ELISA Assay with PDMS Microfluidic Channels Fabricated by 3D Printed Master Mold

Virginia R. Greenberger, Hannah Yang, Tinglu S. Yang, Angela M. Bischof, Paul S. Cremer, Dan G. Sykes*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, USA, dgs12@psu.edu

Received July 25, 2023. Accepted October 2, 2023.

Abstract: Following the emergence of the SARS-CoV-2 (Covid-19) pandemic, interest in understanding antibody diagnostic testing has increased. We describe a quick and inexpensive technique that enabled students to print their own microfluidic devices that can be used to house an immunoassay for detecting a Human Immunodeficiency Virus (HIV) antibody. Both qualitative diagnostic assays and quantitative binding assays were carried out to characterize the HIV interaction with a target antibody. By performing these hands-on low-cost experiments in the analytical chemistry lab course, students were exposed to 3D fabrication, microfluidic technology, surface chemistry, protein-ligand binding affinity studies, and immunoassays within the time frame of two four–hour laboratory periods.

Introduction

Following the first confirmed case of SARS-CoV-2 (Covid-19) in December 2019, the world has faced a public health crisis not seen since the worldwide flu pandemic in 1918–1919 [1]. The current pandemic has altered our daily lives, disrupted workplace routines, forced our social interactions online, and fundamentally changed mainstream educational practices. Early evidence indicates that instructors spend 20–50% additional time preparing and teaching their classes to adopt and enact more flexible and diverse pedagogical practices which are required to accommodate the highly varied learning styles and learning environments of remote students [2, 3]. However, the pandemic has also elevated the public's awareness of public health issues and their intersections with scientific inquiry especially with respect to sample collection and detection methods.

For the analytical chemistry curriculum, the heightened awareness of different testing strategies provides a compelling reason to introduce the chemistry behind clinical testing methods. Broadly speaking, there are two different types of tests – real time diagnostic tests and antibody tests. Two common strategies for diagnosing patients with an active infection are Real Time Polymerase Chain Reaction (RT-PCR) and antigen detection experiments [4–6]. RT-PCR detects the virus' genetic material whereas antigen tests are sensitive to proteins on the virus' surface [7, 8]. Alternatively, an antibody test looks for antibodies that are produced by the immune system in response to a viral invasion and can indicate prior infection. As antibodies can take several days or weeks to develop after infection, RT-PCR, and antigen-based tests are used for active monitoring [9, 10].

Antigen and antibody tests typically employ enzyme-linked immunosorbent assay methods (ELISA) which are important not only for routine clinical testing but also for pharmaceutical therapeutics, forensic, environmental, and toxicological investigations [11–14]. A typical ELISA assay involves "sandwiching" the antibody of interest (i.e., the primary antibody) between its antigen and a secondary antibody which is tagged for detection (Figure 1). The first step is to immobilize the antigen onto the surface of a well plate. Next a blocking agent such as bovine serum albumin is added to each well to block the remaining exposed surface of the glass support to prevent denaturing of the protein antibodies and any non-specific binding to the support [15]. Samples containing the primary antibody are then introduced and bound to the immobilized antigen. Next, an enzyme-linked secondary antibody is introduced that will specifically bind with the primary antibody. The enzyme then reacts with an added soluble reagent inducing a chromogenic, fluorescent, or chemiluminescent signal which is then measured via spectrophotometric methods to determine the presence and quantity of the primary antibody.

The initial interest in developing an ELISA-based activity arose from two issues of programmatic interest: 1) the importance of ELISA as a diagnostic tool and quality control measure in the clinical, environmental, and food industries, among others, and student interest in the application of chemistry to environmental and health related issues, and 2) to introduce student-built devices earlier in the curriculum. Surprisingly, an "ELISA" keyword search for articles in chemical education journals returned a few publications including one on the fabrication of an LED microtiter plate reader which mentions ELISA as a potential application for the device [16]. However, the number of articles increase substantially within the broader science education community and reflect the ubiquitous variety of applications in both academic research and industry. For example, several published instructional laboratory activities employ ELISA for small molecule detection such as biotin [17], arsenic in spiked river water samples [18], or the pharmaceutical digoxin [19]; as a toxicity indicator, for example, characterizing the suppression of hormone secretion by endocrine disruptors [20] or the effect of enzyme inhibitors on proinflammatory cytokine expression [21]; and to detect allergens in foodstuffs [22, 23].

