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An automated ELISA system using a pipette tip as a solid phase and a pH-sensitive field effect transistor as a detector

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Abstract

A fully automated ELISA system was constructed using a pipette tip as a solid phase, urease as a detecting enzyme, and a pH-FET as a detector of urease activity. The inner wall of the end part of a pipette tip was used as a solid phase, and the urease activity of the conjugate, captured after a two-step immunoreaction, was measured by coupling the pipette tip with the pH-FET in a pH-measuring cell. Full automation of the ELISA system was achieved by using a disposable reagent cartridge and three pipetters for all mechanical operations, including sample dilution and B/F separation. This system can treat 60 samples per hour with an assay time of 21 min for all assay configurations. The system was applied to two-step sandwich assays for AFP, CEA, HBsAg, and HBsAb, a two-step competition assay for HBcAb, and a second antibody assay for HTLV-I Ab.

Keywords: Ion-sensitive field effect transistor; ELISA; Urease

1. Introduction

Wide variations of automated ELISA systems have been developed, aiming for higher detection limit and shorter assay time (Gosling, 1990). Solid phases of ELISA systems have changed from low-surface area, such as tubes, beads, or microtiter plates to high-surface area, such as microparticles, magnetized microparticles, or porous membranes (Forrest and Rattle, 1983). Detectors of enzyme activity have also changed from colorimeter and fluorometer to luminometer (Weeks and Woodhead, 1984; Kricka and Thorpe, 1981;

Abbreviations: ELISA, enzyme-linked immunosorbent assay; pH-FET, pH-sensitive field effect transistor; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; HBsAg, hepatitis B virus surface antigen; HBsAb, antibody to HBsAg; HBcAb, antibody to hepatitis B virus core antigen; HTLV-I Ab, antibody to human T cell leukemia virus type I; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetate; PBE, PBS+1 mmol/l EDTA; DTT, dithiothreitol; EMCS, *N*-(ω-maleimidocaproyloxy)-succinimide; BSA, bovine serum albumin.

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Thorpe et al., 1989). These are all optical systems.

On the other hand, only limited efforts have been made to improve the detection limit and assay time of ELISA systems with electrochemical detectors. Meyerhoff and Rechnitz reported an EIA system based on an ammonia gas sensing electrode using urease conjugate (Meyerhoff and Rechnitz, 1980). They adopted a competition assay using polymerized second antibody. The assay time was 1 h, 2 h, and 4 min for the first and second immunoreactions, and for the measurement of urease activity, respectively. A typical detection limit was 10 μg/l for BSA. In 1987, Briggs has reported on a unique ELISA system based on a pH-sensitive light-addressable potentiometric sensor and urease conjugate (Briggs, 1987). This system has been developed further for detecting total DNA (Kung et al., 1990). Since this sensor is of a planar type, they adopted a porous membrane as a solid phase. By placing the membrane close to the silicon sensor, they could measure the urease activity in 1.5 min, and attain the detection limit of 2 ng/l for single-stranded calf thymus DNA. However the immunoreaction and B/F separation were carried out manually, spending 1 h.

In order to improve the detection limit and the assay time of ELISA systems based on potentiometric electrodes, it would be essential to shorten the distance between the wall of the solid phase and the electrode. Close placing of the membrane solid phase and the light-addressable sensor, mentioned above is considered to be an example along this direction.

The pH-FET, invented by Bergveld in 1970 (Bergveld, 1970), was commercialized in 1983 first for the medical monitoring in vivo, making use of its small size (Kohama et al., 1984). This pH-FET has a pH-sensitive gate region on the end part of one side of the chip, which has dimensions of 0.15 mm thickness, 0.45 mm width, and 5.5 mm length (Nakamura et al., 1987). This chip is thin enough to be inserted into a capillary with the inner diameter of 0.55 mm. Paying attention to this point, we undertook to develop a fully automated ELISA system using the inner wall of a pipette tip as a solid phase, urease as a conjugated enzyme, and the pH-FET as the detector of urease activity. This work is considered to be another attempt, following to the work by Briggs mentioned above, to improve the detection limit and assay time of a potentiometric ELISA system. In addition, full automation was also accomplished in the present system, using a reagent cartridge and three pipetters.

