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Immunochromatography Using Colloidal Gold−Antibody Probe for the Detection of Atrazine in Water Samples

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An immunochromatography (ICG) strip test for rapid detection of atrazine in water samples was developed. A monoclonal antibody (MAb) specific to atrazine was produced from the cloned hybridoma cell (AT-1-M3) and used to develop a direct competitive enzyme-linked immunosorbent assay (DC-ELISA) and ICG strip. MAb conjugated to colloidal gold, and that was applied to the conjugate pad of the ICG strip. The visual detection limit for the ICG strip was 3 ng/mL. This test required only 10 min to get results and one step of sample to perform the assay. The results of water samples spiked with 5, 10, 20, and 50 ng/mL of atrazine by ICG strip were in good agreement with those obtained by DC-ELISA. The ICG strip was sufficiently sensitive and accurate to be useful for rapid screening of atrazine in various water samples.

KEYWORDS: Atrazine; herbicide; monoclonal antibody; enzyme-linked immunosorbent assay; immunochromatography

INTRODUCTION

In recent years, modern agriculture has benefited from the use of agrochemicals including insecticides, herbicides, and fungicides (*1*). If we do not use these compounds, agricultural productive capacity is likely to decrease by approximately 10−15%. However, their use can also be of environmental concern due to their persistence or the formation of toxic metabolites. Pesticides have been detected in soil, surface, and groundwater. Consequently, they can contaminate drinking water and agricultural products, presenting an important risk to human health (*2*).

Atrazine is used as a pre- or postemergent herbicide applied to corn fields. This herbicide is very cheap and can be used under rainy conditions since it does not dissolve in water. It degrades slowly with a half-life measured in months or years depending on soil conditions (*3*). Frequently, it runs off agricultural fields into the soil, ground, and surface water, and many food products have become a matter of concern throughout the world (*4*−*6*). Recent papers suggest that atrazine disrupts frog development when atrazine exposure levels are lower than those found in natural water (*7*−*9*). Therefore, there is a need for monitoring residue levels, and legal limits have been established for atrazine in many countries including Australia (20 ng/mL), Canada (60 ng/mL), the European Union (3−60 ng/mL), and the United States (3 ng/mL) (*10*).

Atrazine in agricultural products and foods is mainly analyzed by means of instrumental analysis such as gas chromatography (GC) or high-pressure liquid chromatography (HPLC) (*11*). However, these methods require an extraction step, i.e., sample preparation, and in some cases an enrichment step prior to determination. Therefore, they are mostly laborious and time-consuming, and require complicated cleanup procedures and sophisticated technical equipment (*12*). Many scientists attempted to develop alternative simple and accurate methods for the detection of herbicides in water and agricultural samples. Especially for screening large numbers of samples, enzyme immunoassays are suitable tools for quick and sensitive analysis with high sample throughput (*13*, *14*). In addition to the chromatographic methods currently used, immunoassays can complement the repertoire of analytical techniques in residue analysis, in particular when only small sample volumes are available (*13*). Besides, immunoassay methods are sensitive, cost-effective, easy to perform, and require a small sample volume (*15*). However, such techniques often require long reaction times and involve multiple steps (*16*). The utilization of these immunoassays has been confined to laboratories

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equipped with tools and devices for analysis. Therefore, the convenience and speed of the test have been achieved by a novel concept of immunochromatography (ICG) that depends on the transportation of a reactant to its binding partner immobilized on a membrane surface (*17*, *18*). This recently developed technique, often called strip assay or lateral-flow assay, is based on the immunochromatographic procedure that utilizes antigen and antibody properties and provides rapid detection of an analyte. It combines several benefits including a user friendly format, short assay time, long-term stability over a wide range of climates, and cost-effectiveness. These characteristics make it ideally suited for on-site screening by people who are not skilled analysts (*19*). In the present work, we report on a sensitive and rapid ICG strip using a colloidal gold−antibody probe for the detection of atrazine in various water samples.