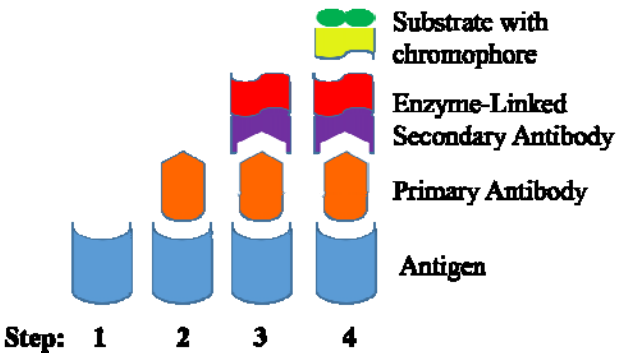

Figure 1. A schematic describing the stepwise addition of ELISA reagents, including an antigen, primary antibody, enzyme-linked secondary antibody, and substrate with chromophore. As drawn, a positive diagnosis would be determined.

Other studies focus on protein detection [24, 25] or characterizing protein-protein interactions [26].

Because ELISA is a robust and relatively straightforward tool, relatively inexpensive commercial kits are available for almost any type of analysis, requiring minimal advanced sample preparation for undergraduate instructional laboratory instructors. Unfortunately, the costs of materials can become unmanageable when scaled up to accommodate larger enrollment laboratory courses typically offered in the first- and second years of a program. Microfluidic devices potentially offer both a time- and cost-saving platform in which to implement ELISA into the undergraduate curriculum. The utility of such devices is evidenced by the more than 20,000 articles tagged in the research literature when using "microfluidics" as the keyword. In contrast, less than 40 articles appear in the science education literature likely because the cost of chip fabrication presents a critical barrier to the incorporation of microfluidics in science curricula [27].

Below, we describe a two-week laboratory investigation which employs ELISA to determine the presence of an HIV-variant antibody using microfluidic devices built by students. During the first of two laboratory periods, student groups (two students per group) rotate through the 3D printing process and a qualitative ELISA experiment. Each group prints their own master using a light-activated resin to create polymerized ridges in the desired spatial pattern; the micron-sized channel patterns are printed directly onto a resin-coated petri dish which requires less than 1 mL of resin per device. Students then cast the stamp by pouring polydimethylsiloxane (PDMS) over the master. The PDMS stamp is set to cure until the next laboratory period.

During the second laboratory period, students poke holes through the cured PDMS stamps at both termini of each channel, and then graft the stamp onto a glass slide after the surfaces of both materials have been flame plasma-treated. Students then perform a quantitative ELISA by micropipetting different concentrations of the target primary antibody into separate channels. The intensity of the green color that develops in each channel is proportional to the concentration of the primary antibody. Students use a cell phone and ImageJ to capture and process an image of their results to construct a calibration curve. As such, the detection hardware/software add no additional manufacturing cost. An Excel program is used to determine the equilibrium dissociation constant for antigen-antibody binding. The total cost to fabricate a single device and conduct both qualitative and quantitative ELISA assays is about $2.50 (based on consumables and not including the initial purchase prices for the 3D printer, UV light, etc.).

Student learning outcomes include a deeper understanding of stereolithographic additive manufacturing, the importance of ELISA as a diagnostic tool, the utility of microfluidic devices compared to traditional ELISA methods, and exposure to simple yet reliable data collection and processing methods to determine antibody-antigen binding affinity constants. Practical skills include solution preparation, the use of micropipettors, and device fabrication using soft lithography. From a programmatic perspective, the device is part of a novel lab-based pedagogy that we have developed over the past twenty years, that is aimed at inspiring and engaging science and engineering students. We have dubbed this on-going initiative as SMILE (small, mobile instruments for laboratory enhancement) [28, 29]. The central premise is that access to low-cost instruments as demonstration and laboratory teaching aids greatly expands the breadth of chemical concepts accessible in a classroom/lab environment and allows a more in-depth exposure of select topics. SMILE engages students to develop low-cost, custom-built instruments that facilitate the practical application of chromatography, electrochemistry, and spectroscopy in undergraduate general and analytical chemistry laboratory courses. We have found that the construction and use of these student-built instruments promote student competency in our courses.

