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Simultaneous quantitation of carbohydrate antigen 125 and carcinoembryonic antigen in human serum via time-resolved fluoroimmunoassay

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ABSTRACT

In clinical diagnosis of cancer, immunology assay with single tumor marker often lead to a false and missed inspection. A quantitative method with a high degree of accuracy, sensitivity, and effectiveness is required for its diagnosis. We developed a dual-label time-resolved fluoroimmunoassay (TRFIA) to simultaneously detect carbohydrate antigen 125 (CA125) and carcinoembryonic antigen (CEA) in human serum to aid the diagnosis and prognosis of gastric cancer. The method was based on a microplate sandwich immunoassay using europium-labeled anti-CA125 antibodies and samarium-labeled anti-CEA antibodies as fluorescent reporters. The assay detection range was widely, and the limit of detection was sufficiently for detecting clinical sample. The intra- and inter-assay coefficients of variation were below 6%, and recoveries ranged from 90% to 110%. No significant statistical difference in sensitivity or specificity was observed between dual label-TRFIA and commercial chemiluminescent immunoassays in serum samples. These results demonstrate the successful development of an effective, reliable, and convenient novel TRFIA method for the simultaneous detection of CA125 and CEA, which can be used for clinical blood screening to monitor the occurrence and development of tumors to facilitate early treatment.

1. Introduction

Several molecules related to the activity or extent of cancer have been extensively studied. In clinical practice, carbohydrate antigen 125 (CA125) and carcinoembryonic antigen (CEA) are useful markers for the diagnosis and early detection of cancer [1] and for monitoring treatment efficacy [2]. CEA is a cell surface oncofetal glycoprotein, and its expression is low in the cells of normal tissues in healthy adults. CEA elevation is primarily used as a tumor marker in various types of screening tests for the early detection of cancer [3,4].

CEA concentrations are particularly high in colorectal carcinoma and gastric cancer, but the elevated concentrations are also found in various benign pathologies and other malignancies, which preclude its use in screening and limit its diagnostic use. However, CEA may be beneficial in the differential diagnosis of gastric carcinoma, preferably in combination with CA125, cause the clinical value of detecting these molecules as tumor markers were widely concerned [5–9]. CA125 is a transmembrane glycoprotein, the largest of the class of membrane-associated mucins to which it belongs [10]. In a healthy population, CA125 serum levels are $< 35\,U\,mL^{-1}$ [11]. Increased CA125 values have been reported in 28% of subjects with non-ovarian malignancies including breast, lung, liver, pancreatic, colon, stomach, biliary tract, cervical, uterine, fallopian tube, and endometrial carcinomas [12,13]. However, CA125 levels are subject to possible interference in the presence of heterophilic antibodies in the serum, similar to other immunoassays [14,15]. In clinical serology diagnosis of gastric cancer, chemiluminescence immunoassay (CLIA) is widely used to detect the concentration of CEA [16] or CA125 [17] in patient serum, however, a single target detection will lead to an inter-group difference which may cause the misdiagnose and lead to the waste of serum. Simultaneous quantitation of CA125 with CEA in serum may thus improve its sensitivity and specificity [18].

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Time-resolved fluoroimmunoassay [19,20] (TRFIA), using lanthanide complex chelates (such as europium [Eu^{3+}] and samarium [Sm^{3+}]) for labeling, is an immunoassay method first reported by Lovgren et al. [21]. Time-resolved fluorometry of lanthanide chelates has been shown to be a successful non-isotopic detection method. Such simultaneous assays have obvious advantages, including rapid analysis, high throughput, small analyte volume, and low cost. Lanthanide can generate strong fluorescence with long decay times, large Stokes shifts, and sharp emission profiles [22,23]. Several detection methods based on TRFIA are well established in the diagnosis of specific targets of the disease [24].

In this paper, we developed a dual-label TRFIA method for the detection of CEA and CA125 using Sm^{3+} and Eu^{3+} labeling. We evaluated the method using dilution and recovery testing, constructed a standard curve, determined the analytical sensitivity and precision, and measured assay specificity [25,26]. Compared with chemiluminescence immunoassay (CLIA), the proposed method exhibits apparent advantages of high sensitivity, short analysis time, and large linear range. Hence, this method can be used for large-scale screening of clinical serum samples.

2. Materials and methods

2.1. Materials and instrumentation

Anti-CEA monoclonal antibodies (McAbs) (clones 5909 and 5910) were purchased from Medix Biochemica. Tris and anti-CA125 McAbs (clones M5807 and M5814) were purchased from Darui Biotechnology. Triton X-100, triglyceride, human hemoglobin (Hb), bilirubin and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Transparent 96-well micro-titration strips were purchased from Nunc (Roskilde, Denmark). Sepharose CL-6B columns were obtained from Pfizer (Chalfont St Giles, UK). The Victor 1420 multilabel counter was a product of PerkinElmer (Waltham, MA, USA), and the Eu^{3+} and Sm^{3+} labeling kits were obtained from PerkinElmer (Turku, Finland). Ultra-pure water, obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used throughout the study. Other chemicals and reagents used were all of analytical grade.

