INTEGRATED ASSAY WITH SAMPLE PROCESSING: PAPER-BASED DEVICE FOR IgM DETECTION

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ABSTRACT

Point-of Care (POC) devices that can perform multi-step processes, are easy to use, and are inexpensive, are needed for use in limited-resource settings. We present an integrated paper-based device that performs an automated immunoassay for the detection of IgM antibodies with on-board sample processing. The device is a two-dimensional paper network (2DPN) consisting of protein-G coated nitrocellulose for flow-through removal of assay interfering IgG, integrated to a nitrocellulose membrane for lateral flow detection of IgM. The 2DPN is appropriate for POC settings with one user-activation step.

KEYWORDS: sample processing, IgG removal, nitrocellulose, lateral flow, immunoassay, diagnostics, point-of-care

INTRODUCTION

Lateral flow diagnostic devices based on immunoassays are available; they involve a simple step of mixing and detection of signal. However, applications that require upstream sample processing such as removal of blood cells and assay-interfering contaminants usually require performing benchtop processes involving multiple steps and centrifugation in a laboratory setting. These are not practical for point-of-care (POC) settings.

We are developing tools to automate sophisticated processes that can be incorporated into disposable devices for use at the POC. One such example is the IgM immunoassay. Elevated disease-specific IgM in blood is indicative of acute infection and thus is important in clinical diagnosis at early stages of several diseases. However, presence of disease-specific IgG in a sample can interfere with the IgM assay, causing false negatives (Fig 1) that lead to missed opportunities to treat patients with active disease. To prevent this erroneous result in IgM immunoassays, IgG removal is necessary. The most common method for IgG depletion from a plasma sample is upstream capture by protein G, which has specific affinity for human IgG.

Previously, we reported on a conventional microfluidic platform consisting of an instrument and a microfluidic card that removed IgG using protein G-coated beads [1] before IgM detection [2]. This platform, though automated, requires pumps to transport fluids through the microfluidic channels. We have also recently developed a two-dimensional paper network (2DPN) format that enables automated multistep reagent delivery without the use of pumps and have demonstrated a signal amplified antigen assay [3]. Presented here, is a first demonstration of an automated IgM assay with integrated IgG removal in the 2DPN format, which can be used for POC serodiagnosis of infectious diseases.

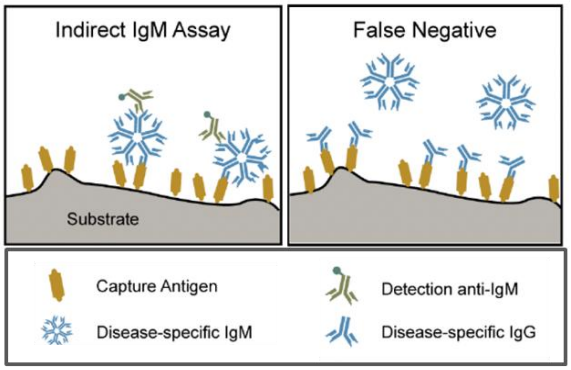

Figure 1: Schematics of the indirect IgM assay format and a potential interference mechanism in which disease specific IgG causes false negatives. IgG "mop-up" would alleviate this problem. Figure adapted from Dean Stevens.

EXPERIMENTAL

The integrated 2DPN IgM assay device consists of an IgG "mop-up" unit made up of a stack of nitrocellulose membranes (450 nm pore size) cut into circles with two projecting arms using a CO_2 laser cutting system. The membranes were incubated with 2 mg/ml protein G in citrate buffer (pH 5.0), washed and dried in a desiccator. Six of the protein G-coated membranes were stacked with the arms stuck on to a Mylar adhesive sheet to hold them together. The membrane stack was then placed directly in contact with a nitrocellulose assay membrane supported on a foldable Mylar laminate card with a cellulose wicking pad at the other end. A glass fiber conjugate pad for buffer supply was placed on the other side of the Mylar device. As a control, a BSA-coated membrane stack was similarly processed and assembled into a device.

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For studying the efficiency of IgG removal, 7 µl of Alexa 488 labeled IgG, at a level present in normal human blood (8 mg/ml), was added to the topmost membrane of the protein G/BSA-coated stack and incubated for 5 minutes. Buffer was added to the glass fiber pad, the adhesive layers of the Mylar card were exposed, and the card folded to activate the flow of the sample. Time-lapse images of the progression of the labeled IgG through the assay membrane to the wicking pad were recorded using a webcam. To quantify the output of fluorophore-labeled IgG, the wicking pads were removed from the card, soaked in buffer overnight, vortexed, and centrifuged to extract the label. The fluorescence signal was measured using a fluorimeter, and IgG depletion calculated and compared to the control.

As a proof-of-principle of integrated 2DPN IgM detection card with IgG depletion, a functional immunoassay was performed. Here, secondary anti-IgM and anti-IgG gold conjugates serving as labels for visible signal detection were dried in the presence of sugars in glass fiber pads and placed below the IgG removal stack in contact with the assay membrane. The assay membranes were striped with anti-IgM and anti-IgG for the capture of IgM and IgG respectively. Normal human plasma was added to the protein G/BSA-coated membranes as the sample. The assay was completed within 30 minutes and the gold signal was imaged using a flatbed scanner.