The aspects of this laboratory which make it unique and suitable for widespread adoption compared to previous work include the cost per device, the ease and minimal time commitment to fabricate the devices, and that students are intimately involved in device fabrication.

Methods

Traditionally, ELISA testing is performed using a 96-well plate. The procedure follows a sandwich method, where the plate is coated with a capture antibody to which the sample is added. Then an enzyme-linked secondary antibody is added, with which a soluble small molecule reacts to create a color change, fluorescent, or electrochemical signal. This method typically uses 50–100 µL of each solution per well (total well volume is approximately 500 µL). After adding each solution, the well plate may need to incubate between 30 minutes and 2 hours. Additionally, the well plate needs to be washed with a generous amount of buffer solution (300–500 µL per well) after adding each solution to remove excess antigen/antibody.

Microfluidic masters are typically made by using photolithography, a common clean room technology but there are many creative workarounds published in the chemical education literature including the use and manufacture of paper-based devices [30–34], acrylate- or PDMS- based resins poured directly over metal tubing shaped into specific patterns [35, 36], and laser printing patterns directly onto a thermoplastic sheet followed by heat-treatment or using a 3D printer to create patterns in a photopolymer resin then using PDMS to cast the stamp [37–40]. Several 3D printing methods are commonly employed but stereolithography (SLA) printers produce smaller, well-defined channels than those produced using fused deposition molding, inkjet/Polyjet, and other 3D technologies [41]. Indeed, the relatively low cost and ease of use make SLA 3D printers increasingly common manufacturing alternatives for microfluidic devices. There are

two significant drawbacks to using SLA printed devices: 1) printing a single device can take several hours and 2) the volume and cost of resin required to build multiple devices may prohibit scale-up to larger enrollment laboratory courses. Additionally, the large excess of unpolymerized resin after printing must be carefully removed by washing and gently rubbing with ethanol or similar solvent so as not to damage the printed master.

With the use of microfluidic devices for ELISA testing, channels are used instead of wells. The patterned channels we used were 300-μm wide, 50-μm deep (measured by a Dial Depth Gauge from Mitutoyo with precision of 0.01 mm), and 2-cm long and solutions were injected via micropipette through an inlet and exit through an outlet, which can be shared or separated for each channel. Using this method, each channel requires only 5 μL of sample solution. The amount of wash buffer used is significantly decreased as well; two flow washes at 5 μL of wash buffer per single flow wash would be sufficient. The total was volume is (5 μL × 2 flow washes × 7 channels) = 70 μL. Because the volume of solution is so small, the incubation period can be reduced to as little as 5 minutes, with a maximum of 15 minutes. The channels are of sufficient size such that any color change is clearly visible and can be photographed with a smartphone device to be analyzed via ImageJ and Excel Spreadsheet. A single AIDS Kit I: Simulation of HIV Detection by ELISA from Edvotek (SKU 271; $129) provides sufficient reagents to be used over the entire two-semester academic year (total number of students = 120, total number of groups = 60) with excess reagent remaining.

Fabrication of Microfluidic Master Mold. The microfluidic devices were fabricated using a soft lithography method and the 3D printed pattern master depicted in Figure 2. The master is used for PDMS replication, in which PDMS is poured over the master, encasing the design and molding to it. After the PDMS is cured, it can be peeled off. Once removed, the PDMS surface that previously contacted the master will be flat with grooves which form the channels. This stamp can be bound to a glass slide via oxygen-plasma treatment, making up the bottom wall of the channels [42]. The oxygen-plasma can be generated by a commercialized plasma cleaner or by a Bunsen burner flame, depending on their availability.

SLA 3D printing creates models in a layer-by-layer photochemical process. A UV laser is focused onto a light-activated resin, following a programmed pattern [43, 44]. As the laser beam is rastered across the sample, the resin photopolymerizes and hardens and becomes the structure that is printed. An SLA 3D printer typically consists of 3 main parts: a resin tray, the printing base, and the UV laser. The object is printed on the base and removed when finished. Usually, a large amount of resin is needed to cover the minimum level in the tray for successful printing. While this method works well for printing most objects, the large amount of resin in the tray is inefficient and takes hours for printing microfluidic channels which require little resin. As such, using the printing base as a platform for PDMS replication is unsuitable. Therefore, we developed a modified method of 3D printing which eliminates the resin tray. Instead, the printing resin is placed directly in a petri dish, which is then fastened onto the printing base. Employing this method, the pattern hardens directly onto the petri dish and serves as the mold for PDMS replication. By eliminating the resin tray and printing directly onto a petri dish, each master mold uses only 0.7 mL of printing resin and can be completed within minutes. The SI contains a complete description of the modified method.

