BIOCHIP JOURNAL, Vol. 1, No. 1, 1-16, March 2007

Mini Review

Immunosensors for Point-of-Care Testing

Se-Hwan Paek[1-3], Joung-Hwan Cho[2], Il-Hoon Cho[3], Young-Kee Kim[4] & Byung-Keun Oh[5]

[1]Department of Biotechnology, Korea University, Jochiwon, Choongnam 339-800, Korea
[2]BioDigit Laboratories Corp., Korea University, Biotechnology Building, Technology Incubation Center, 1, 5-ka, Anam-dong, Sungbuk-ku, Seoul 136-701, Korea
[3]Program for Bio-Microsystem Technology, Korea University, 1, 5-ka, Anam-dong, Sungbuk-ku, Seoul 136-701, Korea
[4]Department of Chemical Engineering, Hankyong National University, Sukjong-dong 67, Ansung, Kyonggi-do 456-749, Korea
[5]Department of Chemical and Biological Engineering, Sogang University, 1, Shinsu-dong, Mapo-ku, Seoul 121-742, Korea
Correspondence and requests for materials should be addressed to S.H. Paek (shpaek@korea.ac.kr)

Accepted 29 January 2007

Abstract

Although immuno-chromatographic assays on membrane strips have been employed at points of care for more than 20 years, their applications have become limited to qualitative analyses of the analytes present at relatively high concentrations in samples. An evolution of technology in this field will be required in order to achieve the capability of detection sufficient for early diagnosis as well as a degree of quantification sufficient to allow for the monitoring of the disease progress, a digital display of analytical results, and automatic recording and correlation in a database. In order to attain proper sensitivity, the colloidal gold normally used as a tracer for colorimetry may be replaced with different signal generators, including fluorophores, magnetic beads, electrochemiluminescent substances, and enzymes. The means by which antigen-antibody binding in the assay can be quantified as a measurable signal also varies, depending on the tracer employed and the transduction technology available. Indeed, we have devoted ourselves for more than ten years to the investigation of combinatorial substitutes consisting of novel immunosensors that fulfill the demands inherent to medical diagnostics. In this review, selected immunosensor technologies developed in our laboratories are introduced, along with their detection principles and analytical characteristics.

Keywords: Immuno-chromatography, Quantification of analyte concentration, Performance characteristics, Signal generators, Transducers

Introduction

Biomarkers including hormones, proteins, and infectious organisms indicating symptoms or diseases of the human body are normally present at rather low concentrations in specimens (e.g., blood, urine, and saliva), and can be measured via biological reactions, including antigen-antibody binding and enzyme-substrate reactions[1-3]. Typically, the receptor, or antibody, binds its counterpart, or antigen, with extremely high specificity as well as high affinity, allowing for good detection capability when utilized as a binding reagent for assays[4,5]. Such antibody characteristics lend themselves to the construction of immuno-diagnostic systems that should prove to be valuable for early disease diagnosis, facilitating effective patient treatment. The majority of diagnostic systems, however, require delicate and precise handling of reagents and equipment, as well as expert knowledge of operation[6-8]. This limits their use in laboratory settings.

With regard to recent immunoassay trends, biomarkers are increasingly being measured at the sites of patient care, including the doctor's office and emergency room, allowing for rapid estimates of the state or progress of an illness, and even at home, for the purposes of self-diagnosis[9,10]. Toward this end, an immuno-analytical system that does not require a complex procedure, is easy to operate, and can be completed in a relatively short period of time is required. A device with such analytical performances may prove possible with the concept of immuno-chromatography, in which a porous membrane strip is utilized as a solid matrix for the immobilization of the antibody (usually), or the antigen when required[11-13]. Upon the absorption of an aqueous sample from the bottom of the strip, the medium transports the analyte, i.e., the substance to be measured, via capillary action through the pores of the membrane to the antibody immobilization site. Antigen-antibody binding occurs here, and the unreacted components are separated by the flow. As this technology utilizes a lateral flow along the membrane strip, the analyte transfer is accelerated in order to complete the reaction in a relatively short time (e.g., on the order of minut-

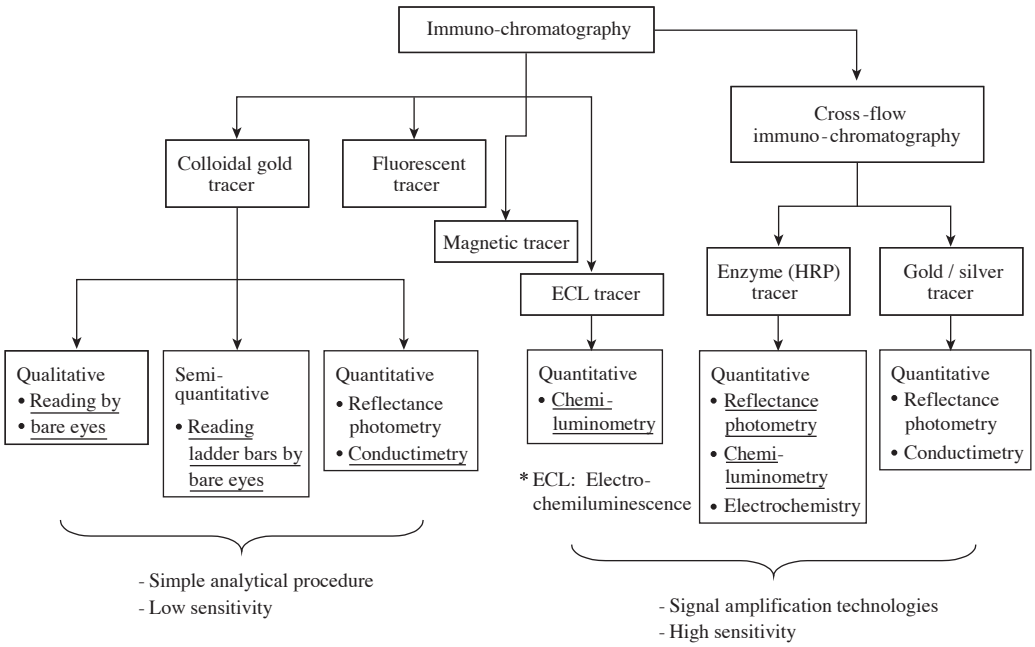


Figure 1. Overview of tracers and transducing technologies used to construct kits and sensors for point-of-care testing predicated on immuno-chromatography. The detection methods demonstrated in our laboratory were underlined and, thus, have been introduced in this review. See text for details.

es)[14,15]. Further, the *in situ* separation allows for one-step analysis, in which the addition of the sample is the only step that the user need perform.

The demands inherent to point-of-care testing (POCT) systems are well reflected by the large demand for self-diagnostic devices, particularly in the context of pregnancy and ovulation[16,17]. As the remote monitoring of personal health status might also, in the near future, be conducted over the internet, POCT systems for a variety of conditions may become an essential element in public health management. However, the home-version rapid test kits allow only for qualitative analyses, generating color signals from colloidal gold tracer that are perceptible to the naked eye (refer to Figure 1)[18]. This feature is definitely insufficient for the analysis of most biomarkers, particularly those of adult diseases, for which quantitative analyses for early diagnosis and progress monitoring are classically constrained. The gold color can be measured either semi-quantitatively by reading the number of ladder bars[19,20], or quantitatively via conversion to optical density using, for instance, a digital camera and attendant software[21,22]. Nevertheless, the test kits tend to exhibit a sensitivity lower than that associated with the traditional enzyme-linked immunosorbent assay (ELISA)[23,24].

Other signal generators evidencing sensitivities comparable to that of the enzyme used in ELISA include fluorophores, magnetic beads, and electro-chemiluminescent (ECL) substances such as ruthenium and osmium (refer to Figure 1). Fluorophores have been extensively employed as tracer materials in a variety of biochips[25-27] and also in immunosensors for the rapid detection of medical biomarkers and agents associated with acts of terrorism[28,29]. Magnetic beads, which were employed originally for the separation of cells[30,31], have now been applied to biomolecular detection applications, via their labeling to different binders (e.g., antibodies)[32,33]. Their detection capability in analytical applications has yet to be confirmed in clinical applications, although the potential for this is increasing with the advent of more sophisticated magnetic sensor technologies. ECL producers also provide a highly sensitive signal due to the low background from the medium and analytical environment under dark conditions[34-36].

As exemplified in ELISA, enzymes can be used as an alternative tracer material, which can be applied to immunosensors for POCT[24,37-39]. The tracer generates an enhanced signal resultant from catalytic action, and also provides different signal types, which are measurable with comparatively simple detectors (e.g., based on photometry[27], chemiluminometry[33], and electrochemistry[34]; refer to Figure 1), depending on the substrate as well as the enzyme used. The enzyme reaction, as a different feature from those of other

tracers, should be conducted separately for signal generation following the completion of the antigen-antibody binding reactions. A standard protocol of the heterogeneous immunoassay, then, requires washing steps for the separation of the immune complexes formed on the solid surfaces from the unreacted reagents. In order to employ this procedure at sites of care, a novel method of cross-flow immuno-chromatography has been developed, in which immunological binding and the enzyme reaction are sequentially conducted[37]. The same concept can also be applied to the silver intensification[40] technique, using colloidal gold as the signal generator.

In the following, selected immunosensor technologies that can be employed for POCT are introduced with regard to their detection principles and analytical characteristics. It should be noted that all of the technologies outlined in this work were developed in our laboratories.

