# Rapid ELISA in Droplet on PDMS Dimple with Nanoliter Reagents Dispensed by Ink-jet Microchip

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IgA ELISA was performed in a droplet using an ink-jet microchip for shortening the reaction time, saving the reaction reagents. Forty-five dimples (500-μm i.d., 100-μm depth) were prepared on a PDMS (polydimethylsiloxane) microchip plate ($30 \times 30$ mm) for the reaction vessels. After the dimple was pored with nanoliter water containing glycerin, an antibody solution was firstly added for immobilization on the dimple, and then nanoliter reaction regents for ELISA (enzyme-linked immunosorbent assay) were added sequentially using an ink-jet chip. A good linear relationship between the fluorescence intensity of Ample®Red and concentration of IgA was obtained with each reaction time of 150 s.

Micro total analysis system ($\mu$-TAS) has been extensively studied in global scale of scientific fields.[1–4] $\mu$-TAS performs a series of analytical operations on small microchips, such as pretreatment including sample extraction and derivatization, separation, and detection. Scientific interest in $\mu$-TAS is mostly derived from the very fast diffusion process arises from its size.[5] The ELISA methods using the microchannel realized decrease in the analysis time. However, the microchannel chips that are widely used for $\mu$-TAS required special connection devices, input/output devices and highly sensitive detection devices because of a tightly closed microflow chamber or flow path. Furthermore, the spreading of sample is caused by dead volume of sample at connection points in microfluidics.

The ink-jet device is paid attention in various fields for dispensing nano and picoliter levels of liquid droplets with accurate control of both the volume and velocity.[6] For that purpose, the ink-jet device can be used for biological samples as a non-contact microdispensing device. Ink-jet devices are applied to fields that include high-throughput screening, genomics,[7,8] and combinatorial chemistry.[9–12] In addition, small droplets from the ink-jet microchip have some advantages, for example, sample is reduced and mixture is made fast on the basis of molecular diffusion. These advantages of the small droplets are utilized for a microarray[13] and nanoliter-scale reactor arrays.[14,15]

In previous study, we reported a surface-reaction system, in nanoliter droplets made by ink-jet device.[16] In the paper, we showed that the reaction time of an enzyme–substrate reaction was shortened from 30 to 3 min due to the size effect. However, accurate control of the shape and the size of droplet which was determined by the hydrophilic patterning, were difficult. The variaties of the size critically affected the reproducibility. Furthermore, previous method has a problem that reaction media contaminate with each droplet when washing. In order to solve these problems, a dimpled PDMS microchip was newly made. The new PDMS chip has microchannel for exclusion of cross-contamination caused by mixing of adjacent droplets. The expected merits of using the new microchip improve reproducibility and prevention of cross-contamination. In this paper, we described an ELISA system using the ink-jet microchip and dimpled PDMS microchip for rapid determination of IgA. The method was successful in reducing consumption of samples and reagents in consequence of all sample introductions by ink-jet besides of wash buffer.

All reagents used in this study were of analytical reagent grade unless otherwise stated. Sodium dihydrogenphosphate, disodium hydrogenphosphate, sodium hydrogencarbonate, and sodium carbonate were obtained from Kanto Chemicals (Tokyo, Japan). Glycerin, 2-amino-2-hydroxymethyl-1,3-propanedial (Tris), sodium chloride, hydrochloric acid, 30% hydrogen peroxide, and pH standard solutions were purchased from Wako Pure Chemical Industries (Osaka, Japan). ELISA grade horseradish peroxidase (HRP) was purchased from Biozyme Laboratories (Blaenavon, Gwent, U.K.). Bovine serum albumin (BSA) was purchased from Calbiochem (San Diego, CA, USA). 10-Acetyl-3,7-dihydroxyphenoxazine (Amplex®Red) was purchased from invitrogen (Carlsbad, California, USA). A human immunoglobulin A (IgA) ELISA quantitation kit was obtained from Bethyl Laboratories Inc. (Montgomery, TX, USA). Water was purified on a Milli-Q system (Nihon Millipore, Tokyo, Japan). All buffers were filtered through a 0.45-μm membrane filter (JHWPO 4700, Nihon Millipore, Tokyo, Japan) and degassed with an aspirator before use.

The microscopic fluorescence analysis system including ejection system used in this study was described in the previous

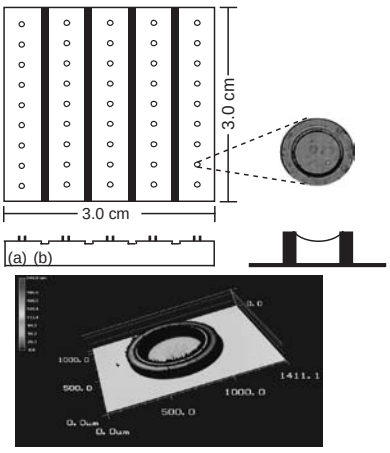


**Figure 1.** Schematic diagram of the PDMS chip with (a) dimples for ELISA, (b) slit for washing solvents, (c) laser microscopic image of the dimple.

paper.[16] Figure 1 shows the design of dimpled microchip. The silicon wafer template was prepared using reactive ion etching by induction coupled plasma (ICP) type. The dimpled PDMS microchip was fabricated by curing the prepolymer components of Sylgard 184 (Dow Corning, MI, USA) on the silicon wafer template. First, an acrylic plate was fenced around the template. The mixture of the PDMS prepolymer and curing agent of 10:1 rate in weight was degassed with a vacuum pump and 8.0 g of the mixture was poured onto the silicon wafer template with positive relief. After curing at 85 °C for 1 h, the PDMS mold with negative relief was peeled off.

