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# Development of Multianalyte Flow-through and Lateral-Flow Assays Using Gold Particles and Horseradish Peroxidase as Tracers for the Rapid Determination of Carbaryl and Endosulfan in Agricultural Products

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Membrane-based competitive immunoassays using gold particles and horseradish peroxidase (HRP) as tracers in flow-through and lateral-flow formats for multianalysis of carbaryl and endosulfan were developed. For gold-based immunoassay, membrane strips were coated with goat anti-rabbit IgG (control line) and carbaryl hapten−ovalbumin (OVA) and endosulfan hapten−OVA (test lines). The visual detection limits for carbaryl and endosulfan were 100 and 10 $\mu$g/L in gold-based assays, respectively. For immunoassay using HRP as tracer, anti-carbaryl and anti-endosulfan antibodies were separately coated on the membrane as test lines, and the visual detection limits were 10 $\mu$g/L for carbaryl and 1 $\mu$g/L for endosulfan. The developed assays used gold particles and HRP as labels, respectively; 10 times enhancement in the visual detection limit using HRP label was obtained in the study. Matrix interference was eliminated by appropriate dilution of sample extracts with buffer. For the validation of the multianalyte assay, the samples were screened by multianalyte gold-based assay and confirmed by HPLC for carbaryl determination and by GC for endosulfan determination. The results of multianalyte gold-based flow-through assay for the determination of carbaryl and endosulfan were in good agreement with the results of instrumental analysis (HPLC with ultraviolet detection and GC with electron capture detection). The developed multianalyte immunoassays for which the results were interpreted visually can be used as convenient qualitative tools for on-site rapid screening of carbaryl and endosulfan simultaneously in agricultural products.

**KEYWORDS: Immunoassay; carbaryl; endosulfan; lateral-flow; flow-through; multianalysis**

## INTRODUCTION

Carbaryl is an *N*-methylcarbamate that has been extensively used as a broad-spectrum insecticide since the 1960s, because of its relatively low mammalian toxicities and low bioaccumulation potentials (*1*). Despite its early introduction, carbaryl is one of the most frequently detected pesticide residues in food analysis worldwide. The presence of traces of carbaryl in agricultural products poses a potential hazard for consumers (*2*). Instrumental analyses such as high-performance liquid chromatography (HPLC) with ultraviolet (UV), postcolumn derivation and fluorescence spectrometry, diode array (DAD), and mass spectrometry (MS) have been used successfully with high sensitivity and reliability for the analysis of carbaryl in different matrices (*3*−*7*).

Endosulfan is a broad-spectrum insecticide and acaricide, which acts as a contact poison to a wide variety of insects (*8*−*10*). It is used primarily on a wide variety of food crops, including fruits and vegetables, as well as on cereals such as rice, maize, sorghum, or other grains (*11*−*13*). Currently, organchlorine residue analysis of compounds such as endosulfan in most developed and developing countries is carried out using gas chromatography (GC) with electron capture detection (ECD) (*14*, *15*).

Detection is performed by chromatographic methods such as GC coupled to an ECD detector for endosulfan and HPLC for carbaryl. These procedures are time-consuming and labor-intensive and do not allow the generation of relevant data in time to prevent contaminated foods from entering retail markets. Recent studies suggest that these requirements can be achieved by using an immunoanalytical screening system (*16*). As it was known that carbaryl and endosulfan show different physicochemical properties, it is necessary to develop simple analytical methodologies devising suitable protocols for the effective treatment and determination of target analytes in samples. The goal of the present work was to develop multianalyte assay formats for the simultaneous determination of carbaryl and endosulfan in vegetable and cereal samples. These formats

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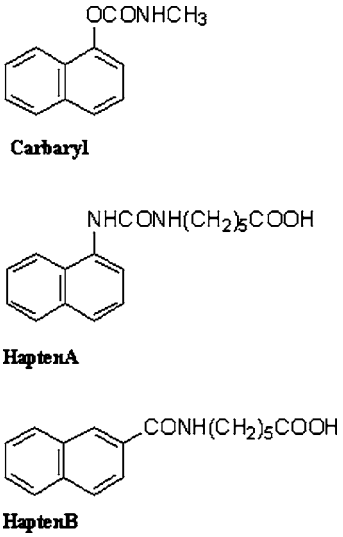


**Figure 1.** Chemical structures of carbaryl and the haptens used for immunogen (hapten A) and enzyme tracer (hapten B).

include flow-through and lateral-flow enzyme-linked immunoassays (ELISAs) and colloidal gold-based flow-through and lateral-flow immunoassays for the rapid detection of carbaryl and endosulfan simultaneously.