The Analytical Concept of Immuno-Chromatography

For POCT, an immuno-chromatography has been developed using membrane strips as solid matrices for antibody immobilization[13]. Tracers employed in this format are normally colloidal gold or Latex beads, the colors of which, as a result of assays, can be detected by the naked eye[12,15,18]. As a distinguishing characteristic of this method from most assays utilizing an incubation mode, the medium flow rate invoked by capillary action through the membrane pores induces the transport of soluble reactants to the immobilized binding partners[41,42]. Under such non-equilibrium conditions, the binding complexes between antigen and antibodies are formed at the solid surfaces, and the unbound reagents are immediately separated by the medium flow. These combined processes of convective mass transfer and binding reactions not only shorten the assay time, but also provide for a one-step sample analysis, without reagent handling[11-13]. Such membrane strip-based analysis techniques have been initially employed for the point-of-care examination of symptoms including pregnancy and ovulation[10,17], and recently for the diagnosis of a variety of diseases, including microbial infections and even acute myocardial infarction[43-45].

In a model of this immunoassay method (Figure 2, left)[37], two antibodies binding distinct epitopes present on an analyte molecule are utilized: one (detection antibody) is labeled with a signal generator (e.g., colloidal gold), and the other (the capture antibody) is immobilized onto a solid surface. The labeled antibody is placed in a dehydrated state into a glass fiber membrane for the fabrication of the conjugate release pad. The capture antibody is dispensed in a line onto a site on a nitrocellulose (NC) membrane (normally,

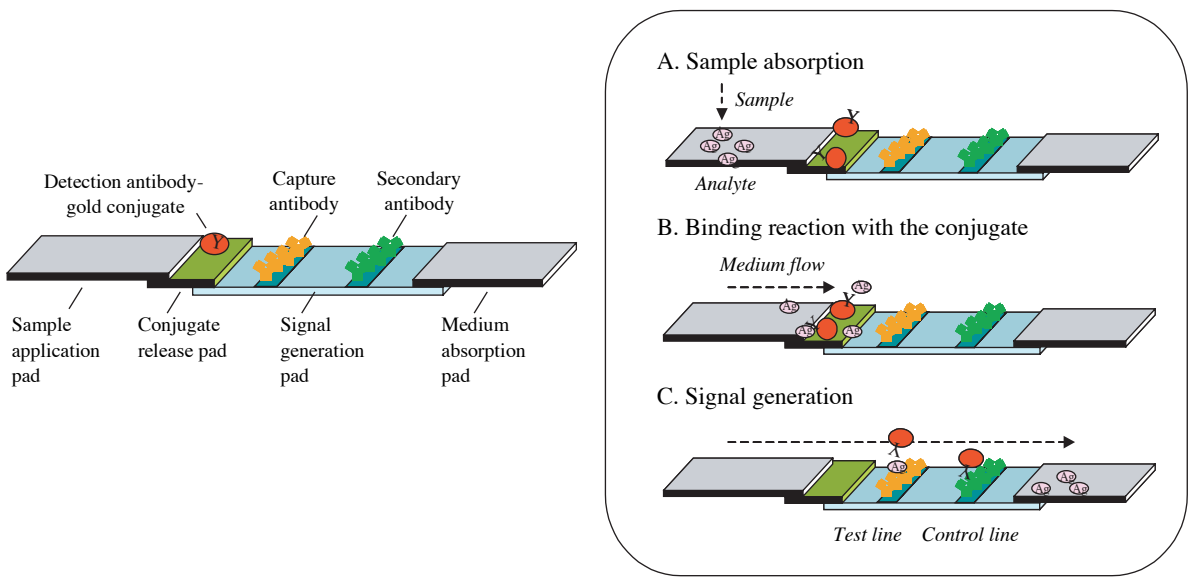


Figure 2. A membrane strip assay system (left) predicated on immuno-chromatography and the concept of analysis (right). The immuno-chromatographic assay system is comprised of four membrane pads for: sample application, antibody conjugate release, signal generation, and continuous, medium absorption. For analysis, a sample containing the analyte is introduced into the strip via capillary action (A) and binding occurs between the analyte and the labeled antibody (B). This binding complex is transferred by the flow to the nitrocellulose (NC) membrane and then allowed to react with the immobilized antibody for signal generation (C).

10 μm pore size) using a micro-dispenser. On the same membrane, a secondary antibody specific to the detection antibody is also dispensed onto a site above the capture antibody. A sample application pad is prepared with a glass fiber membrane which has been pretreated with a hydrophilic substance, such as polyvinyl alcohol. The prepared membrane pads are arranged in order of width from the bottom, in the following order: sample application pad, conjugate release pad, signal generation pad, and a cellulose membrane used as an absorption pad. Finally, a functional immuno-strip is constructed via the partial superimposition of each contiguous membrane strip and fixation on a plastic film, using double-sided tape.

Using the immuno-strips, an analyte can be measured merely by the addition of a sample to the application pad (Figure 2; right, A). The aqueous medium migrates into the conjugate release pad via capillary action, and instantaneously dissolves the labeled antibody. This antibody then participates in the binding reaction to form a complex with the analyte in the liquid phase (right, B). This complex eventually binds to the capture antibody immobilized on the surfaces of the nitrocellulose membranes, which provide a uniform pore and, thus, a liquid-solid interface for reproducible antigen-antibody binding. As the complex includes colloidal gold, a color signal visible to the naked eye is generated in the lower site (test line; right, C). The unreacted conjugate is transferred by the flow to the site at which the secondary antibody is immobilized, and binds to generate a signal from the upper site (control line). The medium absorption pad

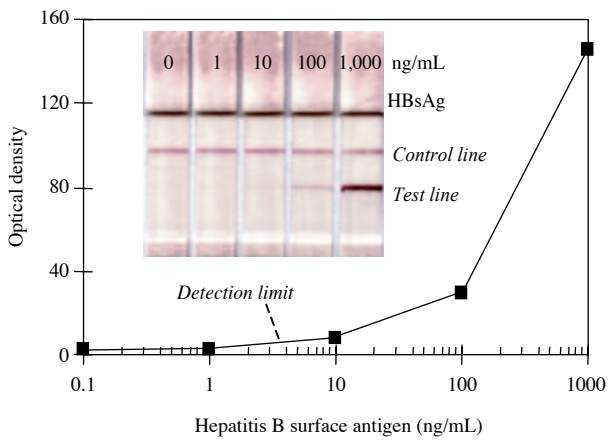


Figure 3. Dose response of the conventional, immuno-chromatographic assay system with the gold label for Hepatitis B surface antigen as a model analyte. Color images of the assay results were captured (inset), and the respective signals were then converted to optical densities in order to plot the dose-response curve.

positioned at the top induces a continuous wicking effect, accumulating the immune complexes with the immobilized antibodies.

Normally, at approximately 10 minutes after the application of the sample, the color signals can be read either by naked eyes (the inset) or by conversions to optical density for quantitation via photometric transduction, and the intensity of the test line is proportional to the dose of the analyte (Figure 3). From the dose-response curve, the detection limit can be determined as the concentration of analyte corresponding to the signal value calculated via the multiplication of the standard deviation of the signal at zero dose by three[37,46]. This capability of the assay is relatively low with other tracers, such as fluorophores and enzymes, and this has significantly limited its analytical application[11,34,37]. For this reason, detection frequently does not cover the clinical range of the minimum analyte concentration required for early disease diagnosis.

Bar Code Immuno-chromatographic Assay

As a non-instrumental approach, the analyte concentration can be semi-quantitatively determined by simply encoding the dose ranges to different numbers of a colored ladder bar, provided that the antibody is immobilized in multiple parallel lines on the signal generation pad[19,47,48]. Although this form of semi-quantitation allows for the direct determination of the analyte concentration, the color may not be clearly confined within the defined areas of each ladder bar. This results in a vague analytical result. This problem can be circumvented via the introduction of a capture technology based on streptavidin (SA)-biotin binding with the highest affinity (a range of 10^{15} L/mol) among the biological reactions thus far known[49,50].

