Field tested milliliter-scale blood filtration device for point-of-care applications

Max M. Gong,^{1,a)} Brendan D. MacDonald,^{2,a),b)} Trung Vu Nguyen,^{3,4,5} Kinh Van Nguyen,³ and David Sinton¹

¹*Department of Mechanical and Industrial Engineering, University of Toronto, 5 King's College Road, Toronto, Ontario M5S 3G8, Canada*

²*Faculty of Engineering and Applied Science, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa, Ontario L1H 7K4, Canada*

³*National Hospital for Tropical Diseases, 78 Giai Phong Street, Hanoi, Vietnam*

⁴*Department of Microbiology, Hanoi Medical University, 1 Ton That Tung Street, Hanoi, Vietnam*

⁵*Department of Clinical Microbiology and Parasitology, Hanoi Medical University, 1 Ton That Tung Street, Hanoi, Vietnam*

(Received 18 June 2013; accepted 25 July 2013; published online 5 August 2013)

In this paper, we present a low cost and equipment-free blood filtration device capable of producing plasma from blood samples with mL-scale capacity and demonstrate its clinical application for hepatitis B diagnosis. We report the results of in-field testing of the device with 0.8–1 ml of undiluted, anticoagulated human whole blood samples from patients at the National Hospital for Tropical Diseases in Hanoi, Vietnam. Blood cell counts demonstrate that the device is capable of filtering out 99.9% of red and 96.9% of white blood cells, and the plasma collected from the device contains lower red blood cell counts than plasma obtained from a centrifuge. Biochemistry and immunology testing establish the suitability of the device as a sample preparation unit for testing alanine transaminase (ALT), aspartate transaminase (AST), urea, hepatitis B “e” antigen (HBeAg), hepatitis B “e” antibody (HBe Ab), and hepatitis B surface antibody (HBs Ab). The device provides a simple and practical front-end sample processing method for point-of-care microfluidic diagnostics, enabling sufficient volumes for multiplexed downstream tests. © 2013 AIP Publishing LLC.

[<http://dx.doi.org/10.1063/1.4817792>]

I. INTRODUCTION

A hallmark of microfluidic point-of-care devices has been the ability to use low sample volumes.¹ There are a number of devices that can produce test results using only a few microliters of fluid.^{2–5} However, larger sample volumes are often required for the detection of analytes with low concentration or the incorporation of multiple diagnostic tests on a single device.

In the case of diseases with dilute markers, larger sample volumes are required to ensure that sufficient analyte is present for reliable detection and quantification. In addition to having a volume with sufficient analyte, transport of analyte to the sensor can also be an issue.^{6,7} Fortunately, these transport issues can be addressed by large volume pumping mechanisms^{8–12} and by nanostructured sensors such as flow-through nanohole arrays that enable rapid transport,^{13,14} and analyte concentration¹⁵ within the sensing element. While sensor design can aid in the collection of analyte from the sample, the need for large initial sample volumes is fundamental for dilute markers.

^{a)}M. M. Gong and B. D. MacDonald contributed equally to this work.

^{b)}Author to whom correspondence should be addressed. Electronic mail: brendan.macdonald@uoit.ca

In cases where a large number of parallel tests are required to properly assess a patient, larger initial sample volumes are also required. For example, to fully assess a patient suffering from chronic hepatitis B, three to four separate immunodiagnostic tests are generally required in addition to three or more biochemistry tests.¹⁶ A comprehensive hepatitis B analysis chip would require all of these tests multiplexed on one device, each requiring on the order of 10–100 μl of sample. Enabling multiplexed tests on point-of-care devices poses new challenges and will require mL-scale upstream sample collection and preparation.