## MATERIALS AND METHODS

**Chemicals and Materials.** Nitrocellulose membranes were purchased from Pierce (Rockford, IL), and nitrocellulose Hi-Flow plus membranes were from Millipore (Bedford, MA). Semirigid polyethylene sheets and adhesive tape were purchased from a local market. Filter paper and analytical grade buffer chemicals were purchased from Hope Biotech Co. Ltd. TEDA, Tianjin, China. Protein A−Sepharose 4B was purchased from Amersham Biosciences (Uppsala, Sweden). Horseradish peroxidase (HRP), keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), 3,3′,5,5′-tetramethylbenzidine, and hydrogen peroxide were purchased from Sigma (St. Louis, MO). Carbaryl and endosulfan were obtained from Chem Service (West Chester, PA).

**Preparation of Immunoassay Reagents.** The synthesis of carbaryl haptens was performed as previously described by Wang et al. (*17*). **Figure 1** shows the chemical structures of carbaryl and the haptens used to develop the immunoassay. The synthesis of endosulfan haptens reported by Lee et al. was accomplished according to their procedures (*18*). The chemical structures of endosulfan and its haptens are shown in **Figure 2**. The haptens were coupled to KLH for use as immunogens or coupled to HRP for use as enzyme tracers.

Antibodies were produced in rabbits as described by Wang et al. (*17*). Female white rabbits were immunized by intradermal and intramuscular injections of haptens conjugated with KLH. IgG from antiserum was purified by protein A−Sepharose 4B affinity chromatography (*19*).

**Preparation of Colloidal Gold and Labeling of Antibodies.** Gold colloids with a mean diameter of 40 nm (checked by transmission electron microscope) were synthesized using the sodium citrate method that has been described in a previous paper (*20*). The colloidal gold solution for labeling antibody was adjusted to pH 9.0 with 0.1 M $K_2CO_3$ or 0.1 M HCl. The optimal concentration of antibody for conjugation was determined. With gentle stirring, 0.82 mg of purified anti-carbaryl antibody was added to 100 mL of pH-adjusted colloidal gold solution, and 0.96 mg of purified anti-endosulfan antibody was mixed with 100 mL of pH-adjusted colloidal gold solution. After overnight incubation at 4 °C, the mixture was centrifuged at 10000 rpm at 4 °C for 30 min, and the pellet was resuspended in 10 mM conjugate storage buffer (2 mM sodium borate containing 1% BSA and 0.1% sodium azide) and diluted for use.

**Gold-Based Flow-through Immunoassay.** *Preparation of Membrane Strip for Flow-through.* Nitrocellulose membrane (Pierce) was cut into sections (2.0 cm × 0.5 cm). Test lines (for carbaryl and endosulfan) were separately coated with their hapten conjugates in a volume of 1 μL containing 1 μg of hapten conjugate with a Camag Linomat5 automatic TLC sampler. The control line was coated with 0.5 μL of anti-rabbit IgG from goat diluted 1/50 in PBS buffer (pH 7.2). The test strips were dried at 37 °C for 15 min. The remaining protein-binding sites of the membrane were blocked by immersing the strips in PBS containing 1% BSA at 37 °C for 30 min. The test strips were washed and dried. The coated test strips were stored in a desiccator at 4 °C.

