Reagent-Loaded Cartridges for Valveless and Automated Fluid Delivery in Microfluidic Devices

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An important problem in the life sciences and in health care is simple and rapid detection of biomarkers. Although microfluidic devices are potentially useful in addressing this problem, current techniques for automating fluid delivery—which include valves and electrophoresis—require sophisticated microfabrication of the chip, bulky instrumentation, or both. In this paper, we describe a simple and reliable technique for storing and delivering a sequence of reagents to a microfluidic device. The technique is low-cost, requires minimal user intervention, and can be performed in resource-poor settings (e.g., outside of a laboratory) in the absence of electricity and computer-controlled equipment. In this method, cartridges made of commercially available tubing are filled by sequentially injecting plugs of reagents separated by air spacers. The air spacers prevent the reagents from mixing with each other during cartridge preparation, storage, and usage. As an example, we used this “plug-in cartridge” technology to complete a solid-phase immunoassay in a microchannel in 2 min with low-nanomolar sensitivity and demonstrate the diagnosis of HIV in 13 min.

Microfluidics offers a powerful platform for the integration of bioassays.1−3 Because of rapid kinetics and the potential for automation, microfluidics can potentially transform routine bioassays into simple and reliable tests for use outside of the laboratory. If the miniaturization is done in an affordable and simple-to-use manner, it can provide tools for the improvement of global health.4,5 At present, however, these microchips suffer from the lack of a simple method for handling multiple reagents. Typically, reagents are manually pipetted into the inlets serially and transported using a vacuum source6−8 or delivered with syringe pumps9−11 this overall process is slow and labor-intensive or expensive and requires bulky instrumentation. Alternative techniques for the delivery of reagents rely on sophisticated chip design, bulky, expensive instruments, or both. The use of valves, for example, requires complicated fabrication of the chip and an external instrument for their operation.12 Similarly, the use of electroosmosis requires a computer-controlled source of high voltages.13−15 Passive forces, such as capillary forces, have been used to drive a sequence of reagents through a valveless microfluidic system but required a complicated microchip design.16 Overall, although these techniques allow for the control of complex fluid delivery schemes, they are generally too expensive and thus not appropriate for point-of-care analysis in resource-poor environments (e.g., in developing countries or outside the laboratory environment).

In this paper, we demonstrate a simple and reliable method for storing reagents for extended periods of time and then delivering sequentially the reagents in a microfluidic device to carry out a bioassay. We use a cartridge, in which reagents are stored and separated from each other by air spacers. Our approach differs from flow injection analysis (FIA), where air bubbles are used to separate a series of samples, while a benchtop instrument is used to operate the valves that dispense the reagents to each sample.17,18 The use of two-phase systems in FIA has also been demonstrated for continuous-flow PCR,19 enzymatic assays,20,21 and a colorimetric assay (where bubbles of aqueous samples were created in a flow of perfluorodecaline—a water-immiscible fluoro-
onto the microscope slide. We made the cartridges by cutting microscope slide in a plasma oxidizer (30 s, 18 W) and prepared nated solvent). To demonstrate the utility of this method for the delivery of reagents, we focus on the detection of biomarkers by heterogeneous immunoassays. We used heterogeneous immunoassays as a model bioassay, because these assays benefit greatly from being integrated on microfluidic platforms. Incubation of samples and reagents under flow conditions in microchips achieves fast transport of molecules to the surface, resulting in short incubation times for heterogeneous reactions. Also, miniaturization of heterogeneous immunoassays— if done in an affordable, portable, and simple-to-use manner— has important applications, including diagnosis of infectious diseases in developing countries, use by first responders in developed countries, and applications for home testing.

