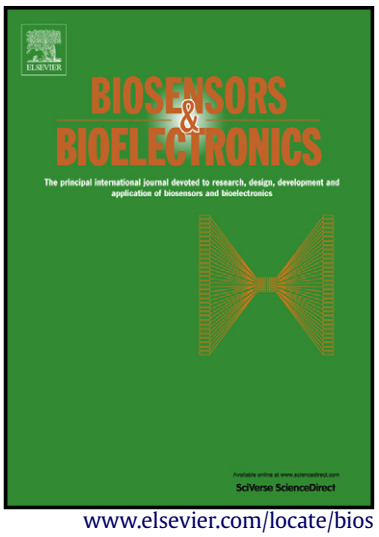

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A portable centrifugal analyser for liver function screening

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Mortality rates of up to 50% have been reported after liver failure due to drug-induced hepatotoxicity and certain viral infections(Gao et al. 2008). These adverse conditions frequently affect HIV and tuberculosis patients on regular medication in resource-poor settings. Here, we report full integration of sample preparation with read-out of a 5-parameter liver assay panel (LAP) on a portable, easy-to-use, fast and cost-efficient centrifugal microfluidic analysis system (CMAS). Our unique, dissolvable-film based centrifugo-pneumatic valving was employed to provide sample-to-answer fashion automation for plasma extraction (from finger-prick of blood), metering and aliquoting into separate reaction chambers for parallelized colorimetric quantification during rotation. The entire LAP completes in less than 20 minutes while using only a tenth the reagent volumes when compared with standard hospital laboratory tests. Accuracy of *in-situ* liver function screening was validated by 96 separate tests with an average coefficient of variance (CV) of 7.9% compared to benchtop and hospital lab tests. Unpaired two sample statistical t-tests were used to compare the means of CMAS and benchtop reader, on one hand; and CMAS and hospital tests on the other. The results demonstrate no statistical difference between the respective means with 94% and 92% certainty of equivalence, respectively. The portable platform thus saves significant time, labour and costs compared to established technologies, and therefore comply with typical restrictions on lab infrastructure, maintenance, operator skill and costs prevalent in many field clinics of the developing world. It has been successfully deployed in a centralised lab in Nigeria.

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Introduction

Liver is the largest solid organ in the body and is largely responsible for metabolism and detoxification.(Gao et al. 2008) Liver function tests are widely used in clinical chemistry to assess therapeutic effects and potential medication-induced liver damage, especially when taking medications for HIV, tuberculosis and cancer.(Landis et al. 2013; Rahmioglu et al. 2009; Vella et al. 2012) Literature reports suggest that a mortality rate of 2 – 28% can be linked with medication-induced liver damage.(Vella et al. 2012) As a result, monitoring of liver function when on certain medications has become common practice in developed countries but can be expensive in poor resource areas. This has prompted local governments and international funding agencies to set up centralised laboratories for liver function monitoring tests especially for HIV patients. Nonetheless, transport logistics and accessibility remains a significant challenge for the majority of these patients. Thus it is very important to develop portable point-of-care (PoC) devices that could be used for liver function screening in the field. Currently, there are only a handful of sample-to-answer PoC devices available for deployment in the field. Recently, Vella *et al.*(Vella et al. 2012) demonstrated an innovative micropatterned paper device for measuring liver function markers from a finger prick of blood.

Over the last two decades, various microfluidic "lab-on-a-disc" (LoaD) platforms have proven to facilitate full integration and automation of laboratory unit operations such as blood separation, metering, aliquoting, mixing, reagent storage and sequential reagent delivery(Ducrée et al. 2007; Duford et al. 2013; Honda et al. 2005; Kim et al. 2013; Robert et al. 2013; Steigert et al. 2007; van Oordt et al. 2013) for applications in bioprocess,(Nwankire et al. 2013) biomedical,(Godino et al. 2013; Park et al. 2012) food allergen(Tortajada-Genaro et al. 2011), pathogen detection(Golden et al. 2013) and environmental monitoring.(Czugala et al. 2012; Hwang et al. 2013) Nevertheless, few examples of portable sample-to-answer systems with optical read-out have been demonstrated.(Abaxis 2012; Lee et al. 2009) A standard computer DVD drive has been proposed for a range of applications including cell counting, food allergen tests and blood analysis.(Imaad et al. 2011; Ramachandraiah et al. 2013; Riegger et al. 2007; Tortajada-Genaro et al. 2011; Yu et al. 2012) The Abaxis Piccolo express is a LoaD type chemistry analyser, commercially available for the use in veterinary and medical diagnostics.(Abaxis 2012) Lee *et al.*(Lee et al. 2009) also demonstrated a portable LoaD system for detection of the Hepatitis B virus. Considering the physical size of these systems, they may not be suitable for in-situ screening in resource-poor settings.