Chandler and his coworkers have carried out an extensive work on a capillary enzyme immunoassay, using a glass capillary as a solid phase and urease as a detecting enzyme (Chandler and Hurrell, 1982; Chandler et al., 1983; Healey et al., 1983). However, it was a semiquantitative assay, because they adopted a visual detection of urease activity using bromocresol purple as a pH indicator.

In the present paper, the basic structure of the automated ELISA system based on a pH-FET is described, and assay performances of two-step sandwich assays for AFP, CEA, HBsAg, and HBsAb, a two-step competition assay for HBcAb, and a second antibody assay for HTLV-I Ab are described.

2. Materials and methods

2.1. Apparatus

The basic structure of the present ELISA system is schematically illustrated in Fig. 1. This system is composed of four parts: a pipetter part, an incubator part, an overflow cell part, and a pumping part. The pipetter part is composed of three pipetters. The first pipetter is used for sample dilution and for starting the first immunoreaction. The second one is for washing the tip after the first immunoreaction and for starting the second immunoreaction. The third one is for washing the tip after the second immunoreaction and for measuring urease activity. Each pipetter moves in the xz plane shown by the dotted lines in Fig. 1. An incubator part is composed of a heating block to keep the reagent cartridges at $37 \pm 0.2°$ C, and a cartridge mover to push the reagent cartridge forward once every 1 min, although only an overview of reagent cartridges is schematically illustrated in Fig. 1.

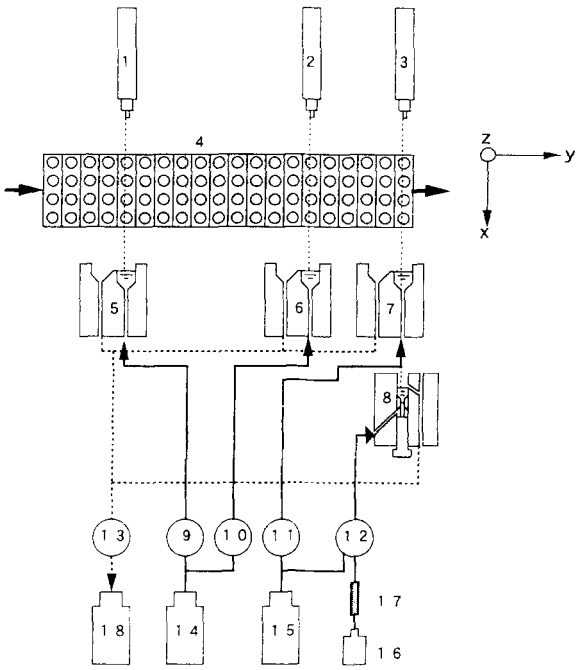

Fig. 1. Basic structure of the present ELISA system: 1, 2, and 3, two-dimensional pipetters; 4, reagent cartridge; 5 and 6, PBS solution cells; 7, ammonium chloride solution cell; 8, pH-measuring cell; 9–12, feeding pumps; 13, wasting pump; 14, 100 mM PBS (pH 7.8); 15, 10 mM ammonium chloride + 154 mM sodium chloride + 0.01% chloramphenycol; 16, 1 M urea; 17, ion exchange column; 18, wasted solution.

The overflow cell part is composed of two PBS solution cells, an ammonium chloride solution cell, and a pH-measuring cell. All of these cells are also kept at $37 \pm 0.2°$ C. The pumping part is composed of five pumps to feed PBS solution (100 mmol/l PBS, pH 7.8), ammonium chloride solution (11.8 mmol/l ammonium chloride + 182 mmol/l sodium chloride + 0.012% chloramphenicol), and urea solution (1 mol/l urea). Urease substrate solution (155 mmol/l urea + 10 mmol/l ammonium chloride + 154 mmol/l sodium chloride + 0.01% chloramphenicol) is prepared by mixing the ammonium chloride solution and the urea solution at the ratio of 84.5:15.5 by pump 12 in Fig. 1, before introducing it into the pH-measuring cell. The urea solution before mixing is treated by an ion exchange column AG501-X8(D) (Bio-Rad Laboratories, Richmond, CA) to remove ammonium and bicarbonate ions produced by spontaneous decomposition of urea, which are anticipated to cause the change of baseline pH and buffering ability of the substrate solution. Operations of the pipetter part, the cartridge mover, and the pumping part are controlled by a computer.

