# PAPER-BASED ELECTROCHEMICAL ELISA

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## ABSTRACT

This paper describes an electrochemical enzyme-linked immunosorbent assay (ELISA) on paper-based microfluidic devices. This paper-based immunoassay method has several advantages over conventional ELISA, such as short assay time, low cost, and high sensitivity. The limit of detection (LOD) of rabbit IgG used as a model system is ~ 3.9 fM; this value is lower than the LOD of paper-based colorimetric ELISA (~54 fM).

**KEYWORDS:** Electrochemical Detection, ELISA, Immunoassay, IgG, Paper

## INTRODUCTION

Enzyme-linked immunosorbent assays (ELISA) have been widely used in biochemistry and clinical diagnosis. This approach combines specificity based on the selective interaction between antigen and antibody, and high sensitivity offered by the amplification of enzymatic catalysis [1]. However, conventional ELISA consumes relatively large quantities of reagents that are usually expensive, limiting its applications in developing countries.

We and others have recently developed paper-based microfluidic technologies for low-cost medical diagnosis and environmental monitoring [2-5]. The paper-based colorimetric ELISA that we recently reported significantly reduced the cost of the assay due to the consumption of less reagents, compared with conventional plastic microtiter plates [4]. However, the sensitivity of the paper-based colorimetric ELISA (LOD for rabbit IgG, 54 fM) is not as high as conventional ELISA (LOD, 4 fM) [6]. This paper presents a low-cost paper-based electrochemical ELISA with improved sensitivity.

## EXPERIMENTAL

We fabricated paper-based microfluidic devices by patterning chromatography paper using the photolithography technique [4]. We screen-printed working and counter electrodes from graphite ink, and a reference electrode from silver/silver chloride ink. Figure 1 shows a photograph of the paper-based microdevice used for electrochemical ELISA. Each device has 4 separate sets of electrodes within their own detection zones (~180 μm in depth).

The electrochemical indirect ELISA that we developed includes five steps: (i) immobilize antigens on SU-8 patterned paper by spotting 4 μL of a solution of rabbit IgG and waiting for 10 minutes, (ii) block to prevent non-specific adsorption of proteins using a blocking buffer [0.05% (v/v) Tween-20 and 1% (w/v) bovine serum albumin (BSA) in PBS], (iii) antibody/antigen binding by pipetting 3 μL of the enzyme-linked antibody, (iv) wash with PBS (three times), and (v) amplify the signal using *p*-aminophenyl phosphate (PAPP, 5 mg/mL) as the electrochemical substrate, before the cyclic voltammetry measurement.

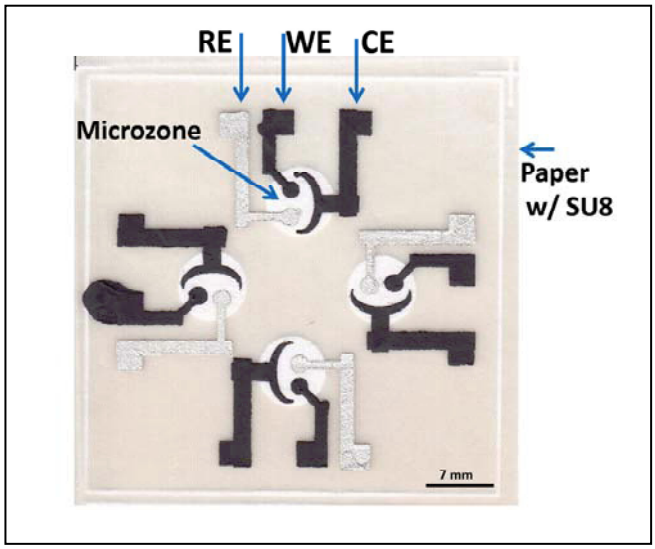


*Figure 1: A representative photograph of a paper-based microdevice with integrated electrodes fabricated by screen printing. Reference electrode (RE) was made from Ag/AgCl ink, whereas working electrode (WE) and counter electrode (CE) were made from graphite ink.*

We used rabbit IgG (25 ng/μL) conjugated with fluorescent tetramethyl rhodamine iso-thiocyanate (TRITC) to assess the immobilization of antigen on paper. 4 μL TRITC-conjugated rabbit IgG was pipetted onto the center of each detection zone, and allowed to immobilize on the paper for 10 minutes before washing 3 times with PBS (6 μL each time).

After dried, the antigen-immobilized microzones in the paper device were imaged by GE Typhoon Trio Scanner. The excitation and emission wavelengths were set to 532 nm and 580 nm, respectively.