*Procedure of Multianalyte Flow-through Colloidal Gold Immunoassay.* Flow-through is an immunoconcentration assay. The flow-through device used in this study (shown in **Figure 3**) was the same as described previously (*20*). In the flow-through device, the filter paper acted as an absorbent actively drawing liquid reagent quickly through the membrane without lateral spreading. All of the reactions were distributed within the spotted zone. One hundred and twenty microliters of endosulfan/carbaryl mixture solutions (0/0 as negative control, 0.01/0.1, 0.1/1.0, and 1.0/10 mg/L) in 5% MeOH (prepared in PBS−0.05% Tween buffer) was mixed with 20 μL of anti-carbaryl PAb colloidal gold conjugate and 20 μL of anti-endosulfan PAb colloidal gold conjugate. After incubation for 5 min, 100 μL of mixtures was added to the test strip that was coated with hapten−ovalbumin (OVA) conjugates and anti-rabbit IgG. After the liquid reagent flowed through the reaction zone (two test lines and control line), different intensities of color on the test lines (carbaryl test line and endosulfan test line) were separately compared with their respective test line of negative

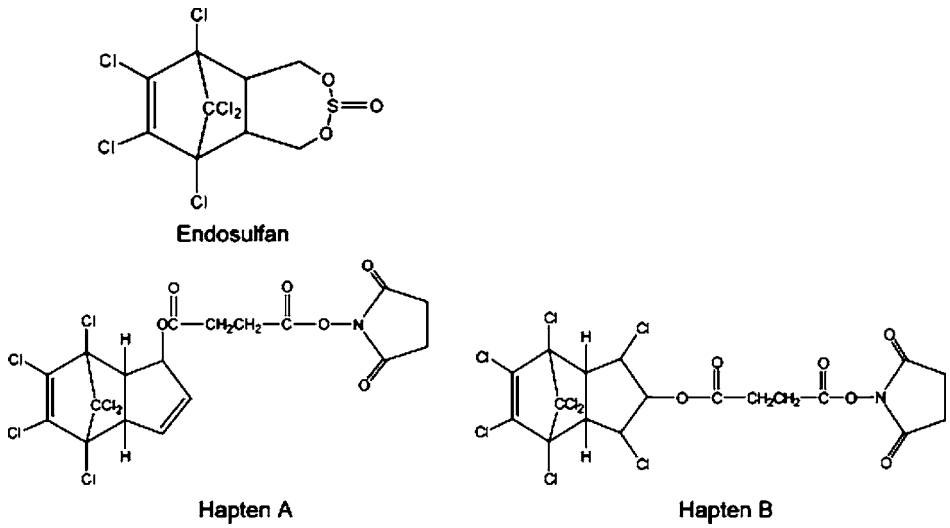


**Figure 2.** Chemical structures of endosulfan and the haptens used for immunogen (hapten A) and enzyme tracer (hapten B).

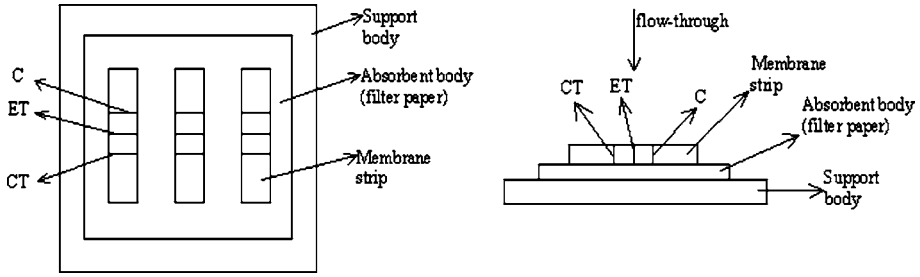


**Figure 3.** Schematic diagram of the analytical device for multianalyte flow-through immunogold assay. C, control line; ET, endosulfan test line (endosulfan hapten–OVA coated); CT, carbaryl test line (carbaryl hapten–OVA coated).

control. The intensity of the color of the test line is the reverse of the concentration of pesticide.

**Gold-Based Lateral-Flow Immunoassay.** *Preparation of Membrane Strip for Lateral-Flow.* The preparation of the membrane strip was the same as that for flow-through assay, except that the membrane from Millipore was chosen and a different size of section (3.0 cm × 0.5 cm) was used.