MATERIALS AND METHODS

Chemicals. Analytical grade atrazine, other related pesticides, and herbicides were obtained from Supelco Inc. (Bellefonte, PA). Complete and incomplete Freund's adjuvant, *N*,*N*-dicyclohexylcarbodiimide, common solvents, and salts were supplied by Aldrich Chemical Co. (Milwaukee, WI). Keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), horseradish peroxidase (HRP), goat anti-mouse IgG, and N-hydroxysuccinimide were purchased from Sigma Chemical Co. (St. Louis, MO). Polyethylene glycol (PEG) 1500 was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Dialysis membrane (Spectra/Por; MW cutoff 6000−8000) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA). The P3-X63-Ag8.653 murine myeloma cell line was obtained from Microbiology Laboratory, Medical College, Gyeongsang National University (Chinju, South Korea). Dulbecco's modified Eagle medium (DMEM), fetal calf serum, and supplements were obtained from Hyclone (Logan, UT). Microculture plates (96 and 24 wells) were obtained from SPL Life Sciences. (Pocheon, South Korea). All other chemicals and organic solvents were of reagent grade or higher.

A sample pad, glass-fiber membrane, conjugate pad, and absorbent pad were provided from Millipore (Bedford, MA). Semirigid polyethylene sheets were purchased from a local market.

The standard solutions of atrazine and cross-reactants were prepared by dilution of stock solutions of these compounds (1 mg/mL in methanol).

Atrazine−protein (KLH and BSA) conjugates were prepared by the method of Thomas (*13*). The atrazine−KLH conjugate was used as an immunogen, and the atrazine−BSA conjugate was used as a capture reagent in the ICG strip. The atrazine−enzyme (HRP) conjugate was prepared by the active ester method according to Wittmann and Hock (*20*). The atrazine−HRP conjugate was used as a competitor in direct competitive enzyme-linked immunosorbent assay (ELISA).

Measurements of optical density for 96 well microtiter plates were performed on a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Richmond, CA). MPM software (version 4.0, Bio-Rad) was used for data processing.

Water Samples. Drinking water, tap water, and surface water were taken from wells, water supply facilities, streams, and rivers in Chinju (Gyeongnam Province, Korea). All samples were centrifuged at 5000 rpm for 5 min, and supernatants were taken and then directly applied to direct competitive (DC)-ELISA and ICG strips. Water samples containing atrazine (5, 10, 20, and 50 ng/mL) were obtained from atrazine negative spring water, tap water, and surface water samples with a known amount of atrazine, derived from 1 mg/mL stock solutions in methanol by artificial spiking. Negative samples were obtained as described above but were not spiked with atrazine.

Production of Monoclonal Antibody (MAb). In order to develop sensitive and specific MAbs against atrazine, the atrazine−KLH conjugate was used to immunize mice. Ten female BALB/c mice (8 weeks old) were immunized with 100 μg of atrazine−KLH conjugate in 0.1 mL of sterilized phosphate-buffered saline (PBS, pH 7.4), which was emulsified with an equal volume of Freund's complete adjuvant. Boost injections were given 2, 4, and 6 weeks later. One week after the third injection, sera were collected from the caudal vein of each mouse. Titers of anti-sera were determined by indirect ELISA. Three days before cell fusion, the mice that produced anti-sera with high titers were given another intraperitoneal boost injection without adjuvant.

Cell fusion was performed by the standard procedures (*21*). Briefly, mouse spleen lymphocytes were fused with myeloma cells at a 10:1 ratio using PEG 1500. The fused cells were distributed in 96 well culture plates at a density of 10^5-10^6 cells/well in 50 μL of DMEM supplemented with 25 μg/mL gentamicin and 20% fetal bovine serum (referred to as s-DMEM). One day after plating, 50 μL of HAT selection medium (s-DMEM supplemented with 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine) was added to each well. Half of the medium in the wells was replaced by fresh HAT medium every other day. During the fused cell selection with HAT medium, the culture supernatants were screened for the presence of antibodies that recognized atrazine by indirect ELISA. ELISA-positive hybridoma cells were cloned by the limiting dilution method. Stable antibody-producing clones were expanded and stored in liquid nitrogen.

Female BALB/c mice, not less than 6 weeks old, were injected intraperitoneally with 500 μL of pristane (2,6,10,14-tetramethylpentadecane), 10 days prior to intrapertoneal injection of 1×10^7 hybridoma cells that had been resuspended in DMEM medium containing 10% fetal bovine serum. Ascites fluid was collected from mice injected with hybridoma cells within 14 days and purified by ammonium sulfate precipitation followed by affinity chromatography on a protein G column (Bioprogen, South Korea). The protein concentration of the purified MAb was determined with the Bio-Rad protein assay kit (Bio-Rad Laboratories). The isotype of cloned MAb was determined with mouse MAb isotyping kit (Roche Applied Science) according to the instructions.