**EXPERIMENTAL SECTION**

**Reagents and Materials.** The photomasks for photolithography were purchased from PageWorks (Cambridge, MA) or CAD Art Services, Inc. (Poway, CA). Negative photoresist SU8 was obtained from Microchem (Newton, MA). Poly(dimethylsiloxane) Sylgard 184 (PDMS) was ordered from Dow Corning (Midland, MI). The MaxiSorp polystyrene substrates were purchased from NUNC (Rochester, NY). Rabbit immunoglobulin G (IgG) and anti-rabbit IgG were obtained from Sigma (St. Louis, MO), and the Alexa-488 donkey anti-sheep and 488-Alexa anti-human IgG were ordered from Molecular Probes (Eugene, OR). The hand-operated pump and the polyethylene tubing (from Intramedic, type PE-60, 0.76/1.22 mm int/ext diameter; and type PE-20, 0.38/1.09 mm int/ext diameter) were purchased from VWR Scientific Products (Pittsburgh, PA). The dye for plug visualization was bought from Waterman (Janesville, WI). The voltage-gated valves were obtained from The Lee Co. (Westbrook, CT), and the PE cartridges were heat-sealed with a Clam Shell Hand Sealer bought from Abbeon Cal Inc. (Santa Barbara, CA). The 250-μL syringe for manually filling the cartridges was purchased from Hamilton (Reno, NV). Fluorescence imaging was carried out with a fluorescence microscope equipped with a CCD camera (ORCA-ER, Hamamatsu Photonics KK, Hamamatsu City, Japan) operated with the Metamorph software (Universal Imaging Corp., Downingtown, PA). The HIV Env antigen gp41 was purchased from Research Diagnostics (Flanders, NJ), and the sera of HIV+ and control patients were obtained from Golden West Biologicals Inc. (Temecula, CA). The data were analyzed with the graphing software Origin, purchased from OriginLab Corp. (Northampton, MA). The movies of plugs entering in the microchannels were acquired with a Phantom 7.0 high-speed camera purchased from Vision Research Inc. (Wayne, NJ). All other chemicals were of analytical grade.

**Determination of Plug-to-Plug Contamination.** Using the rapid prototyping technique, we made a replica in PDMS featuring microchannels (25 mm long, and a cross section of 50 × 50 μm²). We oxidized the PDMS replica and a 5 × 7.5 cm² microscope slide in a plasma oxidizer (30 s, 18 W) and prepared the microfluidic chip by permanently sealing the PDMS replica onto the microscope slide. We made the cartridges by cutting the polyethylene tubing in sections of ~30 cm. Using a source of vacuum, we drew liquid into the cartridge to form a plug of reagent. After each plug of liquid, we drew air into the cartridge to form air spacers. Overall, we prepared the cartridges by loading into the tubing a sequence of plugs composed of the appropriate reagents.

**Movies for Imaging of the Plugs in the Microchannels.** We observed the succession of plugs of blocking buffer (0.05% Tween 20 in phosphate-buffered saline (PBS)) and air spacers in a serpentine-shaped microchannel with a cross section of 50 × 50 μm². The surface of the microchannels was rendered hydrophilic by incubation in blocking buffer for 30 min prior to the injection of the sequence of plugs. The movies (available in the Supporting Information) were acquired at a 300 frames/s, but for clarity, they play 100 times slower than in reality.

**Preparation of the Microchip for the Anti-Rabbit IgG Assay.** The PDMS replicas with microchannels were prepared by rapid prototyping. We processed a first microfluidic design to pattern the stripe of antigen (Figure 1a, one channel, 30 mm long, 200 μm wide, and 60 μm deep), and a second design to carry out the immunoassay (Figure 1b, six parallel channels 50 mm long and 63 μm deep). The latter channels were composed of 5 sections, each 10 mm long, with a width of 500 μm next to the inlet and outlet, 50 μm in the center (where the heterogeneous immunoassay takes place), and 250 μm in the intermediary segments. We chose this geometry to obtain long channels (i.e., where the six inlets and six outlets can be geometrically separated...
from each other) with a limited resistance to flow (i.e., where fluids can be pumped in a hydrodynamic flow with a small pressure drop).