When the PDMS stamp is bound to glass, plasma treatment is required. It is typically performed in a plasma cleaner which is expensive and not available in most teaching labs. Instead, we found that oxygen plasma treatment can be accomplished using a Bunsen burner flame. By passing the PDMS stamp and glass surface over the top of the flame, radicals are formed at each surface which permits the two pieces to bind together and form enclosed channels.

General Procedure. A Peopoly Moai 130 Laser SLA 3D Printer and Peopoly Moai UV Curing Light were purchased from MatterHackers. The Monoprice Rapid UV 3D Printer Resin (1000mL, clear) and CRC 03300 Silicone Mold Release Spray (16oz Aerosol can) were purchased from Amazon. Dow SYLGARD 184 Silicone Encapsulant Clear 0.5 kg PDMS Kit was purchased from Ellsworth Adhesives. In addition to these materials, 9-cm diameter plastic petri dishes were used.

To create the microfluidic master, 0.5 mL resin was coated on a petri dish by manually rotating the dish until evenly spread. The coated petri dish was cured by 405 nm UV light for 2 minutes. Then 0.7 mL resin was placed on the coated petri dish to serve as the resin for 3D printing. The petri dish was attached to the printing base using rubber bands and the design printed with the 3D printer. After printing, the pattern was developed with 95% ethanol and post-cured by 405 nm UV light for 2 minutes. The PDMS (10 g) was mixed at 10 to 1 monomer/crosslinker ratio and degassed prior to pouring over the master which had been treated with a PDMS mold-release spray to facilitate removal of the PDMS stamp from the petri dish. The petri dish was covered and left at room temperature (25 °C) to cure for 2–3 days. Once cured, the PDMS was peeled off and the inlets/outlets were made by poking holes through the stamp at both ends of each channel with an 18-gauge (OD 1.27 mm, ID 0.84mm) syringe needle. Using tweezers, the PDMS stamp and a glass slide were quickly passed over the top of a Bunsen burner flame 2–3 times. Immediately, the PDMS stamp was placed on the glass slide with the channel grooves facing downward. This creates the finished microfluidic device.

For the HIV/AIDS ELISA assay, an AIDS Kit I (Simulation of HIV Detection by ELISA) was purchased from Edvotek. The kit contains all necessary chemicals and solutions: 10X ELISA wash buffer, ELISA dilution buffer, antigen (lyophilized), primary antibody (lyophilized), secondary antibody (lyophilized), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (lyophilized), and ABTS reaction buffer. For the wash buffer, it was diluted 10X and the pH was 8.33. The dilution buffer was used as is from the kit with a pH of 7.4. The ABTS reaction buffer was also used, as purchased, at pH 4.52. Bovine Serum Albumin (BSA, lyophilized powder, ≥96%) was purchased from Sigma Aldrich and prepared at 1 mg/mL in the dilution buffer. Stock solutions of the antigen (~1.4 mg/mL), primary antibody (~1.4 mg/mL), and secondary antibody (~1.4 mg/mL) were prepared in advance of the lab by reconstituting the supplied powders in 7-mL of dilution buffer. Shortly before the lab period, a stock solution of the ABTS substrate was prepared using 10-mL of reaction buffer for a final concentration ~3 mg/mL.

For the qualitative assay, channels were labeled from numbers 1 to 7. Using a 10-μL micropipette, 5 μL of antigen were pushed through all 7 channels. The channels were

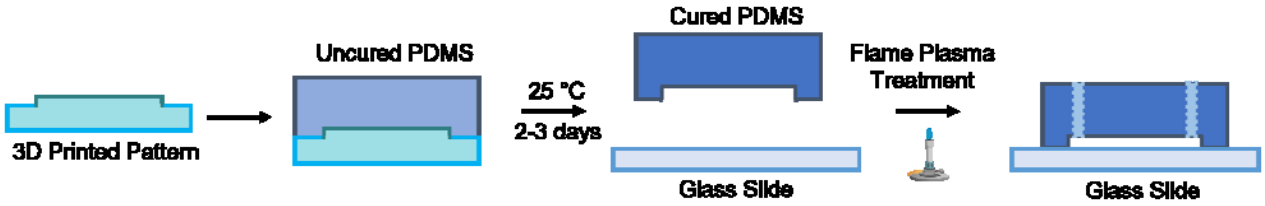

Figure 2. The soft lithography process used to produce PDMS microfluidic devices.