*Procedure of Multianalyte Lateral-Flow Immunogold Assay.* Lateral-flow is an immunochromatography assay. The procedure and analytical device for the multianalyte lateral-flow immunogold assay were the same described previously (*20*), except that after 120 μL of endosulfan/carbaryl mixture solutions had been incubated with antibody−gold conjugates for 5 min, 100 μL mixtures were pipetted into the bottom of the strip (sample application site). After the liquid migrated toward the test lines (carbaryl test line and endosulfan test line) and control line, different intensities of color on the test lines could be observed visually. The colors of the test lines were separately compared with the test lines of the negative control strip.

**Flow-through Enzyme-Linked Immunoassay.** *Preparation of Membrane Strips for Flow-through Enzyme-Linked Immunoassay.* The preparation of the membrane strip and the device for the flow-through enzyme-linked assay was the same as that for the flow-through gold-based immunoassay except that test lines were separately coated with anti-carbaryl antibody and anti-endosulfan antibody, in a volume of 1 μL of solution containing 1 μg of antibody.

*Procedure of Multianalyte Flow-through Enzyme-Linked Immunoassay.* Six hundred microliters of endosulfan/carbaryl mixture solutions (0/0 as negative control, 1/10, 10/100, and 100/1000 μg/L) in 5% MeOH was mixed with 100 μL of enzymatic tracers mixture containing carbaryl hapten−HRP conjugate (1:40000) and endosulfan hapten−HRP conjugate (1:20000). To each test strip was added 100 μL of mixture; care was taken to avoid the liquid flowing outside the reaction zone (coated with carbaryl antibody and endosulfan antibody). After the liquid flowed through the reaction zone, the membrane was thoroughly washed three times with washing buffer. After excess water had been gently shaken off, the membrane was placed in a glass plate. The $H_2O_2$−TMB substrate solution (1.25 mM 3,3′,5,5′-tetramethylbenzidine−1.6 mM hydrogen peroxide in acetate buffer, pH 5.0) was added uniformly over the membrane surface. The colors of the carbaryl test line and endosulfan test line were separately compared with negative control of carbaryl test line and endosulfan test line. As it is a direct competitive assay, the intensity of the color of the test line is the reverse of the concentration of pesticide.

**Lateral-Flow Enzyme-Linked Immunoassay.** *Preparation of Membrane Strips for Lateral-Flow Enzyme-Linked Immunoassay.* The preparation of the membrane for the lateral-flow enzyme-linked immunoassay was the same as that for the lateral-flow gold-based immunoassay except that anti-carbaryl antibody and anti-endosulfan antibody were separately coated on the Millipore membrane as carbaryl test line (CT) and endosulfan test line (ET). Glass fiber membrane was used as enzyme substrate supply pad, and filter paper was used as adsorption pad to provoke the solution flow.

*Procedure of Multianalyte Lateral-Flow Enzyme-Linked Immunoassay.* The analytical device for multianalyte lateral-flow enzyme-linked immunoassay (shown in **Figure 4**) was the same as that for lateral-flow gold-based immunoassay. The procedure was as follows: first, 600 μL of endosulfan/carbaryl mixture solutions was mixed with 100 μL of the enzymatic tracers solution containing carbaryl hapten−HRP conjugate (1:40000) and endosulfan hapten−HRP conjugate (1:20000). One hundred microliters of the liquid reagent was pipetted into the sample application site. After the liquid migrated toward the test lines (antibodies coated), the strips were washed with PBST, and then excess water was absorbed with dry filter paper. Sixty microliters of substrate solution was added to the enzyme substrate supply pad and flowed in vertical direction and horizontal direction, respectively. With the migration of the liquid (substrate solution) over the test lines, a blue band of lines can be observed by the eye.

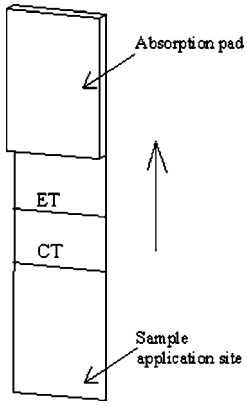


**Figure 4.** Schematic diagram of the analytical device for multianalyte lateral-flow enzyme-linked immunoassay. ET, endosulfan test line (anti-endosulfan antibody coated); CT, carbaryl test line (anti-carbaryl antibody coated).

