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Development of a Test Strip Reader for a Lateral Flow Membrane-based Immunochromatographic Assay

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Abstract A low-cost, simple strip reader system using a linear movement mechanism of CD-ROM deck has been developed to characterize a lateral flow membrane-based immunochromatographic assay. The test strip reader was assembled by a CD-ROM deck and home-made optical head especially designed for immunoassays. The optical head for detecting reflected light from the test strip surface consists of green light-emitting diode, large area silicon photodiode, and anodized aluminum mounting block providing a slit structure for cutting light from the LED. The stepping motor of the deck was operated in the full step mode, whose distance of each reading point is about 0.15 mm. The performance of the strip reader was tested by analysis of HBV (hepatitis B virus) antigen test kit. This strip reader can be useful for inexpensive, disposable, and membrane-based assays that provide visual evidence of the presence of an analyte in a liquid sample.

Keywords: test strip reader, immunoassay, immunochromatographic assay, lateral flow assay

INTRODUCTION

Immunoassays are typically performed to detect molecules in identifying a human subject with a particular disease or condition. They have attractive features, such as high selectivity and affinity, which enable measurements of analytes that have extremely low detection limits. There is an increasing demand for point-of-care immunosensors with rapid clinical interpretations, which can be performed at the physician's office or patient's bedside [1-3]. In the fields of clinical diagnostics, a lateral-flow test kit has become a popular device for the immunoassay of biologically important molecules, such as hormones, antigens, antibodies, toxins and drugs [4-9]. Some commercial products for self-testing are currently available in the market [10-12].

Lateral flow immunoassays utilize a porous membrane for performing the analysis, and the sample is permitted to flow laterally by capillary action from an application zone to a reaction zone on the membrane surface. The lateral-flow test kit usually has an inlet for receiving biological fluids, such as whole blood, plasma, serum, urine, saliva and sweat. After the sample fluid is placed in the sample inlet, the sample flows from the sample pad through embedded reagents, in which specific chemical reactions occur. The reaction product continues to flow through the membrane arriving at the capture reagents. The capture reagents are immobilized on the membrane as a band shape. The captured reaction product generates visually distinguishable color on the bands. Typically two bands are formed on the membrane, one of which is a test band for detecting the sample by its concentration, and the other is the control band for confirming the success of the assay. Sample fluids may continue to flow and can be collected in an absorbent pad [13-15].

While laboratory-based instruments provide accurate and high volume immunoassays within clinical laboratories, the test kit allows immediate test results, minimizes return visits, and enhances the quality of patient care. The test kit does not require a permanent dedicated space, high-priced instruments nor skillful operators. With the test kit, some diseases from the hepatitis B virus (HBV), *Plasmodium falciparum*, and *H. pylori*, or conditions such as pregnancy can be detected in the physician's office or at home [4,9-11].

However, visual interpretation of the bands on the test strip with the naked eye is often prone to human error [16]. In some applications, the assay result may turn from negative to positive or from positive to negative. Recently, some strip readers were developed to digitalize the assay results of specially designed test strips. The strip readers determine or analyze the results of the assays more accurately and remove subjective factors that cause human error. In addition, assay results can be stored and compared with old results to track patient conditions after treatment. For immunochromatographic assay manufacturers, an automated test strip reader offers a useful tool for optimizing product design and controlling manufacturing quality. A conventional strip reader is a type of scanner that illuminates light and