DC-ELISA. MAb solution (100 μL, 1.43 μg/mL in PBS containing 0.01% BSA) was added to microtiter wells (Nunc International, Rockilde, Denmark) and incubated overnight at 4 °C, followed by thrice washing with PBS containing 0.02% Tween 20 (PBST) by using Immuno Wash 12 microplate washer. After the final washing step, the residue washing buffer was discarded. Standard or sample solutions (50 μL) and 50 μL of atrazine−HRP conjugate solution (1:1000 in PBS) were added to the wells. Then, the microtiter plate was rocked on a shaker for 30 s and incubated for 30 min at 37 °C, followed by six washings with PBST. Then, 100 μL of 0.1 M citrate buffer (pH 4.0) containing 0.025% ABTS [2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)] and 0.03% H_2O_2 was added and incubated for 30 min at 37 °C. The enzyme reaction was stopped with 50 μL of 2 M H_2SO_4, and absorbance was measured at 405 nm using a Bio-Rad model 550 microplate reader.

Preparation of Gold−Antibody Probe. Colloidal gold particles with a mean diameter of 40 nm checked by a transmission electron microscope were prepared according to the method of Frens (*22*), and they were used for conjugation with antibody. Briefly, 100 mL of 0.01% tetrachloroauric acid was heated to boiling point, and 1 mL of 1% sodium citrate was rapidly added. The reaction solution was stirred while gently boiling until the color of this solution was changed from purple to reddish-orange. The average particle diameter was measured by a transmission electron microscope. The obtained colloidal gold solution could be stored at 4 °C for several months. The colloidal gold−MAb probe was prepared according to the method of Roth (*23*, *24*). Before conjugation, the minimum amount of MAb for stabilization of colloidal gold solution was determined. Briefly, 0.1 mg of lyophilized MAb was dissolved in 1 mL of 2 mM Borax and the colloidal gold solution was adjusted to pH 8.5 with 0.1 M K_2CO_3. One milliliter of colloidal gold was distributed into each of a series of 1.5 mL tubes. The MAb solution (0−150 μL) was added to each tube. The tubes were shaken for 1 min and then incubated for 5 min at room temperature. One hundred microliters of 10% NaCl was added to each tube and mixed for 1 min. A minimum amount of MAb was evaluated by color change from reddish to blue. If a tube contained a minimum amount of the antibody, the color of the colloidal gold particles was not changed from reddish to blue (*25*). For conjugation, 30 mL of colloidal gold particles was mixed dropwise with 1.2 mL of MAb solution (0.1 mg/mL) in a tube with rapid stirring. After 1 h, 3.1 mL of 10% BSA was added for blocking of residual surfaces of the colloidal