Utilizing the available methods, investigations have been conducted to develop a novel bar code version of the immuno-chromatographic assay[19]. As a key component, a heterobifunctional conjugate, which functions both as the signal generator and capture reagent of the immune complexes simultaneously, was employed. This was synthesized via the biotinylation of the detection antibody-gold conjugate in an optimal ratio toward the antigen-antibody reaction and SA-biotin binding. A membrane pad employed for the production of a bar code signal was generated via the covalent immobilization of streptavidin in a ladder bar pattern on the surface. As the signal should be proportional to the concentration of complex between the heterobifunctional conjugate and the analyte, this system was devised to separate the conjugate remaining unreacted with the analyte prior to signal generation in the assay. To this end, an antigen

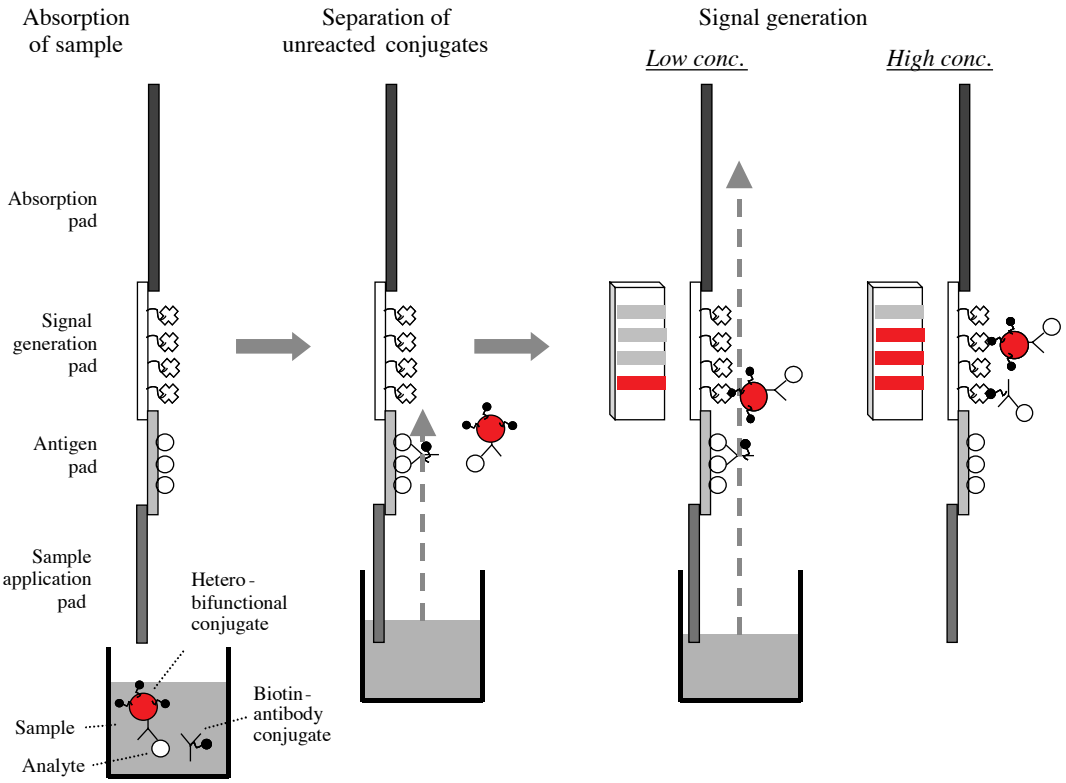


Figure 4. The concept of competitive immuno-chromatographic assays for signal generation in a ladder bar pattern as dose response, which enables us to designate each range of analyte concentration with bar codes. See text for the details of the analytical components and procedure.

pad was fabricated via the chemical immobilization of the antigen, i.e., the analyte to be measured (e.g., microalbumin in renal disease), on the surfaces of the NC membranes[19]. Using these components, a bar code analytical system was constructed using four different membrane strips partially superimposed sequentially, as is shown in Figure 4 (the left).

The bar code pattern of dose response with the heterobifunctional conjugate can be obtained on the basis of competition between the conjugate and a biotinylated antibody for the binding sites of SA immobilized onto the signal pad (Figure 4). The two competing components are added to the sample solution to perform the antigen-antibody reactions with the analyte molecules (4, left). The aqueous solution is absorbed from the bottom of the analytical system via capillary action, then transferred through the antigen pad, in which the unreacted reagents are captured for separation (4, center). When a low concentrations of analyte is in the sample, the total amount of immune complexes forming with the biotinylated reagents are so small that no significant competition occurred on the SA binding sites, and the color may be restricted within the first bar ('Low conc.' in the right of Figure 4). An increase in analyte concentration would generate a substantial quantity of immune complexes, thus resulting in the competitive binding of the biotinylated species to SA immobilized at extended sites ('High conc.'). Thus, a bar code signal representative of a single dose range of analyte can eventually be produced.

In order to demonstrate the proposed concept, the bar-coding of different concentrations of human serum albumin as a model analyte was conducted[51,52]. A single number of the colored ladder bar was designed to correspond specifically to a unique analyte dose range (see Figure 5 for an example). The first ladder bar used as the control signal appeared consistently, regardless of the level of albumin in the sample, and the next bar was generated in the presence of a minimum of 50 μg/mL albumin within a specimen. This semi-quantitative system was capable of consecutively generating additional bars, one by one. From these results, a semi-quantitative system involving the bar-coding of the immuno-chromatographic dose responses can be developed. Under further optimal

conditions, the numbers of the colored bars (2 to 5 digits) after the assays were able to be matched with 30, 60, 90, and 120 μg/mL minimum analyte concentrations, respectively[53,54]. Such dose responses may be suitable for the self-monitoring of a clinical indicator, for example, the urinary concentration of human serum albumin (i.e., microalbumin) necessary for the diagnosis of renal function in diabetic patients[55,56].

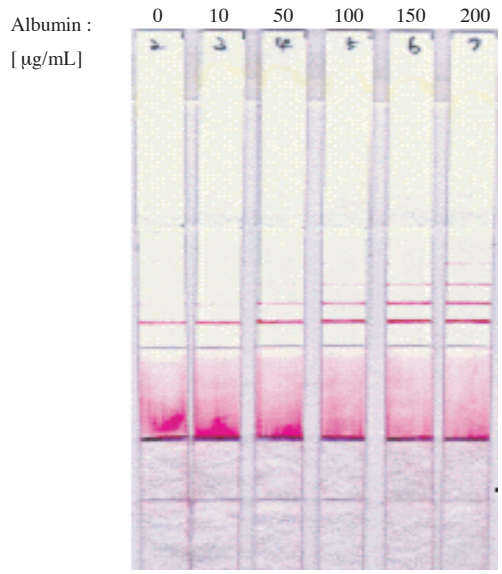


Figure 5. Dose responses of the semi-quantitative analytical system to concentrations of human serum albumin under optimal conditions. In the absence of analytes, only the control line (the first bar at the bottom) appeared. The detection limit of the system was adjusted to 50 μg/mL of albumin in a sample by producing an additional signal bar. The next each bar (five in total) was generated at every 50 μg/mL increment of concentration.

Conductimetric Membrane Strip Immunosensor

As colloidal gold is a metal, the gold signal band forming on the membrane surface in the chromatographic assay allows us to measure the electrical conductivity along the metal particles[57,58]. As the binding reaction proceeds, the particles are accumulated repetitively between the lateral edges of the signal band and, thus, the mean distance among them may be sufficiently short for the formation of an electric circuit. This accumulation effect would cause a variation in conduction in proportion to analyte concentration, and a single event of binding might even control the entire process of signal generation in an exponential pattern.

In order to measure the electric signals from the membrane strips, we have explored thick-film electrodes screen-printed on plastic film, which will be positioned onto the antibody immobilization sites on the NC membranes (Figure 6). The electrodes, which are constructed of silver, were patterned in a planar interdigitated structure in order to ensure a high signal yield[59,60]. These electrodes were combined with the immuno-strip such that the antibody immobilized onto a membrane site was located proximally to the electrode. Electrical contacts extending from the sensor portion were printed onto the same plastic film that functions as an insulator against the aqueous environment. Electrodes of the same pattern can also be formed directly on the surface of the membrane,

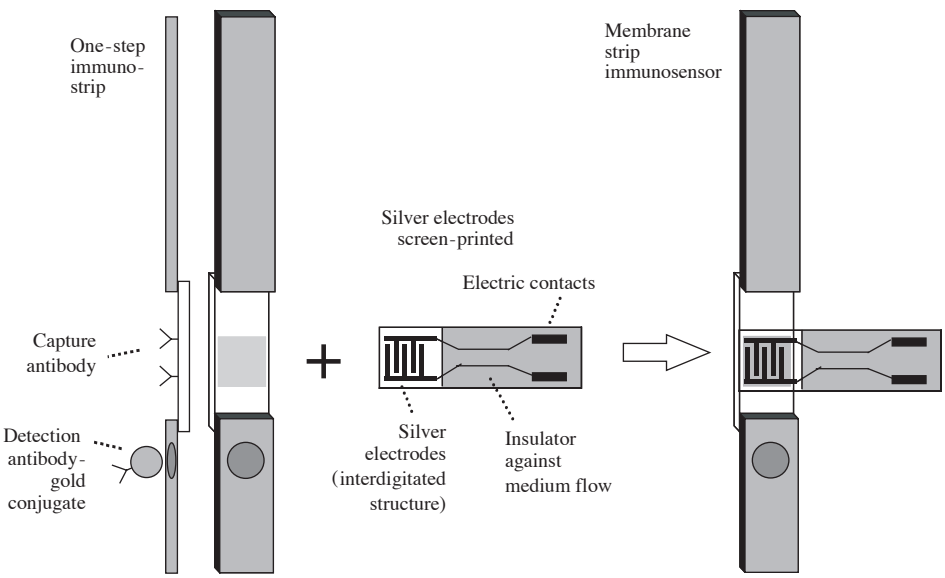


Figure 6. Construction of a conductimetric immunosensor utilizing screen-printed thick film electrodes in an interdigitated structure. The electrodes were printed on a plastic film through a patterned screen (two-by-three interdigit), and combined with an immuno-strip such that the capture antibody is located within the interdigitated area.