incubated for 5 minutes at room temperature. After each incubation period, all 7 channels were washed using 10 µL of wash buffer. Then 5 µL of BSA were added to each channel to block any exposed glass surface, allowed to incubate for 5 minutes, and then washed with 10 µl buffer. Next, 5 µL of primary antibody were added to separate channels: a negative control was added to channel 1, a positive control was added to channels 2–3, donor 1 patient sample was added to channels 4–5, and donor 2 patient sample was added to channels 6–7. The channels were allowed to incubate for 5 minutes at room temperature and washed. Next, 5 µL of secondary antibody solution was added to all 7 channels, then the channels were incubated for 5 minutes at room temperature and washed as before. Lastly, 5 µL of ABTS substrate solution was added to each channel. After a 15-minute incubation period at room temperature, the color change was imaged with a cell phone camera.

For the quantitative binding affinity assay, the concentrations of all solutions were the same as in the qualitative assay except for the primary antibody. From the stock primary antibody solution (1.4 mg/mL), seven different concentration solutions were made: 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. Each solution was used in place of the positive and negative controls and patient samples. Otherwise, the ELISA was performed identically to the qualitative assay. After the final step, the resulting color intensity increased with primary antibody concentration. To find the binding affinity, we took an image of the device using a smartphone and uploaded it into ImageJ. Then, using Excel, the intensities were converted to absorbance and the normalized data points were fit to the Langmuir Binding Isotherm model to find binding affinity.

Results

To demonstrate the successful microfluidic device production, a 9-channel PDMS device was fabricated (Image shown in Figure 3A). The dimensions of the PDMS stamp were approximately 1× 2 cm. The smallest channel width printed was 100 µm, the height was 50 µm (measured by a Dial Depth Gauge from Mitutoyo with precision of 0.01 mm), and the length 2 cm. A 50-µM zinc porphyrin dye solution was prepared and flowed through each channel. The fluorescent microscope image (Figure 3B) clearly showed the correct channel width and spacing.

Qualitative ELISA Assay

For our qualitative evaluation, two unknown samples (donor 1 and donor 2) were tested for HIV/AIDS. A visible color change indicated a positive result whereas no color change indicated a negative result. We used a 7-channel design with separated outlets to avoid possible contamination by backflow. In Figure 4, Channel 1 contained the negative control, channels 2 and 3 contained the positive control, channels 4 and 5 contained unknown donor 1, and channels 6 and 7 contained unknown donor 2. The samples were loaded into multiple channels as a verification check. After testing, channels 2, 3, 4, and 5 displayed a color change from colorless to green, indicating that donor 1 was positive for HIV/AIDS while donor 2 was negative.

In addition to learning about diagnostics, students learn the value of positive and negative controls by carrying out this assay. The positive control helps verify that the test is operating correctly, and that it would diagnose an HIV–positive patient. Combined with a negative control, the dynamic range of the test is established, helping to distinguish positive and negative results.

Binding Affinity Measurements

For the quantitative evaluation, different concentrations of the primary antibody were added to each channel to determine the equilibrium dissociation constant, K_d. The same channel design and procedure were used. The concentrations increased from channel 1 to channel 7: (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). The channels with the primary antibody present showed a color change. The intensity of the color increased with increasing concentration of primary antibody. We took an image of the channels (Figure 5A) using a smartphone and uploaded the picture with ImageJ software, which was used to obtain a brightness value for each channel. The brightness value was calculated using the default RGB weighting factors employed by the "Measure" macro in ImageJ. The brightness value for each channel was converted to absorbance using the value of the 0 mg/mL channel as a reference. Using Excel, the data were fitted to a Langmuir Binding Isotherm model (eq 1):

$$Absorbance = \frac{a \times C}{K_d + C} \quad (1)$$

where a is a factor of proportionality from the data and C is the antibody concentration. The converted absorbance values were plotted against the concentration of the primary antibody (Figure 5B). The orange curve represents the fit of the Langmuir binding isotherm to these points from which the K_d value is calculated.