2.2. pH-measuring cell

A cross-sectional view of the pH-measuring cell, and the sensor coupler region of the cell are shown in Figs. 2 and 3, respectively. The cell body is made of titanium, which has a good heat conductivity and whose ion has no poisonous effect to urease. The pH-sensor body is introduced from the bottom of the cell, and fixed. The pH-FET is the same as that reported previously (Nakamura et al., 1987). A pH-sensitive gate region (9 in Fig. 3) is located at one side and at about 0.5 mm

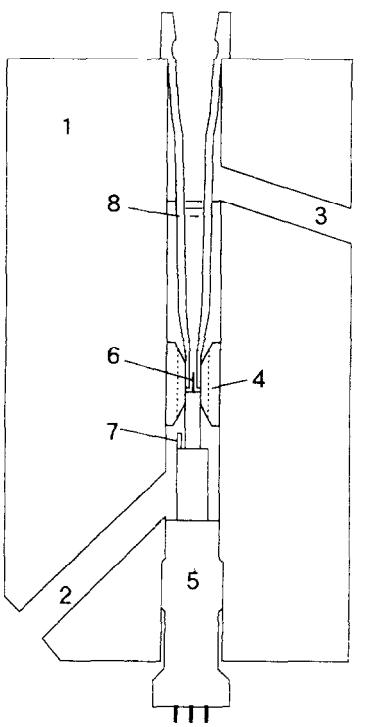

Fig. 2. A cross-sectional view of the pH-measuring cell: 1, cell body; 2, entrance of substrate solution; 3, exit of substrate solution; 4, sensor coupler; 5, sensor body; 6, pH-FET; 7, reference electrode; 8, pipette tip.

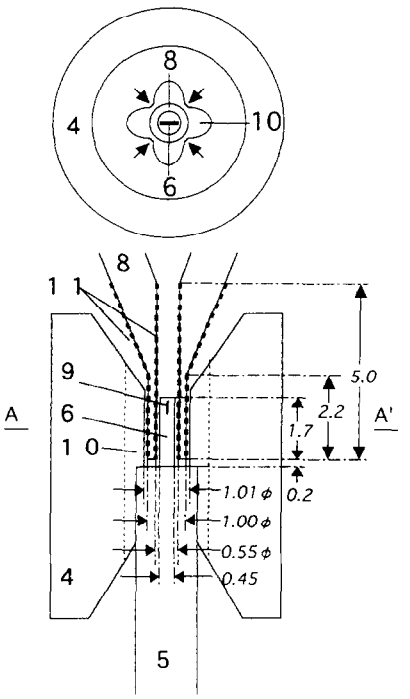

Fig. 3. Top (top) and side (bottom) views of the sensor coupler region of the pH-measuring cell: 4, sensor coupler; 5, sensor body; 6, pH-FET; 8, the end part of pipette tip; 9, gate (pH-sensitive) region; 10, side-flow path for substrate solution; 11, the end part of a pipette tip coated with capturing antibody or antigen layer. Numbers with slanted letters indicate the dimensions in mm. The space between the inner wall of sensor coupler (1.01 mm diam.) and the outer diameter of the end part of the tip (1.00 mm diam.) is exaggerated with respect to the real dimension. Four arrows in the top view indicate the inner edges of the sensor coupler where heavy contamination with conjugate takes place.

from the end of the pH-FET chip. A device called sensor coupler (4 in Fig. 2) is attached at the top of the sensor body. This device has the purpose to guide the pipette tip coming down from the upper mouth of the cell, so that 1.7 mm length of the pH-FET can be placed at the center inside the end part of the pipette tip, as shown in Fig. 3. The sensor coupler has four side-flow paths to equalize the pressure difference between the upper and the lower parts of the cell, caused by insertion of the pipette tip. Also this side flow paths, along with a 0.2 mm space between the end of the pipette tip and the top of sensor body ensures the liquid junction between the gate of the pH-FET and a reference electrode (7 in Fig. 2).

