Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z53016

69451 Weinheim, Germany

An Integrated Approach to a Portable and Low-Cost Immunoassay for Resource-Poor Settings

Samuel K. Sia, Vincent Linder, Babak Amir Parviz, Adam Siegel and George M. Whitesides*

Department of Chemistry and Chemical Biology

Harvard University, 12 Oxford St, Cambridge, MA 02138

*Email: gwhitesides@gmwgroup.harvard.edu

Materials and methods

Rabbit IgG, anti-rabbit IgG (horseradish peroxidase conjugated), anti-rabbit IgG (alkaline phosphatase conjugated), anti-rabbit IgG (gold conjugated), p-nitrophenylphosphate (pNPP), and the silver enhancement kit were obtained from Sigma-Aldrich (St. Louis, MO). AttoPhos was purchased from Promega Corp. (Milwaukee WI). SuperSignal ELISA Femto Max was purchased from Pierce (Rockford, IL). BluePhos phosphatase substrate was purchased from KPL (Gaithersburg, MD). HIV Env antigen (gp41) was purchased from Research Diagnostics (Flanders, NJ). HIV positive serum and control serum were purchased from Golden West Biologicals Inc. (Temecula, CA).

Immunoassays in 96-well microtiter plates were performed using a Tecan Genesis liquid handling robot (Center for Genomics Research, Harvard University). The following Nunc MaxiSorp polystyrene plates were used for the silver reduction and ELISA assays: clear plates for silver reduction and absorbance, black plates for fluorescence and white plates for chemiluminescence. Rabbit IgG (70 μL for each well) in ten-fold dilutions (10 μg/mL to 100 pg/mL, which corresponded to 67 nM to 670 fM) was added to the microwells, except for one row to which PBS was added as a negative control; incubation time was 2 hours. Blocking buffer (100 μL of 0.05% Tween-20 and 1% BSA in PBS) was then added, and left to incubate for 30 minutes. For secondary antibodies, dilutions (30 μL of 0.05% Tween-20 in PBS) of 1:300 anti-rabbit IgG (gold-

conjugated), 1:1000 anti-rabbit IgG (alkaline phosphatase), and 1:1000 anti-rabbit IgG (horseradish peroxidase) were used; incubation time was 1 hour. For ELISA substrates, pNPP (100 uL; 3 minute incubation), AttoPhos (100 uL, used within 1 week of opening; 10 minute incubation), and SuperSignal Femto ELISA (100 uL; after 5 minutes). For silver enhancement, the solutions of silver and initiator (at 4°C) were mixed in a 1:1 ratio immediately before development; it was filtered through a 0.2 μm filter, and 100 uL was added to each well. After a 20 minute incubation, the silver enhancer solution was removed, and each well was washed with water. In general, warming the silver enhancement solution from 4°C to room temperature increased the rate of silver deposition. In between the addition of each new reagent, each well was washed three times with PBS, with the following exception: deionized water was used to wash the wells after incubation with anti-rabbit IgG (gold) and before silver enhancement, in order to avoid precipitation of AgCl. The plate readers used were Spectramax Plus 384 for absorbance measurements, and Spectramax Gemini XS for fluorescence and chemiluminescence measurements.

The output of the optical IC was light transmittance; apparent absorbance values were calculated using the relation $A = -\log(T / T_0)$, where A is the absorbance, and T and T_0 are the transmission of the light through the sample and reference, respectively, to the photodetector. Air was used as the reference in the plate reader, and a blank polystyrene plate was used as the reference for the portable detector.

The absorbance, fluorescence, and chemiluminescence readings (y) were fit to sigmoidal curves using the software Kaleidagraph and the following equation: $y = Ax^n / (B + x^n) + C$, where x is the concentration of the analyte, and A, B, C and n are floating parameters. This equation describes a general sigmoidal curve with the lowest possible number of floating parameters (four). Curve fitting to all four titrations gave correlation coefficients of over 0.99. The readings y for all four titrations were normalized to the same scale (0 to 1) by linearly transforming each data set to achieve the values of $A = 1$ (asymptote as x approaches infinity) and $C = 0$ (y-intercept).

