Clinical Chemistry 67:12
1699–1708 (2021)

Digital Microfluidic Hemagglutination Assays for Blood Typing, Donor Compatibility Testing, and Hematocrit Analysis

Alexandros A. Sklavounos [a,b,†] Julian Lamanna,[a,b,†] Dimpy Modi,[c] Sidharth Gupta,[d] Alex Mariakakis,[d] Jeannie Callum,[c,e,f] and Aaron R. Wheeler [a,b,g,*]

BACKGROUND: Blood typing, donor compatibility testing, and hematocrit analysis are common tests that are important in many clinical applications, including those found in high-stakes settings such as the trauma center. These tests are typically performed in centralized laboratories with sample batching; the minutes that are lost in this mode can lead to adverse outcomes, especially for critical-care patients. As a step toward providing rapid results at the bedside, we developed a point-of-care hemagglutination system relying on digital microfluidics (DMF) and a unique, automated readout tool, droplet agglutination assessment using digital microfluidics (DAAD).

METHODS: ABO and Rhesus blood grouping, donor crossmatching, and hematocrit assays were developed on a portable DMF platform that allowed for automated sample processing. The result of each assay could be determined by eye or automatically with the DAAD imaging tool.

RESULTS: DMF-DAAD was applied to 109 samples collected from different sources (including commercial samples, pinpricks from volunteers, and a hospital blood bank), with perfect fidelity to gold-standard results. Some of these tests were carried out by a nonexpert in a hospital trauma center. Proof-of-concept results were also collected from smaller sample sets for donor compatibility testing and hematocrit analysis.

CONCLUSION: DMF-DAAD shows promise for delivering rapid, reliable results in a format well suited for a trauma center and other settings where every minute counts.

Blood typing, donor compatibility testing, and hematocrit measurements are performed to ensure the compatibility of the donor red blood cells (RBCs), plasma, or platelets with the transfusion recipient and to determine the need for transfusion. These tests are routinely performed in a number of settings, including prior to blood transfusions (1) and organ transplants (2), for the management of trauma patients (3), and for pretransfusion testing for patients with anemia or leukemia, or patients undergoing surgery (4). These tests are typically implemented using one of 2 strategies for analysis. In strategy 1, blood samples are collected, transported to a centralized laboratory, centrifuged to separate red RBCs from plasma, and then batched before evaluation using automated instruments such as the ORTHO VISION® Analyzer (Ortho Clinical Diagnostics), a robotic platform that evaluates samples in multiplexed gel-filled columns. This strategy can add minutes to hours to the test time, which is adequate for many patients but can be life-threatening for those in trauma care and other high-stakes environments, because uncontrolled hemorrhage can lead to death in under 30 min after injury (5–8). Strategy 2 is used in contexts in which centralized laboratories are not available, where portable point-of-care tests are performed directly on whole blood at or near the site that the samples are collected. For example, products like the ELDON™ card (Eldon Biologicals), a test in which the user smears blood onto a flat surface to identify antibody-driven hemagglutination, are often used in remote and emergency scenarios (9). Unfortunately, user mishandling and misinterpretation of results is an Achilles' heel for this strategy, and additionally, these techniques are typically not amenable to integration with modern digital record-keeping

[a]Department of Chemistry, University of Toronto, Toronto, Ontario, Canada; [b]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; [c]Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; [d]Department of Computer Science, University of Toronto, Toronto, Ontario, Canada; [e]Laboratory Medicine Program, University Health Network, Toronto, Ontario, Canada; [f]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [g]Institute of Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.

*Address correspondence to this author at: 80 St George St., Toronto, Ontario M5S 3H6, Canada. E-mail aaron.wheeler@utoronto.ca.
†A.A.S. and J.L. contributed equally.
Received June 12, 2021; accepted August 19, 2021.
DOI: 10.1093/clinchem/hvab180

©American Association for Clinical Chemistry 2021. All rights reserved. For permissions, please email: journals.permissions@oup.com.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

practices. As a result, strategy 2 techniques have failed to win the trust of clinicians and regulatory agencies (10–15), and in most jurisdictions are only approved only for personal use, not for pretransfusion screening (16).

Unfortunately, the limitations of strategy 1 and strategy 2 for blood analysis puts clinicians in the modern trauma care facility in a difficult position. In the absence of rapid tests that that can be implemented at the point of care with high reliability, trauma physicians often rely on blood products from universal blood donors (i.e., group O RBCs and group AB plasma). These products are in chronic short supply and inventories are often below target levels (17). Thus, there is a need for a rapid, reliable point-of-care test that could reduce the dependence on these scarce blood products and, in turn, extend the geographic scope in which emergency transfusions could be safely provided.

The microfluidics community has responded to the limitations described previously with great ingenuity, with a particular emphasis on microfluidic blood typing. Specifically, there are 42 different blood-typing methods reported in the peer-reviewed literature (Supplemental Table 1). While these techniques represent important milestones for the field, a close look suggests that none of them provide relief from the strategy 1 and strategy 2 problems described above. That is, of the 42 reports, 19 rely on large, un-integrated detectors such as microscopes or spectrometers, making them unsuitable for analysis at the point of care (similar to strategy 1). Likewise, 18 of the reports rely on subjective evaluation of visual results and 26 require extensive manual intervention steps (e.g., centrifugation, mixing with reagents) before analysis (similar to strategy 2). All of the previously published microfluidic blood-typing papers shown in Supplemental Table 1 fall into at least one of these categories, with several of them falling into 2. Finally, and most importantly, none of these previous reports includes any data collected outside of the research laboratory by nonexperts, a characteristic that is increasingly becoming important for reports describing methods that are claimed to be useful for point-of-care applications (18).

