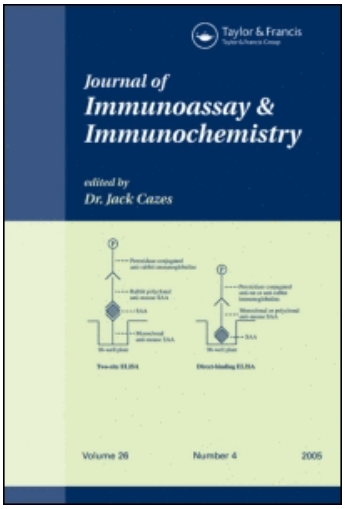

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QUANTITATIVE ANALYSIS OF A PROSTATE-SPECIFIC ANTIGEN IN SERUM USING FLUORESCENCE IMMUNOCHROMATOGRAPHY

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□ *A quantitative analysis of prostate-specific antigen (PSA) in samples of human blood serum by fluorescence immunochromatography using monoclonal antibodies to PSA was developed. The fluorescence immunochromatographic analysis system is composed of anti-PSA-monoclonal antibody (mAb), fluorescence conjugates in detection solution, a immunochromatographic assay strip, and a laser fluorescence scanner. A fluorescence immunochromatographic analysis system was employed to detect PSA on the basis of the area ratio between the control line and the test line of the strip. Under optimal conditions, the area ratio was proportional to PSA concentration ranging from 0.72 to 46.0 ng/mL with a detection limit of 0.72 ng/mL.*

Keywords fluorescence, immunochromatography, monoclonal antibodies, prostate-specific antigen

INTRODUCTION

The prostate-specific antigen (PSA) is a glycoprotein with a molecular mass of approximately 32 kDa consisting of one polypeptide chain, which is produced by the secretory epithelium of human prostate. Prostate-specific antigen has been accepted as the most useful serologic marker for detecting prostate cancer.[1] Therefore, PSA has been suggested as an important tool to screen men older than 50 years and high-risk men such as African Americans and those with a strong family history of prostate cancer.[2]

Immunoassays, with high selectivity and affinity of antibody molecules to their corresponding antigens, have widely been exploited for analytical purposes in the fields of clinical diagnoses.[3–6] Some immunoassays for

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PSA have been reported, such as fluorescence microscopy, surface plasmon resonance technology, lateral flow immunoassay, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA).[7–11] Since radioactive labels in RIA are harmful to the operators, ELISA has been extensively used for PSA detection in clinical diagnoses.[12] Chemiluminescent or fluorescent detection with ELISA, however, requires precise detection devices for miniaturized systems with a small sample volume.[13]

The above methods to measure PSA in serum sample are not simple procedures and are available only at clinical chemistry laboratories. A more rapid and simple method is needed. In this study, we developed a new immunochromatographic assay system for the quantitative analysis of prostate-specific antigen (PSA) in samples of human blood serum. The developed system is easy to use, does not need special equipment, and yields quick results.

In this study, we used a new immunochromatographic assay system using anti-PSA-mAb. In this assay system, an unknown sample containing PSA is simply mixed with the detection solution containing fluorescence-conjugated anti-PSA-mAb and fluorescence-conjugated biotin as an internal standard. PSA in the sample and fluorescence-conjugated anti-PSA-mAb in the detection solution competes for binding to capture the antibody, which was coated at the test line on the detection zone, as they flow laterally from the sample pad to absorption pad. The fluorescence-conjugated biotin in the sample mixture is captured by the streptavidin that was dispensed at the control line on the detection zone. The intensity of the captured fluorescence conjugates on the detection zone is scanned in a laser fluorescence scanner and converted into area value, and the concentration of PSA in the unknown sample is calculated from the standard curve or the equation of the standard curve.

EXPERIMENTAL

Prostate-specific monoclonal antibody (anti-PSA-mAb) was supplied from Boditech Co. (Chunchon, South Korea), and prostate-specific antigen (PSA) was purchased from Sigma Aldrich (St. Louis, MO, USA).

