, reports suggest that a mortality of 2% – 28% can be linked with medication-induced liver damage, for example HIV and tuberculosis [1]. This has prompted local governments to fund routine monitoring of liver function in centralized laboratories; however, owing to a lack of transport logistics, accessibility of such centralized labs remains a significant challenge for the majority of patients. LoaD platforms have proven to facilitate integration and automation of laboratory unit operations [2-3]. Going well beyond our former version [4-5], we have now developed a compact, autonomous and portable LoaD platform for user-friendly, single-step sample-to-answer operation from a finger prick of blood by untrained staff at community health centers.

Figure 1. Image showing loading of blood sample and assay reagents on the portable LoaD system interfacing with an Android tablet. A customized program controls the time modulated spin speed of the disc as well as the wireless acquisition of real-time data. On completion of the full assay protocol, the LED-based system is then used for colorimetric detection of the assay panel.

HARDWARE AND DETECTION SYSTEM

Our previously reported system [5] required a series of cumbersome manual loading steps to distribute the sample into the (six) reaction channels and large benchtop device connected to a separate, stand-alone UV-Vis analyzer. In contrast, we now perform sample aliquoting on-disc and our new Centrifugal Microfluidics Analysis System (CMAS) is now battery powered and integrates a low-cost PEDD detector [6-7]. Using Bluetooth, an Android tablet wirelessly communicates with the device-based microcontroller through a custom designed interface which sets the spindle speed and acquires real-time data from the PEDD (Fig. 1). Emitter LEDs with wavelengths (λ_{max}) at 626 nm (ALB), 405 nm (ALP, GGT), 540 nm (DBIL) and 569 nm (TBIL) were employed; 660 nm LEDs were used for detection.

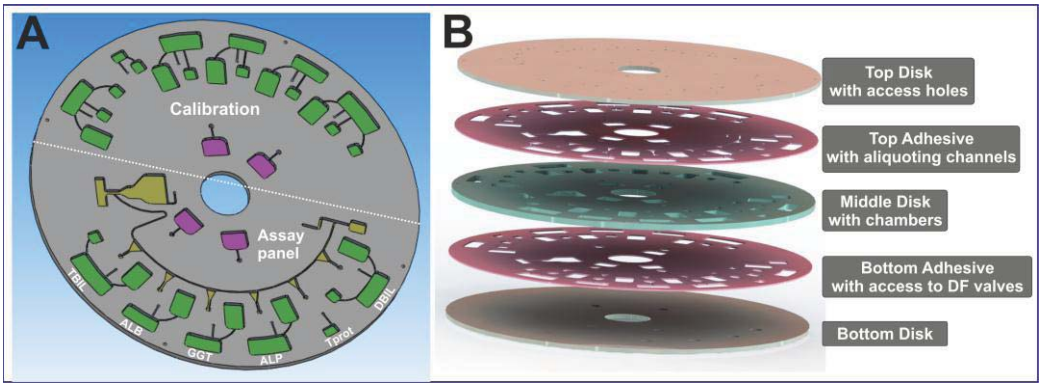

Figure 2. 3-dimensional representation of (A) LoaD platform for both, system calibration and the liver assay panel (LAP) and (B) 5-layer LoaD platform.

ASSAY INTEGRATION

Figure 2 shows the multi-layered LoaD platform comprising of two fluidically independent halves – one for calibration and one for running the assay panel. The unique design of the disc enabled the centrifugo-pneumatic dissolvable film valves [4] on the same radial position to be actuated simultaneously because the liquid reagents in those chambers experience the same centrifugally induced pressure head, P

$$P = \frac{1}{2}\rho\omega^2(r_2^2 - r_1^2) \qquad (1)$$

where ρ, is the density of the liquid, $\omega = 2\pi f$, is the angular frequency at the spin rate f, and r_1 and r_2 are the inner and outer radius of the liquid columns, respectively [8].

The illustration and frame sequence in Fig. 3 represent the steps of the full liver assay panel with ~150 µl blood sample, with other assay reagents pre-loaded on-disc.