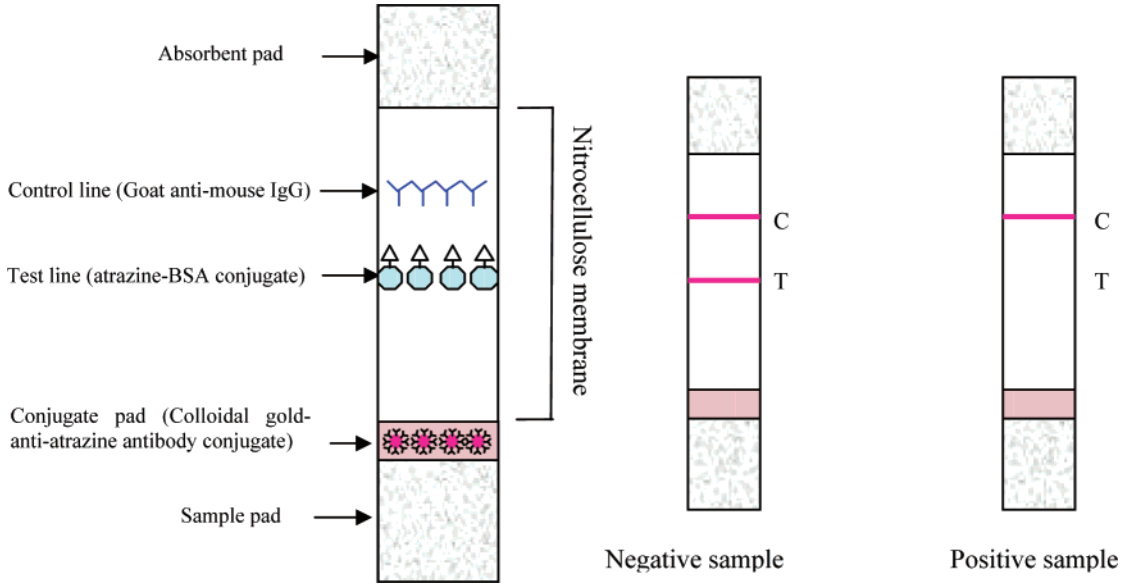


Figure 1. Schematic diagram of ICG strip. C, control line; T, test line.

gold particles and the mixture was incubated for 1 h at room temperature. The mixture was then centrifuged for 15 min at 10000 rpm, and the supernatant was discarded. The pellets were resuspended in 2 mM borate buffer (pH 7.2). The centrifugation was repeated twice, and final pellets were resuspended with 3 mL of 2 mM borate buffer (pH 7.2) containing 1% BSA, 1% sucrose, and 0.05% sodium azide. The colloidal gold−antibody probes were stored at 4 °C before use.

Colloidal Gold-Based ICG Strip. The ICG strip consisted of three pads (sample, conjugate, and absorbent pads) and of one nitrocellulose membrane with test and control lines. A schematic diagram of the ICG strip was shown in **Figure 1**. The sample pad was soaked with 50 mM borate buffer (pH 7.4) containing 1% BSA, 0.5% Tween 20, 5% sucrose, 5% dextrane, and 0.05% sodium azide and then dried for 1 h at 60 °C. Five microliters of colloidal gold−MAb probe (absorbance at 540 nm was 1.5) was applied to an untreated glass-fiber membrane to be used as a conjugate pad. The conjugate pad was dried for 30 min at 37 °C. An absorbent pad was used without treatment. The test line and control line on the nitrocellulose membrane were treated with 3 μL of atrazine−BSA (0.5 mg/mL) conjugate and 3 μL of goat anti-mouse IgG (1.2 mg/mL in PBS), respectively. The treated nitrocellulose membranes were dried for 30 min at 37 °C. All treated pads and the membrane were attached in a semirigid polyethylene sheet.

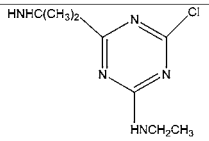
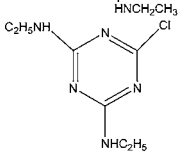
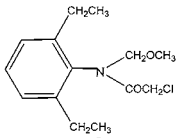
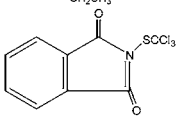
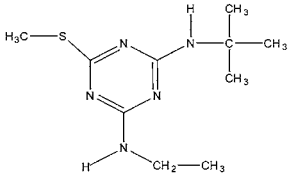
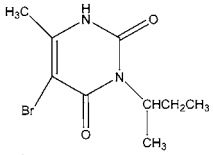
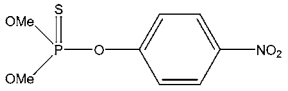
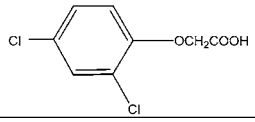
Water samples (150 μL) treated as described above were applied to the sample pad and allowed to migrate up the membrane. After 10 min, test results were evaluated visually. The negative test resulted in two red lines in the test and control region lines. Positive samples gave only one red line in the control region (**Figure 1**).