The PDMS replica for patterning was placed in conformal contact (nonpermanent adhesion, i.e., without plasma oxidation) to the polystyrene substrate, and the channel was filled with a solution of antigen, i.e., 50 ng/mL rabbit IgG in PBS. The antigen was allowed to physisorb onto the polystyrene substrate by incubation for 90 min at room temperature. After incubation, the channels were emptied and rinsed twice with the blocking solution (0.05% Tween 20 in PBS). The PDMS slab was peeled off the polystyrene substrate, and the substrate was rinsed with deionized water (conductivity ≥ 18 MΩ) and dried with a nitrogen gun. Inlets and outlets were punched into the second PDMS slab (for the immunoassay) using a sharpened medical needle with an outside diameter of 1.6 mm (gauge 16G 1⅞). Using this modified needle, we found that the holes in PDMS were large enough to insert PE-60 tubing and small enough to form a tight seal between the cartridge and the PDMS. The second PDMS slab was conformally sealed onto the polystyrene substrate, with its microchannels were oriented orthogonally to the stripe of antigen. The six microchannels were filled with a blocking solution and incubated for at least 2 h to block the surfaces of polystyrene and PDMS, before the assay was carried out.

**Heterogeneous Immunoassay for the Detection of Anti-Rabbit IgG.** The cartridges were loaded with samples and reagents diluted in blocking solution to the appropriate dilution: anti-rabbit IgG (sheep ascites, 2 mg/mL) and Alexa-488 labeled anti-sheep (donkey ascites, 2 mg/mL) at 0.05% Tween in PBS, using the method depicted in Figure 2b. To simulate shipment conditions to an end user, we wrapped cartridges into a 23 × 23 cm² tissue and placed them in an envelope for shipment. We shipped the envelope airborne from Cambridge, MA, to Seattle, WA, and back with FedEx and visually examined the sequence of plugs after reception of the package. Then, we dropped the cartridges 10 times from a height of 1 m and compared them to cartridges not exposed to physical shocks.

**Internal Standards for Quantitative Fluorescence Measurements.** Since we observed daily variations in the response of our detector for the measurement of fluorescence intensities, we used an internal standard (IS) to compensate for these daily variations. For the data in Figure 4c, each curve represents the average of three experimental curves, obtained in three consecutive days. We used as an internal standard the best sigmoidal fit calculated for the raw data obtained for the cartridges with 8 cm plugs, with the formula

\[
y_{\text{raw}} = A_b + (A_1 - A_b)/(1 + (y_{\text{norm}} - B_{\text{cm}} \text{-plug})/x_0)^p
\]

where \(A_1, A_2, x_0, \text{ and } p\) are parameters optimized by the graphing software Origin. We chose to use the sigmoidal fit calculated from all data points (all 6 raw data points for 8 cm-long plugs) because it is less sensitive to experimental error than individual data points (e.g., compared to a fit calculated from only the data points for the highest and lowest concentrations of sample). The curve of the internal standard was then normalized between 0 and 1000 using a relation

\[
y_{\text{norm}} = ay + b, \text{ where } a \text{ and } b \text{ were calculated to obtain a curve that is } y_{\text{norm}} = 0 \text{ at the lowest and } y_{\text{norm}} = 1000 \text{ at the highest concentration of anti-rabbit IgG.}
\]