2.3. Solid-phase pipette tip

The solid-phase pipette tip, moulded by polypropylene, has the shape shown by 8 in Fig. 2. The total length and total inner volume are 48 mm and 200 μl, respectively. Tips were washed in isopropanol before use. The capturing antibodies, antigens, or synthetic peptide, listed in Table 1, were immobilized on the inner and outer surfaces of 5 mm length of the end part of a tip (11 in Fig. 3), by immersing the end part of a tip into the PBS solution (pH 7.4) of each capturing material, for 2 h at 37° C. Concentrations of capturing materials were 50, 200, 50, and 20 mg/l for anti-AFP Ab, anti-CEA Ab, anti-HBsAb, and

Table 1
The list of capturing and detecting antibodies or antigens used for the present system

Analyte	Capturing Ab or Ag	Detecting Ab or Ag
AFP	Anti-AFP poAb [a] (A008, Dako, CA)	Anti-AFP moAb [b] (NB-011, NBL, Tokyo, Japan)
CEA	Anti-CEA moAb (02-1E7-94, Scrips Lab, CA)	Anti-CEA poAb (A115, Dako)
HBsAg	Anti-HBs poAb (B560, Dako)	Anti-HBs moAb (SB 23-06, Scantibodies, CA)
HBsAb	HBsAg (adw, Scrips Lab)	HBsAg (adr, Meiji, Tokyo, Japan)
HBcAb	HBcAg (B-0825, Biogenesis, UK)/anti-HBc poAb (B586, Dako)	IgG fraction of anti-HBc positive human sera
HTLV-I Ab	Synthetic peptide (p19-100c + gp46-175B)-AGP [c]	Anti-human IgG poAb (American Qualex, CA)

[a] poAb, polyclonal antibody.
[b] moAb, monoclonal antibody.
[c] p19-100C:KKPPPPSSPTHDPPDSDPQIPPPVVEPTAPQVL; gp46-175B:KKFLNTEPSQLPPTAPPLLPHSNLDHI.

HBsAg, respectively. The tip for the assay of HBcAb was prepared by an indirect immobilization of HBcAg; 50 mg/l HBcAb in PBS was immobilized first, followed by a treatment of the tip with 1 mg/l HBcAg in PBS. For the assay of HTLV-I Ab, a mixture of synthetic peptides, p19-100C and gp40-175b, was used as a capturing material. These are epitopes of HTLV-I Abs (Kuroda et al., 1990). Since direct immobilization of these peptides on a pipette tip were difficult, their conjugates with α1-acid glycoprotein were coated. The conjugates were prepared, by coupling 2 mg of each peptide with 0.4 mg of α1-acid glycoprotein (Sigma, St. Louis, MO) using 1 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in 1 ml of PBS for 5 h at 4° C. After stopping the coupling reaction with 25 μl of acetic acid, the reaction mixture was dialysed with PBS for 2 days at 4° C. Immobilization of these conjugates were performed at 37° C for 2 h using a PBS solution containing 10 mg/l of each conjugate.

Tips coated with capturing material were washed and blocked with the blocking solution containing 10% sucrose and 1% BSA in PBS (pH 7.4) for 16 h at 4° C. The tips after blocking were drained by centrifuging 90 s at 2000 rpm, followed by vacuum drying at room temperature for 1 h, and stored at 4° C in heat-sealed polyethylene sheet.

2.4. Urease conjugates

The urease conjugates with detecting antibodies were prepared using antibodies listed in Table 1. In each case, 5 mg of the antibody was dissolved in 2.5 ml of 0.1 mol/l PBE (pH 7.0). 10 g/l of EMCS in Dimethylformamide was added into the antibody solution so that the molar ratio of EMCS to antibody was 25. After 30 min reaction at room temperature, unreacted EMCS was removed with Sephadex G-25M (Pharmacia P-L Biochemicals, Milwaukee, WI). 20 mg of urease dissolved in 2 ml of PBE (pH 6.5) was treated by 0.2 ml of 100 mmol/l DTT for 30 min at room temperature, followed by a treatment with Sephadex G-25M column to remove unreacted DTT. Into all of the maleimidized antibody solution, DTT-treated urease were mixed at 1:1 molar ratio, for 18 h at 4° C. The coupling reaction was stopped by adding 1/10 volume of 200 mmol/l mercaptoethanol. The reaction mixture was concentrated into 1 ml by ultrafiltration and purified by Sephacryl S400 H (Pharmacia P-L Biochemicals, Milwaukee, Wisconsin) with the elution buffer being 20 mmol/l phosphate buffer (pH 7.4) containing 0.5 mol/l NaCl, 1 mmol/l EDTA, and 0.05% sodium azide. Collected fractions with high conjugate activity were pooled and diluted with 0.01 mol/l phosphate buffer containing 0.01 mol/l NaCl, 5% BSA, 2% sucrose, 1 mmol/l DTT, 1 mmol/l EDTA, 30% normal goat serum, 3% normal rabbit serum, and 3% normal mouse serum.

