AUTOMATED PAPER-BASED DEVICES BY MICROFLUIDIC TIMING-VALVE FOR COMPETITIVE ELISA

Y.-T. Lai[1], J.-S. Tsai[2], J.-C. Hsu[2], and Y.-W. Lu[1]
[1] Department of Bio-Industrial Mechatronics Engineering,
[2]Department of Entomology,
National Taiwan University, Taipei, Taiwan, R.O.C.

ABSTRACT

We present a timing-valve mechanism on a paper-based device to conduct competitive ELISA procedures. The device consists of multiple channels, where timing-valves regulate each individual liquid flow, to automate sequential steps. The timing-valve is achieved using surfactants to dissolve the hydrophobic barriers and to allow fluid to pass through in a timed duration. Imidacloprid (small molecule pesticide) is tested in our proposed device, while the results validate a simplified fluidic manipulation procedure and permits only single-step application of the sample solution for on-site detection.

INTRODUCTION

Pesticide residue detection has become increasingly important, as more and more evidence shows the relevance between pesticide exposure and other negative outcomes, including neurological disorder, birth defects and fetal death. Although analytical methods to detect pesticide have been demonstrated, they commonly suffer low sample throughput and high equipment cost [1, 2, 3], which prevent themselves for on-site screening applications. On the other hand, paper has been used in biochemical analysis field for hundreds of years. Such application can be seen as pH monitoring or pregnancy testing. It has drawn great attention recently to employ paper as the substrate material of the sensor for the pesticide residue detection.

Meanwhile, one of the most common practices for pesticide residue detection is the enzyme-linked immunosorbent assay (ELISA). ELISA is a specific and highly sensitive method for measurement of antigen or antibody concentration. Typical procedures of ELISA often require a series of steps, which include mixing, washing and incubation – these steps can be tedious and time consuming. To apply ELISA onto a paper-based device, a number of controlling fluid flow methods have been developed. For instance, chemical barriers [4, 5], physical barriers [6, 7], and various fluid paths by geometry pattern have been proposed to control the lateral flow in the paper-based devices [8, 9]. In this study, we utilize wax printing to create different width of hydrophobic barriers to control fluid delay time. Also, building a timing-valves mechanism in our paper-based device.

Timing-valves mechanisms have already been reported in paper-based device for controlling fluid delivery which involves time delay and actuation of multiple fluids. This mechanism play an important role in multistep assays. Martinez et al. reported programmable paper-based device by pressing single-use 'on' push-buttons [10]. Li et al. also reported a method to stop and to promote wicking by manually separating and re-joining two paper channels [11]. While both methods provide timing-valves mechanisms, they require external actuations by hand. Here, we utilize a surfactant solution to reduce the surface tension of hydrophobic barriers to make a built-in valve mechanism. This allows the fluid to stop until the valve is triggered.

EXPERIMENTAL METHODS

Our paper-based device with the timing-valve design to handle sequentially multiple fluidic operations was constructed, as described in Figure 1. The device consists of three areas: (1) analyte input, (2) reaction and (3) detection areas.

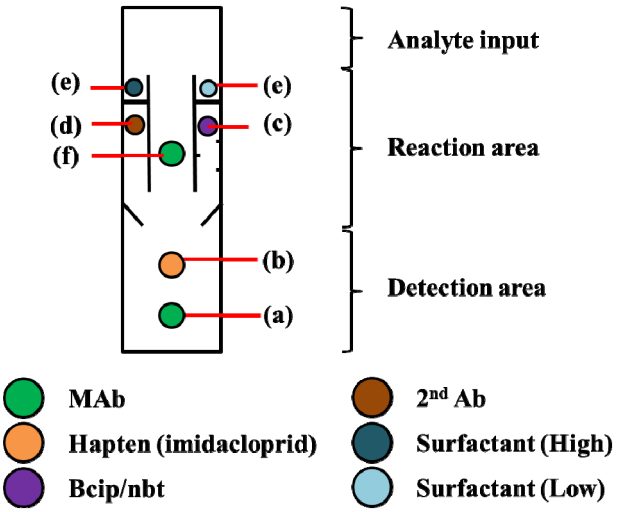

Figure 1: Schematic to show the paper-based ELISA device with our timing-valve mechanism. The device has three areas: analyte input, reaction area and detection area. The analyte was added in the input. The device is prepared before the detection with the following procedures: (a) MAb (antibody) is immobilized in the control region, (b) antigen specific to the target Abs is immobilized in the test region for colorimetric detection (c) bcip/nbt solution to produce color (d) enzyme-linked antibody (2nd Ab) (e) our timing-valve design, which control the timing and sequences of the liquid flow. (f)Mab combine with both tested sample (imidacloprid) and antigen to have competitive reaction.