In this paper, we present for the first time full automation of a 5-parameter liver assay panel (LAP) including plasma separation and serial aliquoting (Al-Faqheri et al. 2013; Mark et al. 2011) from a single, finger-prick blood sample by our unique dissolvable-film based centrifugo-pneumatic valving technology(DUCRÉE et al. 2012; Gorkin III et al. 2012) on portable, battery-powered centrifugal

microfluidic analytical system (CMAS)(Czugala et al. 2013) (Fig. 1A). Colorimetric detection on this 'in-house' developed instrument is based on a low-cost, paired emitter detector diode (PEDD) module (de Vargas-Sansalvador et al. 2011b; Lau et al. 2006). Liver function monitoring tests of the same blood sample were compared between the LoaD platform, benchtop well-plate and standard hospital laboratory tests, respectively. The results show a good correlation with a statistical coefficient of variance (CV), 7.9% across the three tests. As a proof of principle, we report field trials with LAPs on the CMAS device in a centralized laboratory in Nigeria. Although evidently large-scale tests would be needed to validate this approach, our results indicate that our LoaD system can potentially be used for field screening tests in resource-poor settings.

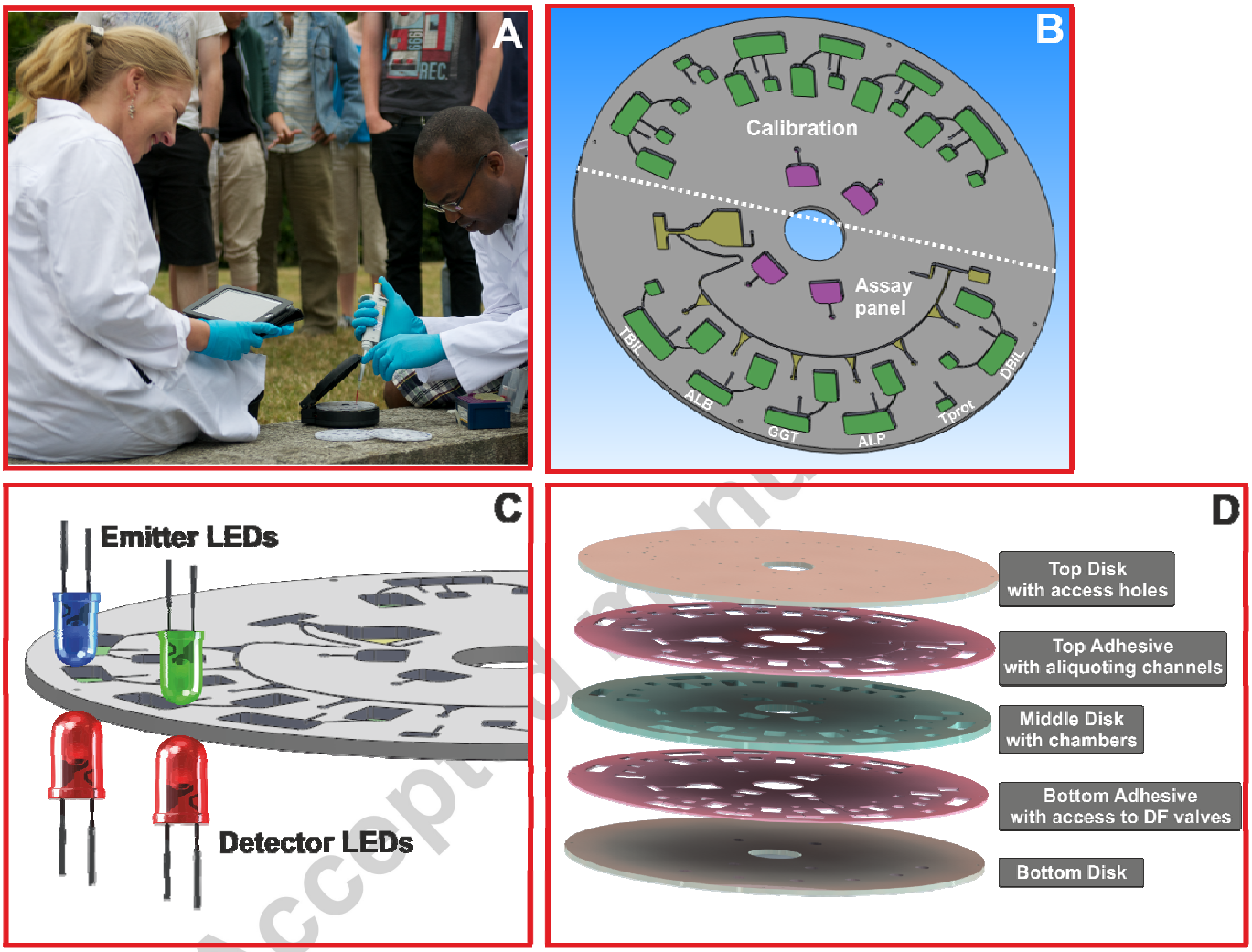

Fig. 1 (A) Image of in-field liver function screening tests on the CMAS. A video of the experimental procedure and advanced fluidic control can be found in the ESI (http://tinyurl.com/CMAS-LiverPanel). (B) Microfluidic disc; calibration standards are performed in the upper half as indicated by the white line, while the assay panel is carried out on the lower half. (C) Schematic illustration of the LoaD platform sandwiched between the emitter and detector LEDs for optical read-out (D) The multi-layered disc platform showing the two, 90-µm thick adhesives sandwiched between three, 1.5-mm thick PMMA discs. The loading and vent holes are located in the top PMMA layer; chambers are cut in the middle PMMA layer, while the microchannels are defined in the adhesive layers.