RESULTS AND DISCUSSION

Production and Characterization of MAbs. Atrazine, like most herbicides, is too small to be immunogenic. In order to overcome the limit of small molecule recognition in an immune response, the atrazine−KLH conjugate was constructed and used as the immunogen instead of atrazine itself. However, immunized animals with atrazine−KLH might induce many kinds of antibodies that recognized not only atrazine but also KLH. For the development of sensitive and specific MAbs against atrazine, cell fusion and cloning were carried out by standard methods (*21*). Anti-sera of six mice in indirect ELISA showed high titer values, and they were used to develop hybridoma cells. After cell fusion and cloning, four cloned hybridoma cells (named AT-1-M3, AT-1-M8, AT-2-M1, and AT-3-M15) showing a positive signal in indirect ELISA were selected. All MAbs produced from cloned hybridoma cells were IgG_1 subclass with κ type light chains. Prior to the competitive tests, the capability of four MAbs to recognize atrazine−BSA conjugate was evaluated by checkerboard titration. All MAbs showed good binding to atrazine−BSA conjugate: Titers were 1/6000, 1/1500, 1/1500, and 1/3000 for AT-1-M3, AT-1-M8, AT-2-M1, and AT-3-M15, respectively. Besides, cross-reactivity of the four MAbs to other pesticides was evaluated. The three MAbs (AT-1-M8, AT-2-M1, and AT-3-M15) showed 53−78% cross-reactivity for simazine, but AT-1-M3 MAb showed 23% cross-reacitivity for simazine (**Table 1**). These indicated that AT-1-M3 MAb was a more specific MAb against atrazine than three MAbs and the titer of AT-1-M3 MAb was highest. Therefore, we selected AT-1-M3 MAb to develop in DC-ELISA and ICG strip. In addition, we thought that the specificity of our MAb was higher than that observed in the previously developed MAb. According to Thomas (*13*), several MAbs showed high cross-reaction with simazine, captan, and terbutryne, whereas our MAb showed little cross-reaction with simazine and no cross-reaction with other pesticides. If this MAb will be empolyed in immunoassay, specificity immunoassays such as ELISA and ICG strips for atrazine detection could be established.

DC-ELISA. In some previous works, the application of a precoating step with anti-mouse immunoglobulins (IgGs) to orient MAb to the competing reagents was described (*2*, *13*). This method was shown to provide the best immunoassay performance for atrazine and other pesticides with the use of MAbs. However, this method required complicated assay procedures and was time-consuming. Therefore, we studied a rapid-coating method of MAb without anti-mouse IgG trapping. Three coating buffers, 0.1 M carbonate buffer (pH 9.0), 0.01 M phosphate buffer saline (PBS, pH 7.4), and 0.01 M PBS containing 0.01% BSA, were evaluated for diluting MAb. Ninety-six well microtiter plates were coated with 100 μL of diluted MAb solution (1.43 μg/mL) using the three coating buffers. The DC-ELISA was carried out as previously described. As shown in **Figure 2**, all of the tested coating buffers showed a high absorption rate of MAb to 96 well microtiter plates. A 0.1 M concentration of carbonate buffer showed a highest signal at 0 ng/mL of atrazine, but sensitivity of ELISA (IC_{50} = 18.5 ng/mL) was lower than other coating buffers. PBS containing 0.01% BSA was shown as a lower signal than 0.1 M carbonate

Table 1. Cross-Reactivity of MAb AT-1-M3 to Atrazine and Related Compounds

Analogues	Structure	Cross-reactivity(%)
Atrazine		100
Simazine		23
Alachor		<1
Captan		<1
Terbutryne		<1
Bromacil		<1
Parathion-methyl		<1
2,4-D		<1

buffer, but the sensitivity of ELISA was highest (IC_{50} = 8.8 ng/mL). In addition, our coating system was compared with another coating system using anti-mouse IgG trapping. DC-ELISA with anti-mouse IgG trapping required 1/15000 dilution of MAb, whereas DC-ELISA without anti-mouse IgG trapping required a high concentration of MAb (1/7000 dilution). The IC_{50} value of coating systems with and without anti-mouse IgG trapping was 8.8 and 9.5 ng/mL, respectively. Although the coating system with anti-mouse IgG trapping was more efficient for MAb consumption, the developed coating system in this study showed high sensitivity. Besides, our coating system was easy to perform and not time-consuming. Therefore, we selected our system for the coating of MAb to 96 well microtiter plates. The typical standard curve of DC-ELISA without anti-mouse IgG is presented in **Figure 3**. The IC_{50} value was 8.8 ng/mL, and the detection limit was 0.5 ng/mL (10% inhibition). We think that this method should become the accurate and detectable method for atrazine in various water samples.