Then all raw data obtained for the 2-, 4-, and 8 cm plugs were normalized between 0 and 1000 using the linear relationship above, and the daily values obtained for \(a\) and \(b\). For the data presented in Figure 5a, there was no sigmoidal curve available for use as IS. Hence, we compensated for daily variations using fluorescein calibrators prepared in a separate microfluidic chip and then normalized the data to be comparable to the scale used in Figure 4c (i.e., the value of the data point at \(t = 0\) in Figure 5a was matched to the value of the data point indicated with a gray square in Figure 4c). The microfluidic calibrator chip consisted of a PDMS replica with four parallel channels (50 mm long, 50 μm wide, and 50 μm deep) permanently sealed on a 5 × 7.5 cm² microscope slide after plasma oxidation. We filled the microchannels with solutions of 0.5, 1, 2, and 4 μM of fluorescein in 50 mM sodium carbonate buffer, pH 9.55. For each daily set of measurements, a new calibrator was prepared using a new microfluidic chip and the appropriate solutions of

with a blocking solution for at least 2 h. Sera from HIV-1-positive patients and healthy donors were diluted 1:1 with the blocking solution and centrifuged at 735g for 3 min. The supernatant was then transferred to a new Eppendorf tube. The assay was carried out by applying a −15 kPa pressure gradient across the microchannel, as described above. The cartridge for the HIV assay comprised plugs of blocking solution (3 × 5 then 10 mm), a plug of detection antibody (120 mm, 488-Alexa anti human IgG, 100-fold diluted in blocking solution), and blocking solution (3 × 5 and 10 mm). Safety consideration: the sera were isolated from blood sample of patients who are HIV+ and are thus potentially infectious.

**Simulation of Physical Shocks.** We filled the PE-20 and PE-60 tubing with 1-, 2-, and 3-cm-long plugs of ink diluted in 0.05% Tween in PBS, using the method depicted in Figure 2b. To simulate shipment conditions to an end user, we wrapped cartridges into a 23 × 23 cm² tissue and placed them in an envelope for shipment. We shipped the envelope airborne from Cambridge, MA, to Seattle, WA, and back with FedEx and visually examined the sequence of plugs after reception of the package. Then, we dropped the cartridges 10 times from a height of 1 m and compared them to cartridges not exposed to physical shocks.
fluorescein. The plot of fluorescence intensity versus fluorescein concentration was fit to a straight line by linear regression. Each data point of fluorescence intensity obtained for the immunoassay was individually treated with the daily result of the linear regression (for $t'_1 - 62$ days), by subtracting the value of the intercept ($b'_i$), then by dividing with the slope ($a'_i$), and then treated with the result of the linear regression obtained on $t'\, 0$ day, by multiplying with the slope ($a_0$) and adding the value of the intercept ($b_0$). The HIV data presented in Figure 6c were obtained within 1 day and were not subjected to normalization or compared against a calibrator.

RESULTS AND DISCUSSION

Use of the “Plug-In Cartridges”. To develop a reliable and simple technique for storing and delivering reagents to a microfluidic device, we filled a commercially available section of flexible polyethylene tubing with a sequence of plugs of fluid using the methods depicted in Figure 2a and b; these plugs of fluid contained the appropriate reagents and washing buffers. We refer to this section of tubing as a “cartridge” (Figure 2c). These plugs are separated by air spacers and thus cannot mix with each other. After connecting the cartridge to a microfluidic device, the user applies a vacuum to the outlet using a simple hand-operated pump; the sequence of reagents is delivered to the microchip with no further intervention from the user. Although air bubbles are sometimes problematic in microfluidic devices because they tend to stick to the channel surface and disturb the flow of reagents, we find that they move through the microchannels very smoothly and reproducibly if we render these channels hydrophilic (for example, by treatment with a detergent, such as Tween 20, or by
plasma oxidation), as illustrated in Figure 2d. Our approach of plug-in cartridge (so named because the cartridge both contains plugs of liquid and is plugged into a microchip by the user) is split into two steps: (i) preparation of the cartridge, which requires technical and human resources to operate benchtop instruments or syringes, and (ii) use of the cartridge to perform the assay, which can take place in an environment without sophisticated instruments and trained staff.