The fluorescence immunochromatographic assay strip consists of a nitrocellulose membrane, a sample pad, an absorption pad, and a backing card. The backing polystyrene card is a support so that the nitrocellulose membrane, sample, and absorption pad are laid on its adhesive side. The nitrocellulose membrane (Millipore HF 180) is the place where the detection zone is located, and the bottom side of the membrane is coated with a plastic thin film. The control line on the detection zone was dispensed with streptavidin (0.38 μg/mL) for the internal standard, and the test line was

coated with anti-PSA-mAb (0.57 μg/mL) for detection of PSA in a sample (Figure 1). The width of the dispensing line was 1 mm, and the dispensing volume was 1 μL/cm. The control and the test line were located 31 mm and 33.5 mm down from the sample pad, respectively. Before being set on the nitrocellulose membrane, the sample pad (S&S 903, 4 × 25 mm) was completely soaked in PBS containing 1% BSA and 0.05% Tween 20, then it was vacuum dried at 50°C for 1 h. The absorption pad (S&S 470, 4 × 20 mm) was set up on the nitrocellulose membrane to remove post-reaction solution that passed through the detection zone. The assembled strip on the polystyrene card was placed into a plastic housing (15 × 90 mm), which was designed to fit to the holder of the laser fluorescence scanner (i-CHROMA, Boditech Med, ChunChon, South Korea). The oval window of the plastic housing for the scanning of detection zone was 15 mm, and the diameter of the sample well for holding 100 μL of sample mixture was 5 mm. In the case when the test line on the nitrocellulose membrane was dispensed with anti-PSA-mAb, the detection solution was a mixture of anti-PSA-mAb-FL conjugate and biotin-BSA-FL conjugate. The concentrations of anti-PSA-mAb-FL and biotin-BSA-FL were 0.22 μg/mL, respectively. Various concentrations of PSA (10 μL) in D.W. and 90 μL of detection solution were mixed well, loaded onto the well of the sample pad on the cartridge, held for 20 min, and scanned in the laser fluorescence scanner. The raw data of scanning was displayed, and the relative intensity of the fluorescence peaks of the test and the control line were converted into

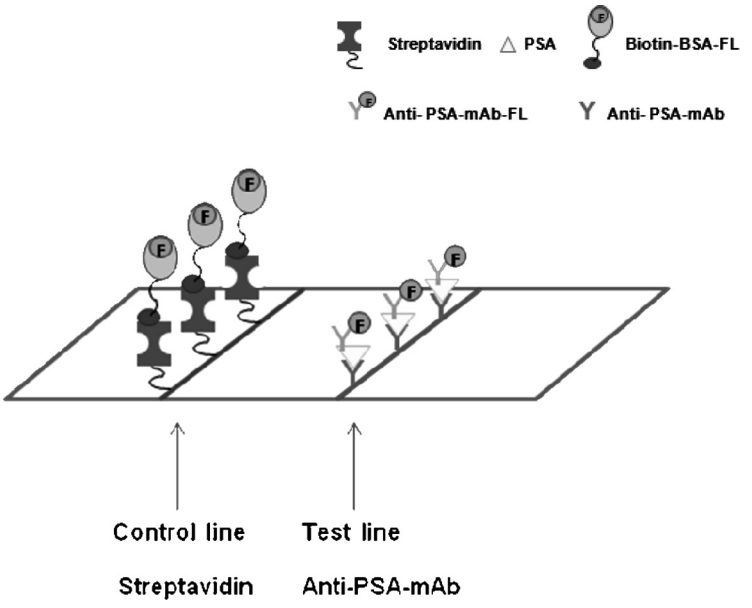

FIGURE 1 A schematic diagram of the fluorescence immunochromatographic assay strip.

the area value (test: A_T, control: A_C) by using the Visual Basic Terminal software program. The ratio of A_T:A_C was plotted against time (sec) or step number, and the equation for the standard curve and correlation value were obtained by using Microsoft Excel.

RESULTS AND DISCUSSION

When the aqueous sample is applied on our fluorescence immunochromatographic strip, two chromatographic lines of fluorescence intensity curves always appear. The fluorescence intensity of the first line (which is also called the test line) is inversely proportional to the concentration of PSA in the serum sample. The second line of fluorescence intensity curves (which is called the control line) is related to the mass transport of sample, and should show a constant value regardless of the concentration of PSA in the sample. This phenomenon results from the way in which the fluorescence immunochromatographic strip is made. Anti-PSA-mAb (0.57 μg/mL) and streptavidin (0.38 μg/mL) were dispensed at the test line and control line, respectively, of an internal standard on the detection zone. A sample mixture (100 μL) containing 10 μL of sample and 90 μL of the detection solution was loaded onto the sample pad of the immunochromatographic assay strip. The detection solution contained the anti-PSA-mAb-FL (0.22 μg/mL) and the biotin-BSA-FL (0.22 μg/mL).