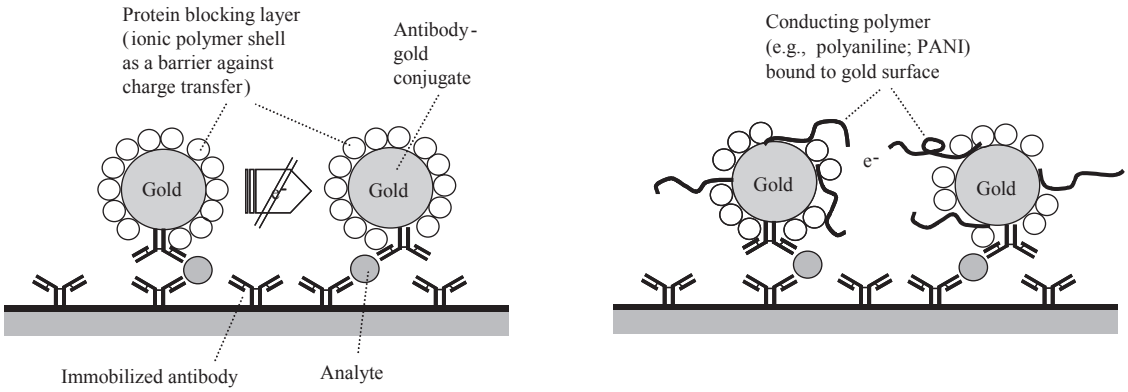


Figure 7. The concept of enhanced electron transfer with a conductive polymer adsorbed onto the surfaces of gold colloids. Whereas the conventional tracer of colloidal gold is unsuitable for the production of the conductimetric signal (left), the modified tracer with polyaniline operating as a molecular wire may transfer detectable electrons (right).

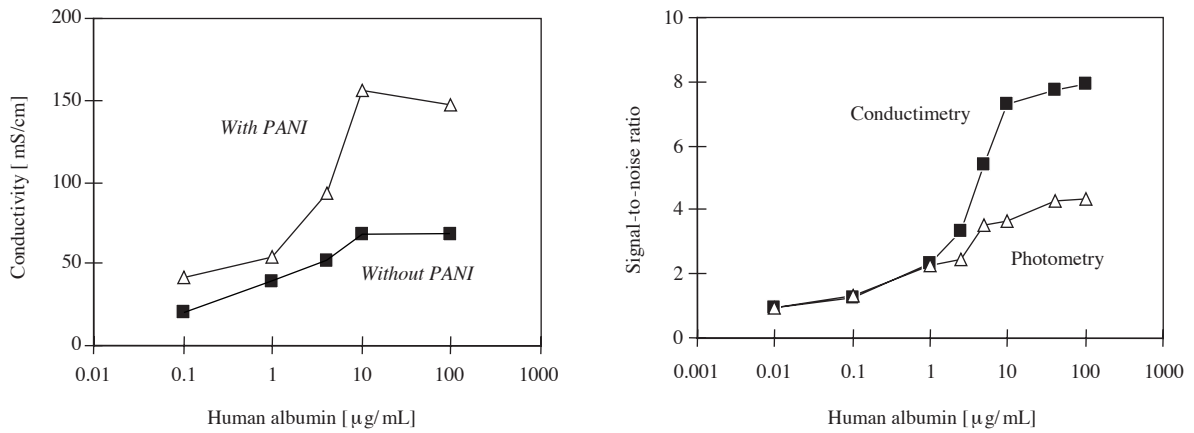


Figure 8. Dose response curves obtained using different tracers of colloidal gold (left) and variable signal generation methods, i.e., conductimetry and colorimetry, from polyaniline (PANI)-bound colloidal gold (right). It has been noted that the tracer with PANI did not interfere with antigen-antibody binding or the generation of color from the gold[66].

using screen-printing technology, upon which the capture antibody was immobilized[61,62].

In a preliminary test, the antigen-antibody binding event occurring between the two electrodes, i.e., the cathode and anode, was measurable as the electrical conductivity and the signal-to-noise ratio determined as a sensitivity indicator, however, was lower than that determined by the colorimetry. Such poor analytical performance resulted from impaired electron transfer along the gold particles, which can be induced by the presence of ionic protein molecules, i.e., immunoglobulin and casein as blocking agents, surrounding each of the particles (Figure 7, left). As protein molecules behave in a fashion similar to that of amorphous semiconductors[63,64], they may function as a barrier against conduction. In an effort to improve this inefficient electron transfer, polymeric conductor molecules (e.g., polyaniline, PANI)[65,66] were introduced to the gold surface to bridge the neighboring particles as to the charge transfer (Figure 7, right)[66]. The degree of protrusion of the polymer strands outside of the surface was controllable by the chemical concentration applied and the molecular dimension.

The concept of molecular wire for electrical connection was evaluated using the constructed conductimetric immunosensor, and its analytical performance under optimal conditions was then compared with that of the conventional colorimetric system (Figure 8). The colloidal gold with PANI bound to its surface evidenced an amplification of the electric signal by a maximum of approximately 3 times that observed with the plain gold without the polymer (8,

left). This indicated that the average distance between the two conducting sites was shortened by the addition of PANI, which consequently augmented electron transfer by hopping[67-69]. It is also conceivable that the presence of the polymer strands on the surfaces augmented the fractal dimension, thereby resulting in elevated interfacial capacitance and, consequently, a gain in conduction[68]. Finally, the novel colloidal gold with PANI was employed as a tracer, to generate both conductimetric and colorimetric signals to compare them with regard to sensitivity expressed in the signal-to-noise ratio (8, right). Although the conductimetric detection, as compared to the results of the colorimetry, consistently enhanced the signal from the immunosensor at a high analyte concentration range[19,56], the detection limit was not significantly different between the two detection methods. This may result from the presence of a barrier against electron transfer, probably due to a wide average distance between gold particles under conditions of low gold density. Nevertheless, the conductimetric immunosensor provides a relative advantage, in that the conduction can be measured using an ordinary electric detector that is stable, accurate, relatively cheap, and simple to operate[70].

Electro-Chemiluminescent Immunosensor

A high sensitivity of the immuno-chromatographic assay may be achieved by employing ECL (Figure 9) tracer combined with a photometric detector such as a charge-coupled device, which has been demonstrated in a variety of analytical systems[34,71,72]. The potentially-portable means of detection can be selected for on-site diagnosis, but its detection capability is inferior to that of a photomultiplier tube (PMT)[73,74]. Such problems may be compensated for via the introduction of a massive transport system of the signal generator, for signal amplification, linked to the antigen-antibody reactions.

An amplification of the ECL signal has, indeed, been achieved via the use of a number of ruthenium molecules encapsulated within a liposome envelope by a freezing/thawing sonication tehcnique[75,76]. The liposome, activated with maleimide groups, was then chemically coupled to the detection antibody and reduced to generate sulfhydryl groups such that the liposome can function as a tracer when incorporated into a novel immunosensor system (Figure 10)[34]. The system was comprised of the antigen pad eliminating the immuno-liposome unreacted with the analyte, and also the signal generation compartment that produces an ECL in proportion to the analyte concentration. The pad was prepared via the immobilization of an antigen (e.g., recombinant *Legionella* antigen) on the surfaces of the NC membranes. The signal generation compartment was also constructed, by positioning two plastic supports with a glass fiber membrane between them. Each support had a carbon electrode (anode) with a large surface area and two-finger patterned-silver electrodes (cathode), respectively, formed via screen-printing.

Such a constructed analytical system has been used to quantitatively determine the concentrations of the analyte (e.g., *Legionella* antigen). The immuno-liposome was combined with an aqueous specimen containing the analyte in order to form the immune complexes, a mixture of which was then absorbed into the membrane strip via capillary action. Upon influx, the immune complexes were passed through the antigen pad and were transported into the glass fiber membrane, in which the liposome particles were burst by detergent pre-located within the membrane. This released ruthenium molecules which were then chemically oxidized on the electrodes to generate an ECL signal detectable by, for instance, a charge-coupled device, as is shown in Figure 9. It is noted that in cases in which the analyte was absent in the sample, the liposome would be captured on the anti-

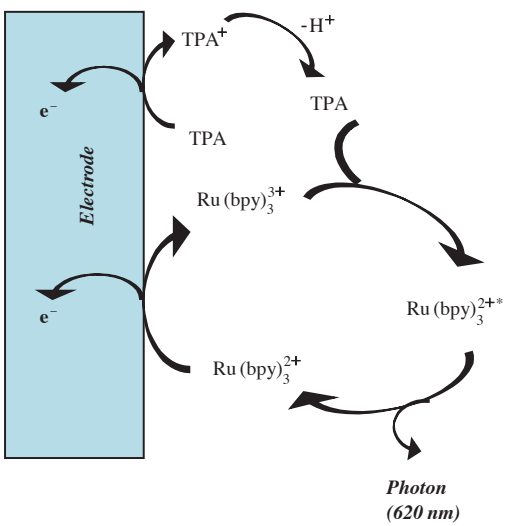


Figure 9. Generation of electro-chemiluminescent (ECL) signals via the electrochemical oxidation of a ruthenium species, e.g., tris (2,2′-bipyridyl) ruthenium (TBR). TBR in its reduced state ($Ru(bpy)_3^{2+}$) is oxidized on the surface of the electrode to $Ru(bpy)_3^{3+}$ which is then converted to an excited state via reaction with tripropyl amine (TPA) radical in solution. This radical is generated via sequential reactions of deprotonation and electrochemical oxidation of TPA on the same electrode. The ruthenium species in the excited state are recycled to the ground state with a co-current release of photons that are detectable at a wavelength of 620 nm.