In the first analyte input area, the analyte was added in this area. The second reaction area had three channels, including left, center and right channels. The center channel was no-delay channel and has no timing-valve. The channel had no wax barrier across the channel and could directly guide the solution flow to the detection

region. The right and left channels were delay channels and had their own timing valves channel. They contained hydrophobic wax barriers across the channel, which prevented the fluid wicking and delayed the solution flow passing through to reach the reaction area. The detection area include two regions: (1) control region, which contained immobilized antibody (Ab) for enzyme-linked detection, and (2) test region, which contained the immobilized specific antigens to the target Abs.

Figure 2 depicted the operation of the timing-valve to sequentially control the flow passing through channels. Once the device was made, the analyte was first added in the input area, laterally flowed through the reaction area.

When the analyte flowed into the area where the surfactant solution was dispensed before the wax barrier, it dissolved the surfactant and reduced the surface tension of the wax barrier, which promoted the wicking ability of the fluid and allowed the fluid to penetrate the barrier. This is the timing-valves mechanism; it can permit the sequential delivery of enzyme-linked antibody (2nd Ab) and bcip/nbt solution to the detection area.

Once the analyte passed through the reaction area and the enzyme reaction was completed. A colored product was formed in the detection area. A visible color change can be observed. The time duration for each valve was determined by the wax-barrier width and the surfactant concentration.

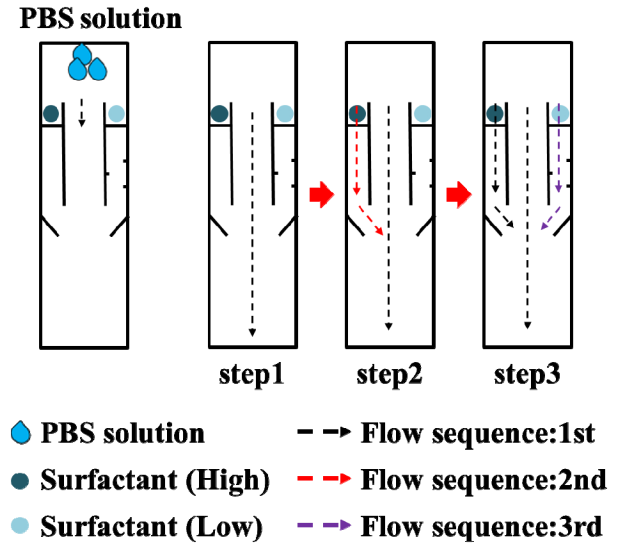

Figure 2: Schematic illustration of time delay step. In the first step, fluid via central channel. Then, left and right channel sequentially opened by dissolving different concentration of surfactant solution.

For the designed pattern, a multiple step process including the capture of MAb, capture of enzyme-Ab2, competitive reaction of sample and MAb, washing off unbound enzyme-linked Ab (2nd Ab), and enzymatic reactions, was performed sequentially by a 3-step timing-valve switch. In the first step, the sample fluid (s) laterally flow through the reaction region, and allowing the competitive reaction happened between the sample and pre-spotted Mab. Then, the flow directly passed through detection region. The enzyme reaction occurred at the detection region. In step two, after the fluid dissolved pre-spotted different concentrations of surfactant solutions, with higher concentration of side channel spent less time allowing flow to penetrate wax barrier and transport 2nd Ab to the detection region. At the last step, the side channel with lower concentrations of surfactant transported bcip/nbt substrate solution to the detection area. Hence, allowing the enzyme reaction happened at the test zone to produce a visible color change. The competitive ELISA procedure was schematically shown in Figure 2.

DEVICE FABRICATION

The patterns in Figure 1 were designed by using AutoCAD and printed on the NC membrane (Nitrocellulose membrane) using a solid ink printer (Xerox, ColorQube8580) with solid wax ink (Xerox, Genuine Solid Ink Black). After printing wax on the surface of the NC membrane, the membrane was baked at 125 °C for 120 seconds to melt the wax into the NC membrane and to form hydrophobic pattern.

In our ELISA protocol, the monoclonal mouse anti-imidacloprid antibody (MAb) and imidacloprid-antigen (antigen) solutions were spotted on the NC membrane in the control and test regions at locations (a) and (b). After drying for 1 hour at room temperature, the NC membrane was blocked to against nonspecific protein adsorption in Bovine serum albumin (BSA) solution for 1 hour and allowed to dry. The BCIP/NBT substrate, Polyclonal goat anti-mouse IgG conjugated with Alkaline phosphatase (2nd Ab) and the MAb were spotted by hand on the NC membrane at locations (c), (d) and (f), respectively. After drying 1 hour at room temperature, different concentrations of surfactant (tween-20) solution was dispensed on NC the membrane at location (e).