To optimize the incubation time of the fluorescence immunochromatographic strip before the detection of the fluorescence signal, six different incubation times (5 min, 10 min, 15 min, 20 min, 25 min, and 30 min) were tried with a 2.3 ng/mL standard PSA solution (Figure 2). From Figure 2, it can be seen that the closest detection value was observed at the incubation time of 20 min.

To evaluate the performance of the fluorescence immunochromatographic strip, a series of experiments was made using standard solutions of 12 different concentrations of PSA in water (0 ng/mL, 0.36 ng/mL, 0.72 ng/mL, 1.44 ng/mL, 2.88 ng/mL, 5.75 ng/mL, 11.5 ng/mL, 23 ng/mL, 46 ng/mL, 230 ng/mL, 2300 ng/mL, 23000 ng/mL). In this experiment, the area value of the test line (A_T) was devided by the area value of the control line (A_C), and the ratios of A_T/A_C were plotted against different concentrations of PSA (Figure 3). From Figure 3, we could observe that the linear dynamic range of the fluorescence immunochromatographic strip is 0.72 ng/mL ~ 46 ng/mL, and the minimum detection level of the strip would be 0.72 ng/mL. The individual replicates, mean, and % CV of all standard samples are shown in Table 1.

Finally, by using fluorescence immunochromatography, we analyzed the content of PSA in human sera samples. Human sera samples are prepared

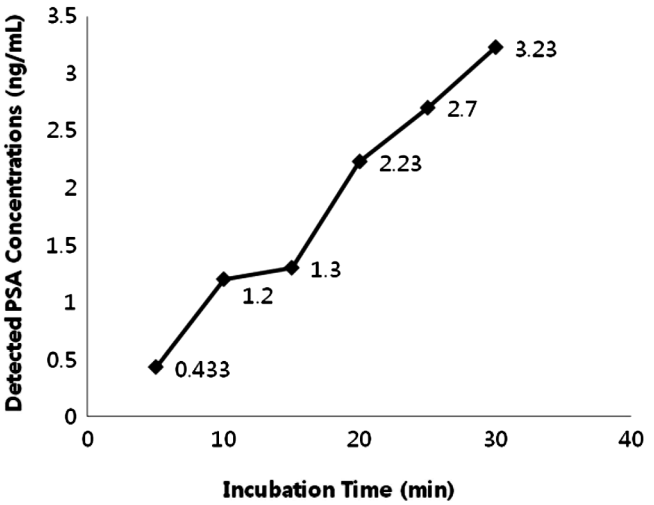

FIGURE 2 Detection values of PSA concentrations as a function of incubation time using a 2.3 ng/mL PSA solution.

as follows: The serum was obtained from a healthy man, and then PSA was spiked to the serum to produce five different concentrations (0.72 ng/mL, 2.3 ng/mL, 4.6 ng/mL, 11.5 ng/mL, 23 ng/mL) of human sera samples. When these five human sera samples are applied on the fluorescence