Here, we introduce a new microfluidic blood-typing, donor testing, and hematocrit-measurement system that overcomes the limitations described previously. The new system relies on digital microfluidics (DMF), a liquid handling technology in which discrete nano- to microliter sized droplets are manipulated on an open array of electrodes through the application of electrical potentials (19, 20). DMF automates routine liquid handling operations such as mixing, splitting, and metering of samples, and can be operated in remote, resource-limited settings using simple and portable instrumentation (21). This system represents a bridge between strategies 1 and 2 described above. The new system can run each of the reported assays within 6 min in a portable, fully automated instrument that features in-line analysis via an inexpensive camera combined with a unique image-processing algorithm that is insensitive to environmental conditions, in a format suitable for the trauma center and other point-of-care settings.

Methods

Detailed methods and procedures are provided in the Supplemental Information file.

Results

DIGITAL MICROFLUIDIC HEMAGGLUTINATION ASSAYS

Multiplexed, microfluidic hemagglutination assays were developed and implemented using a miniaturized instrument (Fig. 1, A) comprising a DMF device for reagent and sample manipulation, an open-source DropBot control system (22) for droplet manipulation on the DMF device, a pump for reconstitution of lyophilized assay reagents, a low-cost webcam for automated analysis, and a laptop computer. The DMF device comprises 2 plates, a bottom plate containing an array of actuation electrodes used to manipulate droplets containing assay reagents or blood samples, and a top plate that acts as a counter electrode. Hemagglutination assays are realized by metering out whole blood droplets onto the array of electrodes, where they are mixed with droplets of agglutination antibodies (blood typing), donor plasma (compatibility), or chemical agglutination agents (hematocrit). For blood typing, anti-A, anti-B, anti-AB, or anti-D antibodies are each mixed with separate droplets of whole blood (Fig. 1, B, Supplemental Movie 1), followed by assessment of the agglutination state of RBCs. The anti-AB mixture provides some internal redundancy as a control. In other experiments, a negative control was included as a test for cold agglutinins that could cause nonspecific agglutination (23). Likewise, for donor plasma compatibility testing, a droplet of whole blood is mixed with a plasma droplet of a potential donor sample, followed by assessment of the agglutination of RBCs. Finally, for hematocrit testing, a chemical agglutination agent is mixed with the blood sample, followed by an assessment of the degree of RBC agglutination. The general procedure for each of these variations is similar, with small variations reported in Supplemental Table 2. In most of the experiments reported here, the different tests were performed separately, but as illustrated in Supplemental Movie 2, they can be run in parallel (on the same device) as needed. The entire process requires less than 6 min to complete.

Because of the need for an operator-proof method that would remove manual steps that could impact test results, we developed techniques to store dried reagents on devices such that they could be reconstituted on-demand, joining a small number of previous reports