Discussion

The HIV ELISA assay demonstrates the utility of microfluidic devices fabricated using this new technique. Students were able to complete both a qualitative diagnostic assay as well as a quantitative determination of an antigen-antibody binding affinity using a socially relevant pathogen. In

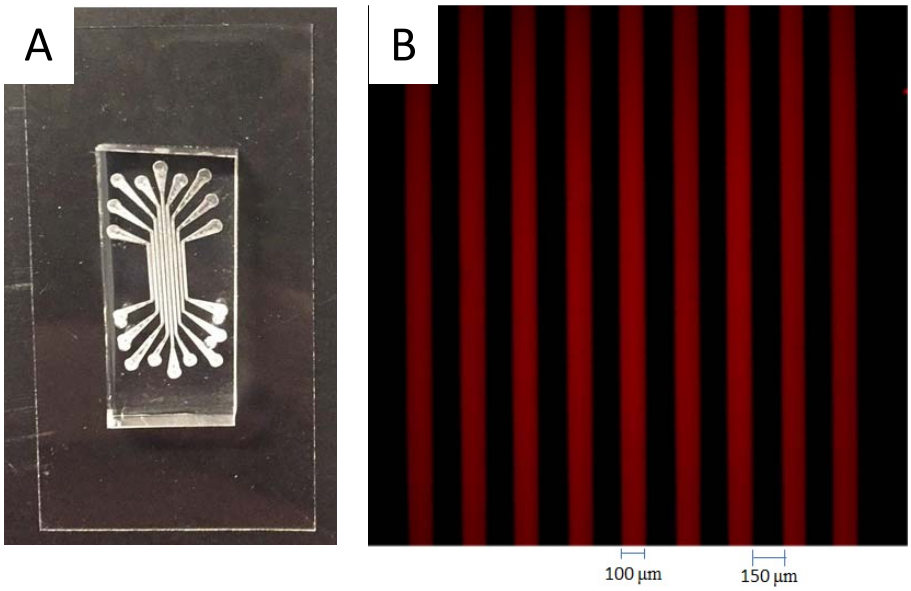

Figure 3. (A) A PDMS microfluidic device fused to glass. **(B)** A fluorescence microscopy image of the channels filled with a 50 µM Zinc Porphyrin dye shows 100 µm channels (red) with 150 µm spacings between.

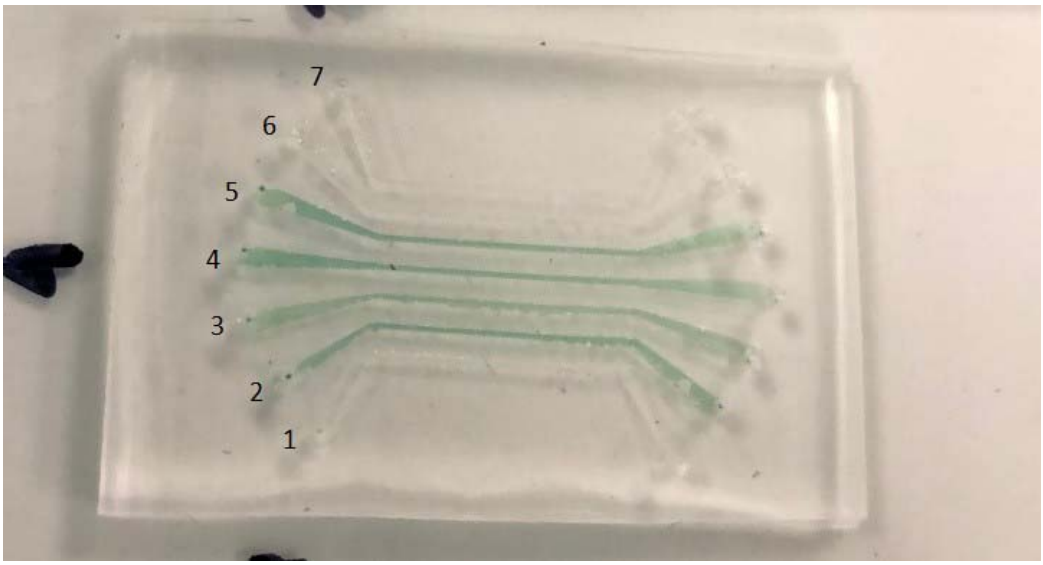

Figure 4. A cellphone image of a qualitative microfluidic experiment. Channel 1 corresponds to a negative control, channels 2–3 to a positive control, channels 4–5 to donor 1, and channels 6–7 to donor 2. Channels 2–5 showed a green response whereas the others did not.