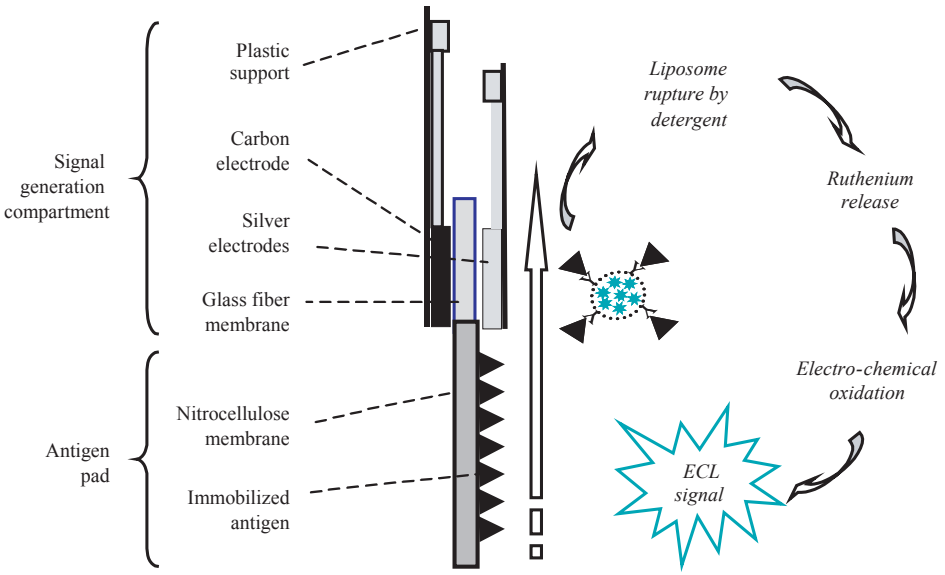


Figure 10. Construction of an ECL immunosensor via the combination of an antigen pad with a signal generation compartment harboring spatially-arranged electrodes screen-printed onto each inner surfaces of the support. A NC membrane of the antigen pad and a glass fiber membrane positioned within the signal generation compartment were superimposed in part. This arrangement allowed for a continuous wicking of an aqueous medium and eventually ECL signal generation in the compartment, as shown.

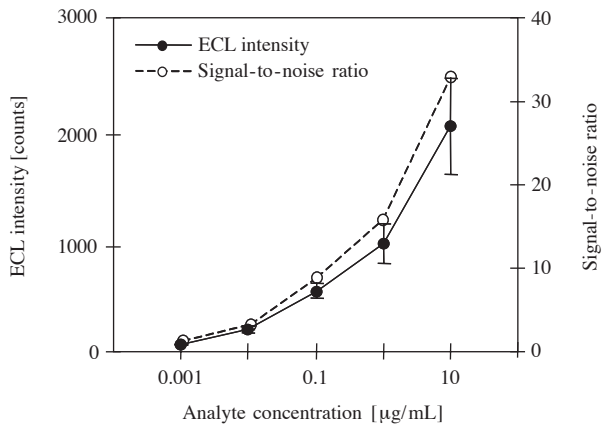


Figure 11. Dose responses of the membrane strip system, based on detection methods using electro-chemiluminometry, to the analyte (e.g., *Legionella* antigen) concentration and the assessment of its analytical performance in terms of signal-to-noise ratio as an indicator of sensitivity. The ECL-producing system evidenced a relatively high sensitivity due to the introduction of the massive transport means of the tracer and low background of ECL.

gen pad, and no beacon would be generated. The ECL signals registered at different concentrations of analyte were found to be proportional to the analyte dose (Figure 11). Signal-to-noise ratios representing detection capability[66] were also generated by dividing the signals by the background. Based on a ratio of greater than 2, the immunosensor detection limit was determined to be 0.002 µg/mL, approximately 10 times lower than that of a colorimetric system which uses colloidal gold as tracer[34]. Such a high degree of sensitivity would result not only from the introduction of the ruthenium liposome transport system, but also from the intrinsically low background of ECL from the environment.

ELISA-on-a-Chip Sensor based on Cross-Flow Chromatography

As mentioned, enzymes used as tracers provide an enhanced signal resulting from catalytic action, as well as variable signals that can be measured on the basis, for example, photometry[12,22], chemiluminometry[34], and electrochemistry[66]. A novel enzyme immunosensor for POCT has been developed via a combination of the method of immuno-chromatography with enzyme signal generation technology[37]. In this sensor, the sequential reactions, namely antigen-antibody binding and the catalytic reaction, have been achieved in a cross-flow mode in the vertical and horizontal directions, respectively. This cross-flow chromatography concept significantly simplified the complex procedure required for ELISA, and also generated a highly sensitive signal.

The sequential flow system was constructed using two groups of membrane pads, vertically-arranged pads and horizontally-arranged pads (Figure 12, left). The pads oriented in the vertical position comprises four different membranes (1 to 4), connected with each other toward capillary action, in a fashion similar to that of the conventional system (see Figure 2), with the exception of an enzyme as a tracer rather than colloidal gold. Two additional membrane pads (5 and 6) used to provide the enzyme substrate after the vertical flow are employed in a horizontal arrangement, and separated spatially from those in the verti-

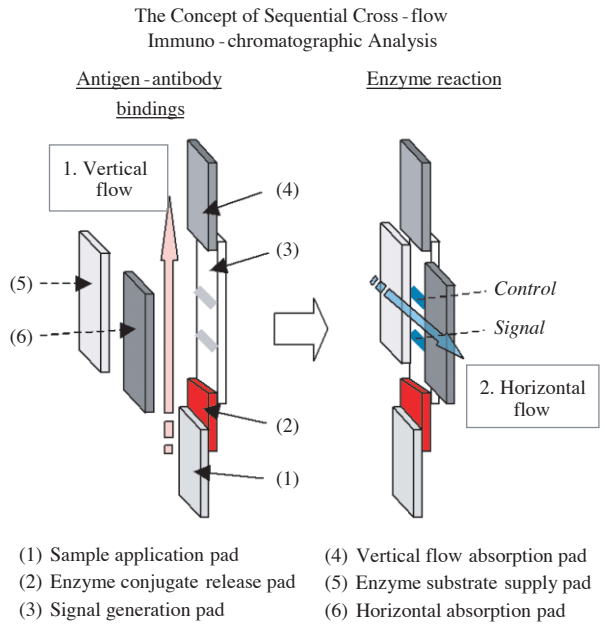


Figure 12. The main components of the cross-flow immuno -chromatographic assay system and its analytical concept. The vertically-arranged pads (1 to 4) are the same as those in the conventional assay as shown in Figure 2, with the exception of the adoption of an enzyme tracer in place of the colloidal gold. Upon sample application, a flow is induced via capillary action and drives the sample in the vertical direction, by which antigen-antibody bindings with the analyte occur (left). The horizontally-arranged pads (5 and 6) are then superimposed on each lateral side of the signal generation pad (3) and supply the enzyme substrate to evoke horizontal flow (right). This consequently generates a signal (e.g., color) from the enzyme tracer bound onto the pad.

cal arrangement as an initial step. At the time of analysis, the bottom of the immuno-strip is immersed in an analyte-containing specimen to absorb and transfer the medium along the strip via lateral flow. After the antigen-antibody reactions are completed, the two horizontally-arranged pads are positioned at each lateral side of the signal generation pad, respectively, and the enzyme substrate solution is added to the supply pad in order to initiate the horizontal flow (Figure 12, right). As the flow passes through the signal generation pad, the enzyme reaction on the signal generation pad is triggered, producing a signal proportionate to the analyte concentration.

Using the concept of cross-flow chromatography, an ELISA-on-a-chip (EOC), a POCT version of ELISA, has been constructed to achieve a semi-automatic shifting of the sequential reaction steps[38]. The chip, which is constructed of plastic, was fabricated via the mechanical etching of the surfaces, to offer two distinct flow channels in both the vertical and horizontal directions (Figure 13, left). The immuno-strip harboring an enzyme tracer (e.g., horseradish peroxidase) was set into the vertical compartment, and the enzyme substrate supply channel and absorption pad were arranged horizontally. In order to analyze a sample using the EOC, medium was added for transfer along the strip via capillary action, and a solution harboring an enzyme substrate was then supplied across the signal generation pad (Figure 13, right). A color signal at the antibody immobilization site was generated in proportion to the analyte concentration, and a control was also used to monitor the consistency of the assay on the same signal generation pad. The color signal was finally quantified with a digital camera based on image capture, and the color density of the image was digitized in the vertical direction using software.

The EOC system has been applied to a variety of detection fields including medical diagnostics (Hepatitis B virus infection[37] and acute myocardial infarction[38]), biodefense (botulinum neurotoxin A[77] and *Bacillus anthraces*), and food tests against microbial contamination. Typically, the dose responses of the sensor have been evaluated using standard samples of cardiac troponin I (cTnI), a commercially available product from Hytest, prepared with human serum (Figure 14, left). The signal measured via the integration of optical densities under peak value after normalization varied in a sigmoidal shape when plotted against the analyte concentration. From the calibration curve, the detection limit of the EOC sensor was determined to be 20 times lower than that obtained from the rapid test kit commercially available[44,45,78]. The novel EOC sensor system was evaluated further for correlation with a commercialized immunosensor, Biosite Triage, using fluorophores as a tracer (Figure 14, right). The samples were prepared blindly by spiking the analytes acquired from different sources, Hytest, Cliniqa, and Randox, with a human serum. The three groups of samples, when the EOC system was utilized, were overestimated relative to those acquired using the commercialized system, although the generated slopes were approximately linear. Such differences may result from different binding characteristics of antibody pairs to cTnI used in the respective system. It has been noted that the analytical performance of the EOC was well correlated with those of clinically-established equipment from Beckman Coulter Access[38].