In the case of HBsAb assay, HBsAg was conjugated with urease through avidin-biotin coupling. Biotin labeling of HBsAg was carried out by reacting 1 g/l HBsAg (subtype adr, Meiji Nyugyo, Tokyo, Japan) with 14.6 mmol/l NHS-biotin (Wako Pure Chemicals, Osaka, Japan) for 4 h at room temperature, followed by the removal of free NHS-biotin with Sephadex G-25M column. Conjugation of urease with avidin was performed by reacting DTT-reduced urease with maleimidized Streptavidin (Boehringer Mannheim, Mannheim, Germany), followed by purification with a gel filtration.

2.5. Reagent cartridge

In order to automate the present ELISA system, a reagent cartridge, shown in Fig. 4 was designed, and moulded with polypropylene. The cartridge has four cups (A, B, C, and D) and a stick board for a bar-cord label. In cup A, sample serum is fed by users. Cup B and C are for sample dilution and the first immunoreaction, respectively. Cup C contains the solid-phase pipette tip. Cup D is for the second immunoreaction, and freeze-dried conjugate is deposited at the bottom of the cup from 10 μl of urease conjugate solution.

2.6. Assay procedure

The assay process of the present system is composed of five steps: (1) pre-heating of the

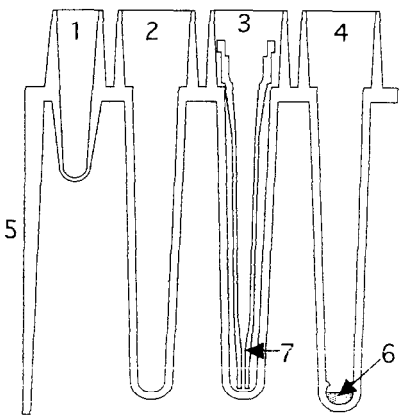

Fig. 4. The reagent cartridge for the present ELISA system: 1, cup A for serum sample; 2, cup B for serum dilution; 3, cup C for the 1st immunoreaction; 4, cup D for the 2nd immunoreaction and also as a container of freeze-dried conjugate; 5, stick board for a bar-cord label; 6, freeze-dried conjugate; 7, pipette tip as a solid phase.

reagent cartridge (5 min), (2) first immunoreaction (10 min), (3) second immunoreaction (5 min), (4) measurement of urease activity, and (5) data treatment (1 min for (4) and (5)). All configurations (two-step sandwich for AFP, CEA, HBsAg, and HBsAb, two-step competition for HBcAb, and second-antibody configuration for HTLV-I Ab) are assayed with the same time schedule.

(1) Preheating of the reagent cartridge. The reagent cartridge with 50 μl of sample or standard serum in cup A is warmed from room temperature to 37° C.

(2) First immunoreaction. The solid-phase tip in cup C is held by the first pipetter probe, and 35 μl of serum in cup A is transferred to cup C. When dilution is necessary, desired amount of solution is also pipetted from PBS solution cell into cup C, and mixed well by repeating suction and emission of the solution for five times. The first immunoreaction is carried out by dropping the pipette tip into cup C, with its end part dipped in the serum at 37° C.

(3) Second immunoreaction. The solid-phase tip after the first immunoreaction is held by the second pipetter probe, and after emitting the serum into cup C, the tip is washed in the PBS solution cell by repeating suction and emission of the solution for 18 times. Then 30 μl of PBS solution is transferred into cup D with washed tip to dissolve freeze-dried conjugate. Dissolved conjugate is mixed well by repeating suction and emission of the solution for 10 times. Then the tip is dropped into cup D to carry out the second immunoreaction at 37° C.

(4) pH measurement. After the second immunoreaction the tip is held by the third pipetter probe, and after emitting the conjugate solution into cup D, it is washed in the ammonium chloride solution cell by four cycles of suction and emission. The washed tip is introduced into the pH-measuring cell in order to couple it with the pH-FET. At the same time, feeding of the substrate solution is stopped, to read the pH change for 20 s. Then the tip is disposed into cup D, and the cell is washed completely by turning on the substrate pump for 40 s.