2.2. Buffers

Labeling buffer solution contained 50 mmol L^{-1} Na_2CO_3-$NaHCO_3$ (pH 8.5) and 0.155 mol L^{-1} NaCl. Elution buffer solution contained 50 mmol L^{-1} Tris-HCl (pH 7.8), 0.9% NaCl, and 0.05% NaN_3. Packing buffer contained 50 mmol L^{-1} Na_2CO_3-$NaHCO_3$ buffer (pH 9.6). Blocking solution contained 50 mmol L^{-1} Na_2CO_3-$NaHCO_3$ buffer (pH 9.6) and 1 g/L BSA. Standard buffer contained 50 mmol L^{-1} Tris-HCl (pH 7.8), 0.2% BSA, and 0.1% NaN_3. Assay buffer contained 50 mmol L^{-1} Tris–HCl (pH 7.8), 0.09% NaCl, 0.02% BSA, 0.05% NaN_3, and 0.05% Tween-20. Washing solution was a Tris-HCl buffered saline solution (pH 7.8) containing 0.9% NaCl, 0.2% Tween-20, and 0.05% NaN_3. Enhancement solution was a 0.1 mol L^{-1} acetate-phthalate buffer (pH 3.2) containing 0.1% Triton X-100, 15 mmol L^{-1} β-naphthoyl trifluoroacetone (β-NTA), and 50 mmol L^{-1} tri-*n*-octylphosphine oxide [27].

2.3. Coating of microwell plates with capture antibody

Anti-CA125 McAb (clones 5910) and anti-CEA McAb (clones M5807) were diluted to a final concentration of 3 mg/L in 200 mL packing buffer co-immobilized in each well (96-well plates), and incubated overnight at 4 °C. After washing, 200 mL blocking solution was added to block the coated surface for 2 h. After the blocking solution was removed, plates were dried under a high vacuum and stored at −20 °C in a sealed plastic bag with desiccant.

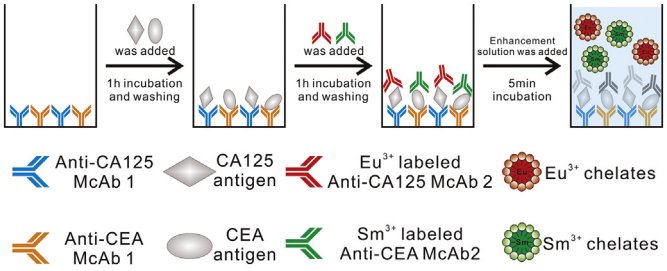

Fig. 1. Experimental operation schematic diagram.

2.4. Lanthanide antibody labeling

CA125 and CEA antibodies were labeled with Eu^{3+} and Sm^{3+} chelates using labeling buffer. CA125 antibody (clones M5814) was gently mixed in 200 mL of labeling buffer with 500 mg of Eu^{3+} chelate in 100 mL of the same buffer (5 mg mL^{-1}). After 22 h of incubation with continuous gentle shaking at 4 °C, free Eu^{3+} chelate and aggregated antibodies were separated from Eu^{3+} antibody conjugates using a 1 cm × 40 cm column packed with sepharose CL-6B, eluted with descending elution buffer, and collected elution product. Concentrations of Eu^{3+} conjugates in collected fractions were measured for fluorescence and diluted with enhancement solution (1,1000). Fluorescence in microtitration wells (200 mL per well) was detected by comparing the signal of samples to that of stock standards diluted at 1:100 in enhancement solution. The fractions from the first peak with the highest Eu^{3+} photon counts were pooled and characterized. Eu^{3+}-labeled CA125 antibodies were rapidly lyophilized under a high vacuum after dilution with elution buffer and stored in 4 °C. CEA antibodies were labeled with Sm^{3+} chelate with same procedure.

2.5. Preparation of antigen working solution

Antigen was diluted to a total concentration of 961.5 ng/mL in CEA and 716.5 ng/mL in CA125 using assay buffer (50 mmol L^{-1} Tris, 0.9% NaCl, 0.5% BSA, pH 7.8). The mixture was used as the antigen working solution.

2.6. Sample preparation

A total of 111 serum samples were provided by Guangzhou Hospital, in which CA125 and CEA values had been measured by Roche CLIA and the patients donated the serum were diagnosed based on characteristic clinical features confirmed by laboratory tests. Samples were stored at −20 °C before use. The study was reviewed and approved by the clinical research ethics committee of the Southern Medical University.

2.7. TRFIA CA125 and CEA detection assay

A dual-label assay was performed using a two-step procedure. To each well containing immobilized antibodies, either 100 μL of standards (50 μL CA125 standards and 50 μL CEA standards) or 50 μL of serum sample were added, followed by 150 μL of assay buffer. Incubation was performed at room temperature for 1 h with continuous slow shaking [28]. After washing four times to remove remaining serum, 250 ng of Eu^{3+}-labeled McAb to CA125 and 900 ng of Sm^{3+}-labeled McAb to CEA was added in a final volume of 100 μL [24]. Incubation was performed at room temperature for 1 h with continuous slow shaking. After six washes, 200 μL of enhancement solution was dispensed into each well. Plates were shaken for 5 min and fluorescence arising from Eu^{3+} and Sm^{3+} was measured using a Eu^{3+}/Sm^{3+} dual-label time-resolved fluorescence measurement program of the Victor™ 1420 multilabel counter. Conditions for Eu^{3+} measurement were as follows: excitation