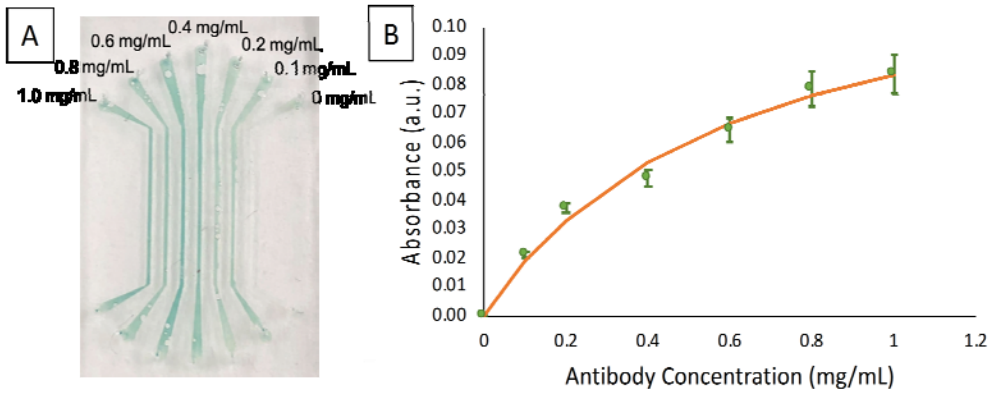

Figure 5. (A) Cellphone image of the quantitative assay for the HIV/AIDS ELISA assay. Varying concentrations of the primary antibody were added to each channel as labeled. **(B)** Absorbance values extracted using ImageJ plotted against the antibody concentration from experimental data. The orange curve represents the best fit to the Langmuir binding isotherm in eq 1.

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future semesters, the assay could be adapted for deactivated SARS-CoV-2 to help students continue to appreciate the impact and utility of immunoassays. By performing these experiments in the analytical lab course, students were exposed to 3D fabrication, microfluidic technology, protein-ligand binding affinity, and immunoassays within the time frame of two four–hour laboratory periods.

The kit from Edvotek includes a positive control, a negative control, and two mock donor samples allowing for a qualitative assay "diagnosing" these donors. In addition to learning about diagnostics, students learn the value of positive and negative controls. The positive control helps verify that the test is operating correctly, and that it would diagnose an HIV–positive patient.

The student calculated K_d values vary from semester-to-semester and whether the lab was "performed" in-person or online. For the in-person experiments values of 5.1 ± 2.8 μM and 3.7 ± 1.3 μM (95% CI) were obtained for the Fall 2020 and 2021 semesters, respectively. For the online semesters, students were provided the same image of a completed quantitative experiment to process using ImageJ and perform the data workup. K_d values of 1.1 ± 0.4 μM, 2.5 ± 0.9 μM, and 2.1 ± 0.9 μM (95% CI) were obtained for the Spring 2020, Fall 2020, and Spring 2021 semesters. Please note that several student groups in Fall 2021 were provided instructor-made devices because their PDMS molds failed to cure. The literature value for binding between HIV antibodies and the HIV antigen is reported to be in the low micromolar to high nanomolar range [45–47]. As such, our inexpensive assay aligned with previous experiments.

Several factors contribute to the large confidence interval but the most common are differences in image quality obtained from student cell phones, issues processing images with ImageJ, poor pipetting skills which include incorrect injection volumes and too fast injection speeds, and leakage between channels arising from poor binding between the PDMS stamp and the glass slide. For the online semesters, students were provided with the same microfluidic device image so any differences in calculated color intensities, and therefore absorbance values, arise from measurement variability along the different transects students selected for ImageJ processing. For example, for the online Spring 2021 semester the range of K_d values calculated for the same image was 0.4–10.7 μM. Although the ranges of values obtained from the in-person semesters are larger than the online semesters, there are significant contributions to the overall variability that are beyond student-related performance. The Supporting Material includes a table comparing K_d values from each semester.