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

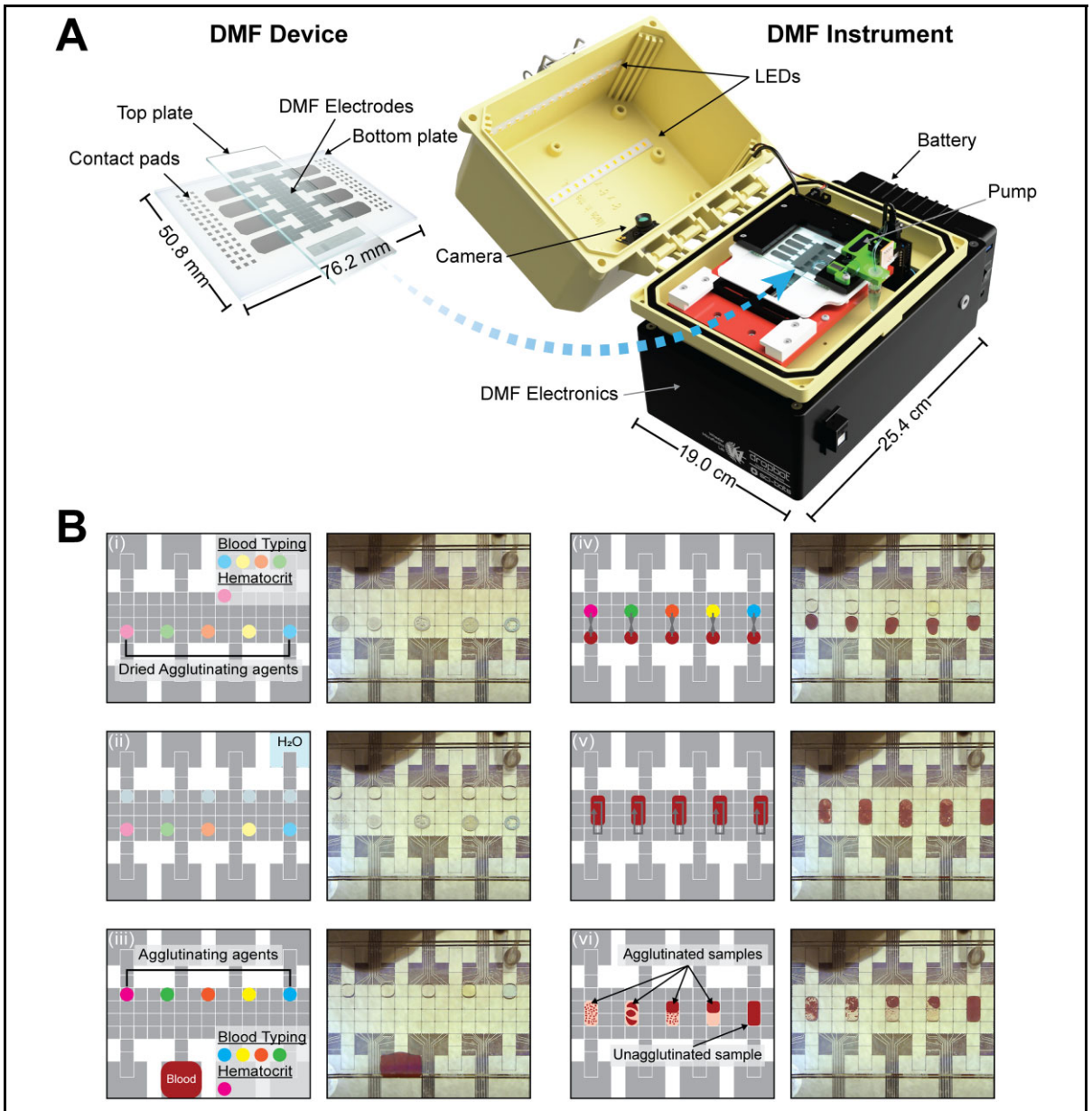

Fig. 1. Digital microfluidics (DMF) instrument and hemagglutination assays. (A) Rendered image of a DMF device and instrument. (B) Cartoons (left columns) and photographs (right columns) illustrating hemagglutination assay steps for simultaneous blood typing and hematocrit measurement. (i) A DMF device has 4 dried blood-typing antibodies (cartoon: blue–anti-A, yellow–anti-B, orange–anti-A, B, and green–anti-D) for the blood-typing assay and a dried chemical agglutination agent (pink) for the hematocrit assay. (ii) Reconstitution solvent is loaded into the reservoir of the DMF device and 5 unit-droplets (cartoon: gray) are dispensed by DMF electrode actuation. (iii) The dispensed droplets are moved to dissolve the lyophilized reagents, while whole blood (cartoon: red) is loaded into a reservoir and 5 unit-droplets are dispensed. (iv) Each blood droplet is merged with a reagent droplet, forming double-unit product droplets. (v) The product droplets are mixed. (vi) Images are captured. See color figure online at clinchem.org.

(24–26) of DMF devices with this feature. Reagents prepared as described here dissolve nearly instantaneously on exposure to droplets of reconstitution solvent (Supplemental Movie 3). In designing the reconstitution system, a micro-pump was integrated in a unique configuration using the DMF controller's capacitance measurements as feedback for managing the output of the pump. Specifically, the pump state and

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

flow rate are controlled by frequency modulation (Supplemental Fig. 1), and a closed-loop control procedure (Supplemental Fig. 2) was developed to allow for automated reservoir filling and refilling, for hands-free operation (Supplemental Movie 4). The dried reagent format is more convenient for end users, but it must be noted that some of the data reported here was collected using liquid reagent format; both are described in detail in the Supplemental Information file.

After the user has loaded the sample onto a device and pressed the appropriate button on the graphic user interface (Supplemental Fig. 3, A), the analysis is implemented automatically, and the results are returned to the operator as a digital patient card (i.e., a stylized window on the laptop screen, as shown in Supplemental Fig. 3, B) and are stored in the computer memory. It should be possible to integrate this type of system with in-line bar-code reading (21) to log patient, device, and assay information into the records. It should be straightforward for the system described here to share results and metadata wirelessly with the hospital's laboratory information management system.

AUTOMATED ANALYSIS OF HEMAGGLUTINATION BY DAAD

To complement the microfluidic method described previously, we developed an image analysis algorithm to assess agglutination state. This approach has been a popular one for microfluidic blood-typing techniques, and common implementations include analyzing the local pixel intensity variance (27–29) or the standard deviation of the pixel intensities of background-subtracted images (30). These methods work reasonably well, but on initial testing they were deemed to be not attractive for the work described here since they required ideal imaging conditions (optimal light, exposure, white balance, and the need to be directly perpendicular to the object), and expensive imaging equipment (i.e., a high-end professional digital single-lens reflex camera with macro lens or microscope setup).

We developed a method that we call droplet agglutination assessment on digital microfluidics (DAAD), which relies on open-source image analysis tools. Specifically, DAAD has the following advantages: it can be applied to any image that is captured under any condition by any camera, the process is fast (in 500 tests with randomly selected images from the pool of all available images reported as mean ± SD, the time required from image to result is 263 ± 4 ms), and has minimal computational requirements because it relies on simple matrix operations.

The first steps of a DAAD analysis for blood typing are represented in Fig. 2, A. An image of a device is corrected for perspective, the location of the droplets is determined automatically (unsupervised detection) and subimages of the droplets are extracted and normalized to the full range of the camera sensitivity. The subimages are then flattened into linear arrays and sorted according to pixel values (Fig. 2, B), and a simple linear fit applied to empirically determined regions of the data is used to determine an agglutination score, which (on comparison to a threshold) yields a binary assignment for agglutination. The process was designed to be platform-neutral, yielding equivalent results regardless of camera, angle, distance, light intensity, or white balance. For example, representative data collected with 2 different cameras under 4 different imaging conditions are shown in Supplemental Fig. 4.

DAAD was calibrated for blood typing on 25 samples of blood evaluated on DMF devices (representing 100 images of droplets with or without agglutination) with blood type verified by gold-standard analysis by the manual tube method (Supplemental Information file). A vertical scatter plot of the agglutination scores for the 100 DAAD-generated agglutination scores sorted by known types is shown in Fig. 2, C, and a receiver operating characteristic curve of this data is shown in Fig. 2, D. From these data, a binary threshold was defined, which was applied to the experiments described below.

DMF-DAAD FOR BLOOD TYPING, DONOR TESTING, AND HEMATOCRIT MEASUREMENTS

Armed with an integrated method, we evaluated performance of DMF-DAAD for blood typing, donor testing, and hematocrit analysis. For blood typing, a validation set of 60 samples (representing 240 measurements of agglutination with the 4 antibodies) was compiled from 32 samples from commercial sources (with blood types given by the vendor but blinded to the DMF operator), 16 finger-stick samples from anonymous donors with known type (with blood types blinded to the operator), and 12 finger-stick samples from volunteers with unknown blood type (thus double blinded, Supplemental Table 3). Each sample was given an identifier and evaluated using DMF-DAAD. The DMF assays included a mix of in-solution and freeze-dried and stored reagents, and images were captured by a random mix of the 2 digital cameras used in this study. Digital patient cards were generated from each sample in the validation set, with examples for each blood type evaluated in the study shown in Fig. 3, A. The DMF-DAAD results (with binary thresholds generated from the unrelated 25-sample calibration set) had an accuracy of 100% (Fig. 3, B). Finally, as a comparison, the same calibration set and validation set of blood-typing results were evaluated by alternative algorithms adapted from previous reports (27–30). DAAD was the only algorithm that exhibited perfect assignment of agglutination to all of the images in both sets (Supplemental Fig. 5).