Preparation of the Cartridges. We prepared the cartridges by cutting commercially available polyethylene (PE) tubing into 30-cm-long units. Two methods are available for injecting the sequence of reagents and air spacers: the use of either a manually operated syringe or, for mass production, the use of an array of computer-operated valves connected to a vacuum source (Figure 2a and b). In the method depicted in Figure 2a, we connected up to six PE cartridges to an array of voltage-gated valves, which were in turn connected to a \( \pm 6 \) kPa vacuum source. To ensure a steady source of vacuum throughout the experiments, we connected the hand-operated pump to a 1-L, round-bottomed flask (acting as ballast). Voltage pulses generated via a home-written Labview program operated the voltage-gated valves. The cartridges and valves were all located on a holder, which fit onto a 96-well plate; in this arrangement, the end of the six cartridges could be dipped simultaneously into a row of 6 wells. Since each row of wells contained a solution of reagent, the cartridges could be loaded with the appropriate solution by moving the holder into the correct row of wells. We observed an increased reproducibility...
to quantify the plug-to-plug contamination and between the reagents would minimize contamination between the three times before incubation with the washing buffer. By analogy microtiter plates, it is common practice to rinse each well at least plug contamination. When performing a heterogeneous assay in plugs, we observed that the plugs left small residues behind them reproducible.

Figure 6. Diagnosis of HIV-1. (a) Schematic representation of the format of the assay. Since the sample is usually not available at the time of the preparation of the cartridges, it cannot be included in the predefined sequence of plugs of the cartridge. Instead, the sample of serum is sipped from an Eppendorf tube into the microchip using a PE tubing (for 7 min in this experiment). The sipper is then removed from the chip, and the reagent-loaded cartridge is inserted into the chip to complete the assay. (b) Fluorescence image of the intersection between the antigen stripe and two microchannels used for the analysis of sera from a HIV-1-positive patient (left) and a control serum (right). (c) Quantification of fluorescence for a triplicate experiment of the HIV-1 assay. The data indicate that the assay is sensitive and reproducible.

in the filling procedure if we filled the tubing first with a solution of washing buffer (0.05% Tween 20 in PBS) before initiating the loading of reagents. With our automated setup, we could fill up to six cartridges in parallel with a standard deviation of 14% in plug length.

The second method depicted in Figure 2b relies on a manually operated Hamilton syringe connected at one end of a single PE tubing. This approach is technically simpler than our former method, allowed for comparable or better control and precision of plug lengths, and is appropriate for the rapid preparation of a limited number of cartridges. When the cartridge preparation was completed, we heat-sealed the tubing end, unplugged the cartridge from the valve/syringe, and heat-sealed the other end. We routinely prepared cartridges containing as many as 10 plugs for the immunoassays described later, and more than 10 plugs could be easily loaded into the cartridges using the same methods.

Plug-to-Plug Contamination in the Cartridge. Using dyed plugs, we observed that the plugs left small residues behind them as they moved into the tubing. Since the subsequent plugs in the sequence collected the residues, this process resulted in plug-to-plug contamination. When performing a heterogeneous assay in microtiter plates, it is common practice to rinse each well at least three times before incubation with the washing buffer. By analogy to this procedure, we hypothesized that three rinsing plugs between the reagents would minimize contamination between the reagent plugs. To quantify the plug-to-plug contamination and determine exactly how many rinsing plugs are needed between reagent plugs, we filled a cartridge with plugs of fluorescein dissolved in 50 mM carbonate buffer (pH 9.5) using the method depicted in Figure 2b. We loaded plugs of fluorescein at a number of concentrations, followed by plugs of buffer (see Figure 3a). We connected the cartridge to the inlet of a microchannel, turned on the vacuum, and recorded the fluorescence intensity of the plugs as they travelled through the microchannel (see Figure 3b). The detector showed a linear response for the dilution series of fluorescein, indicating that the data collected by the detector could be used for quantitative treatment. To quantify the extent of plug-to-plug contamination, we measured the fluorescence signals in the three buffer plugs following a 31 μM fluorescein plug; after background subtraction, we observed fluorescence signals in the three buffer plugs of 7, 0.9, and 0.1% relative to the fluorescein plug. Next we examined the plug-to-plug contamination following a 250 μM fluorescein solution, by examining the fluorescence signals in the six plugs following the fluorescein solution. While the signal of the 250 μM fluorescein plug saturated the detector, we detected fluorescence only in the first three plugs (see Figure 3c). These observations showed that three buffer plugs between each reagent plug were sufficient to prevent cross-contamination between the reagent plugs.