RESULT AND DISCUSSION

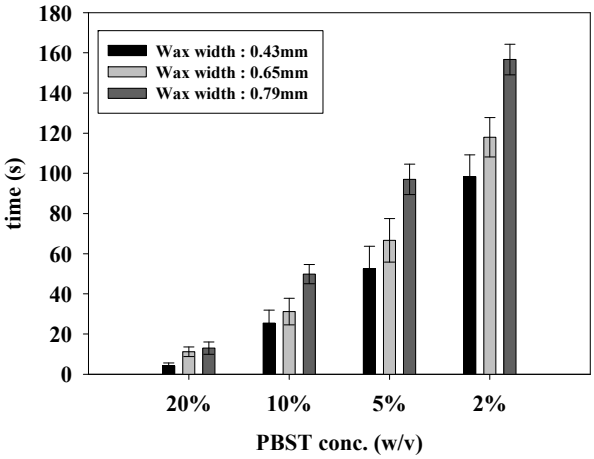

Figure 3: The widths of the wax barriers dissolved by surfactant solution (e.g. tween 20 in PBS solution, or PBST) and the time required to penetrate the wax barriers.

A series of tests using the surfactant solutions at different concentrations to wick through the wax barrier were conducted to characterize the performance of our timing-valves. The concentrations of the surfactant (e.g. tween 20) was sequential diluted in PBS solution from 20% to 1%. Additionally, three different widths of the wax

barriers were designed to measure the delaying time with different concentrations of the surfactant.

To find the optimal concentrations of the surfactant, the pattern of wax was formed by printing wax. The surfactant was dispensed on the front of wax barrier. Then, drying for 1hour to make sure the dispensing surfactant completely evaporated. 100µl of PBS solution was add in the top of the designed pattern. The solution directly wicked to the wax barrier and dissolved the surfactant which spotted on the front of wax barrier.

Figure 3 showed the time for the fluids to penetrate the wax barriers at different widths. Each test strip was printed with a wax barrier and a 0.5µl PBST solution at different concentrations was dispensed on the strip. The time was measured until the fluid wicked through the barriers. The results showed that the time wicked through the barriers decrease with the increasing PBST concentration. As the wax widths increased, the delaying time for fluid to penetrate the barriers increased. The time can be from 2 to 180 seconds, depending on the barrier widths and surfactant concentrations. All the experiments were repeated at least three times for each concentration of Tween 20. It was found that it took much longer time for the PBST solution lower than 2% to wick through the hydrophobic wax barriers. Compared to traditional ELISA, paper-based ELISA (lateral flow) require much less time for reaction. In our experiment, we need 30 to 60 seconds delay time for each timing-valve to open sequentially. Additionally, in order to prevent the solvent evaporation influence the test result. The delay time more than 180 seconds is not used in this study. Thus, PBST lower 2% was not adapted.

Surfactants in different concentrations were applied on the paper to control the fluid wicking through the wax barriers. The sample flows required different time durations to pass through our channels, resulting in the solutions (e.g. bcip/nbt, 2nd Ab...etc) to sequentially arrive in the detection area. Figure 4 showed the SEM image of wax barrier on the paper - which was later dissolved by the surfactant so the liquid flow could pass through in a timed fashion.

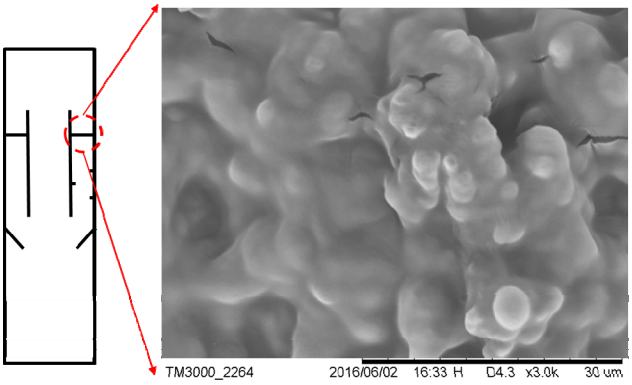

Figure 4: The inset shows the SEM image of the wax barrier on the paper - which is later dissolved by the surfactant so the liquid flow can pass through in a timed fashion.

Competitive ELISA for Imidacloprid detection was conducted. To assess the performance of the paper-based devices, the responses to the concentrations of imidacloprid were obtained under the optimal conditions. Figure 5 (a) illustrated the signals obtained at test and control regions.

Figure 5 (b) showed the image of the competitive ELISA results on automated paper-based devices. The tested sample and antigen were combined competitively with the antibody. The more antigen, the less antibody will be able to bind to the antigen – a competitive reaction. After the competitive reaction for a period of time, secondary antibody labeled with enzyme was added. Then, the consequence was measured by adding a substrate to elicit a chromogenic signal – a visible color change. The signal increased as the analyte concentrations decreased.