2. Materials and Methods

2.1 Integrated microfluidic design and fabrication

The integrated microfluidic disc features two independent fluidic halves; the calibration and assay panel (Fig 1B) designed using SolidWorks 2012 CAD software. The 5-layer disc (Fig. 1C) comprises of three 1.5-mm thick PMMA (poly methyl methacrylate) layers (Radionics, Ireland) and two layers of ~90 μm thin pressure sensitive adhesives (PSA, Adhesives Research, Ireland). Chambers and access holes were machined using a CO2 laser cutter (Zing 16, Epilog USA). The channels in the PSA were patterned using a Craft ROBO knife cutter (Graphtec Corp, USA). The sacrificial valves were fabricated from water dissolvable films (Adhesives Research, Ireland).(Gorkin III et al. 2012) The individually machined disc layers were subsequently aligned and assembled using a custom-made alignment jig and a hydraulic laminator.(Gorkin III et al. 2012)

2.2 Liver Assay Panel

Albumin (ALB) is the most abundant protein in blood plasma and is made by the liver. It is responsible for transporting fat soluble hormones, maintains the acid-base balance and oncotic pressure.(Rhee 2011) A significant reduction in albumin levels would indicate abnormal function of the liver.(Rhee 2011) The assay reagent – Bromo-cresol green is a dye that binds to human ALB, producing a colour change which is detectable at 630 nm. Normal clinical range for ALB is 35 – 50 g/L. Similarly, bilirubin is found in blood plasma, a product of haemoglobin breakdown. It is the water-insoluble product left over as old red blood cells are broken down on daily basis. In the liver, it is made water soluble for excretion. High levels of bilirubin can indicate liver damage, and recently have also been associated with heart failure.(van Deursen et al. 2010) The total bilirubin (TBIL) assay measures all bilirubin in the blood, whether or not it has been metabolised by the liver. The direct bilirubin (DBIL) assay measures the bilirubin that has been made water-soluble by the liver. The TBIL assay initiates a colour change detectable at 578 nm while absorbance for the DBIL assay is at 546 nm. Normal clinical range for TBIL is 0 – 17 μmol/L while that of DBIL is 0 – 4 μmol/L.

Alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) enzymes are quite similar in their determination of liver function. High levels of ALP can indicate biliary obstruction.(Hughes and Jefferson 2008; Vella et al. 2012) High blood plasma GGT levels are associated primarily with liver damage.(Burtis et al. 2008) When there is liver disease, ALP and GGT increase roughly in equal amounts.(Hughes and Jefferson 2008) The ALP and GGT assays depend on the enzymes acting on artificial substrates to give changes in absorbance at 405 nm. The normal adult range for ALP is 30 – 130 U/L, while GGT is 4 – 28 U/L.(Burtis et al. 2008)

2.3 Liver Assay Tests on benchtop, disc platform and standard hospital laboratory

Standard assay reagents for five enzymatic assays on albumin (ALB), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total (TBIL) and direct (DBIL) bilirubin, which are similar to those used in a hospital laboratory setting for these biomarkers of liver function, were purchased from Randox Chemicals, United Kingdom. On the benchtop, the colorimetric LAP was run on a standard transparent 96-well plate according to the manufacturer's instructions. Absorbance measurements were performed using an Infinite M200 spectrophotometric microplate reader (Tecan Group Ltd, Männedorf, Switzerland). Following miniaturisation and optimisation, the assay was transferred onto the LoaD platform. BR411 assay kit was used for total bilirubin (TBIL). For this assay, 20 μl of reagent (r1) 100 μl (r2) and 100 μl (r8) were loaded in chambers c1, c2 and c8 respectively (Fig. 2IA). AB362 assay kit was used for albumin (ALB) assay and 100 μl of (r3) was loaded in chamber c3. The γ-glutamyltransferase (GGT) assay was run using GT 2750 assay kit which required loading 100 μl (r4) in chamber c4. For alkaline phosphatase (ALP), the AP311 assay kit which necessitates the addition of 50 μl (r9) and 100 μl (r5) in chambers c9 and c5 respectively. The direct bilirubin (DBIL) assay used the BR2362 assay kit; 20 μl (r6) and 100 μl (r7) were loaded in chambers c6 and c7 respectively (Fig. 2IA). The calibrator CAL2350, which was reconstituted with 4 ml of ultrapure water, and diluted in series, was used to standardize the assay panel, according to manufacturer's specifications. The full calibration range was performed in triplicate, on separate LoaD platforms. In this study, micropipettes were used to introduce the assay reagents and samples onto the LoaD platform prior to running the tests (Fig 1A). 150 μl of whole blood was obtained from a finger-prick and micro-pipetted into the blood separation chamber, having pre-loaded the assay reagents in their respective chambers (Fig. 1B and 2I). However, in order to account for pipetting, CMAS and disc manufacturing errors; one-point calibration using the calibrator CAL 2350 was run twice daily, on the calibration section of the disc (Fig 1B). This is particularly beneficial for field trips. The entire optimized spinning protocol and absorbance measurements were carried out on the CMAS. On the other hand, for benchtop experiments, sample preparation was carried out by centrifuging whole blood sample in a tube at 10,000 rotations per minute (rpm) for 5 min, after which the blood plasma was taken out and used for the experiments.

