ONE-STEP MICRO-ELISA FOR HIGHLY SENSITIVE DETERMINATION OF TSH

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ABSTRACT

To quantify biomolecules in samples with an enzyme-linked immunosorbent assay (ELISA) with smaller sample volumes more rapidly and easily, we developed a fully automated system called "μELISA™" that uses microfluidics and highly sensitive thermal lens detection. We applied μELISA™ to a one-step sandwich immunoassay of thyroid-stimulating hormone (TSH) for diagnosing thyroid function. We evaluated its performance using human serum samples and demonstrated that it is fast (15 min), highly sensitive (0.1–10 μIU·mL^{-1}), highly precise (<8% variation), and easy to operate. As a result, our system is a potentially powerful and practical diagnostic tool for point-of-care testing (POCT).

KEYWORDS: Microchemical Chip, Immunoassay, Thermal Lens Detection, Point of Care Testing (POCT)

INTRODUCTION

Enzyme-linked immunosorbent assays (ELISA) are widely used to detect cytokines, clinical diagnostic markers, food allergens, and hormones in biological samples. ELISA is a highly specific and sensitive method that uses an enzyme-linked antibody to detect and quantify these antigens. However, it is often expensive, time-consuming (3–24 h), and requires large volumes of reagents and samples as well as complex liquid handling procedures on microtiter plates.

Previously, we developed a microchip-ELISA. In this technique, the antigen-antibody interactions and enzyme reactions are performed on microbeads trapped in a microchannel with a dam structure and the dye products of the enzyme reaction were detected downstream of the dam by using a thermal lens detector (TLD). Compared with conventional ELISA, the microchip-ELISA is faster (10–20 min) and requires a much smaller sample volume (a few microliters) [1]. To facilitate the use of this technique, we developed a prototype of a fully automated immunoassay system by using microfluidics and a highly sensitive TLD. Then, we showed that bead-based microchip-ELISA methods were effective for rapid, sensitive immunoassays [2-4]. Thus, microchip-ELISA systems are expected to be useful for point-of-care testing (POCT).

The thyroid is an endocrine gland in the neck that produces hormones that regulate metabolism. Thyroid disorders exhibit varied symptoms. For example, hyperthyroidism due to Graves' disease is characterized by exopthalmos, tachycardia, excessive sweating, and weight loss. In addition, hypothyroidism due to Hashimoto's thyroiditis presents with bradycardia, fatigue, depression, and low basal body temperature. TSH is an important marker for the clinical diagnosis of thyroid dysfunction. Recently, there has been increasing demand for a sensitive and rapid diagnostic method for TSH.

In this study, we developed a fully automated micro-ELISA system called "μELISA™" that uses a bead-based immunoassay format (Fig. 1). Using this system, we applied the one-step sandwich method for rapid, sensitive, and quantitative determination of TSH.

Figure 1: Fully automated microchip-based "μELISA™" system

THEORY

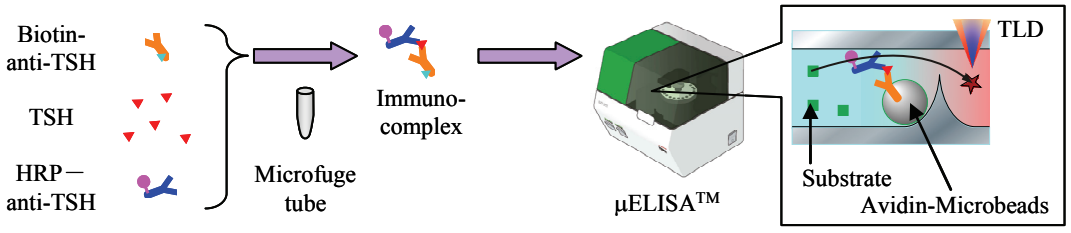

Figure 2: Principle of one-step micro-ELISA on μELISA™

A schematic representation of our one-step micro-ELISA for highly sensitive determination of TSH is illustrated in Figure 2. The analyte (TSH) and 2 antibodies, a biotin-labeled anti-TSH antibody and a horseradish peroxidase (HRP)-labeled anti-TSH antibody, which bind different epitopes, were mixed in a microfuge tube. The resulting immunocomplexes were captured by avidin-conjugated microbeads trapped in a dam structure of a microchannel. Then, for detection, a chromogenic substrate solution for HRP was added using a stopped-flow technique. Finally, the dye products of the enzyme reaction were reflowed downstream of the dam and detected by TLD.

EXPERIMENTAL

All of the components of the μELISA™ system were integrated into a portable unit (253 mm (W) × 200 mm (D) × 222 mm (H)). This system contained a micro-autosampler, a syringe pump, microvalves, a microchip with a dam structure (20 μm depth) in a microchannel (400 μm width and 200 μm depth), a portable TLD, and a controller board. A polyether ether ketone (PEEK) capillary tube injector withdrew aliquots of the samples and reagents from microfuge tubes in a carousel tray and injected them at a predetermined flow rate controlled by a syringe pump. The injector was directly connected to a microchip inlet for these injections. By using microvalves, the flow path for each step of the procedure could be changed with minimal dead volume. We used our previous on-chip TLD for detection. A custom PC software that runs on Microsoft Windows was used to control these devices and analyze the TLD signals.