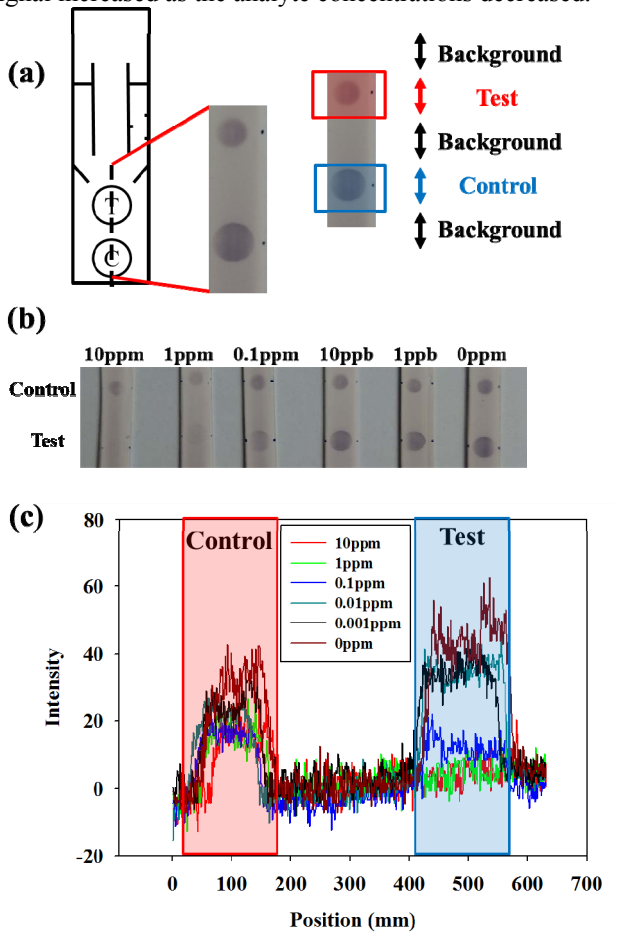

Figure 5: (a) Schematic illustration of the signal was measured at test and control regions. The color in the cross-section on the paper-based device through the test and control regions is quantified. (b) Shows the image of the competitive ELISA results on automated paper-based devices. (c) The signal intensity at different analyte concentrations at 0, 0.001, 0.01, 0.1, 1 and 10ppm can be distinguishable from 0.01ppm to 10ppm.

To further quantify chromogenic signal from our tests, the color in the cross-section on the paper-based device through the test and control regions was obtained by using a smart phone camera (Sony z1) and gray value of the image was quantified by using Image-J. The images were normalized toward each mean of background presented between the signal and control peaks. As shown in Figure 5

(c), the signal intensity in the test region increased as the analyte concentrations decreased. The results showed our device can distinguish Imidacloprid analyte at different concentrations with the limit of detection (LOD) at 0.01ppm.

In order to objectively compare the image intensity on paper from our paper-based device and the optical intensity in solution from traditional ELISA format, Test/Control (T/C) values of the ELISA results were employed. Figure 6 compares the T/C value results from at different Imidacloprid concentrations, showing similar discrimination capability and validating our device applicable for on-site pesticide detection.

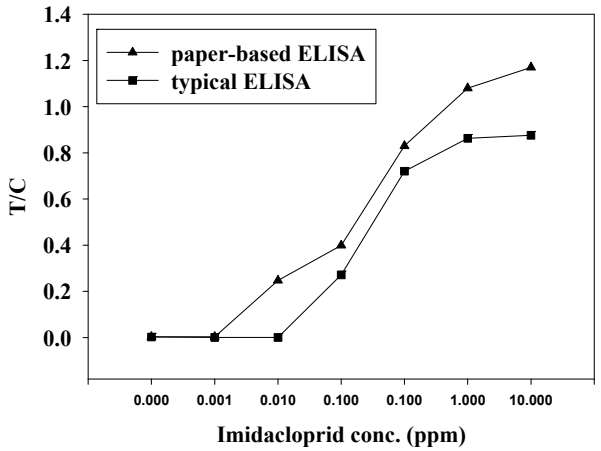

Figure 6: T/C (test/control) values of the ELISA signals from traditional and our automated paper-based ELISA at different Imidacloprid concentrations.

CONCLUSION

The paper-based device with the timing valve mechanism has been successfully developed to delivery fluid sequentially. The mechanism of the timing valve provides a method to conduct multi-step procedure assay by spotting different concentration of surfactant solution. The device can be further improved and provide the potential with a greater variety of clinical or environmental analytes.

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CONTACT

*Corresponding Author's E-mail: yenwenlu@ntu.edu.tw.