



Multi-immunoreaction-based dual-color capillary electrophoresis for enhanced diagnostic reliability of thyroid gland disease



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ABSTRACT

Thyroid-stimulating hormone (TSH) secretion plays a critical role in regulating thyroid gland function and circulating thyroid hormones (i.e., thyroxine (T4) and triiodothyronine (T3)). A novel multi-immunoreaction-based dual-color capillary electrophoresis (CE) technique was investigated in this study to assess its reliability in diagnosing thyroid gland disease via simultaneous detection of TSH, T3, and T4 in a single run of CE. Compared to the conventional immunoreaction technique, multi-immunoreaction of biotinylated streptavidin antibodies increased the selectivity and sensitivity for individual hormones in human blood samples. Dual-color laser-induced fluorescence (LIF) detection-based CE performed in a running buffer of 25 mM Na₂B₄O₇-NaOH (pH 9.3) allowed for fast, simultaneous quantitative analysis of three target thyroid hormones using different excited wavelengths within 3.2 min. This process had excellent sensitivity and detection limits of 0.05–5.32 fM. The results showed 1000–100,000 times higher detection sensitivity than previous methods. Method validation with enzyme linked immunosorbent assay for application with human blood samples showed that the CE method was not significantly different at the 98% confidence level. Therefore, the developed CE-LIF method has the advantages of high detection sensitivity, faster analysis time, and smaller sample amount compared to the conventional methods. The combined multi-immunoreaction and dual-color CE-LIF method should have increased diagnostic reliability for thyroid gland disease compared to conventional methods based on its highly sensitive detection of thyroid hormones using a single injection and high-throughput screening.

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1. Introduction

Thyroid cancer was ranked 13th among the most common cancers (excluding non-melanoma skin cancer) in the world for both genders in 2012 [1–3]. According to Cancer Research UK, thyroid cancer incidence rates have increased since the late 1970s in England [4–6]. Thyroid cancer was the most common cancer in Koreans, comprising 14.2% of cancers in 2014 [7].

Thyroid-stimulating hormone (TSH) secretion plays an important role in controlling thyroid gland function and circulating levels of thyroid hormones (i.e., thyroxine [T4] and triiodothyronine [T3]) (Fig. S1A) [8,9]. Excessive synthesis of thyroid hormones by the

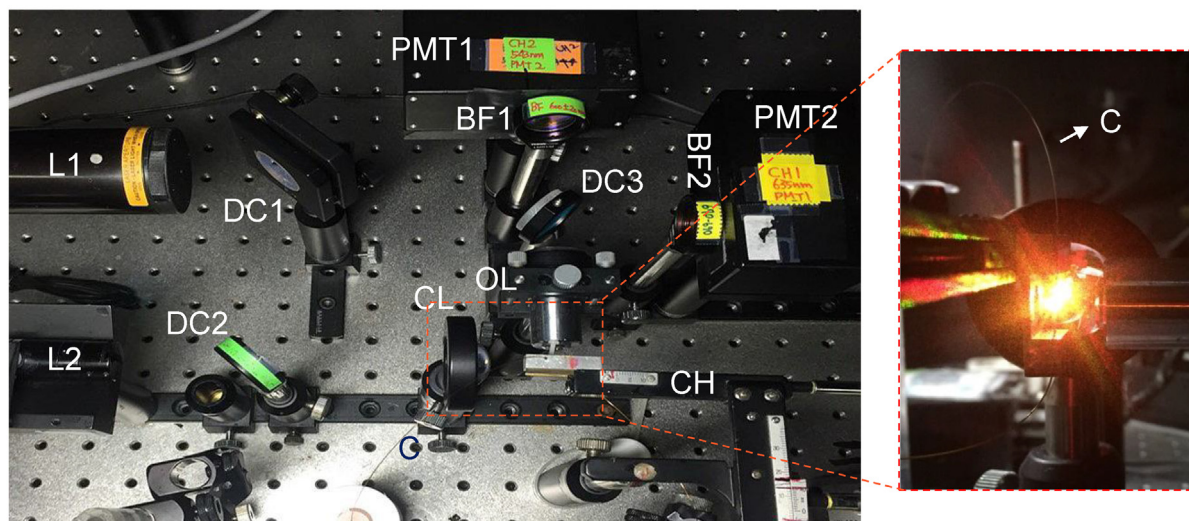
thyroid gland causes hyperthyroidism, which involves symptoms such as heat intolerance, weight loss, diarrhea, and an enlarged thyroid gland (Fig. S1B). On the contrary, lack of thyroid hormones (hypothyroidism) can lead to sluggishness, depression, constipation, and weight gain [10–12]. These disorders are diagnosed by measuring TSH and T4 levels, and measurement of T3 is useful for more accurate diagnosis [13–15]. Normal serum T4 and T3 concentrations are 12–28 pM and 2.8–7.2 nM, respectively. TSH concentrations range from 1.7–29.6 pM. Therefore, development of a sensitive, specific, and reliable method to determine the exact levels of all thyroid hormones in biological samples is of great importance for diagnosing thyroid gland disease [16].