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

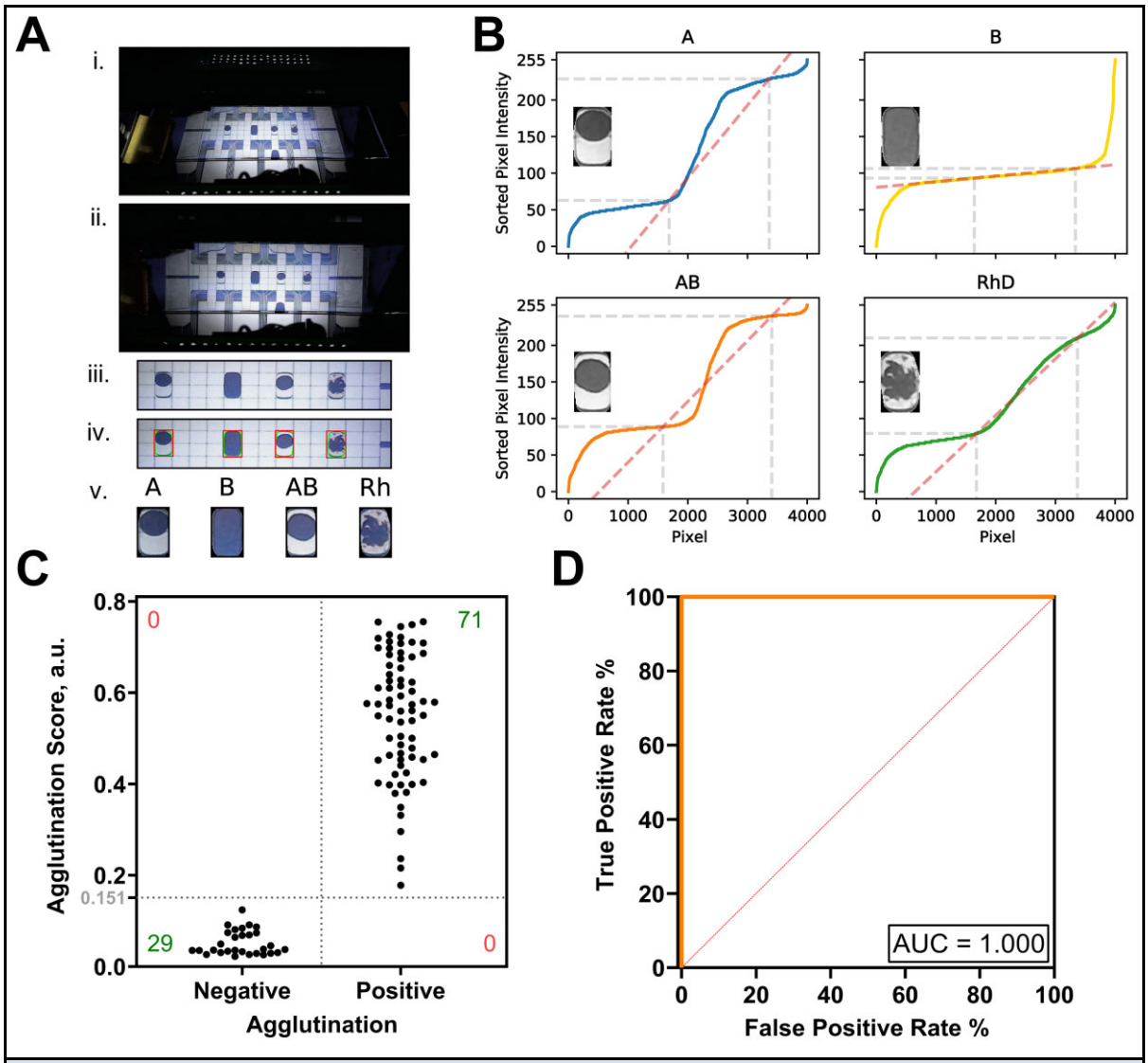

Fig. 2. Droplet agglutination assessment on digital microfluidics. (A) Representative images. (i) A device image is captured at an angle and then (ii) corrected for perspective. (iii) The center of the image is isolated, (iv) region of interest (ROI) images (red boxes) are determined, and (v) assigned to the antibody used for agglutination. (B) Plots of pixel intensity as a function of pixel number for the images in (A), for agglutination with anti-A (top left, blue), anti-B (top-right, yellow), anti-AB (bottom-left, orange), and anti-D (bottom-right, green). Lines are fitted (red dashes) between 42% and at 84% of the pixels (gray dashed lines), and the slopes are defined as agglutination scores. Insets are preprocessed ROI images of the droplets corresponding to each plot. (C) Scatter plot of agglutination scores of 100 ROI images from a calibration set ($n = 25$ samples) including 71 positive (agglutinated) and 29 negative (nonagglutinated) droplets. The empirically determined threshold is shown in gray, and the numbers in green and red indicate the numbers of correct and incorrect assessments, respectively. (D) Receiver operating characteristic curve of the data in C). AUC, area under the curve. See color figure online at clinchem.org.