The ELISA project is the culminating end-of-semester laboratory activity in the sophomore-level analytical chemistry course. The course is offered in both fall and spring semesters with a total academic year enrollment of about 80 students. The lab experiment is supported by a single 50-minute lecture presentation on microfluidic devices (primarily focusing on chromatographic applications) and a short 10- to 15-minute pre-lab lecture (primarily focusing on stereolithography). There is no attempt to assess students' understanding of the theory and use of microfluidic devices or 3D printing technologies based on the lectures. Instead, there are two pre-lab and two post-lab assessments.

The extra-credit pre-lab formative assessment is conducted online a week prior to the lecture presentation on microfluidic devices (and before any work on the lab). Students are asked to answer four free response questions: 1) "What is 3D printing?," 2) "Identify as many applications as you are able in which 3D printing is useful", 3) "What materials are currently being used in 3D printing technologies?," and 4) What is an SLA 3D printer(?), i.e., describe its principle of operation."

Typically, we receive one- to two-sentence responses for each question. Most students know of 3D printing and some have used a 3D printer (mainly polylactic acid, PLA, 3D printers). Most students list two or three applications and the most often cited are hobby/craft and biomedical applications. The commonly listed printing materials are plastics and ceramics, and to a lesser extent, metal. Since the lab was first introduced, only two students knew of and could describe an SLA and its principle of operation.

The pre-lab summative assessment is conducted at the beginning of lab during the first week of the ELISA project. The purpose of the quiz is to ensure each student comes to lab adequately prepared to perform the assigned experiment. Students are asked to answer four free response questions: 1) "Describe the basic ELISA sandwich assay method." 2) "Define the purpose of a positive control," 3) "Define the purpose of a negative control," and 4) "Does the quantitative assay use an external calibration, standard addition, or internal standard method as part of the data analysis?" The final three questions are important to the experimental design and connect to material covered in the first two weeks of the course. Students must receive a 70% or higher on the quiz or risk losing points on their final lab reports. To-date, no student has scored less than 70% on the pre-lab quiz.

The final lab report is handwritten in the laboratory notebook (or type-written during semesters when the course was offered online) and follows the same format as all the other prior lab reports. The report consists of a neat, legible, and well-organized laboratory notebook which accurately reflects the work performed, all calculations, tables, and figures relevant for recording, describing and discussing the data, a discussion centered around the post-lab questions in the lab handout (see the Supporting material), and a concise and pertinent statement of the conclusions.

The final post-lab assessment is a reflection exercise assigned in a subsequent course – an advanced writing-intensive capstone laboratory course in chromatography and electrochemistry. About eight of the eighty students in the sophomore-level analytical course each academic year go on to take the advanced course in their junior or senior year. Students are provided with the following prompt:

"The microfluidics-based ELISA lab was a standalone activity within the CHEM 227 curriculum. Please comment on the success of the microfluidics lab in

1. Generating interest in, and preparing you for, CHEM 425W - Chromatography and Electrochemistry – in which we print and use microfluidic devices for electrophoretic-based separations.
2. Connecting back to concepts introduced in the first few weeks of the semester – QA/QC (positive and negative controls) and methods of quantitation (external calibrations).

Some representative student comments are provided in the Supporting Material.

Conclusion

We have demonstrated a simple, in-house method for producing custom microfluidic devices using an inexpensive 3D printer. The initial investment of all materials costs under $2,000. The cost of each individual device is about $2.50, with the main disposable cost being the PDMS kit. Traditional photolithography techniques used to generate patterns with microscale features are oftentimes technically difficult to work with, expensive, and require the use of hazardous etching chemicals. This requires heavy investments in infrastructure and training, making widespread implementation of microfluidic assays challenging and expensive. The strategy presented here is simple and quick enough to complete over the course of two four-hour lab periods with undergraduate students.

To demonstrate their utility, we implemented a microfluidic ELISA immunoassay. As our world's focus has shifted due to the SARS-CoV-2 (Covid-19) pandemic, understanding viral diagnostics is of heightened interest to students and instructors alike. This set of experiments helps students to appreciate the efficiency of both 3D printing and microfluidic experiments while learning a valuable bioanalytical assay.

Acknowledgements. PSC Acknowledges the National Science Foundation (CHE-2004050 and CHE-2305129) for support. The authors acknowledge the support and permission of Cristina C. to include her laboratory report.

Supporting Material. Included are: Instructor Notes and Student Handout, Grading Rubric, Example Student Lab Report, Student Reflections, Excel File with ELISA Fitting Macro, as well as Source stl, dwg, and gcode files.

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