There are several methods to measure thyroid hormones, including mass spectrometry [17–19], electrochemistry [20], liquid chromatography [21–23], flow injection and sequential injection coupled with different detection methods [24,25] such as chemiluminescence [26] and immunoassays [27]. Although some of these methods are sensitive, they still have critical weaknesses. Some

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(A)



(B)

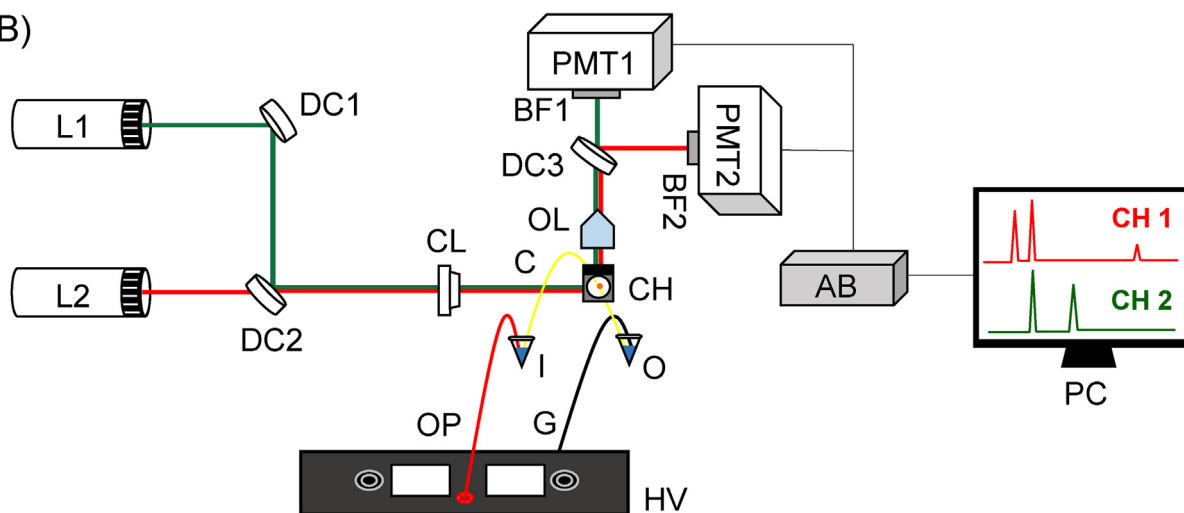


Fig. 1. (A) Photograph and (B) schematic diagram of the CE-LIF system. Abbreviations: L1, 543 nm laser; L2, 635 nm laser; DC 1, dichroic mirror 1; DC 2, dichroic mirror 2; DC 3, dichroic mirror 3; CL, convex lens; C, capillary; I, inlet reservoir; O, outlet reservoir; H, capillary holder; OL, objective lens; BF1, bandpass filter 1; BF2, bandpass filter 2; PMT1, photomultiplier tube 1; PMT2, photomultiplier tube 2; HV, high-voltage power supply; OP, output wire; G, ground wire; AB, amplification board; PC, personal computer.

require a large amount of harmful organic solvent or complex derivatization procedures. Others need expensive instrumentation or involve time-consuming sample clean-up or pre-concentration steps. Also, previous methods measured only T4 and T3 [17–27]. However, to enhance the diagnostic reliability of disease-related thyroid, all of T4, T3, and TSH should be simultaneously measured.

In the previous studies, CE methods measured thyroid hormones [28–30] as they enable precise separation, speed, efficiency, short analysis time, and relatively simple instrumentation. In general, laser-induced fluorescence (LIF) detectors show excellent sensitivity, even with a small amount of sample. However, previous CE-LIF methods developed for thyroid hormones have only utilized one specific light wavelength to excite dyes [11]. These single-wavelength CE approaches could not simultaneously detect various fluorescent dyes with different excitation wavelengths. Sequential injection, separation, and detection of various samples in a single capillary are also too time-consuming, wastes reagents, and results in low throughput. However, several studies have demonstrated multiple detection techniques that are effective strategies

for simultaneous detection of various analytes with high efficiency [31–35].

In this study, we investigated a novel combined multi-immunoreaction and dual-color CE method based on wavelength-dependent LIF detection for the simultaneous detection of T4, T3, and TSH to enhance the diagnostic reliability for thyroid gland diseases. We chromatographically separated a multi-immunoreaction of biotinylated streptavidin antibody-based target molecules (i.e., T3, T4 and TSH) into two different transducers (i.e., photomultiplier tube) using three dichroic mirrors and two emission filters. Although adjacent peaks were not well resolved in a single capillary, the dual-color CE-LIF system enabled simultaneous analysis of various target molecules with high resolution, selectivity, and detection sensitivity. The combination of the biotin-streptavidin reaction-based multi-immunologic reaction and dual-color CE-LIF detection method has several advantages over the conventional immunoassay, including reduced sample consumption, short analysis time, and versatility in developing multiple analyses. The combination of multi-immunoreaction and dual-color CE-LIF assay

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