As described herein, an EOC sensor has been developed and was found to be capable of detecting a minimum of 0.05 ng/mL cTnI as a model analyte, which is highly correlated with the performance of widely-accepted clinical equipment. The detection

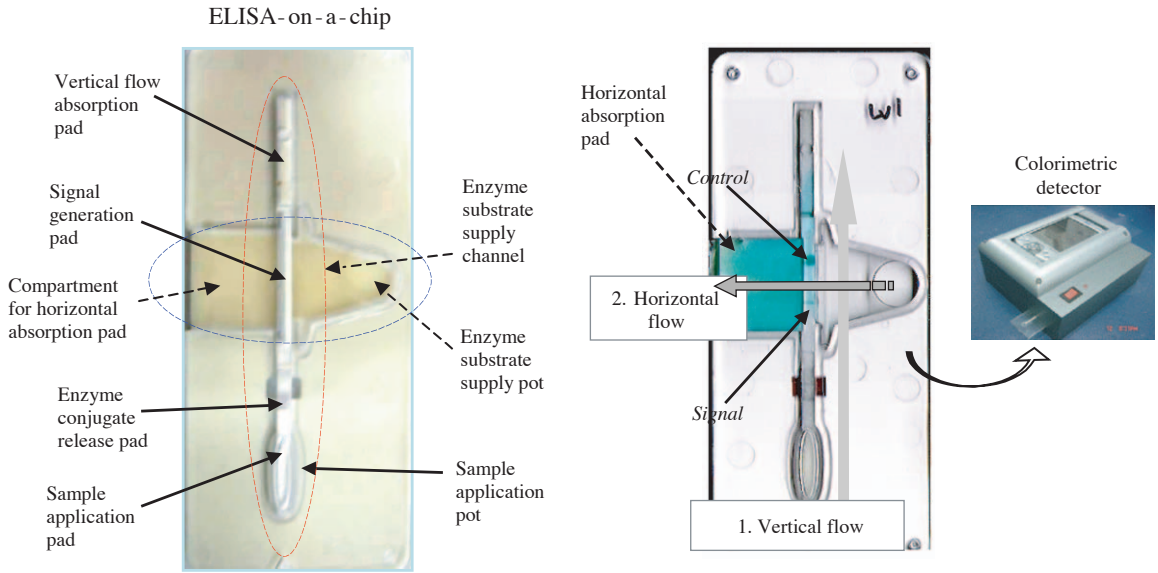


Figure 13. Construction of an ELISA-on-a-chip (EOC) adopting the concept of cross-flow chromatography (left) and its analytical procedures (right). The chip was fabricated by engraving fluidic channels on the surfaces of the plastic in order to hold the immuno-strip and to allow for the supply of the enzyme substrate solution. A sample containing analyte was first laterally eluted in the vertical direction and, after the completion of antigen-antibody bindings, the substrate solution for an enzyme used as tracer was flown in the horizontal direction. The color signal consequently produced was measured using a PDA-based colorimetric detector.

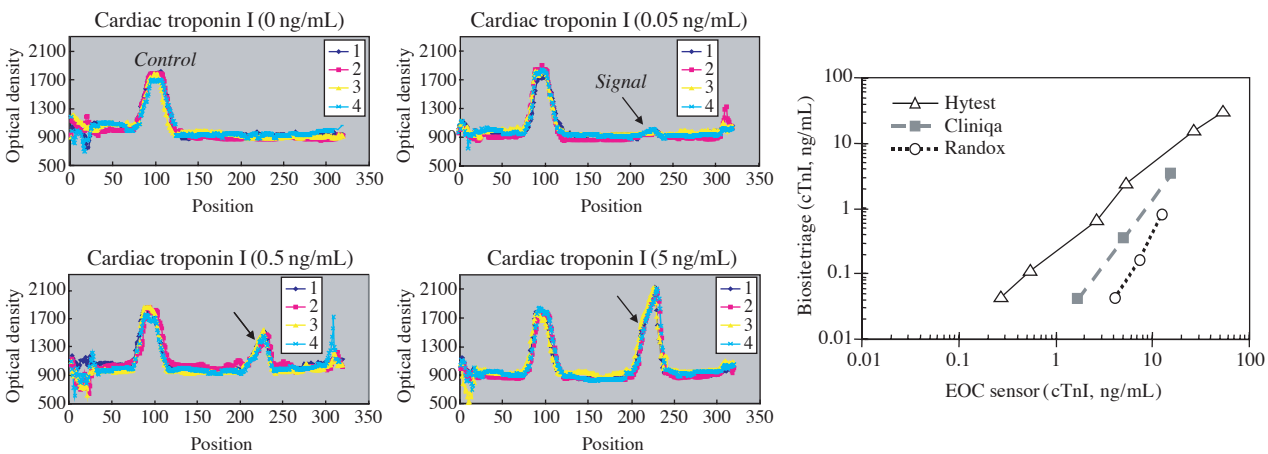


Figure 14. Dose responses of the EOC to an analyte, cardiac troponin I (cTnI) as a specific marker of acute myocardial infarction, (left) and its correlation with a commercialized immunosensor, Biosite Triage (right). To obtain the dose responses, the colored-signal images on the signal generation pads of the chips were scanned using software. The correlation of the EOC sensor system with the commercial one was tested using blindly prepared samples of cTnI from various sources, Hytest, Cliniqa, and Randox.

capability was found to be at least 20 times higher than that of a conventional colorimetric system employing colloidal gold as a tracer, and was also superior to that of the commercially available fluorometric sensor cTnI[79,80]. This feature of EOC may be attributed primarily to the low non-specific binding of the enzyme tracer, the high reproducibility analysis allowed for by the optimal EOC design, and the fact that the fabrication processes optimally realizing the concept of cross-flow immuno-chromatography. The novel immunosensor provides several additional advantages, namely, the generation of variable signal

types that can be measured on the basis of, for example, colorimetry, chemiluminometry, and electrochemistry[38,79], depending on the enzyme-substrate pair employed. Moreover, the sensor can be miniaturized down to one-tenth scale, provided that the horizontal flow is finely controlled using micro-fluidic channels in place of a horizontal flow absorption pad.

Prospects of Immunosensor Development for POCT

As described above, we have evaluated several immuno-analytical systems in different versions of an assay kit and immunosensor, utilizing membrane strip chromatography for their application at points of care. Among the variety of tracers employed in the systems, the enzyme generated highly sensitive, highly reproducible assay results (e.g., 0.05 ng/mL cTnI, complex form from Hytest), provided that it was adopted in an EOC that realized the concept of cross-flow immuno-chromatography (Figure 15, the first column of the list). An immunosensor (a product of i-STAT; Abbott Park, IL, U.S.) using an identical tracer has been commercialized in a miniaturized form via micro-fluidic technology, which also evidenced comparable detection capability (Figure 15, second column). This, however, is capable of measuring a single analyte per assay in the product, probably as the result of limitations in the design of complex fluidic channels and the transducer arrangement. As the miniaturization of the POCT sensor is a trend in technology development in this field, we are scaling the EOC dimension down further, to one-tenth scale, via a top-down approach. To this end, the horizontal absorption pad contained in the EOC (see Figure 13) can first be replaced with micro-channels to control the enzyme substrate supply rate precisely. Such an approach would allow us to install an immuno-strip narrower than 1 mm or a substitute matrix directly impregnated into the plastic surfaces and, thus, to achieve a miniaturized EOC.

Besides enzyme tracers, fluorophores are the most extensively used signal generator for analyses in the biological and medical research fields, as well as for labeling of binders in biochip systems[11,80]. At least two commercialized immunosensor systems from Biosite (San Diego, CA, U.S.) and Response (Burnaby, Canada) are dedicated to the detection of biomarkers associated with disease diagnosis or biodefense (Figure 15, the third column). These biosensors confer a relatively high degree of sensitivity to the kit. However, they require a costly detector that utilizes a precision excitation/emission signal generation process. Thus, this system should evidence long-term durability under severe conditions, and also cannot be used for confirmative analysis without instrumentation. Finally, magnetic beads, which have previously

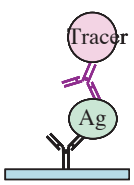
	Cross-flow Enzyme Immuno-chromatographic Sensor	Micro-fluidic Enzyme Immunosensor	Fluorescent Immuno-chromatographic Sensor	Magnetic Immuno-chromatographic Sensor
				
Tracer	Enzyme	Enzyme	Fluorophore	Magnetic beads
Detection limit of cTnI	High (0.05 ng/mL)	High (0.1 ng/mL)	High (0.05 ng/mL)	High (0.1 ng/mL)
Assay time	15-20 min	< 20 min	15 min	15 min
Advantages	Variable types of signal generation	Small sample size	Technology accumulation	Potential pre-treatment using magnetic separation
Disadvantages	Extra time for color signal	Single measurement per assay	Costly instrument No kit formulation	Relatively high cost

Figure 15. Comparison of immunosensor systems typically selected for POCT. They were compared with regard to their analytical performance predicated on the measurement of cardiac troponin I as a specific marker of acute myocardial infarction.

been employed for the separation of cells and proteins, have been recently applied to immunoassays as tracers that generate a highly sensitive signal, as a part of the work of Quantum Design (San Diego, CA, U.S.; Figure 15, the fourth column). The beads can potentially be employed for the pre-treatment of samples *in situ*, in addition to the generation of magnetic signal, but the fact that the system requires a relatively expensive detector may constitute a significant drawback.