## RESULTS AND DISCUSSION

We treated the paper-based devices with an aqueous solution of 2 wt% 3-aminopropyldimethylethoxysilane (APDES) to improve the wettability of electrodes, thus enhancing the electrochemical signals. Then, we used ferrocenecarboxylic acid (1 mM) to evaluate the electrochemical behavior of the microdevice (Figure 2a). After the device was treated with APDES two times, the peak current, $i_p$, from the redox reaction of ferrocenecarboxylic acid increased ~2.1 fold, compared to the peak current when the device was treated with APDES one time. In addition, voltammograms of the redox reactions of potassium ferricyanide indicate a good reproducibility of electrodes (Figure 2b).

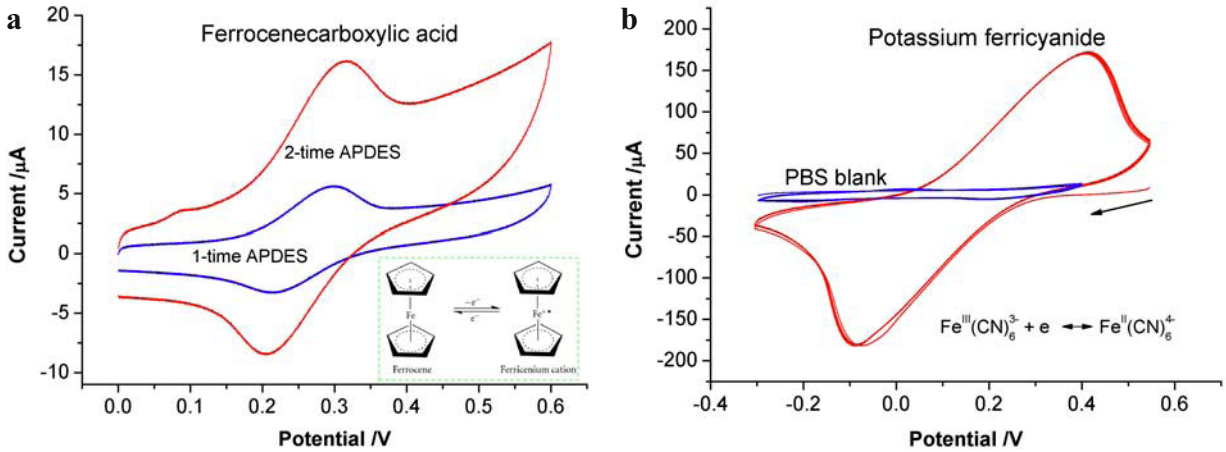


*Figure 2: Characterization of electrodes by cyclic voltammetry. a) Cyclic voltammograms of ferrocenecarboxylic acid (1 mM) in paper-based devices treated once (blue) and twice (red) with 2 wt% APDES in water. The inset shows the redox reaction occurred at the ferrocene part of ferrocenecarboxylic acid. b) 5 cycles of cyclic voltammetry of potassium ferricyanide (10 mM) in PBS, and PBS alone (blank). Potential, vs. pseudo-Ag/AgCl. Scan rate, 100 mv/s.*

We used TRITC-conjugated rabbit IgG (25 ng/μL) to assess the immobilization of antigen on paper. The fluorescence scanning results (Figure 3 inset) did confirm the immobilization of antigens on paper. We also compared the immobilization of TRITC-conjugated IgG on APDES-treated paper with that of non-treated paper. Figure 3 shows that APDES did not cause a significant impact on the antigen immobilization. The immobilization of the antigen on paper-based devices only takes about 10 minutes, compared to 120 minutes or overnight used in the conventional ELISA protocols [6], thanks to the high surface-to-volume ratio offered by macroporous paper.

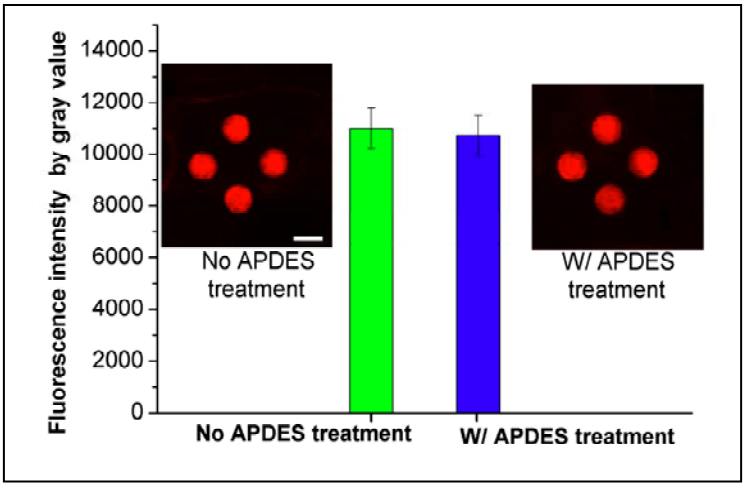


*Figure 3: Effect of APDES on rabbit IgG immobilization on paper. 4 μL TRITC-conjugated rabbit IgG (25 ng/μL) was spotted and kept for 10 minutes on each test zone before washing 3 times with PBS (6 μL each time). The test zone was either treated twice with APDES or not treated by APDES. After dried, the test zones were scanned using a fluorescence scanner (Typhoon Trio, set to 532-nm for excitation and 580-nm for emission). The mean value of the fluorescent intensity of each test zone was measured through ImagJ (N = 12).*