Blood samples were obtained from healthy donors. Approval for use of blood was obtained from the university ethics committee and the donors signed informed consent. The same blood sample was sent through the student health centre to the hospital lab of Mater Misericordiae University Hospital, Dublin for reference tests on the same day. For the preliminary field trial runs, the CMAS was transported to a PEPFAR centralised government laboratory at Abia State University Teaching Hospital (ABSUTH), Nigeria. Calibration and assays were carried out using reagents regularly used at the hospital lab on the LoaD platforms. For this preliminary field trial, three biomarkers (ALB, ALP and Total protein) of liver function were tested.

2.4 Portable centrifugal microfluidic analytical system (CMAS)

CMAS incorporates wireless communication and is powered *via* two 9-V lithium polymer batteries. A Pololu Wixel-based general-purpose programmable module was based on a TI CC2511F32 microprocessor. System control and data acquisition are wirelessly enabled in real time by Bluetooth communication (BlueSMiRF RN-42) with a custom-designed application on an Android tablet (Samsung Galaxy Tab with 7-inch screen, Fig. 1A). The software was programmed for real-time display of the results, internal data storage and data connectivity to the cloud, either *via* a mobile network or WiFi.

The spinning protocol of the disc is controlled by a pulse-width modulated (PWM) motor (Mabuchi Motor RF-500TB). The actual rotational frequency is calculated using an optical switch (Optek OPB830L51) and rotating interrupter. In order to protect the electronics and to minimize interferences from ambient light during the operation of the device, the ProEngineer designed CMAS case was fabricated using a 3D printer (Stratasys, USA) in acetonitrile butadiene styrene co-polymer (ABS).

Table 1 Spectral range, maximum absorbance and emitter LEDs used for the LAP

Assay panel	Wavelength [nm]		
	Spectral range	Max. absorbance	Emitter LED
TBIL	560 – 600	578	569
ALB	600 – 650	626	626
GGT	400 – 420	400	405
ALP	400 – 420	400	405
DBIL	530 – 560	546	540

The colorimetric detection systems are based on a low cost paired emitter detector diode (PEDD). The PEDD device consists of two 5-mm light emitting diodes, one serving as the light source and the other, in reverse bias mode, as the detector.(de Vargas-Sansalvador et al. 2011a; M. O' Toole et al. 2007) A detailed description of the CMAS including the PEDD detection technique can be found in Czugala *et al.*(Czugala et al. 2013) To maximise sensitivity, the emission spectrum of the emitter LEDs is chosen to overlap with the UV-vis absorbance maximum (λmax) of each assay. Therefore, LEDs with λ_{max} at 626 nm (ALB), 405 nm (ALP, GGT), 540 nm (DBIL) and 569 nm (TBIL) were employed (Table 1). A common, 660-nm LEDs served as detector as the LED is sufficiently sensitive through the spectrum below the emission wavelength.(Lau et al. 2004)

3. Results and Discussion

3.1 System integration and automation

Centrifugo-pneumatic DF valves (Gorkin III et al. 2012) sealed by vertical through holes in a 3D, multi-layer microfluidic channel architecture on the disc (Figs 1C & 2A).

Table 2 Full spinning protocol for the LAP.

Step	Rotational Frequency [RPM]	Operation	Duration [min]
1	2000	Blood plasma separation and delivery of first set of assay reagents	5
2	150	Siphon priming by capillary action	2
3	1000	Aliquoting of sample into six separate aliquoting chambers	3
4	2500	Actuation of the DF valves and sample delivery	3
5	2500; 1000	Mixing and incubation by spinning at high and low frequencies	2
6	4000	Delivery of second set of assay reagents	3
7	0	PEDD detection	1[a]

[a] Except for the GGT kinetic assay, whose read out takes 4 minutes.

A detailed spinning protocol employed for the entire LAP after pre-loading all reagents and sample is given in Table 2. The entire assay sequence which involves blood separation, reagent delivery, plasma extraction, aliquoting and metering, sample delivery, mixing, incubation and detection is completed in ~20 min. A colour-coded schematic of the fluidic control and routing for the entire LAP sequence on-disc is given in Fig. 2I. Figure 2II demonstrates with frame sequence images the advanced fluidic control for the LAP, indicating the rotational frequencies and time for each operation. The red dotted arrows in Fig. 2II indicate the fluidic movement for that operation.

In order to enhance contrast in Fig. 2II, whole blood and food dye were used to demonstrate the liquid handling sequence. A video of the full LAP with assay reagents can be found in the ESI. As shown in Fig. 2I, the aliquoting chambers were designed to meter 15 µl of sample for TBIL and DBIL assays and 10 µl for ALB, GGT and ALP assays, respectively.