In this review, immunosensors applicable to POCT have been introduced and characterized with regard to their analytical performance, in particular, detection capability and facility of quantification, using a simple detector. A major constituent of the sensor that controls these two properties at the same time would be the tracer employed for signal generation. Enzymes that have long been served as tracers for ELISA may become a new emerging substitute for colloidal gold, as its separate catalytic reaction for signal generation can now be conducted quite quickly and easily with a biochip. Fluorophores, magnetic beads, and ECL tracers are other alternative labels for immuno-analyses, because of their high conversion yields of antigen-antibody bindings to physically measurable signals. Colloidal gold, which is conventionally employed as a tracer, may still prove valuable provided that an additional enhancement step, such as silver intensification, is conducted. In a future study, we will examine this technique using the same cross-flow chromatography concept.

Acknowledgements

This study was supported by a grant from the Korea Research Foundation (Grant number: D00261) in 2005.

References

1. Obi, T.U. & Ojeh, C.K. Dot enzyme immunoassay for visual detection of peste-des-petits-ruminants virus antigen from infected caprine tissues. *J. Clin. Microbiol.* **27**, 2096-2099 (1989).
2. Nygren, H. & Stenberg, M. Diffusion-in-gel enzyme-linked immunosorbent assay (DIG-ELISA). Quantification of antigen by diffusion over an antibody-coated surface. *Scand. J. Clin. Lab. Invest.* **42**, 355-359 (1982).
3. Yolken, R.H. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. *Rev. Infect. Dis.* **4**, 35-68 (1982).
4. Emanuel, P.A., Takahash, H., Adler, K., Garlick, R.K. & Wands, J.R. Recombinant antibodies: a new reagent for biological agent detection. *Biosens Bioelectron* **14**, 751-759 (2000).
5. Malergue, F. & Chungue, E. Rapid and sensitive streptavidin-biotin amplified fluorogenic enzyme-linked immunosorbent-assay for direct detection and identification of dengue viral antigens in serum. *J. Med. Virol.* **47**, 43-47 (1995).
6. Maia, M., Takahashi, H., Adler, K., Garlick, R.K. & Wands, J.R. Development of a two-site immuno-PCR assay for hepatitis B surface antigen. *J. Virol. Methods* **52**, 273-286 (1995).
7. Liu, M.K. & Pearson, T.W. Detection of circulating trypanosomal antigens by double antibody ELISA using antibodies to procyclic trypanosomes. *Parasitology* **95**(Pt 2), 277-290 (1987).
8. Barbui, C., Motterlini, N. & Garattini, L. Health status, resource consumption, and costs of dysthymia. A multi-center two-year longitudinal study. *J. Affect. Disord.* **90**, 181-186 (2006).
9. Lowell, M. Consumer trends in self diagnosis, home testing and over the counter medication: implications for the registered nurse. *Kans. Nurse.* **70**, 1-2 (1995).
10. Daviaud, J. *et al.* Reliability and feasibility of pregnancy home-use tests: laboratory validation and diagnostic evaluation by 638 volunteers. *Clin. Chem.* **39**, 53-59 (1993).
11. Kim, Y.M., Oh, S.W., Jeong, S.Y., Pyo, D.J. & Choi, E.Y. Development of an ultrarapid one-step fluorescence immunochromatographic assay system for the quantification of microcystins. *Environ. Sci. Technol.* **37**, 1899-1904 (2003).
12. Sato, I. *et al.* Rapid detection of semenogelin by one-step immunochromatographic assay for semen identification. *J Immunol Methods* **287**, 137-145 (2004).
13. Paek, S.H., Lee, S.H., Cho, J.H. & Kim, Y.S. Development of rapid one-step immunochromatographic assay. *Methods* **22**, 53-60 (2000).
14. Takeda, T., Yamagata, K., Yoshida, Y., Yoshino, K. & Nomura, T. [Evaluation of immunochromatography-based rapid detection kit for fecal Escherichia coli O157]. *Kansenshogaku Zasshi* **72**, 834-839 (1998).
15. Kashiwagi, S. *et al.* [Evaluation of immunochromatography assay technique for detection of antibody to hepatitis B surface antigen (HBsAg)]. *Kansenshogaku Zasshi* **69**, 297-302 (1995).
16. Scalise, D. Poised for growth. Point-of-care testing. *Hosp. Health. Netw.* **80**, 77-83 (2006).
17. Eichner, S.F. & Timpe, E.M. Urinary-based ovulation and pregnancy: point-of-care testing. *Ann. Pharmacother* **38**, 325-331 (2004).
18. Qiaojia, H. *et al.* Qualitative bedside assay of increased human serum myoglobin by sandwich dot-immunogold filtration for the diagnosis of acute myocardial infarction. *Clin. Chim. Acta.* **273**, 119-130 (1998).
19. Cho, J.H. & Paek, S.H. Semiquantitative, bar code

version of immunochromatographic assay system for human serum albumin as model analyte. *Biotechnol. Bioeng.* **75**, 725-732 (2001).
20. Fidanza, F., Gentile, M.G. & Porrini, M. A self-administered semiquantitative food-frequency questionnaire with optical reading and its concurrent validation. *Eur. J. Epidemiol.* **11**, 163-170 (1995).
21. Conrad, C.C. *et al.* Chemiluminescent standards for quantitative comparison of two-dimensional electrophoresis western blots. *Proteomics* **1**, 365-369 (2001).
22. Malek, A.M., Izumo, S. & Alper, S.L. Quantitative densitometric analysis using a commercially available handheld CCD digital camera. *Biotechniques* **22**, 1150-1153 (1997).
23. Shamsuzzaman, A.K., Akhter, S., Shamsuzzaman, S.M. & Siddique, A. Comparison between ELISA and ICT- MycoDot in adult pulmonary tuberculosis. *Mymensingh Med. J.* **15**, 33-39 (2006).
24. Oles, K.S., Penry, J.K. & Dyer, R.D. Evaluation of an enzyme immunochromatography method for carbamazepine: a comparison with enzyme-multiplied immunoassay technique, fluorescence polarization immunoassay, and high-performance liquid chromatography. *Ther. Drug. Monit.* **11**, 471-476 (1989).
25. Askari, M., Alarie, J.P., Moreno-Bondi, M. & Vo-Dinh, T. Application of an antibody biochip for p53 detection and cancer diagnosis. *Biotechnol. Prog.* **17**, 543-552 (2001).
26. Sun, B. *et al.* Microminiaturized immunoassays using quantum dots as fluorescent label by laser confocal scanning fluorescence detection. *J. Immunol. Methods* **249**, 85-89 (2001).
27. Mendoza, L.G. *et al.* High-throughput microarray-based enzyme-linked immunosorbent assay (ELISA). *Biotechniques* **27**, 778-780, 782-776, 788 (1999).
28. Peruski, A.H., Johnson, L.H. & Peruski, L.F., Jr. Rapid and sensitive detection of biological warfare agents using time-resolved fluorescence assays. *J. Immunol. Methods* **263**, 35-41 (2002).
29. Ogert, R.A., Brown, J.E., Singh, B.R., Shriver-Lake, L.C. & Ligler, F.S. Detection of Clostridium botulinum toxin A using a fiber optic-based biosensor. *Anal. Biochem.* **205**, 306-312 (1992).
30. Wynick, D. & Bloom, S.R. Magnetic bead separation of anterior pituitary cells. *Neuroendocrinology* **52**, 560-565 (1990).
31. Ossendorp, F.A., Bruning, P.F., Van den Brink, J.A. & De Boer, M. Efficient selection of high-affinity B cell hybridomas using antigen-coated magnetic beads. *J Immunol Methods* **120**, 191-200 (1989).
32. Aytur, T. *et al.* A novel magnetic bead bioassay platform using a microchip-based sensor for infectious disease diagnosis. *J. Immunol. Methods* **314**, 21-29 (2006).
33. Fan, A., Lau, C. & Lu, J. Magnetic bead-based chemiluminescent metal immunoassay with a colloidal gold label. *Anal. Chem.* **77**, 3238-3242 (2005).
34. Yoon, C.H. *et al.* Development of a membrane strip immunosensor utilizing ruthenium as an electro-chemiluminescent signal generator. *Biosens. Bioelectron* **19**, 289-296 (2003).
35. Semlitsch, G., Buchinger, W., Reiterer, E., Binter, G. & Rainer, F. [Measurement of free triiodothyronine (FT3) using an electrochemiluminescence immunoassay in patients with autoantibodies to triiodothyronine]. *Acta. Med. Austriaca.* **27**, 54-55 (2000).
36. Sano, M. & Tatsumi, N. [Electro chemiluminescence immunoassay]. *Rinsho. Byori.* **44**, 1076-1079 (1996).
37. Cho, J.H., Paek, E.H., Cho, I.H. & Paek, S.H. An enzyme immunoanalytical system based on sequential cross-flow chromatography. *Anal. Chem.* **77**, 4091-4097 (2005).
38. Cho, J.H., Han, S.M., Paek, E.H., Cho, I.H. & Paek, S.H. Plastic ELISA-on-a-chip based on sequential cross-flow chromatography. *Anal. Chem.* **78**, 793-800 (2006).
39. Bhattacharyya, A. & Klapperich, C.M. Design and testing of a disposable microfluidic chemiluminescent immunoassay for disease biomarkers in human serum samples. *Biomed. Microdevices* (2006).
40. Chiao, D.J., Shyu, R.H., Hu, C.S., Chiang, H.Y. & Tang, S.S. Colloidal gold-based immunochromatographic assay for detection of botulinum neurotoxin type B. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.* **809**, 37-41 (2004).
41. Biagini, R.E. *et al.* Rapid, sensitive, and specific lateral-flow immunochromatographic device to measure anti-anthrax protective antigen immunoglobulin g in serum and whole blood. *Clin. Vaccine. Immunol.* **13**, 541-546 (2006).
42. Zhang, G.P. *et al.* Development of an immunochromatographic lateral flow test strip for detection of beta-adrenergic agonist Clenbuterol residues. *J. Immunol. Methods* **312**, 27-33 (2006).
43. Watanabe, T. *et al.* Development of a simple whole blood panel test for detection of human heart-type fatty acid-binding protein. *Clin. Biochem.* **34**, 257-263 (2001).
44. Alhashemi, J.A. Diagnostic accuracy of a bedside qualitative immunochromatographic test for acute myocardial infarction. *Am. J. Emerg. Med.* **24**, 149-155 (2006).
45. Pagani, F., Serena, C., Bosio, C., Cuccia, C. & Panteghini, M. Evaluation of a rapid bedside immunochromatographic assay for detection of cardiac troponin I in whole blood. *Clin. Chem. Lab. Med.* **39**, 458-459 (2001).
46. Gaylor, D.W., Lutz, W.K. & Conolly, R.B. Statistical analysis of nonmonotonic dose-response relationships: research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. *Toxicol. Sci.* **77**, 158-164 (2004).
47. Pfab, T. *et al.* Rapid immunochromatographic strip test for the detection of albuminuria and brief literature review on albuminuria screening. *Eur. J. Med. Res.* **11**, 3-6 (2006).