Use of Liquid Spacers. We also investigated the use of water-immiscible liquids, such as perfluorodecalin (PFD), instead of air as spacers between the plugs of reagent. We observed that, compared to aqueous solutions, PFD wetted hydrophilic microchannels much more poorly. When a PFD plug entered the microfluidic channel, it only partially flushed the aqueous solution out of the microchannel. As a result, the incubation times and washing efficiency were irreproducible; for this reason, we used air plugs rather than PFD plugs to separate reagents in subsequent experiments. Moreover, because the volumetric flow rate through a microchannel is inversely proportional to the viscosity of the fluid, air travels ~250 times faster than PFD for the same pressure gradient (the values for viscosity at ambient temperature are ~20 μPa·s for air and ~5 mPa·s for PFD). As demonstrated by the experiment in Figure 3, although the air spacers in the cartridge had a length similar to that of the plugs of reagent, the time elapsed between two reagent plugs in the microfluidic device was less than 10 s. For comparison, a spacer of similar length made of a PFD took several minutes to travel through the microchannel (data not shown). Analyses could, as a result, be accomplished most quickly using air spacers, rather than PFD spacers.

Heterogeneous Immunoassay for Anti-Rabbit IgG. We characterized the plug-in cartridge technique with an immunoassay based on the detection of anti-rabbit IgG. Using previously published methods, we prepared a microfluidic chip that had one stripe of immobilized antigen (rabbit IgG) positioned perpendicular to a set of microchannels (Figure 1). We loaded the cartridges with the following sequence of plugs: sample (sheep anti-rabbit IgG), wash, detection antibody (488-Alexa labeled anti-sheep IgG), and wash. To run the assay, we connected the cartridges (each with a different concentration of sample) to the


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microchannel inlets and applied a −15 kPa vacuum at the outlets to deliver the reagents into the microfluidic device (Figure 4a). After all the plugs had traveled through the microchannel, we detected a clear fluorescence signal on the stripe of rabbit IgG, and no fluorescence was detected outside of the stripe (Figure 4b).

The incubation times for reagent can be changed by varying the lengths of the reagent plugs. We varied the length of the reagent plugs from 2 (9 μL) to 8 cm (36 μL), resulting in total assay times of 120−465 s. The assay showed only a minimal loss in sensitivity when run in the most rapid format tested and exhibited a limit of detection comparable to that observed in other on-chip immunofluorescence assays (~1 nM),7,11,12 as illustrated in Figure 4c.

**Long-Term Storage of the Cartridges.** For storage and transportation (e.g., from the time of manufacture to the point of application by the end user), the cartridges should retain their functionality over time, preferably at room temperature. To test the long-term stability of the ready-to-use cartridges, we filled 70 cartridges with 8-cm-long plugs of 170 nM sheep anti-rabbit IgG and 488-Alexa labeled anti-sheep IgG (the same concentrations of reagents as that of the data point indicated with a gray square in Figure 4c). Immediately after the preparation of the cartridges, we performed the assay in parallel on two different chips using six cartridges and recorded the fluorescence signal arising from the immunocomplex. The remaining cartridges were split into three batches, each stored at a different temperature: 4 °C, room temperature (~20 °C), and 37 °C. Later, we repeated the immunooassay on two freshly prepared chips with four cartridges from each batch. We observed no reduction in the performance of the assays after storing the cartridges for two months, either at 4 °C or at room temperature (Figure 5a). For the cartridges stored at 37 °C, the observed loss of signal was likely due to significant losses of water from evaporation, and not from loss of activity of the antibodies, as after 25 days, the length of the 8-cm-long plugs shrunk by more than 50%. The liquids in the plugs had completely evaporated after 62 days of storage at 37 °C. Although we did not investigate in detail the long-term stability of the antigens physisorbed on the microchip, there exist numerous studies on the use of stabilizers, such as trehalose, for long-term storage of dried proteins.28