A number of on-chip sample collection and preparation devices have been developed to incorporate the separation process. Traditionally plasma is separated from whole blood through centrifugation; however, this approach does not translate well to point-of-care diagnostics. Most previous on-chip separation devices are designed for finger prick collection and have input blood capacities ranging from 1 to 300 μl .^{2,5,17–24} Obtaining blood from a finger prick in an average adult finger provides between 10 and 20 μl ;²⁵ therefore, there is a practical limit on the amount of blood that can be collected from a patient through finger pricks. Successful blood filtration was demonstrated in a laboratory setting using animal (mouse, rat, and rabbit) blood,^{17,19,20,23,26} and human blood.^{5,18,21,22,27} For testing with human blood samples the blood was typically spiked with analyte and/or diluted to facilitate the separation process.^{5,18} Most notably, Homsy *et al.*²² developed an on-chip whole blood filtration element that was validated for clinical studies by measuring the adsorption of interleukins through the filtration element. Their device was capable of separating 12 μl of plasma from 100 μl of undiluted whole blood in approximately 10 min. Importantly, the work of Homsy *et al.*²² demonstrated the suitability of microfluidic on-chip whole blood filters as sample preparation units for clinical studies, for low plasma volume output ($\sim 10 \mu\text{l}$). On-chip sample preparation with mL-scale whole blood and in-field efficacy with clinical blood samples have not been demonstrated.

Commercial blood filtration systems are also available, such as the in-line Blood Filter (Bemedical Filtration Corp., Plano, TX, USA) and Rapid Plasma Separation Device (patent pending—Advanced Microdevices Pvt. Ltd., Ambala Cantt, India). The in-line Blood Filter is designed for use in cardiopulmonary bypass procedures to remove microemboli greater than 40 μm in diameter (e.g., aggregates of platelets and red blood cells). It is not suitable for the removal of individual red and white blood cells (average sizes of 7 μm and 15 μm in diameter, respectively), which is required for blood plasma production. In contrast, the Rapid Separation Device is designed specifically for plasma production; however, its sample volume capacity and plasma output are low ($< 1 \text{ ml}$ and $< 25 \mu\text{l}$, respectively).

In this paper, we present a low cost and equipment-free blood filtration device capable of producing plasma from mL-scale blood samples and demonstrate its clinical application for hepatitis B diagnosis. We report the results of in-field testing of the device with undiluted, anticoagulated whole blood samples from patients at the National Hospital for Tropical Diseases in Hanoi, Vietnam. The plasma generated by the device is compared with plasma generated by centrifugation for red and white blood cell counts, liver enzyme and metabolite levels (e.g., alanine transaminase, aspartate transaminase, urea, and creatinine), and hepatitis B antigen and antibody levels (e.g., hepatitis B “e” antigen, hepatitis B “e” antibody, and hepatitis B surface antibody) related to hepatitis B examinations. The diagnostic tests selected for this study represent the standard hepatitis B panel performed at the hospital. This device provides a practical front-end sample processing method for point-of-care microfluidic diagnostics, enabling integration with multiplexed downstream tests and dilute analyte detection tests.

II. EXPERIMENTAL

A. Device design and fabrication

The blood filtration device is designed as a front-end modular sample preparation unit with the capacity to be integrated with downstream on-chip components or as a cartridge into a universal diagnostics system. This approach, as envisioned by our granting agency Grand Challenges Canada in partnership with the Bill and Melinda Gates Foundation, leverages the

“plug and play” nature of a universal diagnostics system where the user can tailor the system to a specific need or application by exchanging different cartridges.

The device consisted of a bottom layer of poly(methyl methacrylate) (PMMA) with hydrophilic channels to transport the plasma, a membrane layer for blood filtration, a layer of silicone rubber to prevent leakage, and a top layer of PMMA which provided structural support and formed the top of the syringe port and collection area, as shown in Fig. 1. The syringe port is shown offset from the chip to facilitate location of the input port during insertion, and it is analogous to the patient-to-chip syringe interface presented in our previous work.¹² In practice, the syringe port can be oriented vertically such that the syringe is inserted into the device without requiring the operator to manually brace the device, as shown in Fig. 1(a). This approach ensures safe, hands-off, bench-top operation of the device. To facilitate testing in the hospital where blood samples were provided in vials, a simple opening in the top PMMA layer was provided for pipetting, in lieu of the syringe port. This method was used at the National Hospital for Tropical Diseases for all subsequent testing of the devices with patient samples. Plasma was collected from the extraction port. In a universal diagnostics system, an integrated device would not require an extraction port since it could be connected directly to another component for downstream on-chip diagnostic testing.

