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Electronic immunochromatography embedding RFID sensor

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1. Introduction

Immunochromatographic assay is becoming a standard tool in point-of-care-testing (POCT) [1–3]. It utilizes solid-phase immune reactions in a porous membrane to detect disease-marker protein or infectious pathogens. The major advantages of this tool are that measurement results can be obtained within 10 minutes even outside the laboratory without the need for specialized equipment or extensive training. When POCT is compared to a laboratory assay such as an enzyme-linked immunosorbent assay (ELISA), however, it is pointed out that sensitivity is relatively poor and the assay only gives qualitative results because a color change of the membrane caused by immune aggregation is detected by visual observation [1,3]. In the present study, a novel sensitive and quantitative POCT device—incorporating a chemiluminescent reaction and radio-frequency-identification (RFID) sensor chips [4] into an immunochromatographical test strip—with easy operability was developed.

2. Experimental procedures

A schematic cross section of the electronic immunochromatography and a block diagram of the RFID sensor chip are shown in Fig. 1. A primary antibody (monoclonal antibody #6601, Medix Biochemica) was immobilized in a test zone of the membrane (polyethersulfone), which functions as a lateral-flow-type test strip. To evaluate the test strip, antigen spiked samples were prepared by diluting recombinant hCG (Rohto Pharmaceutical) with a dilution buffer. In the first step of the measurement procedure, a mixture of antigen and secondary antibody labeled by alkaline phosphatase (AP) was applied to the membrane. Antigen was captured by the primary antibody, forming a sandwich complex consisting of primary antibody, antigen, and secondary antibody (monoclonal antibody #5008, Medix Biochemica). In the second step, dioxetane-based chemiluminescence-substrate solution was flowed, resulting in a chemiluminescence reaction with AP on the sandwich complex. Chemiluminescence detection by the RFID sensor chips placed on the test zone (with primary antibody) and control zone (without antibody) was started a few minutes before the substrate application. The digital outputs of the sensor chips were transmitted wirelessly to a reader. A “signal” corresponding to the net chemiluminescence intensity reflecting antigen concentration was determined by measuring the difference between the output of the test chip and that of the control chip.

As shown in Fig. 1, the sensor chip consists of three functional blocks: an RF front-end, a signal-processing circuit consisting of an amplifier/13-bit ADC, and a photosensor. These blocks are integrated on a 2.5-by-2.5-mm silicon chip with an antenna coil. Control commands and power to drive the sensor chip are transmitted from a reader unit by RF wave (13.56 MHz). As the sensor chip directly contacts the test zone of the membrane, chemiluminescence can be collected at high efficiency without need for a lens. To evaluate device performance, human chorionic gonadotropin (hCG) was used as a test analyte. For the primary and secondary antibodies, mouse monoclonal antibodies, which recognize different epitopes of hCG, were employed.

Figure 2 shows a conceptual image of the measurement procedure by electronic immunochromatography. The test strip (shown in Fig. 1) is packaged in an optically shielded plastic case. The measurement process consists of three steps: introduction and incubation of sample and reagent;

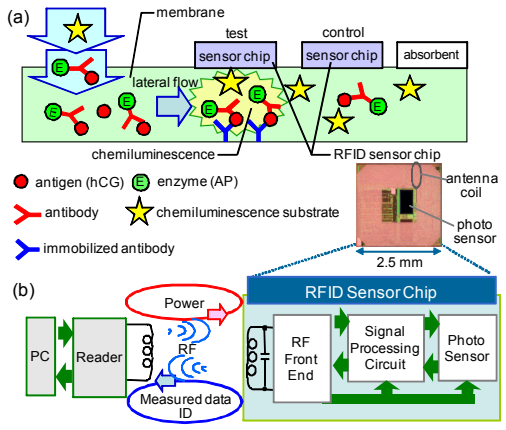

Fig. 1 Schematic of electronic immunochromatography: (a) cross section of test strip, (b) block diagram of RFID sensor chip.

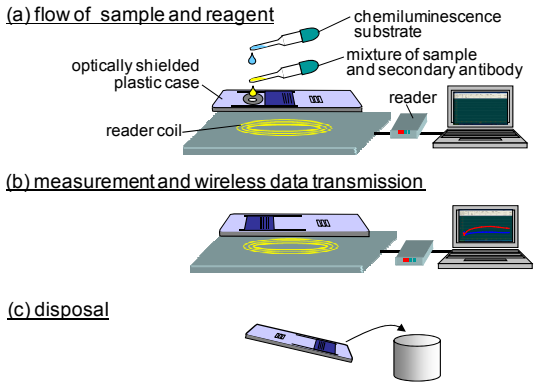

Fig. 2 Conceptual image of biomarker measurement by electronic immunochromatography.

introduction of chemiluminescence substrate followed by signal detection and transmission of measurement data; and disposal of the used test strip. Since the communication protocol is based on a universal standard (ISO15693), the reader system is readily available. This procedure provides a highly sensitive and quantitative diagnosis that can be used at patient bed sides without sacrificing the simple operation procedure or portability of conventional immunochromatography.