We next evaluated the potential application of the new platform to blood donor–recipient crossmatching, an extra step that is performed in some settings to ensure compatibility immediately prior to transfusion. Specifically, the first step in all transfusions is to determine the blood type of the recipient according to the ABO/RhD system, to be able to identify acceptable blood types for the donor (e.g., B+ donors for a B+ recipient). In some cases, an additional compatibility step may be performed, in which plasma from a potential donor is tested directly for agglutination with the recipient's blood. An example of a mock crossmatching test

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

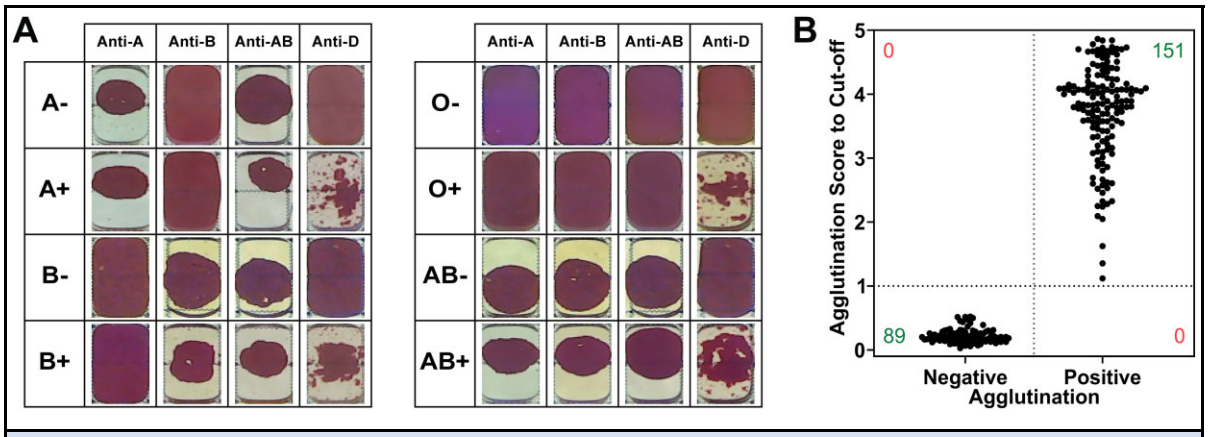

Fig. 3. Validation of DMF blood-typing method. (A) Representative digital patient cards from the validation set ($n = 60$). (B) Scatter plot of ratios of agglutination scores for the 240 ROI images in the validation set relative to the binary threshold (cutoff) established from the calibration set. Numbers in green and red indicate the numbers of correct and incorrect assessments, respectively. See color figure online at clinchem.org.

executed by DMF hemagglutination and analyzed by DAAD is shown in Supplemental Fig. 6. In this example, 2 of 4 potential donors were found to be compatible with a potential recipient. A more extensive crossmatching dataset is shown in Supplemental Fig. 7. In this collection of tests, 6 samples from donors were collected, and each whole blood sample from a recipient was cross-matched with plasma samples from the others, as mock donors (Supplemental Fig. 7, A). To confirm these results, the opposite experiment was then performed, in which each plasma sample from a donor was cross-matched with whole blood samples from the others (Supplemental Fig. 7, B). The 2 datasets represent the inverse of each other, suggesting promise for using DMF-DAAD for this niche application.

Finally, the techniques developed for blood typing and donor testing were adapted to measuring hematocrit. In initial experiments, addition of a nonspecific chemical agglutination agent (Merquat-100) to blood was found to cause the formation of an agglutinate with size that correlated with the RBC count and size, and thus, the hematocrit. DAAD was modified for these experiments to generate a quantitative hematocrit score (Fig. 4, A), as opposed to the binary agglutination score/threshold used for blood typing and donor compatibility. Calibration data for the DAAD hematocrit score (Fig. 4, B) for samples with a range of known hematocrits (20–60%) showed a correlation of $R^2 = 0.9990$ between the values. The method was then applied to evaluate 12 donor whole blood samples with unknown hematocrit. A Bland–Altman analysis (Fig. 4, C) showed that the DMF-DAAD measurements fell within the 95% confidence interval of the gold-standard microhematocrit method (detailed in the Supplemental Information File).

DMF-DAAD BLOOD TYPING AT THE POINT OF CARE

A pilot study was conducted to evaluate the performance of the new technique when operated on-site in Canada's largest trauma treatment center, located in Sunnybrook Health Sciences Centre. These experiments were conducted using the DMF-DAAD instrument running on battery power, operated by Sunnybrook staff who were previously naïve to microfluidic technologies. A set of 24 blood samples submitted for routine pretransfusion testing to the Sunnybrook blood bank were evaluated in a double-blind study by the gold-standard ORTHO VISION Analyzer and by DMF-DAAD. On un-blinding, there was perfect agreement between the 2 techniques (Supplemental Table 4) as well as with results generated by visual inspection of DMF devices. In other experiments, the performance of a popular commercial, portable blood-typing test, the ELDON card system, was evaluated. Representative results collected from each of the 3 techniques (the ORTHO VISION Analyzer, DMF-DAAD, and the ELDON card) are shown in Supplemental Table 5.

Discussion

We developed a portable digital microfluidic system to carry out hemagglutination assays. The system includes multiple technical advances relative to the only DMF system previously reported to be useful for analysis outside of the laboratory (21), including the addition of a micro-pump that is managed in a unique closed-loop system relying on droplet capacitance measurements for automated, in-line dissolution of stored, lyophilized reagents, and a custom image analysis algorithm for rapid analysis of agglutination state.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