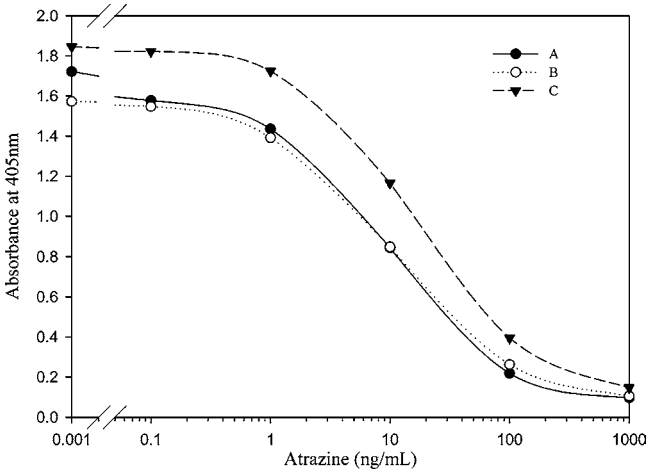


Figure 2. DC-ELISA standard curves for atrazine using different buffers for the dilution of MAb. The labels A, B, and C show 0.01 M PBS containing 0.01% BSA (IC_{50} = 8.8 ng/mL), 0.01 M PBS (IC_{50} = 10.6 ng/mL), and 0.1 M carbonate buffer (IC_{50} = 18.5 ng/mL), respectively.

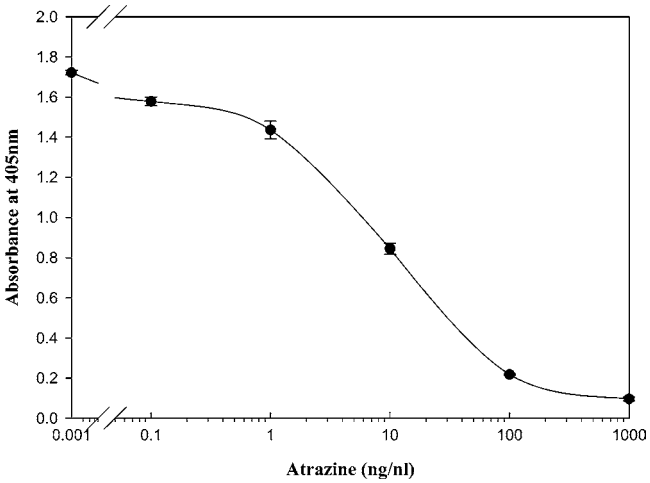


Figure 3. DC-ELISA standard curve for atrazine detection in buffer with 10% methanol. The procedures are described in the text. Each point of the curve represents the mean ± SD (standard deviation) of n = 4 assays on the same day.

Labeling of the Antibody with Colloidal Gold. Colloidal gold has been generally used as an immunospecific probe for immunocytochemistry and immunoblotting (*26*, *27*). Many scientists recognized that colloidal gold particles could be applied in immunoanalysis, biosensors, gene therapy, and DNA computation (*28*). The size of the colloidal gold particles is directly dependent on the amount of sodium citrate used in its preparation process. The strength of color is closely related to the size and quality of the colloidal gold particles. In some previous papers, they represented that 40 nm colloidal gold particles offered maximum visibility due to the least steric hindrance in the case of IgG conjugation (*29*). Therefore, the 40 nm colloidal gold particles were selected in our study. Spherical gold particles with a diameter of 40 nm formed when 1 mL of sodium citrate was added to 100 mL of 0.01% gold chloride solution (**Figure 4**). With the increase of sodium citrate volume up to 2, 3, and 4 mL, the diameter of the colloidal gold particles uniformly decreased to 20, 15, and 10 nm, respectively. With 1−4 mL of the sodium citrate, the color of colloidal gold was reddish or red-orange. However, when the amount of sodium citrate added was 0.5 mL, the color of the obtained colloidal gold particles was purple and the diameter of the particles was >100 nm.