**Extraction of Residues.** A rapid extraction method was used as described by Wang et al. (*21*). The food sample was treated by mixing 5 volumes of methanol and was shaken by hand for 5 min.

**Instrumental Analysis.** Methods and sample cleanup procedures with an SPE cartridge for HPLC and GC analyses were the same as described in the papers by Wang et al. (*21*, *22*).

The samples of rice, oat, carrot, and green pepper were purchased from a local supermarket, and incurred residues of pesticides were detected by HPLC and GC. Once the samples were confirmed to contain the carbaryl and endosulfan at less than 5 and 0.1 μg/kg, respectively, they were used for the confirmation study for the immunoassay.

## RESULT AND DISCUSSION

**Feasibility of Multianalysis of Carbaryl and Endosulfan.** It was known that carbaryl and endosulfan show different physicochemical properties, and their haptens show great differences in chemical structure. The different corresponding coating antigens for each pesticide could be fixed at different sites as respective test lines on the strip. As shown in **Figure 5**, using the flow-through gold-based assay, when the mixture of two gold−antibody conjugates was added to the reaction zone, both test lines (carbaryl line and endosulfan line) and control line had color development. When gold−antibody (specific to endosulfan) conjugate was added to the test lines, only the color of the endosulfan test line appeared, and no color

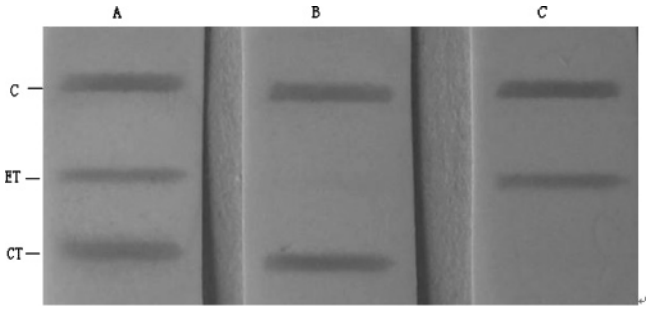


**Figure 5.** Illustration of multianalyte flow-through gold-based immunoassay: upper line (C), control line (goat anti-rabbit IgG); middle line (ET), endosulfan test line (endosulfan hapten–OVA coated); lower line (CT), carbaryl test line (carbaryl hapten–OVA coated); (**A**) the mixture of two gold–antibody conjugates was added; (**B**) anti-carbaryl antibody colloidal gold conjugate was added; (**C**) anti-endosulfan antibody colloidal gold conjugate was added.

developed in the carbaryl test line. It was also found that there was no color development in the endosulfan test line when the gold−antibody conjugate (specific to carbaryl) was added. The result was the same when using the lateral-flow gold-based assay. For the multianalyte colloidal gold immunoassay and single pesticide (carbaryl or endosulfan) colloidal gold immunoassay, the visual detection limits of carbaryl and endosulfan were the same for both formats (data shown later). The existence of endosulfan (or carbaryl) did not affect the binding between carbaryl (or endosulfan) and anti-carbaryl (or endosulfan) antibody gold conjugate as seen by a comparison of the visual results of the single-pesticide gold-based immunoassay and the multianalyte gold-based immunoassay. It could be concluded that there was no cross-reaction for multianalysis of carbaryl and endosulfan using the colloidal gold-based immunoassay. It should be feasible to detect multiple pesticides by combining different conjugates in one NC membrane.

**Optimization of Colloidal Gold Immunoassay.** The main purpose of the multianalytical assay was to allow visual evaluation for each test line simultaneously. It was used as a qualitative tool to detect contaminations at only a threshold level. For these the color intensity of each test line must be high enough to be seen and enable observation of difference in color intensity between negative control and samples. Experiments were carried out to determine the optimal conditions for flow-through and lateral-flow colloidal gold immunoassays, and the results were as follows: carbaryl hapten−OVA and endosulfan hapten−OVA coated on the membrane at 1 $\mu$g/strip separately, anti-carbaryl PAb colloidal gold conjugate and anti-endosulfan PAb colloidal gold conjugate and carbaryl/endosulfan mixture solution in the ratio of 1:1:6, and incubation time of 5 min. For flow-through colloidal gold immunoassay of a single pesticide, the visual detection limit of carbaryl was 100 $\mu$g/L and the visual detection limit of endosulfan was 10 $\mu$g/L. As shown in **Figure 6**, color appeared at both test lines if they contained no carbaryl and endosulfan (negative control); 100 $\mu$g/L of carbaryl and 10 $\mu$g/L of endosulfan caused a slight but distinguishable difference compared with negative control. It can be concluded that the multianalyte flow-through colloidal gold immunoassay developed in this study had the visual detection limits for carbaryl and endosulfan at 100 and 10 $\mu$g/L, respectively. For multianalyte lateral-flow gold-based immunoassay, the visual detection limits of carbaryl and endosulfan were the same as for the flow-through assay (data not shown).