The bottom layer was constructed of 3 mm thick PMMA micromachined by a CO₂ laser (Universal Laser Systems Inc., Scottsdale, AZ, USA). The channels were cut in the pattern shown in Fig. 1(b) using the laser, which resulted in V-shaped channels approximately 350 μm wide and 900 μm deep. After laser cutting, the PMMA pieces were placed in an oven to anneal

FIG. 1. Schematics of the blood filtration device. (a) Assembled chip shown with syringe for injecting patient blood sample. The syringe port can be oriented vertically such that the syringe is inserted into the device without requiring the operator to manually brace the device. (b) Expanded device illustrates details for each layer.

at 85 °C for at least 30 min. To facilitate the transport of the filtered plasma along the channels and away from the bottom of the filtration membrane, the channels were coated with Pluronic® F-108 (Sigma-Aldrich Co. LLC., St. Louis, MO, USA), which is hydrophilic and reduces protein adsorption.²⁸ Contact angle tests with deionized water demonstrated a decrease in the contact angle from 75° for native PMMA to 25° for the PMMA coated with Pluronic® F-108. The coatings were tested for longevity and no measurable decrease in capillary flow performance was found over a 4 week testing period; therefore, the coatings were deemed suitable for devices being shipped to Vietnam. Immediately following 1 min of oxygen plasma treatment (PDC-32G Harrick Plasma, Ithaca, NY, USA), an aqueous solution of 0.01 g/ml Pluronic® F-108 was applied directly into the channels by a pipette (approximately 70 μ l). The coated PMMA pieces were then baked in an oven at 85 °C for at least 16 h (usually overnight). After baking, the PMMA pieces were partially covered with tape, so that only the coated channels were covered, to prepare for the bonding stage, which is described below.

The membrane selected for this device was the GR VIVID™ Plasma Separation Membrane (Pall Corporation, East Hills, NY, USA). It is a hydrophilic asymmetric polysulfone membrane designed for plasma filtration from whole blood. The membrane is approximately 330 μ m thick and the capacity of blood filtration is listed by the manufacturer as 40–50 μ l/cm² membrane area. The membrane was cut into the shape shown in Fig. 1(b) using a scalpel and a stencil. The resulting membrane area for this device was approximately 15 cm². The specified loading volume of blood is 750 μ l; however, we found from experimenting with volumes up to 1 ml of blood that a membrane of this size is capable of filtering more blood than the specified amount. The efficiency of the membrane for plasma yield is listed as greater than 80%.

A silicone layer was placed on top of the membrane to prevent leakage and to seal the syringe port. The silicone rubber sheet was 1.6 mm thick (McMaster-Carr, Elmhurst, IL, USA). The pattern shown in Fig. 1(b) was micromachined using the CO₂ laser after which the silicone was washed thoroughly with water and isopropanol. To seal the membrane, the method of Maltezos *et al.*²⁵ using PDMS was attempted; however, it was not possible to prevent PDMS in liquid form from transporting along the membrane surface and into the pores, which clogged the membrane and blocked blood transport. This difference in performance could be due to a difference in the membrane material; the specific membrane used by Maltezos *et al.*²⁵ is no longer obtainable from Pall Corporation. Here, a silicone layer was employed to provide an effective seal when bonded using the procedure described below.

The top PMMA sheet was constructed of 1.5 mm thick PMMA, which was micromachined by the CO₂ laser. After laser cutting the PMMA pieces were placed in an oven to anneal at 85 °C for at least 30 min. The roles of this layer are to (1) form the top portion of the syringe port, (2) seal the blood chamber, (3) provide confinement of the patient sample, and (4) provide structural support for the device. For testing in the hospital where blood was provided in vials, this top PMMA sheet was cut in the same pattern as the silicone rubber layer so the blood could be applied directly onto the membrane by a pipette.