Limits of detection were calculated according to the IUPAC definition: three times the standard deviation of the blank sample ("noise") divided by the slope ("sensitivity"). In samples with no rabbit IgG (i.e. negative controls), the methods that exhibited the least to most noise were (after normalization of the signal from 0 to 1): 0.006 for absorbance of pNPP, 0.014 for chemluminescence of SuperSignal ELISA Femto Max, 0.023 for silver (using the portable detector), and 0.066 for fluorescence of AttoPhos. The methods that showed the highest to lowest sensitivities, which were measured as slopes of the best-fit curves in the middle of the linear working range of detection (signal of 0.50), were (in normalized units per 100 pM of analyte): 0.193 for chemiluminescence, 0.121 for fluorescence, 0.078 for silver, and 0.035 for absorbance.

To prepare immunoassay samples for analysis by AFM, holes (4 mm in diameter) were punched in a PDMS slab, and the PDMS slab was placed onto a polystyrene surface. Immunoassays were carried out in individual PDMS wells. After silver

development, the PDMS slab was removed, and the samples on the flat polystyrene substrate were analyzed by tapping mode AFM. AFM was performed with a Dimension 3100 Scanning Probe Microscope (Digital Instruments, Santa Barbara, CA) in tapping mode, using silicon probes (Si #MPP-111000; NanoDevices, Santa Barbara, CA) at a scan rate of 0.35 Hz. Streaking was observed for samples with the largest silver grains, which suggested that the silver grains were loosely bound to the surface.

The microfluidic device was fabricated in PDMS using published procedures in soft lithography [17]. The dimensions of the microchannels were 2 mm in width and 130 μm in height. We initially patterned the polystyrene surface with a stripe of HIV Env antigen (10 μg/mL) by filling a PDMS channel (conformally sealed onto the polystyrene plate) with the antigen solution. After an overnight incubation, we emptied the channel, removed the PDMS slab from the polystyrene surface, and rinsed the surface with deionized water. We covered the stripe of antigen with an unstructured slab of PDMS, and oxidized the remaining surface of polystyrene with oxygen plasma[17]. After removal of the plasma-protective PDMS slab, we sealed another microfluidic channel (also freshly plasma-oxidized) orthogonally to the antigen stripe. The dimensions of these microchannels were 2 mm in width and 40 μm in height; the width of the channel must be large enough to register a signal with the portable detector. To avoid sagging of the PDMS, pillars (which took up 12% of the surface area) were included in the channel design. We carried out the anti-HIV antibody assay in the microfluidic channels with the following incubation times: 1 to 4 hours for blocking, 10 minutes for samples, 10 minutes for gold-labelled anti-human IgG, and 13.5 minutes for silver enhancement solution.

After 6.5 minutes of silver enhancement, we exchanged the silver solution with a freshly prepared one. We removed the PDMS microchannel above the initial stripe of antigen before measuring the optical density of the silver film. The HIV assay in microwells were performed with the following incubation times: overnight for HIV Env antigen, 2 hours for blocking, 3 hours for samples, 1 hour for gold-labelled anti-human IgG, and 10 minutes for silver enhancement solution.

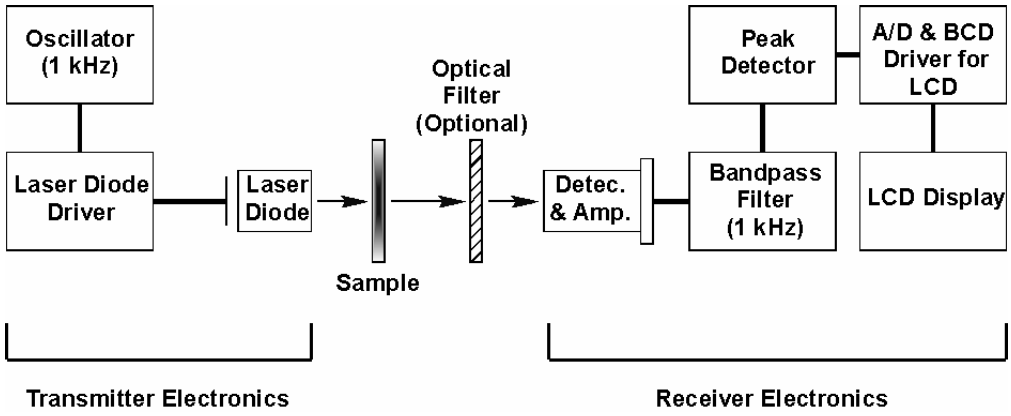
For each concentration of rabbit IgG and each dilution of human serum, triplicates of the immunoassay were performed, and average values and standard deviations were calculated.