**Multianalyte Enzyme-Linked Immunoassay.** It was also found that the multianalysis of carbaryl and endosulfan by using

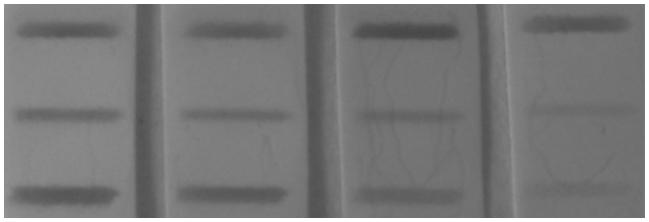

**Figure 6.** Flow-through immunogold assay: (upper line) control line (goat anti-rabbit IgG); (middle line) endosulfan test line (endosulfan hapten–OVA coated); (lower line) carbaryl test line (carbaryl hapten–OVA coated). Endosulfan/carbaryl mixture solutions were, from left to right, 0/0, as negative control; 0.01/0.1, 0.1/1.0, and 1.0/10 mg/L. The color intensities of 0.01 mg/L for endosulfan and 0.1 mg/L for carbaryl were clearly distinguishable, by seven persons, from that of negative control.

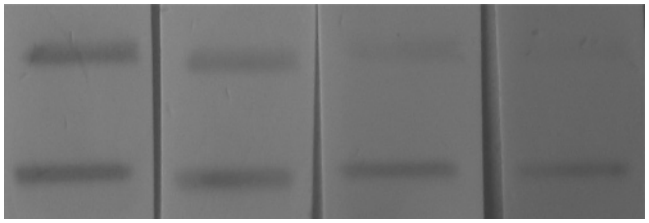

**Figure 7.** Flow-through enzyme-linked immunoassay: (upper line) endosulfan test line (anti-endosulfan antibody coated); (lower line) carbaryl test line (anti-carbaryl antibody coated). Endosulfan/carbaryl mixture solutions were, from left to right, 0/0, as negative control; 0.001/0.01, 0.01/0.1, and 0.1/1 mg/L. The color intensities of 0.001 mg/L for endosulfan and 0.01 mg/L for carbaryl were clearly distinguishable, by seven persons, from that of negative control.

enzyme-linked immunoassays was feasible (data not shown). First, the optimal immunoreagent concentrations to be used in the flow-through test for a single pesticide were determined by optimization experiment. Singh and Jang reported that (*23*) the sensitivity of a membrane-based assay could be controlled to a desired level by adjusting the amount of immobilized antibody on the solid support. Decreasing the immobilized antibody concentration increased the sensitivity of assay within certain limits. The purpose of the qualitative assays was to allow visual evaluation for each test line (CT and ET); the color intensity of each line must be high enough to be seen and low enough to enable observation of difference in color intensity between negative control and samples. **Figure 7** shows optimal multianalyte flow-through ELISA, developed under the following conditions: anti-carbaryl antibody and anti-endosulfan antibody coated at 1.0 $\mu$g/strip separately, the mixture of enzymatic tracer (the dilution of carbaryl hapten−HRP 1:40000, the dilution of endosulfan hapten−HRP 1:20000). As shown in **Figure 7**, 10 $\mu$g/L of carbaryl and 1 $\mu$g/L of endosulfan caused a slight but distinguishable difference in color compared with the negative control. Thus, the visual detection limits for carbaryl and endosulfan were 10 and 1 $\mu$g/L, respectively, using the flow-through enzyme-linked immunoassay.