The layers of the device were bonded using (3-aminopropyl)trimethoxysilane (APTMS, 97%, Sigma-Aldrich Co. LLC., St. Louis, MO, USA). An aqueous mixture of 5% v/v APTMS was pre-heated to 85 °C in an oven. The bottom PMMA layer was treated with oxygen plasma for one minute then the pre-heated APTMS was applied to the surface with a pipette (approximately 80 μ l), taking care not to apply fluid on the tape-covered channels. The coated PMMA pieces were baked in an oven at 85 °C for at least 5 min, and the tape was removed. The same procedure was applied to the top PMMA layer. The silicone layer was treated with oxygen plasma for 1 min on each side and the complete unit was assembled as shown in Fig. 1(b). The layers were compressed in a heated press (Carver, Wabash, IN, USA) for at least 40 min at 85 °C under a force of approximately 1300 N. The assembled chip was manually checked for bonding quality then wrapped in aluminum foil and sealed in a plastic bag until use. The time between manufacture in the lab at the University of Toronto and testing at the National Hospital for Tropical Diseases was between seven and twelve days. After shipping, no changes in the sealing or wettability were detected.

B. Blood cell count, biochemistry, and immunology testing

Device testing was undertaken at the National Hospital for Tropical Diseases in Hanoi, Vietnam. Patient blood samples were delivered in vials and the volume was approximately 2 ml of blood. In most cases, 3 ml of blood were drawn from the patient and the tests ordered by their doctor required 1 ml, leaving $2 \text{ ml} \pm 0.5 \text{ ml}$ of blood available for testing. These volumes varied due to variability between patients and the practices of individual health care providers. Since there was a high probability that the samples were infected with hepatitis B and a possibility with HIV or other tropical diseases, extreme care was exercised during the testing. Only hospital-based medical personnel handled the samples and performed the tests. The testing focused on hepatitis B, an infectious inflammatory illness of the liver. Hepatitis B is prevalent in Vietnam and the National Hospital for Tropical Diseases specializes in diagnosis, treatment, and care for patients infected with the hepatitis B virus.

Biochemistry testing was performed for alanine transaminase (ALT), aspartate transaminase (AST), urea, and creatinine levels. ALT and AST are enzymes associated with liver parenchymal cells; therefore their levels in plasma provide an indication of liver health. The blood urea nitrogen test is a measure of the amount of nitrogen in the blood in the form of urea. Ammonia is converted into urea by the liver; therefore, urea levels in plasma can indicate the ability of the liver to remove ammonia from the blood stream. Chronic hepatitis B infection can cause liver failure and inhibit the liver from removing ammonia from the blood, which can lead to severe health problems, such as the development of hepatic encephalopathy. Hepatitis B can also result in kidney damage resulting from the deposition of immune complexes in kidney tissue, which leads to increased toxin levels in the blood. Creatinine is one such toxin, which is primarily filtered out of blood by the kidneys. Patients with hepatitis B and high levels of creatinine may be recommended for dialysis to reduce the toxin levels in their blood stream. Testing of these four biomarkers is essential for diagnosing the health status of hepatitis B patients.

Immunology testing yielded positive or negative readings for hepatitis B “e” antigen (HBeAg), hepatitis B “e” antibody (HBe Ab), and hepatitis B surface antibody (HBs Ab). A positive test result for HBeAg 3 to 6 weeks after onset of symptoms indicates an acute active infection at its most infectious period, and means that the patient is infectious. Persistence of HBeAg beyond 10 weeks shows progression to chronic infection and infectiousness. During the acute stage of infection the conversion from HBeAg to HBe Ab indicates that the patient is combating the infection. A positive test for HBs Ab 1 to 4 months after onset of symptoms indicates recovery and subsequent immunity to hepatitis B. HBs Ab can neutralize hepatitis B and provide protection against infection. For this reason, a positive HBs Ab test could also indicate that the patient received a vaccination for hepatitis B. Collectively, these three immunodiagnostic tests reveal much about the hepatitis B infection, particularly with respect to the time of infection and the patient’s immune system response.