Avidin-conjugated microbeads were prepared by passive adsorption of streptavidin (Wako Pure Chemical Industries, Ltd., Japan) onto polyvinylbenzene microbeads (40 μm diameter). Anti-TSH antibodies and TSH were purchased from Fitzgerald Industries International (USA) and AspenBio Pharma (USA), respectively. Biotin- and HRP-labeled anti-TSH antibodies were prepared by using a Biotin Labeling Kit - NH_2 and a Peroxidase Labeling Kit - NH_2, respectively (Dojindo Molecular Technologies, Inc., Japan). The SureBlue TMB 1-component peroxidase substrate kit (KPL, Inc., USA) was used as a chromogenic substrate for HRP. TSH standard samples were prepared by mixing CRM470 serum standard (Eiken Chemical Co., Ltd., Japan). One percent bovine serum albumin (BSA) in PBS(pH 7.4) was used as the running buffer.

The amount of TSH in the sample was measured with μELISA™. Briefly, 5 μL of a solution containing 1 $\mu g \cdot mL^{-1}$ biotin-labeled anti-TSH antibody and 1 $\mu g \cdot mL^{-1}$ HRP-labeled anti-TSH antibody were mixed with 20 μL of analyte at room temperature for 10 min. Subsequently, 10 μL of this mixture was injected into the microchip at 5 $\mu L \cdot min^{-1}$ to bind the immunocomplexes to avidin-conjugated microbeads that were placed in the dam beforehand. Then, 10 μL of HRP substrate was injected in the microchip at 10 $\mu L \cdot min^{-1}$ and incubated at 30 °C for 2 min with a stopped-flow technique. To detect the resulting dye product, additional 10 μL of HRP substrate was reflowed into the microchip at 10 $\mu L \cdot min^{-1}$. The microbeads were washed with running buffer between each step. All of these procedures were automated and could be performed repeatedly.

RESULTS AND DISCUSSION

The performance of μELISA™ was calibrated using 0.1–5 $\mu IU \cdot mL^{-1}$ TSH standards. The calibration curve was fitted with a 4-parameter logistic model with a correlation coefficient of 0.99 (Fig. 3).

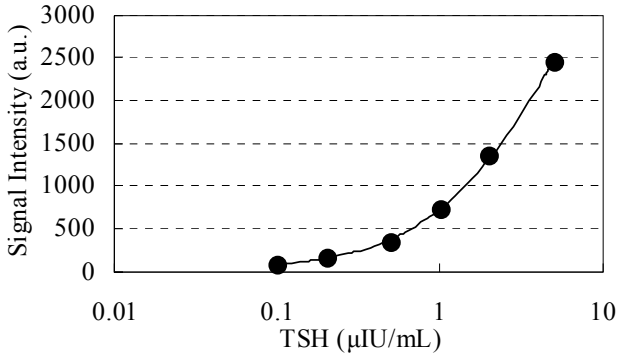

Figure 3: Calibration curve of TSH

The precision of μELISA™ measurements was determined by measuring a sample of known concentration (4 replicates). Since the coefficient of variance between the theoretical and measured values was less than 8%, the measurements were reproducible (Fig. 4). The lower limit of detection (LOD) is derived from the calibration curve and measured as mean + 2 (SD) with reference to the blank; the LOD for TSH on μELISA™ was calculated to be 0.033 mIU•mL-1. This value was much less than the minimum normal level of TSH (0.3 $\mu IU \cdot mL^{-1}$ [5]). These results suggested that the sensitivity of TSH measurement with μELISA™ is sufficient for diagnosing thyroid function.

Finally, we tested 38 human serum samples to determine the correlation between μELISA™ and enhanced chemiluminescence assay (ECLIA) (Elecsys® TSH; Roche Diagnostics K.K., Japan). We found good correlation between the 2 methods (r = 0.9552 and Sy/x = 2.892) (Fig. 5).

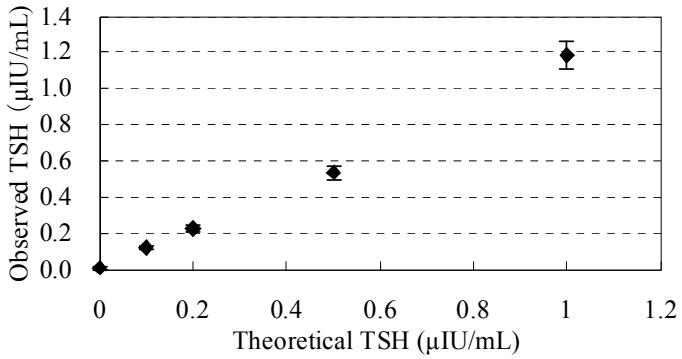

Figure 4: Comparison between theoretical and observed concentrations of TSH

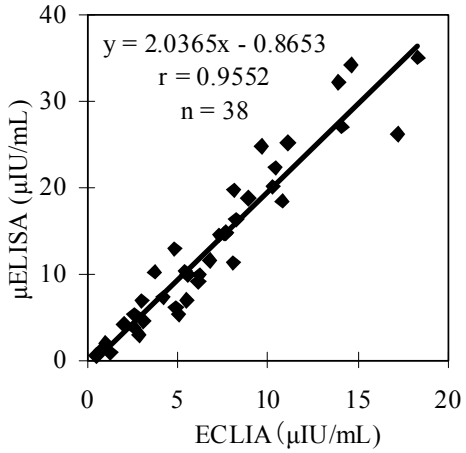

Figure 5: Correlation of serum TSH concentrations between ECLIA and μELISA methods

CONCLUSION

In conclusion, our μELISA™ system is a potentially practical tool not only for POCT of TSH but also for other immunoassays. Since μELISA™ only requires minimal amounts of reagents and is inexpensive, it can be used to detect biomolecules in small sample volumes or high-throughput analyses. Moreover, this method can be used for other applications, including environmental analyses, food evaluations, and drug screenings. Finally, the microfluidics-based control system used in μELISA™ can be useful for other systems, such as reactors, chromatography systems, and integrated systems.

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