The electronic circuit consisted of a transmitter section and a receiver section. In the transmitter section, a 1 kHz oscillator modulated the light output of a laser diode. We used a red semiconductor laser diode (Sharp GH06510B2A; normally used for optical data storage applications such as DVD); it emitted at a wavelength of 654 nm with a maximum power of 10 mW. The laser output went through the sample to the receiver section. We used an optical IC (Sharp IS455; normally used in photocopy machines) to detect and amplify the signal. IS455 provided a linear output current with respect to the input illuminance (1 μA per lux). (The dimensions and costs of the red laser diode and the optical IC were 5.6 mm and $10, and 5.0 mm and $2, respectively.) The signal was then filtered by a second-order bandpass filter centered at 1 kHz, and its amplitude registered by a peak detector. The output of the peak detector was connected to an Analog/Digital converter that also encoded the output into binary coded decimal (Intersil

ICL7106). The signal was displayed by a 3.5 digit liquid crystal display, which provided an output readout range from 0 to 1999. The entire circuitry was operated with either a 9 V battery or a single polarity 5 V source, which was inverted with a CMOS voltage converter (Intersil ICL7660) to create a ± 5 V supply. To reduce the noise in the system, we used pulse modulation of the optical signal at 1 kHz to filter the noise power in the frequency spectrum; as a result, only the portion of the optical noise that fit in the pass band of the receiver filter contributed to the overall noise detected. The system could also function without the signal modulation (i.e. at direct current), albeit with higher sensitivity to optical noise from the surroundings.

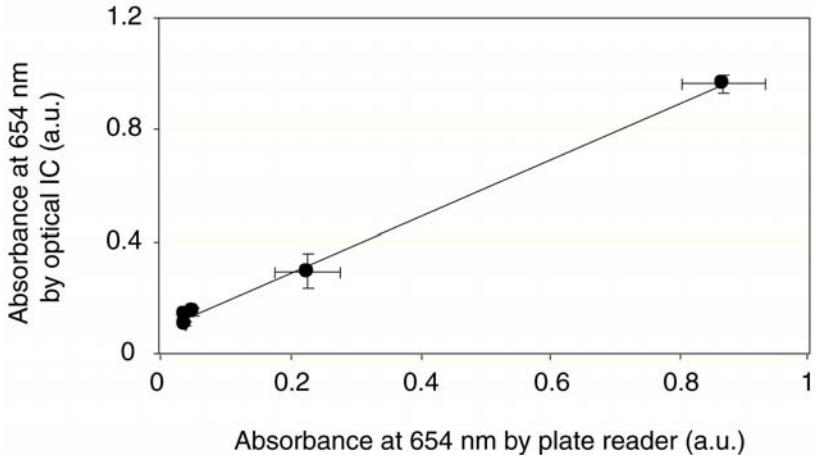
The laser diode and optical IC were placed on two separate circuit boards that were held at a fixed orientation to ensure consistent alignment of the light path from the light source to the photodetector. Between the light source and photodetector, a glass plate was placed. A black transparency, with a pinhole aligned with the light path, was placed on the glass plate to block the transmission of stray light that did not enter the sample. To record a measurement, a polystyrene plate (either a 96-well plate or a plate with a microfluidic device) was placed onto the glass plate. The sample was aligned to the light path by roughly placing the sample over the pinhole, and finely adjusting the x and y position of the polystyrene plate until a maximum transmittance was achieved. The reading from the liquid crystal display was recorded.

Supplementary Figure 1. Optical detection device: schematic of the circuit.



See experimental section for details of the circuit.

Supplementary Figure 2. Comparison of absorbance readings of BluePhos using a an optical IC and a UV-visible spectrophotometer plate reader.



Absorbance of microwells containing different concentrations of BluePhos, which absorbs maximally at 600 nm, as measured by a UV-visible absorbance plate reader and the optical IC described in this study. A direct ELISA was performed on 0.67 pM to 0.67 nM of rabbit IgG as the analyte, using an anti-rabbit IgG conjugated to alkaline phosphatase and BluePhos as the phosphatase substrate. Measurements with both devices were made at 654 nm. The best fit line by linear regression is shown (correlation coefficient of 0.998, slope of 1.01, y-intercept of 0.08). Error bars are standard deviations of measurements of three different microwells.

In this assay, in which the colorimetric product is a homogeneous solution in the microwell, the two detection methods resulted in almost perfect agreement (correlation coefficient of 0.998). Thus, inhomogeneity of silver deposition on the surface may have

contributed to the imperfect agreement between the two measurement methods, such that different parts of the same well were sampled by the laser diode and by the plate reader (correlation coefficient of 0.996).