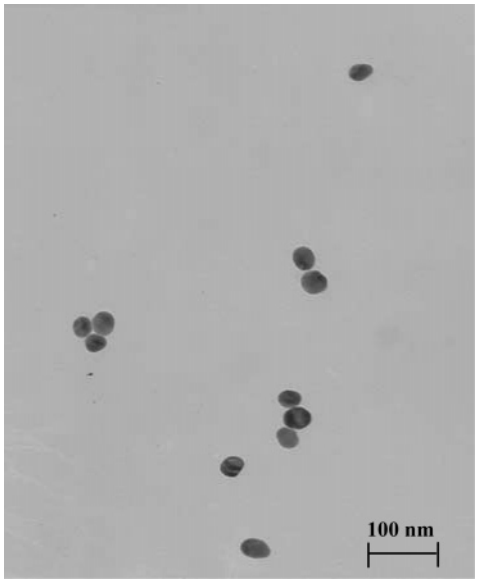


Figure 4. Colloidal gold observed by transmission electron microscope (110000×). The diameter of those particles was 35–40 nm.

Table 2. Determination of Minimum Amount of AT-1-M3 MAb for the Conjugation with Colloidal Gold Particles[a]

	atrazine standard concentration (ng/mL)			
	0		1000	
amount of AT-1-M3 MAb (μg/mL)	test line	control line	test line	control line
2	±	+	±	+
3	+	+	−	+
4	+	+	−	+
5	+	+	−	+

[a] Key: +, an obvious red band was observed; ±, a faint band was observed; and −, no band was observed.

For conjugation of colloidal gold and MAb, a minimum amount of antibody should be needed for the stabilization of colloidal gold particles. The minimum amount of MAb was determined by adding NaCl to colloidal gold particles containing different amounts of MAb. After reaction with NaCl, a colloidal gold solution containing minimum MAb could be kept reddish or red-orange color. In our experiment, 3 μg of MAb was confirmed to be the minimum amount for stabilization of colloidal gold. However, we selected 4 μg of MAb for the conjugation because 3 μg of MAb in our study often caused coagulation of gold particles during the conjugation (**Table 2**).

ICG Strip. The main objective of the ICG strip was qualitative detection of atrazine contamination at threshold levels. For this, the color intensity of the test line must be high enough to be seen and enable observation of difference color intensities between negative controls and positive controls. For the development of a sensitive ICG strip, we checked to determine the optimal immobilization amount of atrazine−BSA conjugate as a capture reagent and the optimal amount of colloidal gold−MAb probe as the detector reagent. The optimal concentration of capture reagent and the amount of detector reagent were selected as a clear appearing in the negative control with the shortest time and comparison of the intensity of color between positive samples and negative samples, and the positive sample could be easily distinguished from the negative sample by eye. The optimal conditions for ICG strips were as follows: 1.5 μg of atrazine−BSA conjugate was treated in the test line on the membrane, and 5 μL of colloidal gold−MAb probe was sprayed on the conjugate pad.

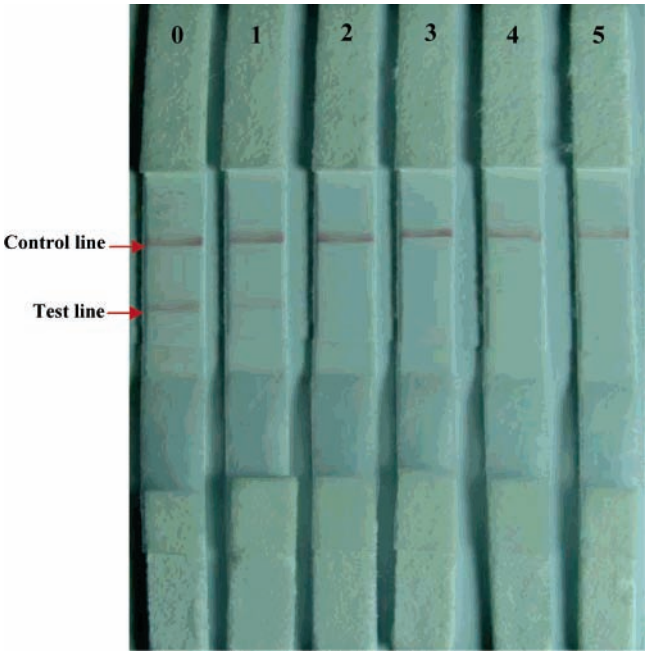


Figure 5. Detection limit of ICG strip for the detection of atrazine. The tests were run four times at room temperature using distilled water samples spiked with atrazine standard. The labels (0–5) show the concentrations of atrazine (ng/mL).