The procedure for blood filtration using our devices was performed by a designated doctor at the hospital. Blood was first delivered to the membrane by a pipette. The amount of blood delivered was 0.8–1 ml, in one continuous batch. A clean pipette was used to collect the plasma from the extraction port, withdrawn in small ($\sim 20 \mu\text{l}$) batches at a steady, unhurried pace, until there was no plasma. For manual extraction, the collection time was dependent on the efficacy and speed of the pipette withdrawal and varied considerably. For an integrated device, where manual extraction is unnecessary and continual wicking to downstream components is assumed, we have used the experimental observations to estimate a delivery time of $\sim 5 \text{ min}$ for the plasma volumes used in our study. Plasma collection is shown in Fig. 2.

The blood remaining in the vial ($\sim 1 \text{ ml}$) was separated using a centrifuge (Hettich Universal 320, Tuttlingen, Germany) and plasma was extracted from the vial with a pipette. The plasma from the device and the centrifuge were labeled and taken for the hematology, biochemistry, and immunology testing. The hematology testing (CBC) was performed for red blood cell (RBC) and white blood cell (WBC) levels. The same technician tested all of the samples. The volume requirements for each of the tests are summarized in Table I. The specifications of the methods used for the testing are summarized in Table II.

FIG. 2. Collection of plasma from a device during testing at the National Hospital for Tropical Diseases, Hanoi, Vietnam.

III. RESULTS AND DISCUSSION

A different patient blood sample was used to test each of the 34 devices. The collected plasma output was subsequently tested for remaining red and white blood cells, biochemistry, and immunology. Results for each patient and each test were compared with traditional centrifugation sample preparation, as described below.

A. Volume of filtered plasma

The maximum theoretical yield for 800 μl of whole blood on the membrane is 350 μl of plasma, with a membrane efficiency of 80%. The device has a dead volume of $\sim 70 \mu\text{l}$; therefore, the maximum theoretical yield for the device is 280 μl of plasma. The volume of plasma produced from each of the 34 devices is summarized in Fig. 3. There is a wide range of results, and we attribute the discrepancy to both inter-patient and inter-device variation, specifically the deviation in fluid properties between patient blood samples (i.e., viscosity), the manual plasma extraction process, and the inconsistencies of the hydrophilic coating inherent to individual devices. The engineering factors can be overcome with refined manufacturing and downstream integration, but some variation is unavoidable due to inherent differences in fluid properties of patient blood samples.

TABLE I. Volume of plasma required for tests commonly performed on hepatitis B patients at the National Hospital for Tropical Diseases.

Test type	Analyte	Plasma volume (μl)
Hematology	RBC/WBC	50
Biochemistry	ALT	10–15
Biochemistry	AST	10–15
Biochemistry	Urea	10–15
Biochemistry	Creatinine	10–15
Immunology	HBeAg	35
Immunology	HBe Ab	35
Immunology	HBs Ab	40

TABLE II. Methods and test kits used for testing.

	Cell count	Biochemistry	Immunology
Company	Sysmex - Japan	Olympus - Japan	Hitachi – Japan
Model	XS 1000i	AU400	Elecsys 2010
Test kits	1. Cell Pack (Japan) 2. Stomatolyser-4DL (Singapore) 3. Stomatolyser-4DS (Singapore) 4. Sulfolyser (Japan)	1. AST (OSR6109) 2. ALT (OSR6107) 3. Urea-Urea nitrogen (OSR6134) 4. Creatinine (OSR6178)	1. HBeAg 2. Anti-HBe 3. Anti-HBs (ROCHE)

B. Blood cell count results

Blood cell counts were performed to obtain a quantitative measure of the effectiveness of the devices at filtering out both red and white blood cells from the whole blood. The samples were analyzed successfully except for sample number 11, in which there was an error for the cell counter. The results for the red blood cell counts are summarized in Fig. 4. The device outperformed the centrifuge in 31 out of 34 cases, with lower red blood cell counts. In comparison to the whole blood levels, shown in the inset of Fig. 4, the device filtered out an average of 99.9% of the red blood cells. Most biochemistry tests require cell-free plasma (i.e., 99% or better removal of red blood cells) to minimize unwanted matrix effects.²⁹ The results of the red blood cell counts indicate that the device can produce plasma with purity suitable for biochemistry testing. The results for the white blood cell counts are summarized in Fig. 5. The device output was comparable to that of the centrifuge process in most cases with the exception of three anomalous results (these higher white blood cell levels did not impact the biochemistry and immunology results, as detailed later). In comparison to the whole blood levels, shown in the inset of Fig. 5, the device filtered out an average of 96.9% of the white blood cells. Collectively, these results demonstrate successful filtration of both red and white blood cells using the device, with output levels comparable to (and in the case of red blood cells, improved over) the current centrifugation process.