This disc was designed such that the radial positioning of the DF valves to be actuated simultaneously is the same.

The centrifugal pressure at the DF valves (Madou et al. 2006) depends on the mean radial position $\vec{r}$, length Δr and density ρ of the liquid plug and the angular velocity ω of the disc.

$$P = \rho\omega^2 \overset{G}{r} \Delta r \qquad \text{(Eq 1)}$$

So for the same upstream channel geometry which determines the burst frequency of the DF valves as well as the pressure P (Eq 1), the equally aliquoted liquid volumes are released simultaneously if placed at the same radial position.

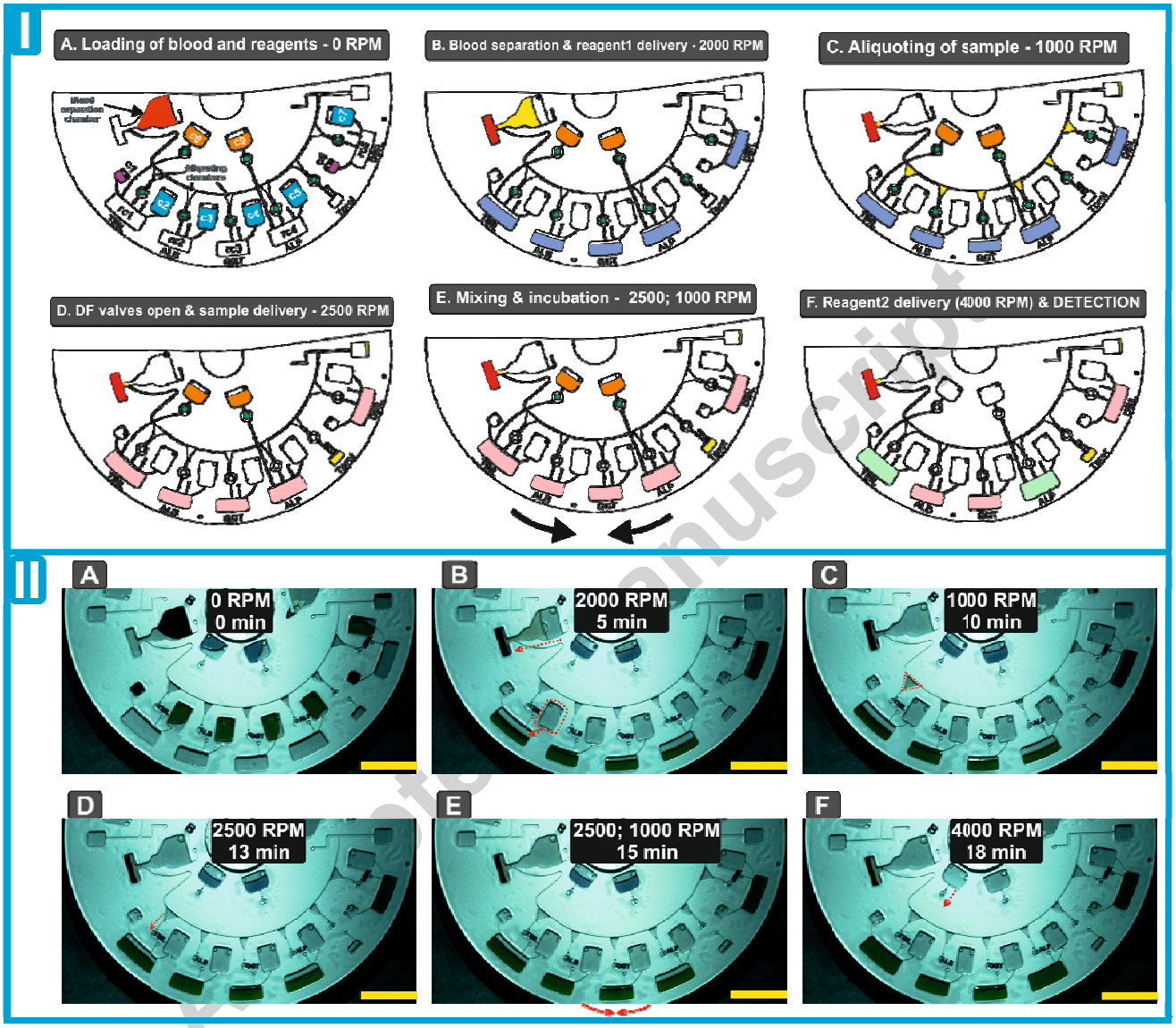

Fig. 2 (I) Schematic of full fluidic sequence of the assay (A) Start – Loading of blood sample and all assay reagents in their respective chambers (B) 5 min – Blood separation and reagent delivery into the reaction chamber at 2000 rpm (C) 10 min – Siphon priming and aliquoting of extracted plasma into 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 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 images of the entire fluidic operation as fully described in (I) above. These images were obtained using real blood samples and food dye to enhance contrast. The yellow scale bar measures 20 mm. The red dotted arrows indicate fluidic movement for that particular operation.