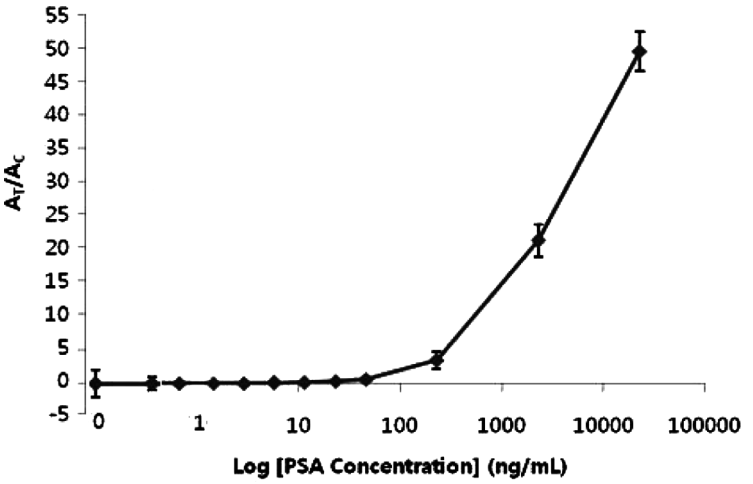

FIGURE 3 A_T/A_C were plotted against different concentrations of PSA. Each point on the graph represents the mean value, and error bars represent standard deviation values of three independent experiments. Twelve different concentrations (0 ng/mL, 0.36 ng/mL, 0.72 ng/mL, 1.44 ng/mL, 2.88 ng/mL, 5.75 ng/mL, 11.5 ng/mL, 23 ng/mL, 46 ng/mL, 230 ng/mL, 2300 ng/mL, and 23000 ng/mL) of PSA standard samples were used.

TABLE 1 Ratio of A_T/A_C and Mean, % CV, of Different Concentrations of PSA

	Ratio of A_T/A_C				
PSA (ng/mL)	1	2	3	Mean	CV (%)
0	0	0	0.003	0.001	173.21
0.36	0.008	0.004	0.006	0.0060	33.33
0.72	0.015	0.015	0.017	0.0157	7.35
1.44	0.028	0.029	0.027	0.0280	3.57
2.88	0.0061	0.055	0.059	0.0583	5.25
5.75	0.098	0.097	0.101	0.0987	2.11
11.5	0.1875	0.182	0.197	0.1889	4.02
23	0.365	0.369	0.394	0.376	4.18
46	0.655	0.655	0.684	0.6647	2.52
230	3.978	3.412	3.329	3.5730	9.88
2300	24.043	21.256	19.888	21.729	9.75
23000	50	50	50	50.0000	0

immunochromatography, detected PSA concentrations in the fluorescence immunochromatography and PSA concentrations in human sera samples correlate very well (Table 2) and show an excellent correlation coefficient (R^2) of 0.9983 (Figure 4).

The aim of this study was to apply a fluorescence immunochromatography method with our monoclonal antibodies that is sensitive enough for quantitative analysis of PSA in human blood samples. The performance of fluorescence immunochromatography with our monoclonal antibodies was satisfactory. Since the fluorescence immunochromatography assay is easy to perform and its quantitative range is within PSA concentrations in human blood samples, it shows a potential to be a powerful tool in quantitative analysis of PSA in human blood samples.

We anticipate that this assay will be used in clinical practice in the following way: A standard curve is not required in each batch run of samples because the standard curve will be stored in the fluorescence scanner's software. The acceptance criteria for a batch run will be 8% CV. A batch constitutes more than three samples. The optimum incubation time of test

TABLE 2 Quantitative Analysis of PSA for Human Sera Samples Using Fluorescence Immunochromatography

Spiked PSA concentrations (ng/mL)	Ratio of A_T/A_C	Detected PSA concentrations (ng/mL)
0	0.0115	0.20
0.72	0.01375	0.70
2.3	0.04525	2.30
4.6	0.0769	4.25
11.5	0.1835	11.3
23	0.3275	20.7

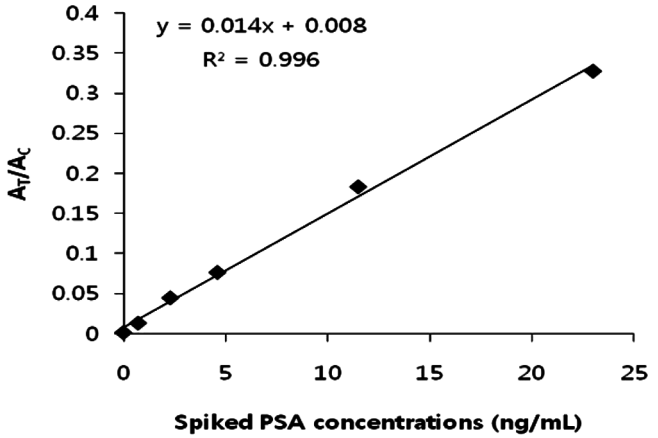

FIGURE 4 Correlation curve of A_T/A_C ratios and spiked PSA concentrations in human sera samples.

samples would be exactly 20 min, since the standard curve data stored in the fluorescence scanner would be obtained with an incubation time of 20 min.

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