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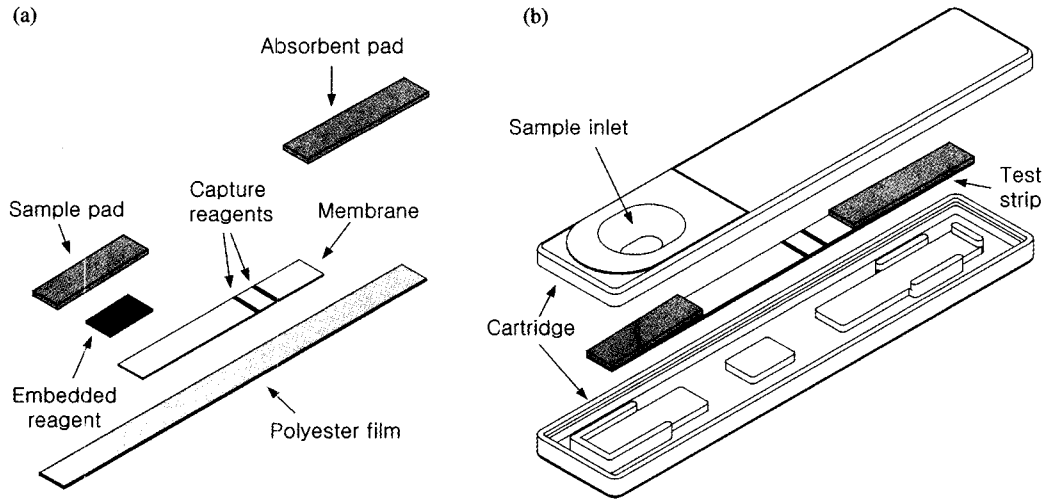

Fig. 1. Schematic diagrams of (a) a typical test strip and (b) a lateral-flow test kit.

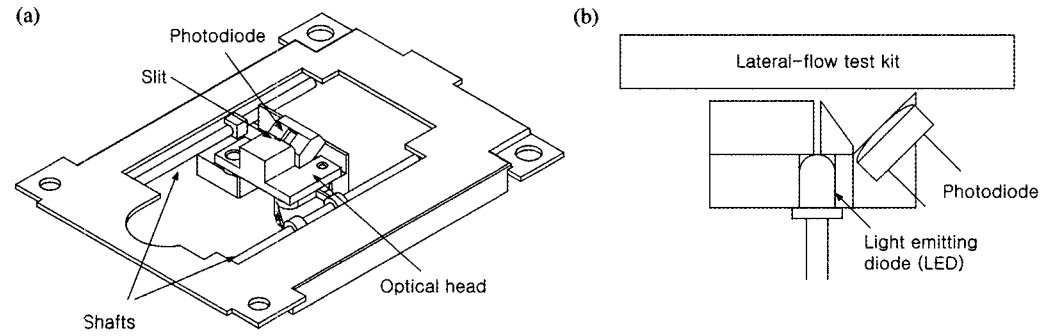

Fig. 2. Schematic diagrams of (a) the mechanical parts of the strip reader and (b) cross-sectional view of the optical head.

measures the attenuation of the reflected light from the strip surface according to the longitudinal position. Typically, a light is perpendicularly illuminated on the strip and a photo-detector is located on the same side of the light source at a certain distance. Unfortunately, commercial strip readers are only available for specially designed test strips. They are not designed to use for general purposes and are not compatible for different test strips.

In this study, we have developed a low-cost strip reader using a linear movement mechanism of a standard CD (compact-disk)-ROM (read-only memory) deck for general purposes. The performance of the strip reader was tested by analysis of HBV antigen test kit. The CD-ROM deck has two moving parts, one for the linear movement of the optical pickup and the other for the rotation of the compact disk. Since the CD-ROM deck is very simple and ubiquitous nowadays, low-priced linear translation, which is needed for scanning the strip surface, can be achieved.

MATERIALS AND METHODS

Standard Hepatitis B surface antigen (HBsAg) and the test strip for HBsAg used for the immunoassay were contributed by the Biotech Research Institute, LG Life Sciences Ltd. (Daejeon, Korea). Fig. 1 shows the schematic diagrams of a lateral-flow test kit used for the experiments. Generally, lateral-flow test kits have two or three reagent bands on the strip, which are optimized for the immunoassay protocols. One band acts as a control band for color discrimination.

