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Antigen detection based on background fluorescence quenching immunochromatographic assay

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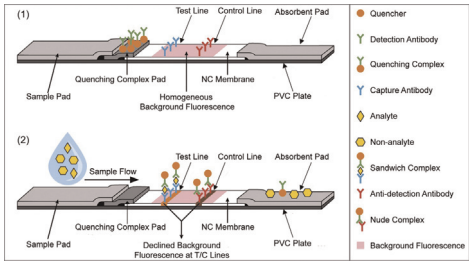
HIGHLIGHTS

- bFQICA is a novel background fluorescence quenching based immunochromatographic assay.
- Fluorescence donor in quenching is fluorescein coated on entire nitrocellulose membrane.
- bFQICA measurement is based on a unique background vs. specific fluorescence ratio.
- bFQICA is exceptionally sensitive and of combined qualitative and quantitative capabilities.

GRAPHICAL ABSTRACT



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ABSTRACT

Gold immunochromatographic assay (GICA) has been around for quite a while, but it is qualitative in the vast majority of applications. A fast, simple and quantitative GICA is in call for better medicine. In the current study, we have established a novel, quantitative GICA based on fluorescence quenching and nitrocellulose membrane background signals, called background fluorescence quenching immunochromatographic assay (bFQICA). Using model analyte alpha-fetoprotein (AFP), the present study assessed the performance of bFQICA in numerous assay aspects. With serial dilutions of the international AFP standard, standard curves for the calculation of AFP concentration were successfully established. At 10 and 100 ng mL^{-1} of the international AFP standard, the assay variability was defined with a coefficient of variance at 10.4% and 15.2%, respectively. For samples with extended range of AFP levels, bFQICA was able to detect AFP at as low as 1 ng mL^{-1}. Fluorescence in bFQICA strips stayed constant over months. A good correlation between the results from bFQICA and from a well-established Roche electrochemiluminescence immunoassay was observed in 27 serum samples ($r = 0.98$, $p < 0.001$). In conclusion, our study has demonstrated distinctive features of bFQICA over conventional GICA, including utilization of a unique

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fluorescence ratio between nitrocellulose membrane background and specific signals (F1/F2) to ensure accurate measurements, combined qualitative and quantitative capabilities, and exceptionally high sensitivity for detection of very low levels of antigens. All of these features could make bFQICA attractive as a model for antigen-antibody complex based GICA, and could promote bFQICA to a broad range of applications for investigation of a variety of diseases.

1. Introduction

Immunochromatographic assays, also called lateral flow tests or simply strip tests, are based on eluent motion along the membrane resulting in the formation of specific immune complexes that are detected as stained bands [1,2]. Enzymes, stained latexes and quantum dots are used as labels in these systems; however, in the overwhelming majority of cases, gold nanoparticles (GNPs) are utilized [2–5]. Osikowicz et al. [6] used, for the first time, gold immunochromatographic assay (GICA) for detection of human chorionic gonadotropin (hCG) at an early stage of pregnancy. Since then, GICA has widely been applied to many fields such as clinical diagnosis [7,8], veterinarian medicine [9], drug monitoring [10] and food security [11,12] because of its simplicity, rapidity, and cost effectiveness. Nonetheless, applications of GICA have mostly been qualitative or semi-quantitative. A recent study [13] has demonstrated quantitative GICA with a computational model of signals obtained in transformation of reflective optical to digital signals. The method used in this study, however, is image processing-based that might be problematic in detection of samples with high background, such as samples from patients with jaundice. In order to improve the quantitative performance of GICA and to be free of background interference, we have established a novel, quantitative GICA assay based on fluorescence quenching and nitrocellulose membrane background signals, called background fluorescence quenching immunochromatographic assay (bFQICA). In existing fluorescence quenching based GICAs, fluorescence donors are usually pre-conjugated fluorescent materials on capture antibodies, and the quenching occurs between gold particles and antibodies [14,15]. In our new bFQICA assay, the fluorescence donors are fluorescein that are pre-coated on the entire nitrocellulose membrane and quenching occurs between the gold particles and nitrocellulose membrane, which represents an innovative approach that is unique from all existing GICAs. As alpha-fetoprotein (AFP) is a very important clinical biomarker and is widely utilized in assay development, the present study chose AFP as a model analyte to assess the performance of bFQICA through establishment of standard curves, tests for limit of blank (LoB), variability, detection time, fluorescence stability and concentration recovery, and correlation of measurements with a well-established method such as Roche electrochemiluminescence immunoassay (ECLIA).