Details of the disc design including the radial positions and chamber dimensions can be found in the ESI. As shown in Fig. 2IA, the capillary valves for chambers (c1 – c7) containing reagents (r1 – r7), that would be delivered first to the reaction chambers (rc1 – rc5) were placed at a radius of 47 mm. The DF valves for the aliquoting chambers that would aliquot and meter the sample volumes were at a radius of 44 mm. The second set of reagents (r8, r9) due to be delivered to their respective reaction chambers (rc1, rc4) after mixing and incubation were positioned at a radius of 24 mm. This unique design enabled the full integration and advanced fluidic control of the entire LAP.

Mixing and incubation was carried out by unidirectional shake-mode rotation alternating between high (2500 rpm) and low (1000 rpm) frequencies. In good agreement with literature reports,(Noroozi et al. 2010) we observed that on-disc mixing reduced incubation times across

the assay panel by a factor of 2 when compared with benchtop well-plate experiments.

3.2 Calibration of the Liver Assay Panel

The aim of this calibration study was to evaluate the capability of the CMAS device to detect the concentrations of the assay in the clinically relevant and linear range. Dilution series of the standard calibrators supplied with the assay kits were obtained. On a separate disc which was pre-loaded with the respective assay reagents, the diluted standard was introduced instead of the blood plasma. The mixing and incubation times matched those of the full assay panel.

The discharge time (detector signal) as a function of the biomarker concentration is presented in Fig. 3A-E. The discharge times for each concentration were calculated as an average of 180 data points (3 data points per second). The calibration samples were run in triplicate (n = 3). The results demonstrate very good correlation with a R2 of 0.98 (TBIL), 0.99 (ALB), 0.99 (GGT) and 0.996 (ALP), respectively. Good linear response was obtained for the three runs and for each concentration over the ranges: 0 – 54.9 µmol/L for TBIL, 0 – 54.9 U/L for ALB, 0 – 48 U/L for GGT and 0 – 159 U/L for ALP. The end-point assays for TBIL, DBIL, ALB and ALP were read out about 60 s after completion of the assay. However, for GGT, which is kinetic assay, signal was detection "on-the fly" over an interval 4 mins. The slope of the GGT calibration curve was multiplied by 1158, according to the standard assay protocol, in order to obtain the absolute read-out.