(5) Data treatment. The output (source potential) of the pH-FET was read and stored in CPU during the above mentioned 20 s at 0.1 s interval. The maximum changing rate of the source potential ($\Delta V/\Delta t$, mV/s) is calculated from these 200 data.

2.7. Samples and standard sera

Each of standard sera for six analytes was prepared by diluting each of the standard grade AFP (HA-1, Nippon Biotest Laboratories, Tokyo, Japan), CEA (Scripps Laboratories, San Diego, CA), HBsAg (subtype ad, Paul Ehrlich Institut, Berlin, Germany), pooled HBsAb, IgG fraction of HBc-positive sera, or THLV-I positive serum, with normal human serum (no. 1121 Nesco Bio, Tokyo, Japan). Sample sera for these items were generously granted by Kurashiki Central Hospital and Okayama Red Cross Blood Center. All analytes except for AFP and HTLV-I Ab were assayed without sample dilution. Sample sera for AFP were diluted on the system three times by PBS solution. In the case of HTLV-I Ab, which

Table 2
Assay precision of the present ELISA system

Analyte	Within-run		Between-run	
	Concentration (μg/l)	CV (%)	Concentration (μg/l)	CV (%)
AFP	10	7.5	20	7.9
	50	2.6	50	7.9
	100	5.6	100	6.9
CEA	2.5	10.2	2.5	9.3
	30	6.0	30	7.4
	50	3.8	50	2.5
HBsAg	0.25	10.3	0.25	9.4
	0.50	4.2	0.50	10.1
	1.00	3.6	1.00	9.0
HBsAb	PC	5.9	L	10.6
			H	6.4
HBcAb	PC	2.6	L	6.0
HTLV-I Ab	PC	7.4	L	8.7
			H	8.3

PC: positive control serum; L: low titer serum; H: high titer serum.

has a second antibody configuration using anti-human IgG conjugate, suppression of non-specific adsorption of human IgG on the solid phase was very difficult. Therefore sample sera which had been diluted five times with goat serum in advance, were fed for the ELISA system, and assayed without further dilution. In order to evaluate the degree of the carryover in the present system, solutions of AFP with 0 μg/l and 1 mg/l were assayed alternately.

3. Results

Table 2 shows the data of the within-run and between-run precision for six analytes. All within-run CVs are calculated from five replicates. All between-run CVs are calculated from five points, representing five individual analyses performed on 5 different days.

The correlation between the present and conventional systems was obtained using IMx system (Abbott Laboratories, IL) as a reference system, for all analytes except for HTLV-I Ab. For HTLV-I Ab, a microplate ELISA kit (K Assay HTLV-I, Medical and Biological Laboratories, Nagoya, Japan), which uses the same synthetic peptides as in the present system as the capturing material, was selected as a reference kit (Washitani et al., 1991). Obtained correlation equations and correlation coefficients for quantitative assays were as follows.

AFP ($n = 139$) $y = 1.030x - 9.5$ (μg/l) $r = 0.991$

CEA ($n = 100$) $y = 0.847x + 1.45$ (μg/l)

$r = 0.969$

Here, x and y are the values obtained for the IMx system, and the present system, respectively. For other (qualitative) assays, clinical sensitivity and specificity are shown in Table 3.

The detection limit of the present system was determined for AFP, CEA, and HBsAg. The detection limit was defined as the concentration corresponding to AV + 2 SD of signal intensities at 0 μg/l. Here AV and SD mean an average

Table 3
Sensitivity and specificity of the present ELISA system

	Sensitivity	Specificity
HBsAg	88/88 = 100%	98/101 = 97.0%
HBsAb	40/40 = 100%	50/50 = 100%
HBcAb	37/37 = 100%	60/62 = 96.8%
HTLV-I Ab	50/50 = 100%	49/50 = 98.0%

Table 4
Mean values (Av.) and standard deviations (SD) of signal intensities (mV/s) for AFP, CEA, and HBsAg at low concentration ($n = 5$ for all cases)

Concentration (μg/l)	AFP		CEA		HBsAg	
	Av.	SD	Av.	SD	Av.	SD
0	0.030	0.007	0.043	0.005	0.101	0.010
0.25	ND [a]	ND	ND	ND	0.174	0.012
0.50	0.048	0.005	0.130	0.013	0.227	0.006
1.00	0.073	0.006	0.201	0.012	0.348	0.030
2.00	0.110	0.005	0.289	0.015	ND	ND

[a] ND: not determined.