2. Materials and methods

2.1. Key reagents and preparation

AFP antigen was acquired from WHO International Laboratory Biological Standards (Copenhagen, Denmark), and the corresponding anti-AFP monoclonal antibodies (including detection antibodies and capture antibodies) and anti-detection antibodies were from HyTest Ltd. (Turku, Finland). GNPs (20 ~ 30 nm) were made through trisodium citrate reduction of hydrogen tetrachloroaurate (III) hydrate ($HAuCl4{\cdot}3H_2O$), a method pioneered by Frens [16], and labeling of GNPs with AFP detection antibodies was carried out as following: 10 μL of 1.5 mg mL^{-1} AFP detection antibodies were added in 1 mL of GNPs for 30 min, and then 100 μL of 10 mg mL^{-1} BSA and 100 μL of 1% PEG20000 were added in the GNP mixture for additional 10 min. Lastly the GNP mixture was centrifuged at 15,000 rpm for 45 min at 4 °C, and the supernatant was discarded. The resulting pellet was re-suspended in 30 μL of remnant solution for use in downstream experiments.

2.2. Fluorescein and antibodies coating on nitrocellulose membrane

Hi-Flow Plus HF135 nitrocellulose (NC) membrane (Cat #SHF1350225) was purchased from Millipore (Billerica, Massachusetts, U.S.A.), and it was in house coated with fluorescein at Shanghai Simp Bio-Science Co., Ltd. (Shanghai, China). As the maximal emission of fluorescein is at 521 nm, which is overlapped with the absorbance wavelength of GNPs (250–650 nm), we chose fluorescein as the source of fluorescence in the bFQICA assay. Although the technical details of the NC membrane coating with fluorescein were not disclosed in the manuscript due to commercial reason, the coating has to be homogeneous and firm so that the background signal remains constant and high enough to accommodate significant fluorescence drop produced by quenching when samples with high levels of AFP were applied. After coating with fluorescein, AFP detection antibody-labeled GNPs (named yet as quenching complex) were fixed at the quenching complex pad, while AFP capture antibodies and anti-detection antibodies were immobilized at Test Line and Control Line, respectively, during fabrication of NC membrane strips (Fig. 1A), according to the manufacturer's instructions at http://www.millipore.com/techpublications/tech1/tb500en00.

2.3. Instrument fabrication and functions

The bFQICA dedicated fluorescence readers were in-house manufactured at Shanghai Simp Bio-Science Co., Ltd. (Shanghai, China) and they consist of several core parts: an optical sensor, a scanning platform and its stepping system, and a signal processing system (Fig. 1B). The function of the optical sensor was to acquire excitation light, namely the beam spot, and to convert the fluorescence signal of the strip into electrical signal. The stepping motor drives the scanning platform that moves the test strip against the beam spot. The beam spot scanned longitudinally through the strip, and passed through Control Line and Test Line sequentially. The dynamic fluorescence intensities were read in the scanning process.

2.4. bFQICA principal and fluorescence quenching

At first, 60 μL of serum sample (or AFP calibrator) were added into the sample port on the test strip. In the capillary action, the sample was flowing toward the absorbent pad and re-dissolving pre-coated quenching complexes in the quenching complex pad. While the sample was moving forward with the quenching complex, as specific sandwich complexes formed at the Test Line, fluorescence quenching was fired on site. Excess quenching complexes conjugated with anti-detection antibodies to form nude complexes at the Control Line where fluorescence quenching occurred once again on the NC membrane. The un-conjugated quenching complexes continued moving forward, along with