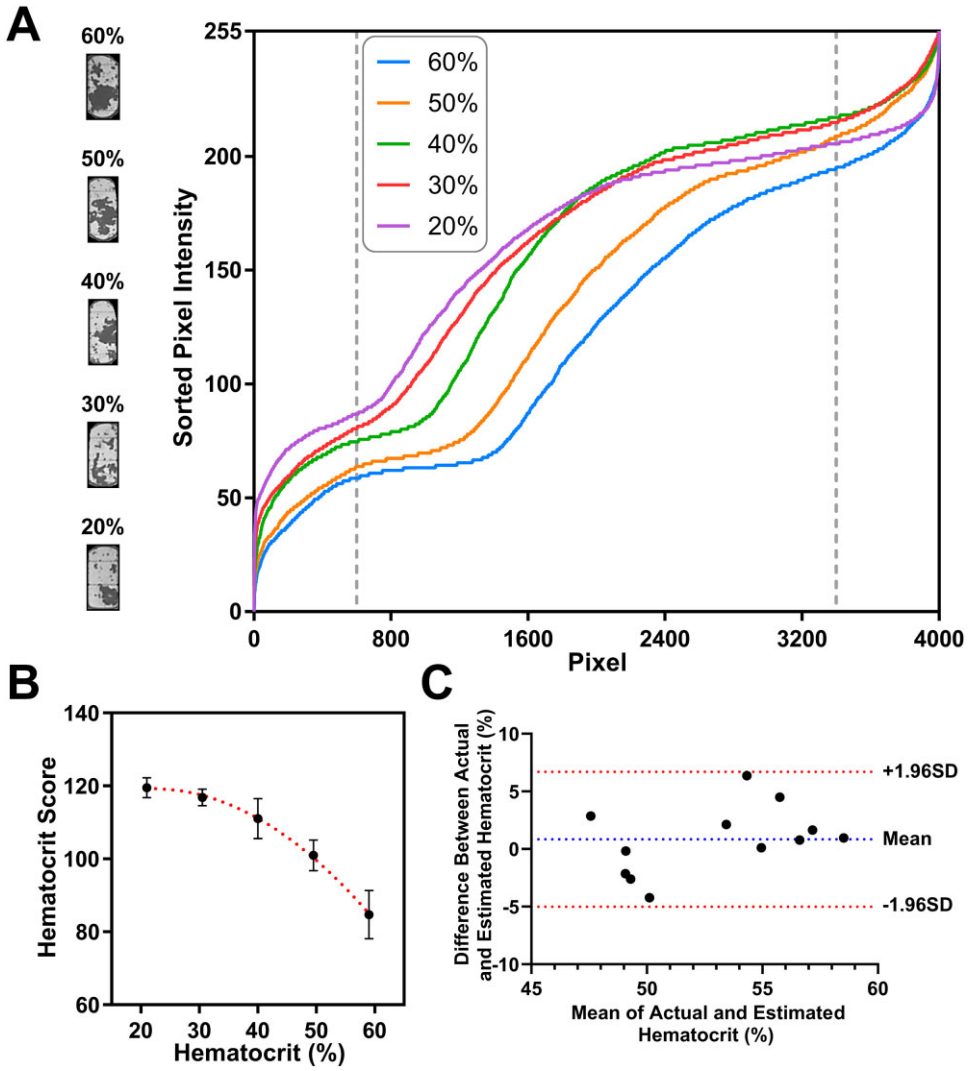

Fig. 4. DMF hematocrit determination. (A) Representative ROI images (left) of agglutinated blood samples with varying hematocrit values, and plots of pixel intensity as a function of pixel number (right) for each of the images, with purple, red, green, orange, and blue line colors corresponding to 60%, 50%, 40%, 30%, and 20% hematocrit, respectively. The integrals of the pixel intensities between 15% and 85% of the total number of pixels (vertical dashed lines) were defined as the hematocrit scores. (B) Calibration curve of DAAD hematocrit scores (markers) as a function of known hematocrit values. Error bars represent ±1 SD for $n = 3$ experiments per condition. (C) Bland-Altman plot comparing hematocrit measurements from 12 finger-prick whole blood samples made using the reference technique (actual hematocrit) and the digital microfluidic technique (estimated hematocrit). The mean difference between the 2 methods (blue dotted line) and ±1.96 SD (red dotted lines) is shown. See color figure online at clinchem.org.

We are aware of 3 previous reports (26, 31, 32) of agglutination applications implemented by DMF. Two reports (31, 32) describe agglutination assays for biomarker detection, and were implemented in open (single-plate) DMF devices. The open format is useful for method development and sample accessibility, but is not compatible with the type of portable analyzer desired for the work described here. Specifically, in single-plate DMF devices, the sample is exposed directly to the environment posing risks to the sample (e.g., evaporative losses) and the user (biosafety concerns) and droplets can be displaced with minimal forces (e.g., an accidental shake of the instrument). The third report (26) describes a lectin-based agglutination procedure for separating plasma from blood, which is functional rather than the analytical assays introduced here. Beyond DMF, there are number of microfluidic methods that have been developed for blood-typing donor

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

compatibility testing or hematocrit measurements (Supplemental Table 1). These represent important steps forward, but none fit the characteristics that we sought for this work—that is, a system that can perform any combination of blood-typing, donor compatibility testing, and hematocrit analysis (separately or in parallel), a system that does not require any user sample manipulation before analysis, a system that matches the performance of the state-of-the-art, and a system that can truly be used at the point of care, without requiring a large/external instrument to analyze the results.

Our DMF hemagglutination method satisfies the criteria outlined above. The unique pancake shaped droplets in DMF devices (each droplet is around $4.0 \times 2.0 \times 0.2$ mm for the devices used here) combined with the gentle mixing that is inherent for DMF operation rapidly concentrates the agglutinates and makes visual determination of agglutination straightforward. But as noted, a key goal for this work was to develop a completely automated sample-to-answer analysis system, which was achieved via a new image analysis algorithm (DAAD). The DMF-DAAD method was calibrated for blood typing on 25 blood samples with known type and was then used to analyze 60 blood samples in a validation set, with an accuracy of 100%. An additional set of 24 samples from a hospital blood bank was also evaluated, in a hospital trauma-center setting in which the DMF-DAAD system was operated by a user without previous microfluidics experience, again with 100% accuracy.

Finally, proof-of-concept results were generated for using the same system for donor plasma crossmatching, an application of current interest given the use of convalescent plasma to treat COVID-19 (33) and hematocrit analysis, assays that can be performed separately or in parallel (on the same device). In donor crossmatching experiments, a series of 6 mock recipients were matched correctly to mock donors. In hematocrit experiments, a Bland–Altman analysis showed that the new hematocrit measurements lie within a 95% confidence interval of conventional, gold-standard analyses. This performance is likely sufficient for many applications, but if needed, it should be able to be improved by pairing DAAD with capacitance measurements to better monitor the volume of the blood and reagents to account for minute changes in droplet volume, noting that hematocrit is a volume-dependent measurement (34), as well as morphological changes of RBCs over time (35).