The test strip reader was assembled by a CD-ROM deck and home-made optical head especially designed for the immunoassays. As shown in Fig. 2, a linear movement mechanism of the CD-ROM deck was used to move the optical head. The CD-ROM deck mechanism was contributed by the Digital Media Research Laboratory, LG Electronics Inc. (Seoul, Korea). The optical head for detecting reflected light from the test strip surface consisted of a green light-emitting diode (LED; LPG-315CWH; Apro Systems, Seoul, Korea), large area silicon photodiode (VTP8045; Perkin-Elmer, Fremont, CA, USA), and anodized aluminum mounting block that provides a slit (0.5 mm width, 2 mm length) structure for cutting light from the LED. The optical pickup unit from the CD-ROM deck was modified for mounting the opti-

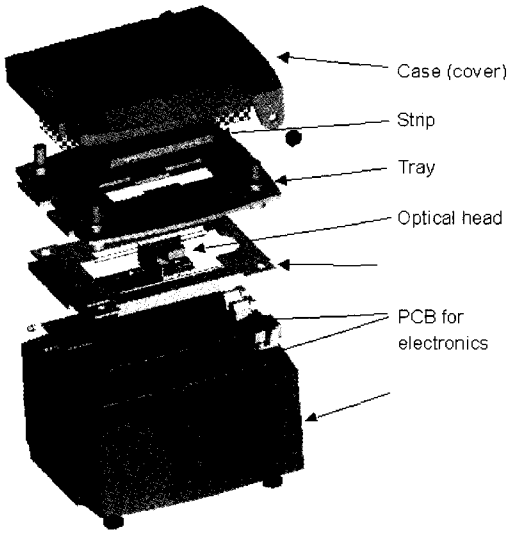

Fig. 3. The overall picture of the assembled test strip reader.

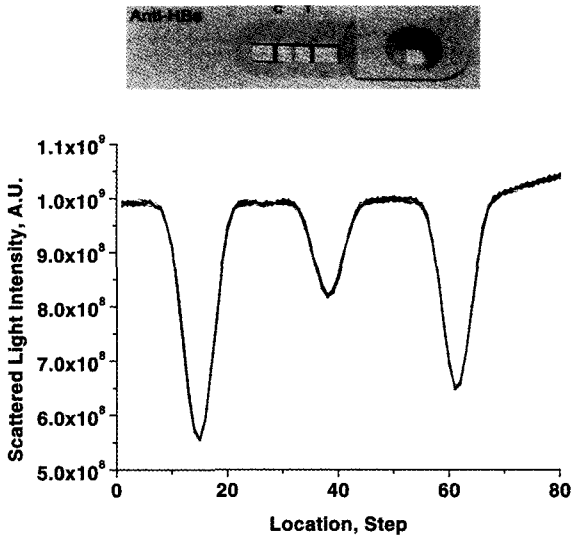

Fig. 4. Reproducibility of the test strip reader (n=10).

cal head. The light from the LED was shaped like a wide rectangular with a slit and illuminated on the surface of test strip, and the reflected light was partly collected with a photodiode. The current generated from the photodiode was converted to a voltage signal with an OP-Amp (TLC084, Texas Instrument, Dallas, TX, USA). Then the voltage signal was digitalized by a 24-bit analog-to-digital converter (ADC; ADS1252; Texas Instrument). The stepping motor of the deck was operated in the full step mode, whose distance of each reading point was about 0.15 mm. A microcontroller (PIC16C74A) was used for the stepping motor control, voltage signal collection, and communication with a computer. Fig. 3 shows the overall picture of the assembled test strip reader. The reflected light was measured and summated 100 times for each step and transferred to a computer. The program for communicating with the microcontroller in the strip reader was built with LabView 5.1 (National Instrument, Austin, TX, USA).

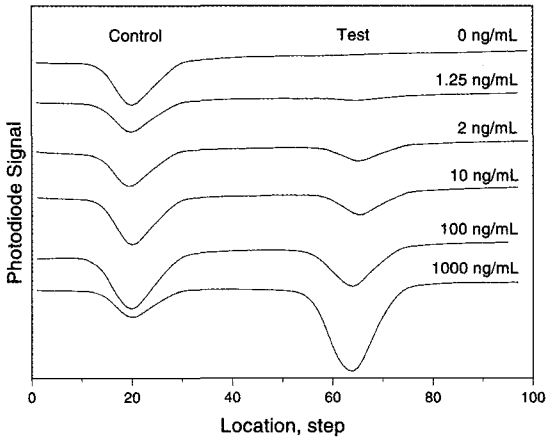

Fig. 5. Series of strip reader scans from the HBV antigen test kits of different concentration. The antigen concentration is written on the upper right-hand corner of each graph.