FIG. 3. Volume of plasma collected for the 34 devices tested. Initial raw blood volumes were 0.8–1 ml, with a maximum expected yield of 280 μ L. Variability is largely due to the deviation in fluid properties between patient blood samples (i.e., viscosity), the manual plasma extraction process, and the inconsistencies of the hydrophilic coating inherent to individual devices.

FIG. 4. Comparison between the red blood cell counts of the plasma filtered with the devices and separated using a centrifuge. The inset shows the red blood cell counts of the whole blood prior to filtration. The device outperformed the centrifuge machine in 31 out of 34 cases, with lower red blood cell counts. The device filtered out an average of 99.9% of the red blood cells.

C. Biochemistry results

Biochemistry testing was performed to demonstrate the capability of the devices as upstream blood filtration units for downstream biomolecular diagnostics. For each patient/device, the biochemistry results using plasma obtained from devices were compared to those obtained from the centrifuge. The reference values used by the National Hospital for Tropical Diseases and test precision information for the four biomarkers investigated in this study are listed in Table III. The objective of this testing was to assess the degree of molecular adsorption in the device, and particularly any impact on diagnostic outcome. Due to the high internal

FIG. 5. Comparison between the white blood cell counts of the plasma filtered with the devices and separated using a centrifuge. The inset shows the white blood cell counts of the whole blood prior to filtration. The device filtered out an average of 96.9% of the white blood cells.

TABLE III. Reference ranges and test precision for biochemistry tests at the National Hospital for Tropical Diseases, Hanoi, Vietnam.

Biomarker	Reference range	Standard deviation
ALT	≤ 40 U/L	0.79 U/L
AST	≤ 37 U/L	1.06 U/L
Urea	2.5–7.5 mmol/l	0.15 mmol/l
Creatinine	Male: 62–120 μ mol/l Female: 53–100 μ mol/l	2.29 μ mol/l

surface area of the device, some molecular adsorption by the device is expected; however, it is critical that levels of adsorption do not preclude downstream detection, and the degree of adsorption is consistent and predictable.

The results for the biochemistry tests are presented in Fig. 6, which illustrates that the levels from the devices were consistently lower than those from the centrifuge. In Fig. 6, some of the samples have no biochemistry results displayed since there was an insufficient amount of plasma to perform the test. In order to examine the relevance of the reduced biomolecule levels, the reference ranges listed in Table III were scaled according to the difference between the mean values of the two data sets. For ALT, the levels using plasma from the device were, on average, 63% of those from the centrifuge, AST were 87%, urea were 94%, and creatinine were 28%. The reference ranges for the centrifuge from Table III are shown in Fig. 6 by dashed lines, and the scaled ranges for the devices are shown as dot-dashed lines. Urea and creatinine have two dashed lines and two dotted-dashed lines to represent the lower and upper values of their reference range and scaled range, as shown in Figs. 6(c) and 6(d), respectively. These relative reference ranges enable comparison of the diagnoses resulting from the plasma derived from the device versus the centrifuge.