In summary, the use-characteristics of DMF-DAAD represent an advance for trauma care

Table 1. Operating characteristics for 3 blood-typing systems: strategy 1 systems such as the ORTHO VISION Analyzer (column 2), the new DMF-DAAD system (column 3), and strategy 2 systems such as the ELDON card (column 4).

	Centralized	Portable	
	ORTHO VISION Analyzer[a]	DMF-DAAD[a]	ELDON card[a]
Time to result	30 min + sample transit, centrifugation, and reporting time	<6 min	5–10 min
Accuracy of test results[b]	100%	100%	80%
User interventions required	sample centrifugation and loading	sample loading	solvent loading, sample loading, sample homogenization, reagent mixing, incubation
Interpretation of results	automated	automated	manual
Instrument cost, size ($w \times d \times h$), and weight[c]	$137 300, 107.4 × 77 × 88.9 cm, 190 kg	<$7000, 25.4 × 19 × 23 cm, 4.5 kg	N/A
Price/test[d]	$1.58	<$3	$26.65

[a]This table represents the authors' best efforts to compare commercial products (i.e., the ORTHO VISION and the ELDON card) to a noncommercial method (i.e., DMF-DAAD), a proposition that is at best difficult.
[b]Accuracies are from FDA filing (ORTHO VISION), the results for 109 samples reported here (including the 25-sample calibration set, the 60-sample validation set, and the 24-sample set evaluated at Sunnybrook hospital) (DMF-DAAD), and from reference (13) (ELDON card).
[c]Instrument costs are in Canadian dollars from a quotation obtained from the vendor in July 2020 [ORTHO VISION], and an estimate for the Cost of Goods for the home-built system described here, including a commercial version of the DropBot (SciBots Inc.), the pump (Servoflo Corp.), the camera, the battery, and other custom components [DMF-DAAD]. Note that this analysis is for one instrument and thus does not reflect discounts expected for high-volume manufacture.
[d]Test prices are in Canadian dollars from purchases from the vendors in July 2020 (ORTHO VISION and ELDON card), and an estimate for Cost of Goods for a low-cost version of the devices used here, reported previously (36) as US$1.74, plus the reagent costs for one test ($0.02 for solvent and anti-A, anti-B, anti-A, B, anti-D, and negative control) (DMF-DAAD). Note that this analysis is for one device and thus does not reflect discounts expected for high-volume manufacture.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

applications, improving on the portability-limitation of strategy 1 systems, and mitigating the risk of operator misuse and misinterpretation of strategy 2 systems (Table 1). These results represent only the first of several interesting steps for DMF-DAAD. For example, strategy 1 platforms like the ORTHO VISION Analyzer typically report degrees of agglutination for blood typing rather than the binary scoring system reported here for DMF-DAAD. To address this deficit, we are augmenting the DAAD algorithm with machine learning (Supplemental Table 6) to provide scaled agglutination scoring for blood typing with promising prediction-accuracy relative to manual scoring (Supplemental Table 7; Supplemental Fig. 8). Outside of trauma, given that hemagglutination is widely used for other applications, including assays for the presence of host antibodies to SARS-CoV-2 (37, 38), the system reported here has promise for delivery of rapid, reliable agglutination results in a format suitable for the point of care.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: DMF, digital microfluidics; DAAD, droplet agglutination using digital microfluidics; RBCs, red blood cells

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Abbott Diagnostics, and Canadian Blood Services. A.A. Sklavounos was supported by a graduate fellowship from the Centre for Research and Applications in Fluidic Technologies (CRAFT); J. Lamanna was supported by a graduate scholarship from the Canadian Institutes for Health Research (CIHR); A.R. Wheeler was supported by the Canada Research Chair Program.
Expert Testimony: None declared.
Patents: A.A. Sklavounos, J. Lamanna, and A.R. Wheeler are coinventors on a patent application (PCT/CA2020/050592) that describes some of the techniques reported here.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.
Previous Presentations: None.

Acknowledgments: We thank Prof. Rodrigo Fernandez-Gonzalez and Dr. Michael D.M. Dryden for fruitful discussions. We thank Dr. M. Dean Chamberlain for assistance with interpretation of gold standard blood-typing results performed at the University of Toronto and fruitful discussions. We thank Dr. Ryan Fobel, Dr. Christian Fobel, and Joshua Dahmer for technical support.