RESULTS AND DISCUSSION

Reproducibility of the Scanning Results

Prior to analysis of the HBV (Hepatitis B virus) antigen test kits, we have tested the assay for accuracy. The sample strip used for the experiment consisted of a three band system used for confirming the test, which indicate control, low, and high concentration of the analyte. Although each band can be detected with the naked eye, the colors of the two bands on the strip were not easily distinguishable. Because of operator error and subjectivity, the manual measurements caused CVs (coefficient variables) to be in the range of 5~10% [15]. However, the scattered light intensity of the strip reader showed three independent peaks. The data obtained from the strip reader were almost the same results after 10 scanning experiments, indicating CVs less than 1% (Fig. 4). The scanning speed was 6 mm/sec, and the overall scanning range was programmable in the range of 37 mm.

Scanning of the HBV Antigen Test Kit

The performance of the strip reader was tested by analysis of the HBV antigen test kits. Standard HBV antigen sample solution with a known concentration was diluted to the proper concentration and put into the sample inlet of the test kit. After air-drying, the test kit was scanned with the test strip reader. Peak height and area of the scanned data was calculated with the Microcal Origin 5.0 software (Microcal Software, Northampton, MA, USA) after obtaining the smoothed value for each data point using the 5-point Savitzky-Golay filter method. Fig. 5 shows series of scanned data of a lateral-flow test

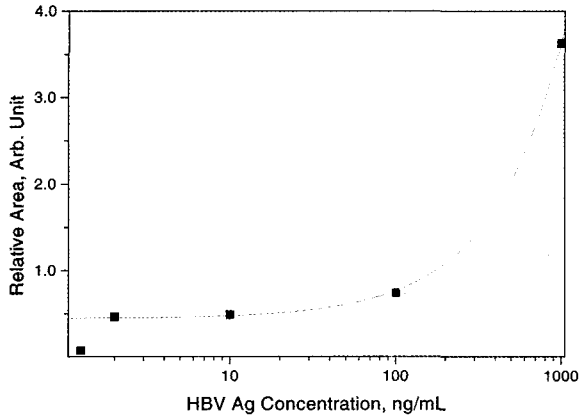

Fig. 6. Plot of the relative peak area (Test/Control) vs. HBV antigen concentration.

kit for different HBV antigen concentrations. The photodiode signal in this figure means scattered light intensity in the arbitrary unit. As can be seen, the intensity of the test band increases as the antigen concentration increases from 1.25 to 1,000 ng/mL. However, the control band did not show the same intensity of the color against all range of the sample concentration. It is clear that the peak height increases as the antigen concentration increases. Because light from the LED is not focused, a wide illuminated light on the strip makes a wide peak. Since the typical distance between the test strip bands are larger than 6 mm, there is no overlap between the control and test bands. The peaks obtained from the strip reader were integrated, and the results were plotted against the antigen concentration after being divided by area of the control band (Fig. 6). The solid line in Fig. 6 is a linear fitting line for the antigen concentration from 2 to 1,000 ng/mL. The relative peak area (Test/Control) was linearly related to the antigen concentration in this range. The relative peak area had better linearity in comparison to the peak area of the test band. Because of the deviation of the sample condition and/or the reagent amount on the strip, the control band does not always give equivalent results. By numerical compensation, the strip reader can improve the quality of the test.

Since infectious diseases, such as HBV, can be diagnosed by detection of the antigen or antibody, sensitivity of the assay is important. The strip reader was better than looking at the material with the naked eye. A test band of 1.25 ng/mL, which is the tiniest in our experimental set, can be barely detected with the naked eye. Digitalized data acquired with the strip reader can provide precision and reliability in the immunoassay.

CONCLUSION

The low-cost, simple strip reader composed of a CD-ROM deck module and optical head was evaluated by analysis of the HBV antigen test strips. The primary result of our system is allowing a simple and rapid one-step immunochemical measurement method. This strip reader has the advantage of giving quantitative self-test results through a small and inexpensive method. The signal sensitivity of the strip reader meets the prerequisites for portable immunodiagnostic devices. The strip reader can be useful reducing the time required to measure and record the performance data and can also improve the accuracy of such measurements.

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