An analysis of the diagnostic results from the biochemistry tests allows for a qualitative understanding as to the effectiveness of the devices for upstream plasma separation for each of the biomarkers. In Fig. 6(a), we see that for ALT the centrifuge results indicate 8 patients above the reference range, and the device results correlate well with those of the centrifuge with the exception of one disagreement above the scaled range (sample #6 in the upper left quadrant) and two disagreements below the scaled range (samples #16 and #19 in the lower right quadrant). In Fig. 6(b), we see that for AST the centrifuge results indicate 9 patients above the reference range, and the device results correlate well with only one disagreement above the scaled range (sample #12). In Fig. 6(c), we see that for urea the centrifuge results are similar to the device results with agreement for the test below the reference range (sample #3). Considering the error precision of the urea test, there were only a few results below the scaled range where the device disagreed with the centrifuge results (samples #1, #7, #8, #12, and #17). In Fig. 6(d), we see that there is little correlation between the centrifuge and device results for creatinine (reference ranges and scaled ranges shown on plot are for males), where ten results for the device disagreed with those of the centrifuge (i.e., two results above the bounds of the scaled range but within the bounds of the reference range and eight results below the bounds of the scaled range but within the bounds of the reference range). These results indicate that the device adsorbs a significant and inconsistent amount of creatinine. Collectively, these results demonstrate that for ALT, AST, and urea the device adsorbed a consistent and predictable amount of the biomolecules and is thus suitable as an upstream plasma filtration unit for downstream testing of these biomarkers.

D. Immunology results

Immunology testing was performed to demonstrate the suitability of the device for use with hepatitis B immunoassays. Specifically, antigens and antibodies associated with hepatitis B were compared for each patient's plasma prepared by both the device and the centrifuge. For the 29 immunology tests performed, only the HBe Ab test for sample #23 resulted in a

FIG. 6. Comparison of the biochemistry testing results between plasma derived from the devices and the centrifuge for the four biomarkers, (a) ALT, (b) AST, (c) urea, and (d) creatinine. Reference ranges for the centrifuge are shown by the dashed lines, and reference ranges for the devices—scaled to accommodate adsorption levels averaged over all samples—are shown by dotted-dashed lines (scaled 63% for ALT, 87% for AST, 94% for urea, and 28% for creatinine). Urea and creatinine have two dashed lines and two dotted-dashed lines to represent the lower and upper values of their reference range and scaled range, respectively.

differing test result between the plasma obtained from the device and the centrifuge. Both of these results were close enough to the cut-off value that under normal clinical conditions a follow-up test would be recommended. In this case, it is likely that the test result from the device is more accurate since the patient was positive for HBeAg, and thus unlikely to also be positive for HBe antibodies. Collectively, these 29 test results demonstrate that the device is effective for use as an upstream plasma filtration component for downstream hepatitis B immunodiagnostic testing.

IV. CONCLUSIONS

A device capable of filtering plasma from mL-scale blood samples was presented. A batch of devices was tested in the field with clinical blood samples from patients at the National Hospital for Tropical Diseases in Hanoi, Vietnam. Hematology testing confirmed that the devices were capable of filtering out red and white blood cells, and the plasma collected from the devices contained lower red blood cell counts than plasma obtained from a centrifuge. Biochemistry testing demonstrated that the devices can be used as upstream plasma filtration components for testing ALT, AST, and urea levels. Immunology testing demonstrated that the devices can be used as upstream plasma filtration components for testing HBe antigens and antibodies, and HBs antibodies. The device provides a simple and practical front-end sample processing method for point-of-care microfluidic diagnostics, enabling integration with multiplexed downstream tests.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from Grand Challenges Canada (Grant No. 0005-02-02-01-01). We would also like to acknowledge the assistance and support of the staff at the National Hospital for Tropical Diseases in Hanoi, Vietnam. Most notably, testing by Dr. Van Dinh Trang is gratefully acknowledged. The authors also gratefully acknowledge infrastructure funding from the Canada Foundation for Innovation (CFI) and on-going support from the Natural Sciences and Engineering Research Council of Canada (NSERC).