References

1. Callum JL, Barret, J. Obstetric and Intrauterine Transfusion. In: CD Hillyer, LE Silberstein, PM Ness, KC Anderson, JD Roback, editors. Blood Banking and Transfusion Medicine. 2nd Ed. Philadelphia (PA): Churchill Livingstone; 2007. p. 496–509.
2. Reddy MS, Varghese J, Venkataraman J, Rela M. Matching donor to recipient in liver transplantation: relevance in clinical practice. World J Hepatol 2013;5:603–11.
3. Branco BC, Inaba K, Doughty R, Brooks J, Barmparas G, Shulman I, et al. The increasing burden of phlebotomy in the development of anaemia and need for blood transfusion amongst trauma patients. Injury 2012;43:78–83.
4. Sharma S, Sharma P, Tyler LN. Transfusion of blood and blood products: indications and complications. Am Fam Physician 2011;83:719–24.
5. Pati HP, Singh G. Turnaround Time (TAT): difference in concept for laboratory and clinician. Indian J Hematol Blood Transfus 2014;30:81–4.
6. Hawkins RC. Laboratory turnaround time. Clin Biochem Rev 2007;28:179–94.
7. Spinella PC, Cap AP. Prehospital hemostatic resuscitation to achieve zero preventable deaths after traumatic injury. Curr Opin Hematol 2017;24:529–35.
8. Vanderspurt CK, Spinella PC, Cap AP, Hill R, Matthews SA, Corley JB, et al. The use of whole blood in US military operations in Iraq, Syria, and Afghanistan since the introduction of low-titer Type O whole blood: feasibility, acceptability, challenges. Transfusion 2019;59:965–70.
9. Daniel Y, Sailliol A, Pouget T, Peyrefitte S, Ausset S, Martinaud C. Whole blood transfusion closest to the point-of-injury during French remote military operations. J Trauma Acute Care Surg 2017;82:1138–46.
10. Najafpour Z, Hasoumi M, Behzadi F, Mohamadi E, Jafary M, Saeedi M. Preventing blood transfusion failures: FMEA, an effective assessment method. BMC Health Serv Res 2017;17:453.
11. Strauss R, Downie H, Wilson A, Mounchili A, Berry B, Cserti-Gazdewich C, et al. Sample collection and sample handling errors submitted to the transfusion error surveillance system, 2006 to 2015. Transfusion 2018;58:1697–707.
12. Migeot V, Ingrand I, Salmi LR, Ingrand P. Reliability of bedside ABO testing before transfusion. Transfusion 2002;42:1348–55.
13. Bienek DR, Perez NM. Diagnostic accuracy of a point-of-care blood typing kit conducted by potential end users. Military Med 2013;178:588–92.
14. Ingrand P, Surer-Pierres N, Houssay D, Salmi LR. Reliability of the pretransfusion bedside compatibility test: association with transfusion practice and training. Transfusion 1998;38:1030–6.
15. Giebel F, Picker SM, Gathof BS. Evaluation of four bedside test systems for card performance, handling and safety. Transfus Med Hemother 2008;35:33–6.
16. Eldon Biologicals A S. FDA Filings. https://fda.report/Company/Eldon-Biologicals-A-S (Accessed April 2021).
17. Williamson LM, Devine DV. Challenges in the management of the blood supply. Lancet 2013;381:1866–75.
18. Nayak S, Guo T, Lopez-Rios J, Lentz C, Arumugam S, Hughes J, et al. Integrating user behavior with engineering design of point-of-care diagnostic devices: theoretical framework and empirical findings. Lab Chip 2019;19:2241–55.
19. Pollack MG, Fair RB, Shenderov AD. Electrowetting-based actuation of liquid droplets for microfluidic applications. Appl Phys Lett 2000;77:1725–6.
20. Cho SK, Moon H, Kim C-J. Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. J Microelectromech Syst 2003;12:70–80.
21. Ng AHC, Fobel R, Fobel C, Lamanna J, Rackus DG, Summers A, et al. A digital microfluidic system for

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021

serological immunoassays in remote settings. Sci Transl Med 2018;10:eaar6076.
22. Fobel R, Fobel C, Wheeler AR. DropBot: an open-source digital microfluidic control system with precise control of electrostatic driving force and instantaneous drop velocity measurement. Appl Phys Lett 2013;102:193513.
23. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. Blood 2013;122:1114–21.
24. Yang H, Luk VN, Abelgawad M, Barbulovic-Nad I, Wheeler AR. A world-to-chip interface for digital microfluidics. Anal Chem 2009;81:1061–7.
25. Newman S, Stephenson AP, Willsey M, Nguyen BH, Takahashi CN, Strauss K, et al. High density DNA data storage library via dehydration with digital microfluidic retrieval. Nat Commun 2019;10:1706.
26. Sista RS, Ng R, Nuffer M, Basmajian M, Coyne J, Elderbroom J, et al. Digital microfluidic platform to maximize diagnostic tests with low sample volumes from newborns and pediatric patients. Diagnostics 2020;10:21.
27. Huet M, Cubizolles M, Buhot A. Real time observation and automated measurement of red blood cells agglutination inside a passive microfluidic biochip containing embedded reagents. Biosens Bioelectron 2017;93:110–7.
28. Huet M, Cubizolles M, Buhot A. Red blood cell agglutination for blood typing within passive microfluidic biochips. High Throughput 2018;7:10.
29. Kline TR, Runyon MK, Pothiawala M, Ismagilov RF. ABO, D blood typing and subtyping using plug-based microfluidics. Anal Chem 2008;80:6190–7.
30. Castro D, Conchouso D, Kodzius R, Arevalo A, Foulds IG. High-throughput incubation and quantification of agglutination assays in a microfluidic system. Genes 2018;9:281.
31. Yoon JY, You DJ. Backscattering particle immunoassays in wire-guide droplet manipulations. J Biol Eng 2008;2:15–2.
32. Rastogi V, Velev OD. Development and evaluation of realistic microbioassays in freely suspended droplets on a chip. Biomicrofluidics 2007;1:14107.
33. Malani AN, Sherbeck JP, Malani PN. Convalescent plasma and COVID-19. JAMA 2020;324:524.
34. Fernandes SC, Baillargeon KR, Mace CR. Reduction of blood volume required to perform paper-based hematocrit assays guided by device design. Anal Methods 2019;11:2057–63.
35. Murray LP, Baillargeon KR, Bricknell JR, Mace CR. Determination of sample stability for whole blood parameters using formal experimental design. Anal Methods 2019;11:930–5.
36. Dixon C, Ng AHC, Fobel R, Miltenburg MB, Wheeler AR. An inkjet printed, roll-coated digital microfluidic device for inexpensive, miniaturized diagnostic assays. Lab Chip 2016;16:4560–8.
37. Alves D, Curvello R, Henderson E, Kesarwani V, Walker JA, Leguizamon SC, et al. Rapid gel card agglutination assays for serological analysis following SARS-CoV-2 infection in humans. ACS Sens 2020;5:2596–603.
38. Kruse RL, Huang Y, Smetana H, Gehrie EA, Amukele TK, Tobian AAR, et al. A rapid, point-of-care red blood cell agglutination assay detecting antibodies against SARS-CoV-2. Biochem Biophys Res Commun 2021;553:165–71.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1699/6377222 by University of Toronto user on 01 December 2021