48. Oberpenning, F. *et al*. Semi-quantitative immunochromatographic test for prostate specific antigen in whole blood: tossing the coin to predict prostate cancer? *Eur. Urol*. **43**, 478-484 (2003).
49. Wu, S.C. & Wong, S.L. Engineering soluble monomeric streptavidin with reversible biotin binding capability. *J. Biol. Chem*. **280**, 23225-23231 (2005).
50. Walker, B. *et al*. The synthesis, kinetic characterization and application of a novel biotinylated affinity label for cathepsin B. *Biochem. J*. **283(Pt 2)**, 449-453 (1992).
51. Choi, S. *et al*. A rapid, simple measurement of human albumin in whole blood using a fluorescence immunoassay (I). *Clin. Chim. Acta*. **339**, 147-156 (2004).
52. Renard, J. & Vidal-Madjar, C. Kinetic study of the adsorption of human serum albumin on immobilized antibody using the split-peak effect in immunochromatography. *J. Chromatogr. A*. **661**, 35-42 (1994).
53. Pegoraro, A.A. *et al*. Evaluation of a new fluorescent dye method to measure urinary albumin in lieu of urinary total protein. *Am. J. Kidney. Dis*. **35**, 739-744 (2000).
54. Kessler, M.A., Meinitzer, A., Petek, W. & Wolfbeis, O.S. Microalbuminuria and borderline-increased albumin excretion determined with a centrifugal analyzer and the Albumin Blue 580 fluorescence assay. *Clin. Chem*. **43**, 996-1002 (1997).
55. Parikh, C.R., Fischer, M.J., Estacio, R. & Schrier, R.W. Rapid microalbuminuria screening in type 2 diabetes mellitus: simplified approach with Micral test strips and specific gravity. *Nephrol. Dial. Transplant*. **19**, 1881-1885 (2004).
56. Leong, S.O., Lui, K.F., Ng, W.Y. & Thai, A.C. The use of semi-quantitative urine test-strip (Micral Test) for microalbuminuria screening in patients with diabetes mellitus. *Singapore Med. J*. **39**, 101-103 (1998).
57. Osawa, T. *et al*. The advantage of the osmium conductive metal coating for the detection of the colloidal gold-conjugated antibody by SEM. *J. Electron. Microsc* (*Tokyo*). **48**, 665-669 (1999).
58. Walther, P. *et al*. Detection of protein A gold 15 nm marked surface antigens by backscattered electrons. *Scan. Electron. Microsc*. 1257-1266 (1984).
59. Tomcik, P. & Bustin, D. Voltammetric determination of iodide by use of an interdigitated microelectrode array. *Fresenius J. Anal. Chem*. **371**, 562-564 (2001).
60. Niwa, O., Xu, Y., Halsall, H.B. & Heineman, W.R. Small-volume voltammetric detection of 4-aminophenol with interdigitated array electrodes and its application to electrochemical enzyme immunoassay. *Anal. Chem*. **65**, 1559-1563 (1993).
61. Susmel, S., Guilbault, G.G. & O'Sullivan, C.K. Demonstration of labeless detection of food pathogens using electrochemical redox probe and screen printed gold electrodes. *Biosens. Bioelectron*. **18**, 881-889 (2003).
62. Wang, J., Pamidi, P.V. & Rogers, K.R. Sol-gel-derived thick-film amperometric immunosensors. *Anal. Chem*. **70**, 1171-1175 (1998).
63. Rinaldi, R. *et al*. A protein-based three terminal electronic device. *Ann. N. Y. Acad. Sci*. **1006**, 187-197 (2003).
64. Fink, H.W. & Schonenberger, C. Electrical conduction through DNA molecules. *Nature*. **398**, 407-410 (1999).
65. Tseng, R.J., Huang, J., Ouyang, J., Kaner, R.B. & Yang, Y. Polyaniline nanofiber/gold nanoparticle nonvolatile memory. *Nano. Lett*. **5**, 1077-1080 (2005).
66. Kim, J.H. *et al*. Conductimetric membrane strip immunosensor with polyaniline-bound gold colloids as signal generator. *Biosens. Bioelectron*. **14**, 907-915 (2000).
67. Carell, T., Behrens, C. & Gierlich, J. Electrontransfer through DNA and metal-containing DNA. *Org. Biomol. Chem*. **1**, 2221-2228 (2003).
68. Yoo, K.H. *et al*. Electrical conduction through poly (dA)-poly (dT) and poly (dG)-poly (dC) DNA molecules. *Phys. Rev. Lett*. **87**, 198102 (2001).
69. Tenelsen, K. & Schreiber, M. Low-temperature many-electron hopping conductivity in the Coulomb glass. Physical Review. B. *Condensed Matter*. **52**, 13287-13293 (1995).
70. Yagiuda, K. *et al*. Development of a conductivity-based immunosensor for sensitive detection of methamphetamine (stimulant drug) in human urine. *Biosens. Bioelectron* **11**, 703-707 (1996).
71. Zhan, W., Alvarez, J. & Crooks, R.M. A two-channel microfluidic sensor that uses anodic electrogenerated chemiluminescence as a photonic reporter of cathodic redox reactions. *Anal. Chem*. **75**, 313-318 (2003).
72. Pantano, P. & Kuhr, W.G. Characterization of the chemical architecture of carbon-fiber microelectrodes. 2. Correlation of carboxylate distribution with electron-transfer properties. *Anal Chem* **65**, 2452-2458 (1993).
73. Wu, X.Z., Lingyue, M. & Akiyama, K. Chemiluminescence study of active oxygen species produced by TiO_2 photocatalytic reaction. *Luminescence* **20**, 36-40 (2005).
74. Huang, X.J., Pu, Q.S. & Fang, Z.L. Capillary electrophoresis system with flow injection sample introduction and chemiluminescence detection on a chip platform. *Analyst*. **126**, 281-284 (2001).
75. Kim, N.H., Park, H.M., Chung, S.Y., Go, E.J. & Lee, H.J. Immunoliposomes carrying plasmid DNA: preparation and characterization. *Arch. Pharm. Res*. **27**, 1263-1269 (2004).
76. Zelphati, O. *et al*. Stable and monodisperse lipoplex formulations for gene delivery. *Gene. Ther*. **5**, 1272-1282 (1998).
77. Han, S.M. *et al*. Plastic enzyme-linked immunosorbent assays (ELISA)-on-a-chip biosensor for botulinum neurotoxin A. *Analytica Chimica Acta* In press.
78. Mainet-Gonzalez, D. *et al*. Preliminary evaluation of a rapid, qualitative one step immunoassay of cardiac

Troponin I in the acute myocardial infarction diagnosis. *Invest. Clin.* **45**, 221-242 (2004).
79. Peetz, D. *et al.* Method comparison of cardiac marker assays on PATHFAST, StratusCS, AxSYM, Immulite 2000, triage, elecsys and cardiac reader. *Clin. Lab.* **52**, 605-614 (2006).
80. Apple, F.S. *et al.* Simultaneous rapid measurement of whole blood myoglobin, creatine kinase MB, and cardiac troponin I by the triage cardiac panel for detection of myocardial infarction. *Clin. Chem.* **45**, 199-205 (1999).