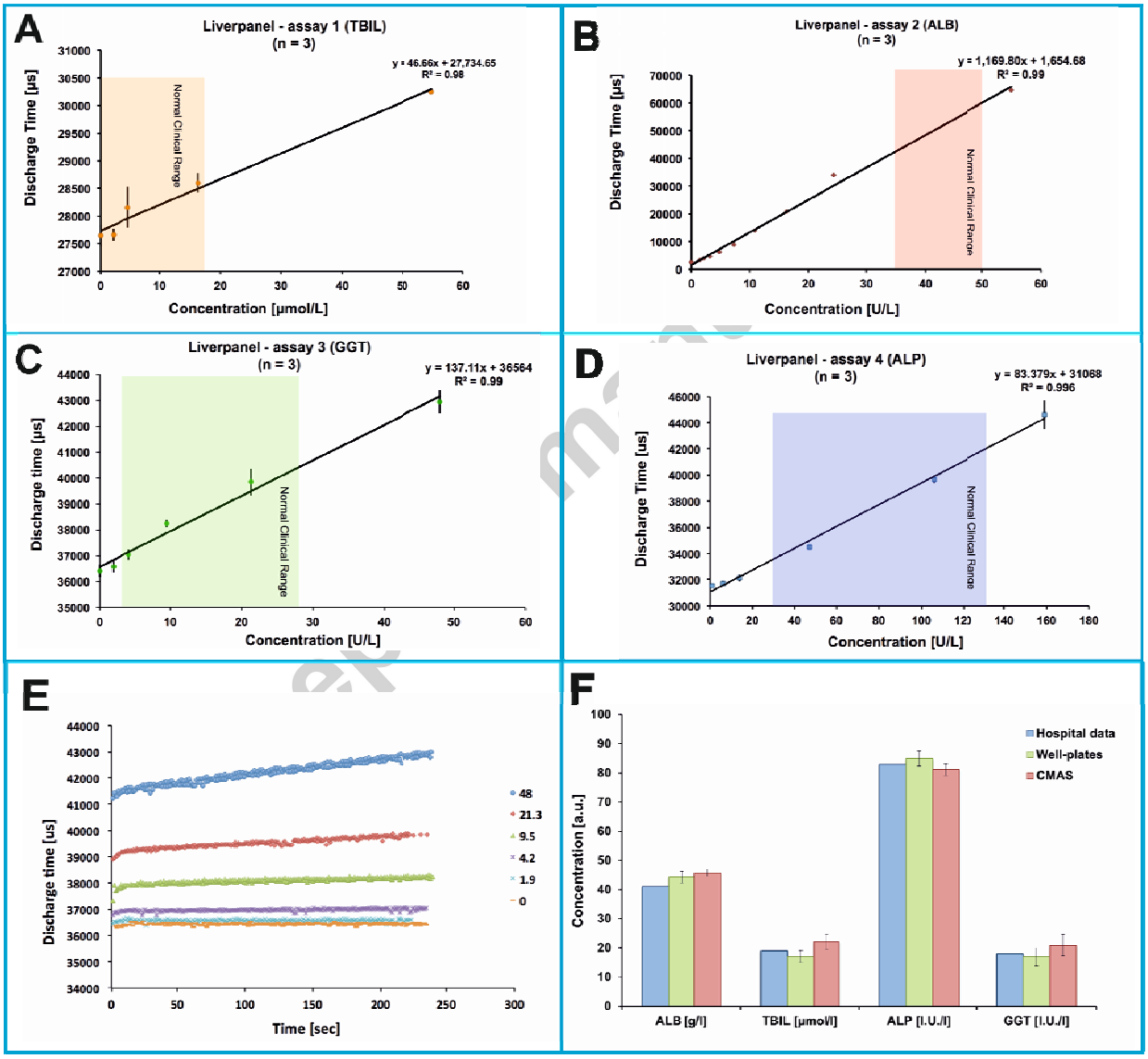

Fig. 3 Calibration curves for (A) Total bilirubin (B) Albumin (C) Gamma glutamyl transferase and (D) Alkaline phosphatase. (E) Graph of the kinetic assay read-out of GGT at different concentrations. The R2 values 0.98 (TBIL), 0.99 (ALB), 0.99 (GGT) and 0.996 (ALP) obtained for their respective linear fitted curves; demonstrate the sensitivity and accuracy of the CMAS device for this assay panel. The highlighted areas in the graphs indicate the normal clinical range. Each error bar is a standard deviation of three separate runs. (F) Benchmarking of LAP tests with a given blood sample on the same day at a standard hospital laboratory, benchtop well-plate and CMAS portable device. The tests were run in triplicates on the CMAS and benchtop plate reader, respectively.

3.3 Liver function tests

Once the calibration method was established, we analysed the liver panel biomarkers in blood samples. As shown in Fig. 3F and Table S3 (see ESI), the results obtained using the CMAS platform are well within the error bars of the well-plate reader and hospital laboratory tests. An unpaired two sample t-test with unequal variance was used to compare the mean of the measurements obtained using the CMAS and benchtop well-plate reader with a null hypothesis of equal means. The two-tailed t-test with 8 degrees of freedom gave a p value of 0.94 and t-statistic of 0.078. While failing to reject the null hypothesis, the results suggest that there is no statistical difference between the mean of the measurements (with 94% certainty of equivalence) obtained using the CMAS and the benchtop well-plate reader. A similar statistical comparison between CMAS and hospital laboratory results with 14 degrees of freedom gave a p value of 0.92 and t-statistic of 0.106, suggesting no statistical difference between the means of both measurements (with 92% certainty of equivalence).
Results of the in-situ preliminary trials from a government-funded centralised laboratory at ABSUTH, Nigeria are given in Table 3. Using reagents at the lab, three liver assays (ALB, ALP and Total protein) were run on our portable LoaD system. The results shown in Table 3 are only preliminary and indicative; yet, they demonstrate that our system has the potential to be deployed for on-field screening tests and in community health centres.

Table 3 Comparison of preliminary test results obtained from the CMAS and a centralised hospital in Nigeria.

	CMAS (Nigeria)	Centralised lab (Nigeria)	Reference range
ALB [g/L]	46 ± 5.2	37	37 - 54
ALP [U/L]	42 ± 6.4	35	25 - 92
Total protein [g/L]	69 ± 3.7	57	60 - 77

4. Conclusions and Outlook

We have developed a fully integrated and automated centrifugal microfluidic platform and a low-cost LED-based centrifugal microfluidic analytical system. A liver assay panel comprising of five independent enzymatic assays that determine liver function was integrated on this system. Our uniquely designed microfluidic disc enabled the running of the complete assay panel from single loading of whole blood sample obtained from a finger prick of blood.
In comparison to the Tecan plate reader, the CMAS platform provides comparable results within a handheld mobile device, which in turn allows for a true point-of-care diagnostic. Furthermore, our system was deployed to a remote centralized laboratory in Nigeria, a Sub-Saharan African country for preliminary field trials. The results indicate that this device can potentially be used for on-field screening tests, thus helping patients to get efficient and appropriate treatments.
The level of full integration and automation on the CMAS platform makes it amenable for on-field screening tests by quickly trained personnel. Furthermore our system has minimal reagent requirements (e.g. 100 µl versus 1 ml of assay reagent for the TBIL assay, thus notably reducing the cost per test on the CMAS platform. Portability, which is the hallmark of our system when compared to benchtop and other systems (e.g. Abaxis 2012; Lee et al. 2009) lends it for *in-situ* routine monitoring and on-field screening. Such high, on-site availability is vital for early detection, permitting timely change of medication and efficient treatment of fatal (liver) disease. Other significant advantages include increased sample throughput due to minimal human intervention and shorter incubation times. Results can also be transferred to remote locations, displayed in real time and stored on web-databases for location independent access. As a result of all these features, our system allows for de-centralised testing which is particularly important in low-resource economies where significant transportation and logistics challenges exist.
We will further optimise the LoaD platform, carry out further validation tests, expand the scope of assay panel and make this rugged, portable, multi-purpose and cost-efficient point-of-care diagnostic available to resource-poor economies.