Table 5
Signal intensities for a continuous measurement of 0, 0, 1, 1, 0, and 0 mg/l AFP

Concentration of AFP (mg/l)	Signal intensity (mV/s)
0	0.036
0	0.045
1	16.996
1	15.039
0	0.039
0	0.038

value and a standard deviation (n = 5). Signal intensities at low concentration for these three analytes are shown in Table 4. AV + 2 SD at 0 μg/l were 0.044, 0.053, and 0.121 mV/s, and the detection limits determined from the data shown in Table 4 were 0.35, 0.09, and 0.13 μg/l for AFP, CEA, and HBsAg, respectively.

Table 5 shows signal intensities for the alternate measurements of 0 μg/l and 1 mg/l AFP, which was carried out to check the degree of carryover of the present system.

4. Discussion

To the author's knowledge, the present system is the first trial of full automation of an electrochemical ELISA system. The assay time of the present system is 21 min as the sum of 5, 10, 5, and 1 min for preheating of sample, 1st immunoreaction, 2nd immunoreaction, and pH measurement, respectively. The assay speed is 60 samples/h. Assay performance, such as within-run CVs, between-run CVs, detection limits, and correlation with the conventional ELISA kits are satisfactory for all of six analytes. These results demonstrate that electrochemical ELISA can also be automated with the comparable performance to optical systems. For example, the detection limit for CEA, 0.09 μg/l with the present system is comparable to or better than those reported for most advanced chemiluminescent ELISA systems (0.086 μg/l with Luminomaster LEIA 2000 (Sankyo, Tokyo, Japan), 0.5 μg/l with ACS 180 (Ciba Corning, USA), 0.06 μg/l with Lumipulse 1200 (Fujirebio, Tokyo, Japan), 0.3 μg/l with Amerlite System (Amersham) (Tsuji and Sugano, 1992).

High sensitivity and short assay time were attained by using a pipette tip with the inner diameter of 0.55 mm as the solid phase, and a pH-FET with 0.45 mm width and 0.15 mm thickness as a detector. Coupling of the tip and the pH-FET results in a short distance between the inner surface of the tip and the gate of pH-FET (0.2 mm). This in turn makes it possible to measure the pH change inside the tip in 20 s. A thinner tubular solid phase would also contribute to shorten the diffusion time of analyte molecules to the wall of the solid phase, shortening the time necessary for immunoreactions. In fact, it has been confirmed that the immunoreaction between the antibody on the wall of solid phase and the analyte molecules in solution reaches equilibrium in 10 min (data not shown).

Another reason for high sensitivity of the current system is the weak buffering ability of the substrate solution. The degree of pH change of substrate solution due to decomposition of urea depends on the concentration of ammonium chloride used as a buffer reagent. One order decrease of the concentration of ammonium chloride causes 2.5–3.0 times increase of $\Delta V/\Delta t$ (data not shown). However too low concentration of ammonium chloride, such as 1 mmol/l, causes instability of the pH of the substrate solution. Therefore 10 mmol/l was selected as an optimum concentration.

One of anticipated disadvantages of using an electrochemical detector compared to using an optical detector is the contamination of the electrode. Indeed, the inner wall of the sensor cou-

pler, indicated by four arrows in the top view of Fig. 3, which contacts inevitably with the outer wall of the end part of pipette tip, is heavily contaminated by urease conjugate. However it was found that the contamination of this part has no influence on the pH of the solution inside the tip, once the tip and the pH-FET are coupled. Contamination of the pH-FET itself with urease has severe influence, of course. However this was prevented by designing the dimensions and the shapes of the sensor coupler, sensor body, and the pipette tip so that direct contact between the pH-FET and the tip should never occur (Fig. 3). The results in Table 5 clearly show that the signal intensities for 0 μg/l AFP are not affected by the preceding measurement of AFP of very high concentration. This indicates that the carryover of analyte or conjugate is negligible not only in the pH-measuring cell but also in washing cells. Other disadvantages of an electrochemical detector are the drift of the baseline potential and the change of the sensitivity. The source potential of the pH-FET drifted 2–5 mV for 1 week at the working conditions. However, this has eventually no effect on the measurement of $\Delta V/\Delta t$, because every measurement finishes in 20 s. On the other hand, the change of pH sensitivity was less than $\pm 1\%$ during 1 year. Therefore this has also no effect on the assay precision.

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