**Comparison of Flow Paths (Vertical and Horizontal) for Supplying the Enzyme Substrate in Lateral-Flow ELISA.** In this experiment, the format of supplying the enzyme substrate in the lateral-flow enzyme-linked immunoassay for multianalysis was an immunoanalytical system based on sequential cross-flow chromatography as described by Cho et al. (*24*). We carried out such sequential processes employing chromatographic analysis, using two crosswise-arranged membrane pads in vertical and horizontal directions. The vertically arranged pad was the same as in the lateral-flow gold-based immunoassay for multianalysis except that anti-carbaryl antibody and anti-

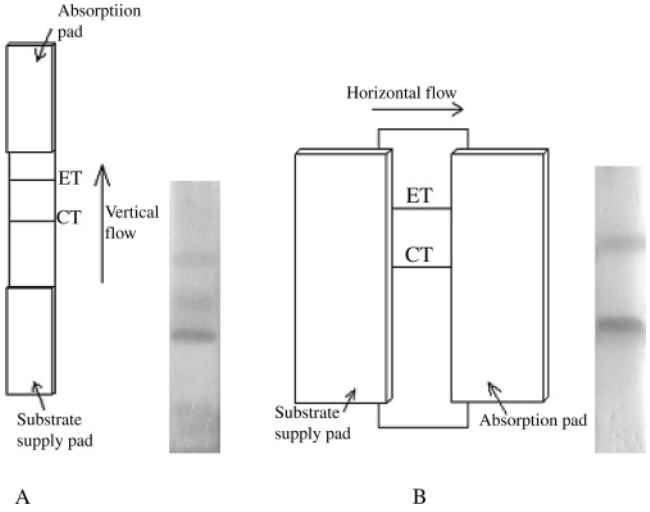


**Figure 8.** Various flow paths for supplying the enzyme substrate solution: (**A**) vertical flow direction; (**B**) horizontal flow direction.

endosulfan antibody were coated on the membrane as carbaryl test line (CT) and endosulfan test line (ET), with the exception of the use of HRP as tracer. While the solutions containing carbaryl/endosulfan mixture solutions and enzymatic tracers (mixtures of carbaryl hapten−HRP and endosulfan hapten−HRP) migrated toward the test lines (CT and ET), the competition assay occurred simultaneously. Cho et al. (*24*) reported that different flow paths for supplying the enzyme substrate affected signal generation and the results observed by eye. As shown in **Figure 8**, it was found that the straight horizontal flow path for substrate solution produced a uniform signal of color, and the background noise was low. While the substrate solution flowed in a vertical direction, the background noise was high. However, the background noise increased and the color bands diffused when the reaction time of the enzyme substrate was in excess of 1 min for both flow paths of substrate solution in the lateral-flow ELISA. It might be unsuitable for antibodies to coat steadily on the Hi-Flow plus chromatographic membrane in the lateral-flow ELISA when the enzyme substrate solution was added for signal generation. Therefore, the format of the lateral-flow ELISA for multianalysis of carbaryl and endosulfan was not preferable. More work should be carried out for lateral-flow ELISA, for example, how to ensure the antibody coats the membrane steadily and whether some additive can be added to reduce the background noise. Therefore, the format of flow-through ELISA was chosen.

**Comparison of Sensitivity for Immunoassay Using HRP as Tracer and Gold as Label.** The one-step immunochromatographic assay employed tracers such as gold colloids, which produce a detectable color signal by eye without the addition of substrate. In the immunoassays using HRP as tracer, the enzyme reaction should be carried out separately for signal generation following the completion of antigen−antibody binding. For gold-based multianalyte assays, the detection limits of carbaryl and endosulfan were 100 and 10 $\mu$g/L, respectively. However, for enzyme-linked multianalyte assays, the detection limits of carbaryl and endosulfan were 10 and 1 $\mu$g/L, respectively. The sensitivities were enhanced 10-fold compared to that of gold label.