- ¹G. M. Whitesides, *Nature* **442**, 368 (2006).
- ²L. Gervais and E. Delamarche, *Lab Chip* **9**, 3330 (2009).
- ³W. Dungchai, O. Chailapakul, and C. S. Henry, *Anal. Chem.* **81**, 5821 (2009).
- ⁴Z. K. Wang, S. Y. Chin, C. D. Chin, J. Sarik, M. Harper, J. Justman, and S. K. Sia, *Anal. Chem.* **82**, 36 (2010).
- ⁵I. K. Dimov, L. Basabe-Desmonts, J. Garcia-Cordero, B. M. Ross, A. J. Ricco, and L. P. Lee, *Lab Chip* **11**, 845 (2011).
- ⁶P. E. Sheehan and L. J. Whitman, *Nano Lett.* **5**, 803 (2005).
- ⁷T. M. Squires, R. J. Messinger, and S. R. Manalis, *Nat. Biotechnol.* **26**, 417 (2008).
- ⁸S. S. Wang, X. Y. Huang, and C. Yang, *Microfluid. Nanofluid.* **8**, 549 (2010).
- ⁹M. Moscovici, W.-Y. Chien, M. Abdelgawad, and Y. Sun, *Biomicrofluidics* **4**, 046501 (2010).
- ¹⁰W. Wu, T. L. Kieu, and N. Y. Lee, *Analyst* **137**, 983 (2012).
- ¹¹G. Li, Y. Luo, Q. Chen, L. Liao, and J. Zhao, *Biomicrofluidics* **6**, 014118 (2012).
- ¹²M. M. Gong, B. D. MacDonald, T. V. Nguyen, and D. Sinton, *Biomicrofluidics* **6**, 044102 (2012).
- ¹³F. Eftekhari, C. Escobedo, J. Ferreira, X. B. Duan, E. M. Girotto, A. G. Brolo, R. Gordon, and D. Sinton, *Anal. Chem.* **81**, 4308 (2009).
- ¹⁴C. Escobedo, A. G. Brolo, R. Gordon, and D. Sinton, *Anal. Chem.* **82**, 10015 (2010).
- ¹⁵C. Escobedo, A. G. Brolo, R. Gordon, and D. Sinton, *Nano Lett.* **12**, 1592 (2012).
- ¹⁶A. S. F. Lok and B. J. McMahon, *Hepatology* **45**, 507 (2007).
- ¹⁷J. Moorthy and D. J. Beebe, *Lab Chip* **3**, 62 (2003).
- ¹⁸S. Thorslund, O. Klett, F. Nikolajeff, K. Markides, and J. Bergquist, *Biomed. Microdevices* **8**, 73 (2006).
- ¹⁹S. Choi, S. Song, C. Choi, and J. K. Park, *Lab Chip* **7**, 1532 (2007).
- ²⁰X. Chen, D. F. Cui, C. C. Liu, and H. Li, *Sens. Actuators, B* **130**, 216 (2008).
- ²¹J. S. Shim, A. W. Browne, and C. H. Ahn, *Biomed. Microdevices* **12**, 949 (2010).
- ²²A. Homsy, P. D. van der Wal, W. Doll, R. Schaller, S. Korsatko, M. Ratzer, M. Ellmerer, T. R. Pieber, A. Nicol, and N. F. de Rooij, *Biomicrofluidics* **6**, 012804 (2012).
- ²³C. Y. Li, C. Liu, Z. Xu, and J. M. Li, *Biomed. Microdevices* **14**, 565 (2012).
- ²⁴S.-H. Liao, C.-Y. Chang, and H.-C. Chang, *Biomicrofluidics* **7**, 024110 (2013).
- ²⁵S. J. Vella, P. Beattie, R. Cademartiri, A. Laromaine, A. W. Martinez, S. T. Phillips, K. A. Mirica, and G. M. Whitesides, *Anal. Chem.* **84**, 2883 (2012).
- ²⁶G. Maltezos, J. Lee, A. Rajagopal, K. Scholten, E. Kartalov, and A. Scherer, *Biomed. Microdevices* **13**, 143 (2011).
- ²⁷H. W. Hou, H. Y. Gan, A. A. S. Bhagat, L. D. Li, C. T. Lim, and J. Han, *Biomicrofluidics* **6**, 024115 (2012).
- ²⁸M. Amiji and K. Park, *Biomaterials* **13**, 682 (1992).
- ²⁹C. Blattert, R. Jurischka, I. Tahhan, A. Schoth, P. Kerth, and W. Menz, in *Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, San Francisco, CA (IEEE, New York, 2004).