All the reactions were performed in the dimples of PDMS microchip. Anti-hIgA solution, BSA solution, antigen solution, diluent buffer, second anti-IgA labeled with HRP solution, and Amplex®Red solution were dispensed 50 droplets (ca. 17 nL) in each dimple. All solutions ejected from ink-jet microchip were contained 30 wt % glycerin to prevent evaporation.[16,17] Ten μg/mL antibody solution in 0.1 M carbonate buffer (pH 8.3) was kept in a constant-humidity box (80%) for 30 min, washed for three times with phosphate buffer and wiped off with western blotting paper. The blocking operation with the blocking solution (1% BSA in 0.1 M carbonate buffer containing 30 wt % glycerin) was carried out in the same way. In the next step, human IgA antigen standards (312.5 ng/mL in 0.05 M Tris buffer containing 30 wt % glycerin) and diluent buffer (0.05 M Tris buffer containing 30 wt % glycerin) were mixed in the dimple. In this operation, six different combinations of droplets for the antigen and diluent buffer (0 + 50 for control, 1 + 49, 2 + 48, 4 + 46, 6 + 44, and 8 + 42, respectively) were examined. The PDMS microchip was stored in the constant-humidity box (80%) for 30 min and washed for three times with phosphate buffer, and then, wiped off with western blotting paper. Then, fifty droplets of the secondary antibody solution (100 ng/mL in 0.05 M Tris buffer containing 30 wt % glycerin) was injected in the dimple. Thereafter, the PDMS microchip was stored in the constant-humidity box (80%) for 10 min and washed for three times with phosphate buffer and wiped off with western blotting paper. Finally, fifty droplets of substrate solution were injected in the dimple. The Amplex®Red reacts with hydrogen peroxide, in combination with HRP, to produce the highly fluorescent oxidation product, resorufin. The enzyme substrate reaction was proportional to the reaction time before 60 s and reached equilibrium after 195 s during the droplet assay. The calibration curve of IgA standard solution was linear in the range from 0 to 50 ng/mL over the reaction time of 105 s. The slope of the calibration curve improved with the increasing of reaction time. However, the relative standard deviation (RSD) values showed high values (15%<) over the reaction time of 165 s. In addition, the correlation coefficient values showed the best value ($R^2 = 0.969$) at the reaction time of 150 s.

Based on these results, we decided that the reaction time for the enzyme–substrate reaction was 150 s. Figure 2 show the calibration curve of IgA. The fluorescence intensity increased with the increasing of the concentration of IgA. A slight increase of the fluorescence intensity in the blank (0 ng/mL of IgA) was also observed. This is due to the self-oxidation of Amplex®Red into resorufin or the non-specific adsorption of secondary antibody. Briefly, sufficient sensitivity was unavailable in the method because the area of reaction field toward the sample volume is very small. Immobilization of enzyme inner surface of dimples would improve the dynamic range. We have reported an ELISA system for detection of analytes in nanoliter water pool coupling with the ink-jet microchip. This result indicates that the ELISA using the PDMS had dimples provided advances in linearity and reproducibility. This is due to the fact that the contamination associated with the washing operation was avoided and the size of area for reaction was determined. Furthermore, this ELISA system is expected to improve the reaction rate, sensitivity, and decreasing the cost of analysis. And it would be available in a wide variety of analytical fields.

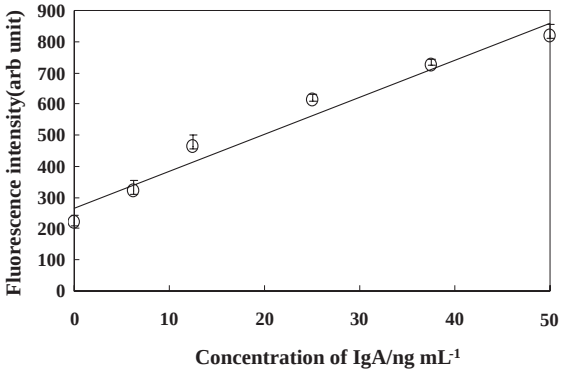


**Figure 2.** Calibration curve of IgA. Conditions: concentration of IgA; 0, 6.25, 12.5, 25, 37.5, and 50 ng/mL, enzyme–substrate reaction time, 150 s, laser irradiation time: 5 s, other ELISA conditions were described in the text.

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