On a given chip, we observed small variations (i.e., typically less than 5%) between the signals obtained for the cartridges stored under the same conditions. We found, however, that the chip-to-chip variations were sometimes as high as 25% using cartridges stored under the same conditions. This observation suggested that the major source of irreproducibility in the immunooassay results arose from chip-to-chip variation in the functionality over time, preferably at room temperature. To test the stability of the cartridges to physical shocks, such as those that may occur during shipment and handling in the field. To mimic the physical shocks during shipment to an end user, we sent a set of cartridges for an airborne, coast-to-coast round trip with a commercial carrier and then repeatedly dropped the cartridges from a height of 1 m (Figure 5b). This procedure resulted in no degradation in the shapes of the plugs in PE tubing with an inner diameter of 0.38 mm (type PE-20). Although shipment of cartridges made of tubing with an inner diameter of 0.76 mm (type PE-60) caused no degradation to the sequence of plugs, repeated dropping of the cartridges caused the plugs to break into smaller plugs, leading eventually to the mixing of reagents. We hypothesize that, in cartridges with small inner diameters, the plugs were insensitive to physical shock because of high viscous drag (the viscous drag that opposes the motion of the plugs scales as 1/r).

**Diagnosis of HIV.** We demonstrated the use of our plug-in cartridge technique for the detection of anti-HIV-1 antibodies in human serum. In a microchip prepared with a stripe of HIV-1 antigen (gp41), the anti-HIV-1 antibodies in the serum of an infected patient bind to the gp41 and are in turn detected using labeled anti-human IgG. Because the sample is typically not available at the time of the preparation of the cartridge, we used an additional section of PE tubing connected to the inlet of the microchannel (hereby referred to as "sipper") to inject the serum into the device. The sipper was then disconnected from the inlet of the microchip, and a cartridge preloaded with the following reagents was in turn connected to the inlet: wash, detection antibody (488-Alexa anti human IgG), and wash; the total assay time was 13 min (Figure 6a). In triplicate experiments, assays of HIV-1-positive sera produced a clear and reproducible signal, compared to that of control sera (Figure 6b and c). This fluid delivery technique can thus be used for a clinically relevant immunooassay and can, in principle, also be used for immunooassays using other amplification chemistries.8

**CONCLUSION**

Because the plug-in cartridge method overcomes the labor-intensive processes and the complicated instrumentation associated with the delivery of multiple reagents in microfluidic assays, it is well-suited for applications where simplicity and rapidity benefit the user.20 For heterogeneous immunooassays, this approach has the potential to simplify automation for conventional high-throughput assays such as ELISA (current methods use complicated robots for dispensing liquids). Moreover, because the cartridges and the reagents are stable on storage and transportation, this technique may provide a simple method for reagent delivery for point-of-care diagnostics. The combination of this plug-in cartridge technique with a portable immunooassay,8 which provided sensitive analysis with little instrumentation, could serve as the basis for a portable, sensitive, and low-cost microfluidic chip that is simple to operate and provides rapid analysis. Such an application may be simple enough to be used in both developed and developing countries.

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SUPPORTING INFORMATION AVAILABLE

Two movies showing (i) the arrival of plugs of buffer in a serpentine-shaped microchannel filled with air and (ii) the arrival of an air spacer in the same microchannel filled with buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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