3. Results and Discussion

Figure 3 shows CCD images of chemiluminescence emission in the membranes with different antigen concentrations. In the test zone, a mouse anti hCG antibody was immobilized as in the case shown in Fig. 1. Unlike the control zone in Fig. 1 where no antibody exists, a rabbit anti-mouse antibody was immobilized in a positive control zone in Fig. 3. The chemiluminescence substrate solution was introduced from the top right position in the images. The images were taken 200 seconds after the substrate solution was flowed. It was confirmed that the intensity of the test zone increased according to antigen (hCG) concentration and that the intensity of the positive control zone decreased complementally to that of the test zone.

It should be noted that the transport behavior of the chemiluminescent substrate was substantially limited compared to that of a colorimetric precipitating substrate. Hence, chemiluminescence intensity was examined in terms of substrate transport length d, which is defined as the distance between the test zone and the dispensing position of the substrate. Figure 4 illustrates chemiluminescence intensity measured by CCD for two values of d. The intensity for d of 12 mm is about 12% of that for d of 1 mm.

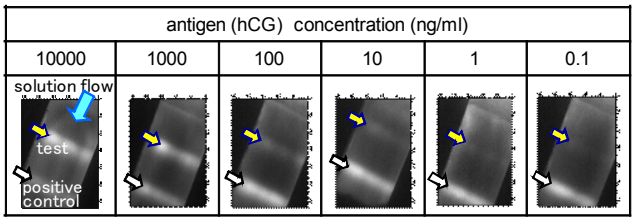

Fig. 3 CCD images of chemiluminescence emission in the membranes applied with different antigen concentrations.

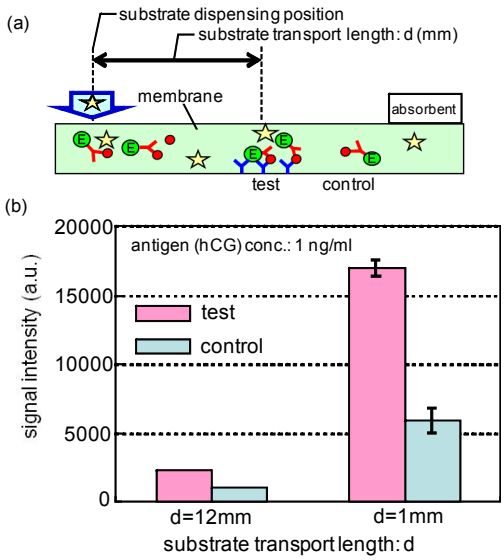

Fig. 4 Chemiluminescence intensity measured by CCD for two cases of d. (a) substrate transport length: d in the test strip, (b) relationship between signal intensity and d.

Chemiluminescence of samples with different hCG concentrations was measured by an electronic immunochromatography technique using the experimental setup shown in Fig. 1 with substrate transport length d of 1 mm. The obtained calibration curve of hCG concentration is shown in Fig. 5. The lower limit of detection is 0.07 ng/ml, which is lower than that of conventional visual observation (1 to 10 ng/ml) by one order or more. Moreover, the signal output increases with increasing hCG concentration within the range of 0.01 to 10 ng/ml. These results represent a quantitative assay covering the range from 0.01 to 10 ng/ml.

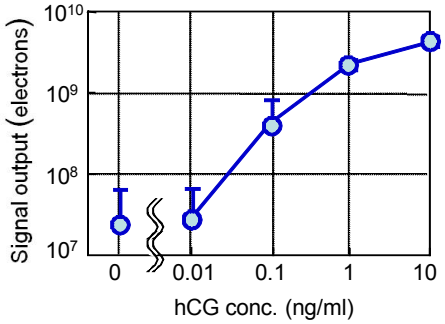

Fig. 5 Calibration curve of hCG measured by electronic immunochromatography.

4. Summary

Electronic immunochromatography applying an RFID sensor chip was used for detecting a biomarker protein. Experiments using hCG as a standard sample showed that quantitative and highly sensitive measurement of biomarker protein is possible with this method. This technique will make it possible to construct a portable POCT device with both high performance and simple operability.

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