After 6 µL of the electrochemical substrate, *p*-aminophenyl phosphate (PAPP), was spotted onto the test zones, the enzyme alkaline phosphatase catalyzed the enzymatic conversion of PAPP to *p*-aminophenol. *p*-aminophenol can be oxidized at a low potential of ~0.1 V (*vs.* Ag/AgCl pseudo-reference electrode). Figure 4a shows a typical response of electrochemical ELISA using rabbit IgG as the targeted antigen (6.7 pM); an obvious peak of oxidation occurs at ~0.1 V due to the oxidation of *p*-aminophenol. The analysis of a lower concentration of IgG (67 fM) for the evaluation of the sensitivity of our method still shows a well-defined oxidation peak, compared with the bank (Figure 4b). The LOD is estimated to be 3.9 fM, demonstrating the higher sensitivity of paper-based electrochemical ELISA than that of the colorimetric ELISA (54 fM) reported previously [4]. Furthermore, the sensitivity of our electrochemical ELISA is similar to the sensitivity of conventional ELISA; however, our method consumes less reagents (~19 fold less) and takes less time than the conventional method [6].

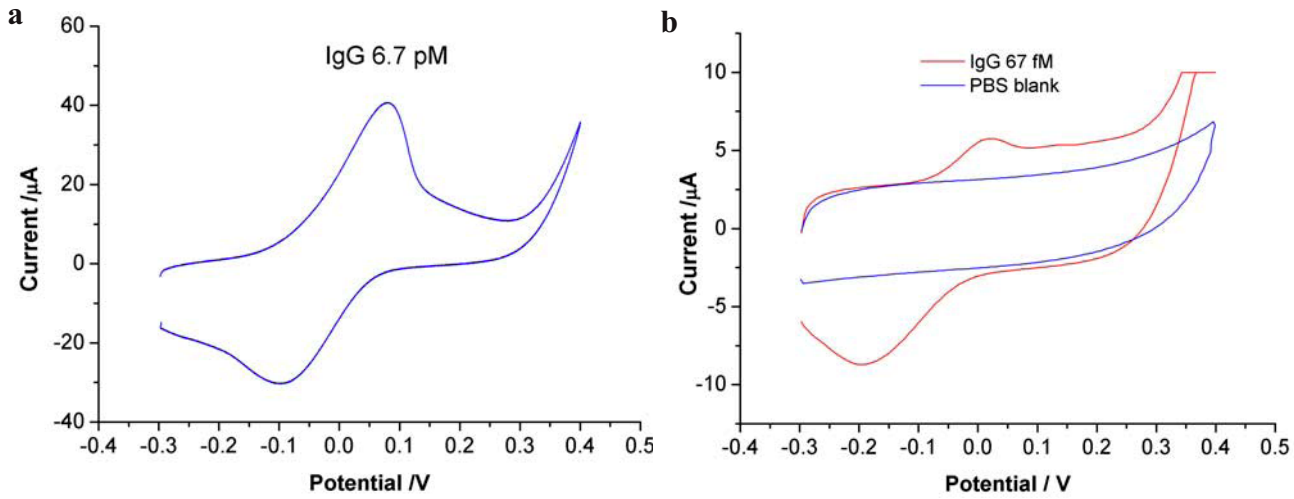


*Figure 4: Paper-based electrochemical ELISA. a) Cyclic voltammogram of 6.7 pM (1 ng/mL) rabbit IgG on paper. b) Cyclic voltammogram of 67 fM (10 pg/mL) rabbit IgG on paper and the PBS control. Other conditions are as in Figure 2.*

## CONCLUSION

We have developed a low-cost, paper-based electrochemical immunoassay that has high sensitivity. This paper-based electrochemical ELISA has five advantages: 1) it has higher sensitivity than colorimetric assay; 2) it is highly quantitative; 3) it is low cost, and consumes less reagent; 4) It is fast due to the high surface-to-volume ratio provided by the macroporous structure of paper materials; and 5) this method does not require heavy and expensive extra facilities, such as optical scanners. Therefore, this electrochemical immunoassay is suitable for sensitive medical diagnosis or pathogen detection in developing countries, such as the early detection of HIV.

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