Table 3. Cross-Reactivity of the ICG Strip to Other Pesticides[a]

		concentration (ng/mL)						
compound		0	1	5	10	50	100	500
atrazine	test line	+	±	−	−	−	−	−
	control line	+	+	+	+	+	+	+
simazine	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
alachor	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
captan	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
terbutryne	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
bromacil	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
parathion-methyl	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+
2,4-D	test line	+	+	+	+	+	+	+
	control line	+	+	+	+	+	+	+

[a] Key: +, an obvious red band was observed; ±, a faint band was observed; and −, no band was observed.

The detection limit is defined as the concentration of atrazine in the solution that causes a complete invisibility of the test line. A series of dilutions of atrazine standard (0−5 ng/mL) were tested by ICG strips. As shown in **Figure 5**, the results of this test were judged within 10 min after the reaction started. Two red lines on the membrane indicated that the atrazine concentration was below the detection limit. If one red line was shown in the control line, the atrazine concentration was above the detection limit. The detection limit was estimated as 3 ng/mL for ensuring the detection results because the concentration of 2 ng/mL atrazine was shown as a weak red line in the test line. The specificity of the ICG strip was evaluated in comparison to other pesticides. As shown in **Table 3**, two clear red lines were observed in the test and control lines on various

Table 4. Results of Spiked Water Sample Analysis by DC-ELISA and ICG Strip

		DC-ELISA		ICG strip	
sample	spiked atrazine concn (ng/mL)	detected atrazine conn (ng/mL)	recovery (%)	test line	control line
drinking water ($n = 4$)	0	ND[a]	NC[b]	+[c]	+
	5	4.2 ± 0.4	84 ± 8	–[d]	+
	10	8.8 ± 0.4	88 ± 4	–	+
	20	19.1 ± 0.3	96 ± 2	–	+
	50	48.2 ± 2.4	96 ± 5	–	+
tap water ($n = 4$)	0	ND	NC	+	+
	5	4.6 ± 0.3	92 ± 6	–	+
	10	9.1 ± 0.3	91 ± 3	–	+
	20	18.6 ± 0.2	93 ± 1	–	+
	50	43.0 ± 1.3	86 ± 3	–	+
surface water ($n = 4$)	0	ND	NC	+	+
	5	4.1 ± 0.2	82 ± 4	–	+
	10	8.5 ± 0.2	85 ± 2	–	+
	20	17.6 ± 0.3	88 ± 2	–	+
	50	46.0 ± 3.0	92 ± 6	–	+

[a] ND, not detected. [b] NC, not calculated. [c] Key: +, an obvious red band was observed. [d] Key: –, no band was observed.

concentrations of other pesticides. This indicates that the developed ICG strip had a high specificity to atrazine without cross-reaction for other pesticides.

Sample Analysis. The water samples spiked with atrazine standard were determined to validate the reliability of ICG. The atrazine negative samples determined by HPLC were spiked with 5, 10, 20, and 50 ng/mL of atrazine and then analyzed by DC-ELISA and ICG. The recovery by DC-ELISA ranged from 78 to 101.2%. In the ICG test, samples without atrazine spiked were judged negative, but positive results were obtained from the samples spiked with various atrazine concentrations (**Table 4**). A total of 50 water samples obtained from wells, water supply facilities, streams, and rivers in Chinju (Gyeongnam Province, Korea) were treated as described above. All water samples were analyzed by DC-ELISA and ICG. No positive water samples were observed (data not shown). Because of the prohibition of atrazine by the World Health Organization and the U.S. EPA in the 1990s, this pesticide is not used by Korean farmers nowadays.

In conclusion, the ICG strip with a colloidal gold−MAb probe was developed for the detection of atrazine in various water samples. The detection limit was 3 ng/mL. The results observed for water samples spiked with atrazine standard by the ICG strip were in good agreement with those obtained by DC-ELISA (**Table 4**). The ICG strip is easy to perform, and results can be obtained within 10−15 min without the need for expensive equipment, washing, and/or separation steps. Moreover, with respect to its overall speed and simplicity, the assay is superior to other immunoassays, such as radioimmunoassay and ELISA. The method has a potential as a rapid, cost-effective on-site screening tool for pesticide contamination in agricultural and environmental samples. The assay can be applied, for example, by water inspection centers and/or beverage companies, for the preliminary screening of water samples for pesticide residues.

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