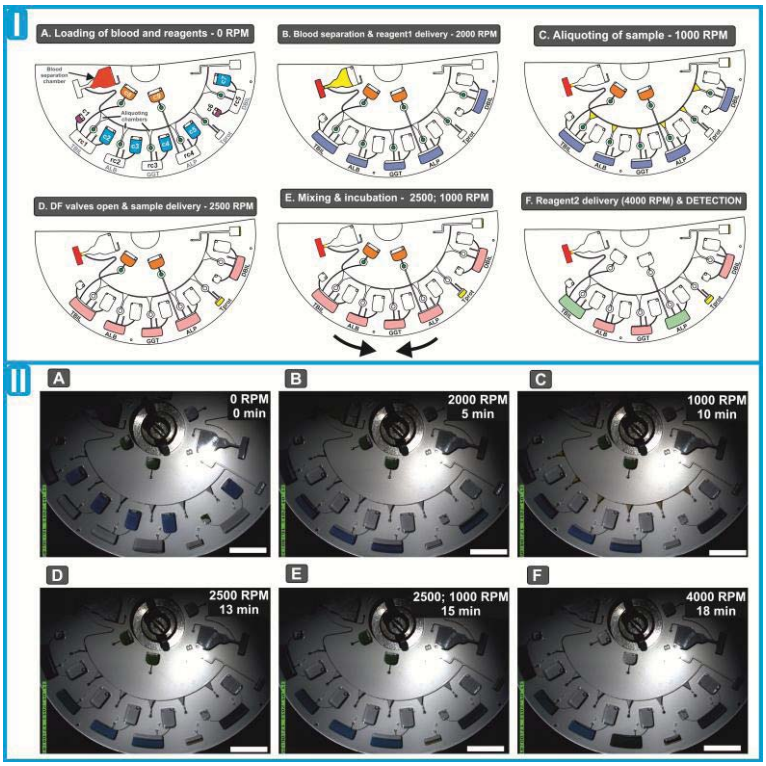

Figure 3: **(I)** Schematic of full fluidic sequence of the assay (A) Start – Loading of a single blood sample and all assay reagents in their respective chambers (B) 5 min – Blood separation and reagent delivery into the reaction chamber at 2000 rpm (C) 10 min – Siphon priming and aliquoting of extracted plasma into their respective sample collection chambers at 1000 rpm (D) 13 min – Actuation of DF valves and delivery of aliquoted samples to the reaction chambers by increasing the rotational frequency to 2500 rpm (E) 15 min – Mixing/incubation of the samples with reagents by alternating spinning at high (2500 rpm) and low (1000 rpm) frequencies (F) 17 min – Delivery of final assay reagents, final mixing and detection. **(II)** Frame sequence of the entire liquid handling operation as fully described in **(I)** above. These images were obtained using real blood samples and food dye to enhance contrast. Scale bar is 20 mm.

The corresponding calibration curves obtained from this system are shown in Fig. 4. The test results were benchmarked with a well plate reader (Tecan) and on intravenous blood sent on the same day to a hospital laboratory. The results (Table 1) show very good correlation with a CV of 7.9% averaged over the individual assay experiments.

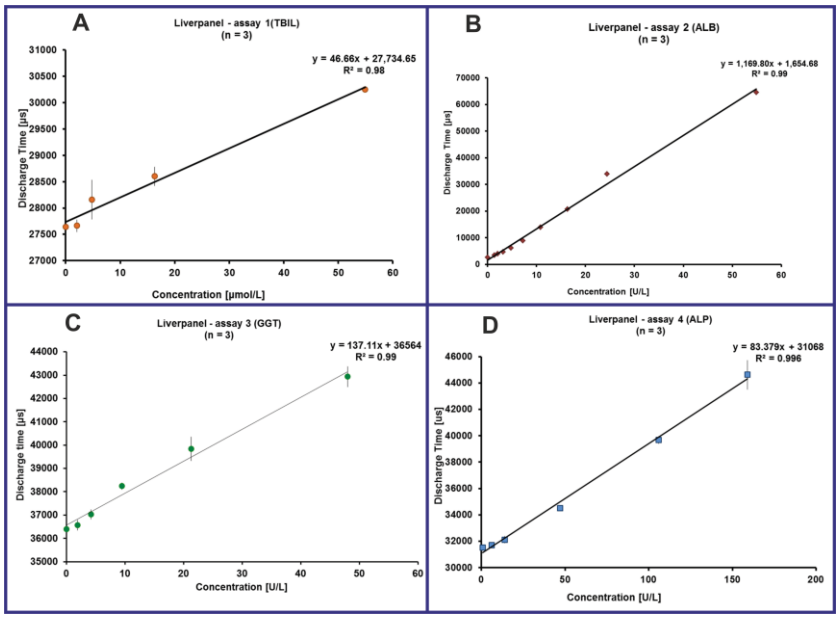

Figure 4: Calibration curves for (A) Total bilirubin (B) Albumin (C) Gamma glutamyl transferase and (D) Alkaline phosphatase. The high R^2 of the linear fitted curves demonstrate the sensitivity and accuracy of the CMAS device for this assay panel. The high R^2 of the curve fits after three runs demonstrate the reproducibility of the platform.

Table 1. Comparison of liver assay panel results obtained using blood obtained on the same day and tested in a hospital lab, well plate reader and CMAS portable device.

Liver assays	Hospital clinic data	Well plate reader (n=3)	CMAS (n=3)	Normal clinical range
ALB [g/l]	41	44.3 ± 2.0	45.77 ± 1.23	35 – 50
TBIL [µmol/l]	19	17.0 ± 2.1	22.07 ± 2.53	5 – 24
ALP [I.U./l]	83	84.9 ± 2.7	81.15 ± 2.02	30 – 130
GGT [I.U./l]	18	16.9 ± 3.1	20.78 ± 3.67	11 – 67

CONCLUSION AND OUTLOOK

We presented the first fully integrated, portable and autonomous centrifugal analyzer for the single-step automation of a 5-parameter liver assay panel. We plan to extend the range of clinically relevant assay panels, test formats and detection schemes in the future. Furthermore, work is on-going for the deployment of this battery-powered portable device and our LoaD platform for preliminary on-field screening tests in the remote parts of sub-Saharan Africa.

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