**Reliability of Flow-through Gold-Based Immunoassay and Analysis of Food Samples by Test Strip.** The reliability of multianalyte flow-through gold-based immunoassay for the analysis of food samples was tested. Matrix interference was eliminated by 20-fold dilution of sample extracts with PBS−0.05% Tween buffer for the gold-based assay. Samples were spiked with carbaryl /endosulfan at three levels (0/0, 10/1, and 20/2 mg/L) and extracted with methanol as described under Materials and Methods. The samples were screened by multianalyte gold-based assay and confirmed by HPLC for carbaryl determination and by GC for endosulfan determination. The results are shown in **Table 1**. In general, the visual results of the multianalyte flow-through assay for the determination of carbaryl and endosulfan were in good agreement with the results of instrumental analysis (HPLC and GC).

In conclusion, the multianalyte strip assays developed in this paper offer a portable analytical system that can be used for the fast qualitative or semiqualitative analysis of carbaryl and endosulfan in food samples simultaneously. The result was obtained within 15 min (rapid extraction and assay time). The cutoff levels for carbaryl and endosulfan were, respectively, 100 and 10 $\mu$g/L in gold-based assays. The sensitivities were enhanced 10-fold using HRP as tracer in immunoassay: the cutoff level for carbaryl was 10 $\mu$g/L and that for endosulfan 1 $\mu$g/L. A one-step immunoassay using gold as tracer is more convenient for on-site determination, although it showed relatively low sensitivity compared with the assay using enzyme as tracer. For both formats using gold as label or HRP as tracer, the interpretation of the result was visual and can be used as a convenient qualitative tool for rapid screening to determine if

**Table 1.** Comparison of Results Obtained by Flow-through Gold-Based Immunoassay and Instrumental Analysis

| matrix | carbaryl fortification level (mg/L) | visual results ($n = 3$) flow-through gold-based assay for carbaryl test line[a] | results of HPLC confirmation of concentration of carbaryl[b] (mg/L) | endosulfan fortification level (mg/L) | visual results ($n = 3$) flow-through gold-based assay for endosulfan test line[c] | results of GC confirmation of concentration of endosulfan[d] (mg/L) |
|---|---|---|---|---|---|---|
| rice | 0 | −, −, − | 0 | 0 | −, −, − | $<0.1 \times 10^{-3}$ |
| | 10 | −/+, +, −/+ | 7.32 | 1 | +, −/+, −/+ | 0.8 |
| | 20 | +, +, + | 13.45 | 2 | +, +, + | 1.54 |
| oat | 0 | −, −, − | 0 | 0 | −, −, − | 0 |
| | 10 | +, −/+, + | 8.36 | 1 | −/+, +, −/+ | 0.73 |
| | 20 | +, +, + | 14.28 | 2 | +, +, + | 1.31 |
| carrot | 0 | −, −, − | $<5 \times 10^{-3}$ | 0 | −, −, − | 0 |
| | 10 | −/+, −/+, + | 6.97 | 1 | −/+, −/+, −/+ | 0.64 |
| | 20 | +, +, + | 13.04 | 2 | +, +, + | 1.23 |
| green pepper | 0 | −, −, − | 0 | 0 | −, −, − | 0 |
| | 10 | −/+, −/+, −/+ | 6.21 | 1 | −/+, −/+, + | 0.72 |
| | 20 | +, +, + | 12.37 | 2 | +, +, + | 1.38 |

[a] −, absence of carbaryl; +, presence of carbaryl. [b] HPLC confirmation for all samples was carried out according to the method described in a previous paper (*21*). [c] −, absence of endosulfan; +, presence of endosulfan. [d] GC-ECD confirmation for all samples was carried out according to the method described in a previous paper (*22*).

the concentration of contaminate was above the established cutoff level. The developed on-site multianalyte assay was reliable for analyzing endosulfan and carbaryl in agricultural commodities.

## ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; PAb, polyclonal antibody; HRP, horseradish peroxidase; OVA, ovalbumin; KLH, keyhole limpet hemocyanin; BSA, bovine serum albumin; SPE, solid-phase extraction; HPLC, high-performance liquid chromatography; GC, gas chromatography; ECD, electron capture detection; HPLC, high-performance liquid chromatography; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 0.05% Tween-20; NC, nitrocellulose membrane; CT, carbaryl test line; ET, endosulfan test line.

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