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Notes

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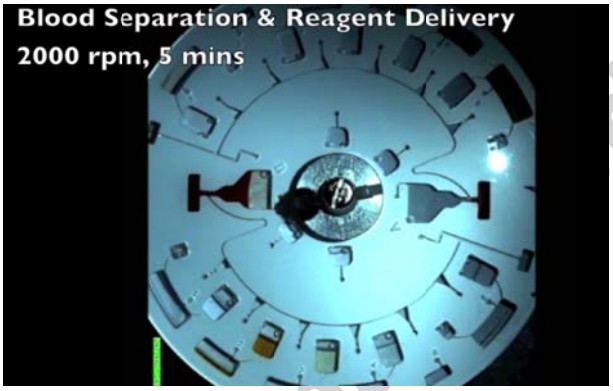
Electronic Supplementary Information (ESI)



S-1: A video of the full LAP with assay reagents. Video file can be found at http://tinyurl.com/CMAS-LiverPanel.

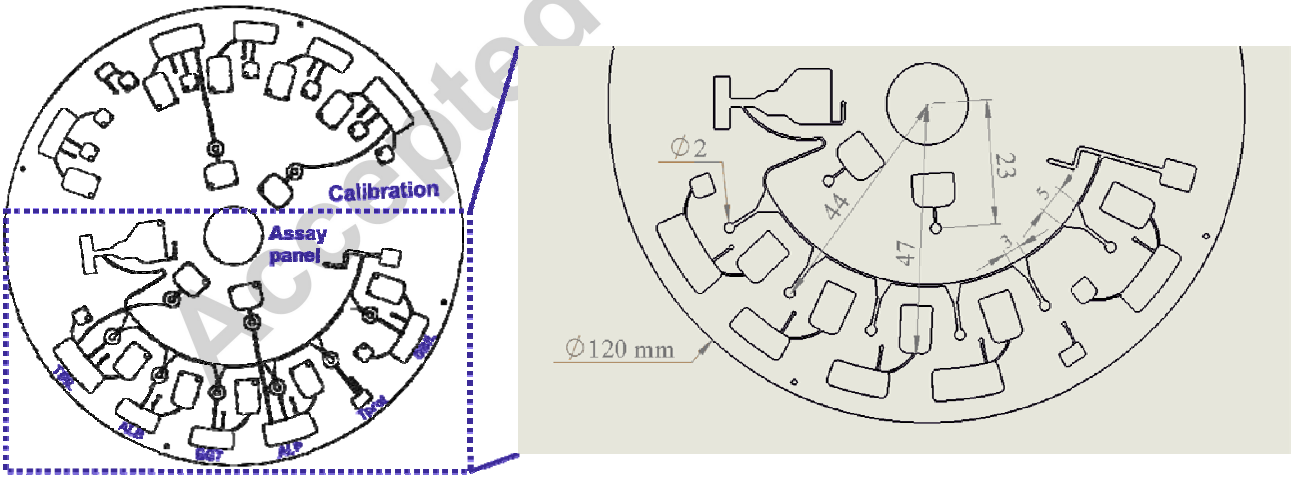


Fig. S1: Details of the LoaD platform design indicating the equi-radial positions of the valves that enable the advanced fluidic control on the platform.

Table S1 Results obtained using the CMAS platform, Well-plate reader and hospital laboratory tests for liver function monitoring tests.

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

Table S2 Raw data obtained using the CMAS platform to run the calibration standard tests.

ALB assay

Concentration [g/l]	Average discharge time [µs] (n = 3)	StDev (n = 3)
0	2585	0.03
1.4	3474	5
2.1	4019	1
3.2	4662	2
4.8	6117	11
7.2	8877	9
10.8	13892	71
16.3	20778	13
24.4	33882	86
54.9	64600	124

ALP assay

Concentration [I.U. /l]	Average discharge time [µs] (n = 3)	StDev (n = 3)
0.8	31521	110
6.2	31718	205
14.0	32121	265
47.1	34521	140
106.0	39680	288
159.0	44619	1108

TBIL assay

Concentration [µmol/l]	Average discharge time [µs] (n = 3)	StDev (n = 3)
0	27641	1617
2.1	27663	195
4.8	28158	115
16.3	28602	372
54.9	30254	181

GGT assay

Concentration [I.U./l]	Average discharge time [µs] (n = 3)	StDev (n = 3)
0	36403	244
1.9	36574	225
4.2	37032	207
9.5	38244	146
21.3	39837	511
48	42933	444

Highlights

- We present a portable, battery-powered, fully integrated and automated microfluidic analyser
- The compact centrifugal system parallelized a multi-parameter liver assay panel from whole blood
- The portable device was deployed for liver function screening tests at a HIV clinic in West Africa
- Results demonstrate the system has the ruggedness for *in-situ* liver function screening