

# Ph 調整系を組み込んだ Lab on Paper による全自動 ELISA の高感度化

## Improvement of Sensitivity for Automated ELISA

### on Lab-On-Paper by Ph Changing System

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## 1. Introduction and Objective

Despite of high sensitivity of ELISA technique, it takes relatively long analysis time due to multistage procedure. In previous work, we developed automated paper-based devices for automatic multistep sequencing of sandwich ELISAs. In the device, however, the enzymatic reaction between alkaline phosphatase (ALP) and BCIP/NBT substrate require alkaline medium, this may limit its application for real samples. In this work, automatic pH changing system on lab-on-paper devices for enzyme-linked immunosorbent (ELISA) has been developed in order to improve their sensitivity and make the technique much more applicable to real samples.

## 2. Experimental

Figure 1 shows the schematic illustration of the automated paper-based device with pH changing system for the sandwich ELISA. The prepared substances composed on the devices of (a) the control zone, which contains the immobilized Ab that picks up free (Ag unbound) enzyme-linked detection Ab to confirm that the test has operated correctly, (b) the test zone, which contains the immobilized specific Abs to the target Ag (forming a sandwich ELISA) and shows a colored band for positive test samples, (c) enzyme-linked detection antibody (the second antibody) that is allowed to bind to the antigen, (d) substrate mixture that reacts with the enzyme label to generate an insoluble colored product, and (e) alkaline reagent. After immersing the device into a sample solution, the multi-steps of ELISA were automatically executed at the test line where the substrate region was prepared with alkaline condition and the sample was loading with neutral pH.

## 3. Results and discussion

The pH changing system on automated paper-based device was successfully applied to demine hCG in 50 mM Tris buffer pH 7.4. The limit of detection for hCG is 3.7 mIU/mL (Figure 2) which is lower than previous work (8.7 mIU/mL). Enhancing the sensitivity is due to pH 7.4 improves antigen-antibody binding capability. As the results, this method is applicable to a variety of multi-step process and can be extended to a greater variety of clinical or environmental analytes.

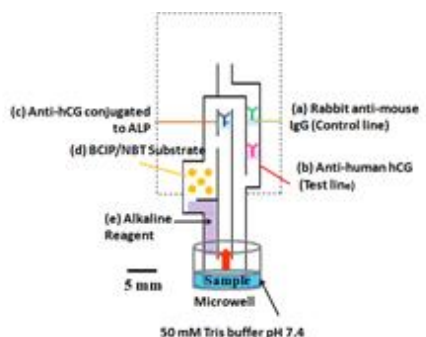


Figure 1. Schematic illustration of the automated paper-based device with pH changing system for the sandwich ELISA.

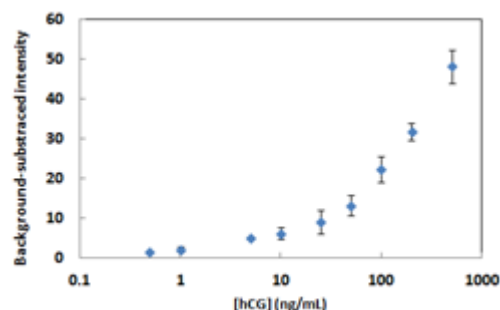


Figure 2. Calibration plot of the hCG ELISA results from 1–1000 ng/mL hCG in Tris buffer solution pH 7.4 on the automated paper-based device with pH changing system.