RESULTS AND DISCUSSION

Figure 2 shows the components of an integrated 2DPN IgM assay card. The key features of this design are a) the use of a stack of 450 nm pore-size nitrocellulose membranes coated with protein G for IgG "mop-up", b) integration of the IgG removal with IgM detection by overlaying the protein G-coated stack in direct contact with a lateral flow assay membrane and including glass fiber pad with dry secondary gold-labels, and c) housing of the paper networks and other components in a foldable Mylar and adhesive card. The small pore size of the membrane for IgG removal provides a high surface area for the high capacity binding of the protein G and subsequent efficient removal of IgG present in the blood (~ 8 mg/ml). We used at least six protein G-coated membranes in the stack to ensure effective IgG removal. The device requires the addition of sample and buffer at the appropriate locations and then a folding step. The sample flows vertically through the stack protein G stack allowing the binding of IgG to different layers and then laterally through the assay membrane to the detection zone for IgM detection. The advantage of a foldable card is that it can be easily closed for automated sample processing and immunoassay with one user-step activation.

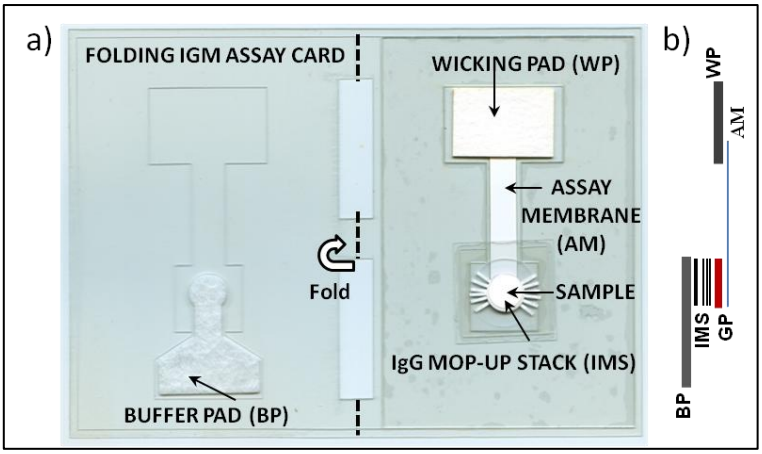

Figure 2: a) Foldable 2DPN IgM assay card with a stack of six nitrocellulose membranes coated with protein G for IgG "mop-up" overlaying the assay membrane via secondary gold label pad (GP). The assay is operated by adding sample over the topmost layer of the stack and folding the card to bring the buffer pad in contact with the stack. b) Side-view of the layers.

Figure 3a shows time-lapse images of Alexa 488-labeled IgG flowing through the device containing protein G/BSA-coated stack. The protein G-coated stack was successful in removing IgG, visualized as an intense signal at the region of the stack, whereas in the BSA-coated stack, the labeled IgG progressed through the device and was collected in the wicking pad. Figure 3b shows the images of individual membranes removed from the stack after a run. The different layers of the protein G stack show varying levels of bound IgG with decreasing intensity from top to the bottom of the stack. The BSA-coated membranes on the other hand did not significantly bind the labeled IgG. Using this device format we obtained ~95 % efficiency in IgG removal with the protein G stack as determined by fluorimetry.

Figure 4 shows an image of a functional immunoassay performed using the integrated device to demonstrate IgG removal and the IgM detection. IgG removal from human plasma by the protein G-coated stack was successful with the gold signal seen only for the IgM. As expected, the device with the control BSA-coated stack showed both the IgG and IgM signals. The total assay was completed in 30 minutes. Future work will integrate plasma separation, IgG removal, and the immunoassay into the 2DPN device for the serodiagnosis of diseases from a whole blood sample.

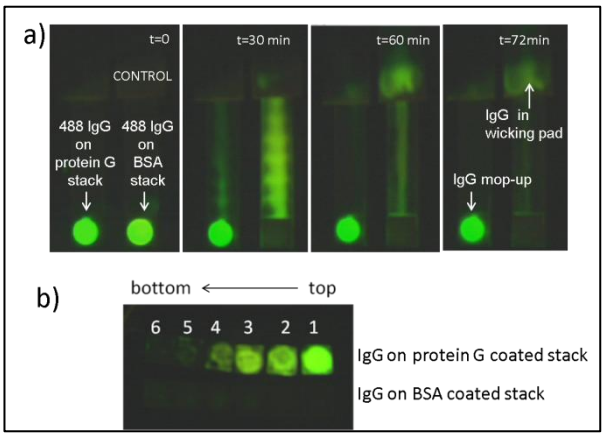

Figure 3: a) Time-lapse images of Alexa 488 labeled IgG flowing through the protein G-coated membrane stack in the 2DPN assay card. A BSA-coated membrane stack was used as a control. Arrows indicate successful IgG removal by the protein G-coated stack, whereas in the control, IgG collects in the wicking pad. b) Individual membranes from the protein G and BSA-coated membrane stacks indicate labeled IgG levels in each case.

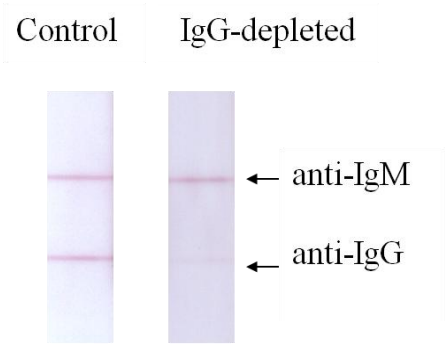

Figure 4: Scanned images of a functional IgM assay membrane showing IgG depletion and IgM capture. Normal human plasma flowing through the protein G-coated membrane stack showed signal only for IgM, indicating successful IgG "mop-up" (right), whereas BSA-coated membrane stack used as a control, showed signals for both IgG and IgM (left).

CONCLUSION

We have demonstrated an automated IgM assay with integrated IgG removal in a 2DPN device, which can be used for serodiagnosis of many infectious diseases. This device format, with one user activation step, can be used for removal of a variety of assay interferents, and is rapid, inexpensive, and appropriate for use in low-resource POC settings.

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