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Development and Validation of a Gold Nanoparticle Immunochromatographic Assay (ICG) for the Detection of Zearalenone

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A monoclonal antibody (mAb)-based gold nanoparticle immunochromatographic assay (ICG) for zearalenone detection was developed, optimized, and validated. The detection limits of ICG optimized with appropriate amounts of zearalenone−bovine serum albumin and gold nanoparticle−mAb to zearalenone were 2.5 ng/mL and 30 μg/kg for the standard solution and spike sample, respectively, and a weak cross-reaction for α-zearalenol and β-zearalenol was observed. The assay required only 15 min to obtain results and one step to perform the assay. In validation, the results obtained from spiked corn (10, 20, 30, 50, and 100 μg/kg) and naturally contaminated corn samples by the ICG were in good agreement with those obtained by direct competitive enzyme-linked immunosorbent assay (DC-ELISA) and high-performance liquid chromatography (HPLC). Therefore, the results obtained in this study could be used as basic research for the development of zearalenone−ICG, and the ICG developed could be a useful on-site screening tool for the rapid detection of zearalenone in corn without special instrumentation.

KEYWORDS: Zearalenone; immmunochromatographic assay; validation; gold nanoparticle; corn

INTRODUCTION

Fungi and their mycotoxins often contaminate foods and agricultural products. *Fusarium* species are probably the most prevalent toxin-producing fungi found on cereals grown in the temperate regions of America, Europe, and Asia. *Fusarium* toxins have been known to cause a variety of toxic effects in animals and humans (*1*).

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)presorcylic acid lactone] is a secondary metabolite and a nonsteroidal estrogenic mycotoxin produced by *Fusarium* fungi (*2*). Although zearalenone has a relatively low acute toxicity (oral LD_{50} value = 20 g/kg of bw) after oral administration in animals, it has been associated with early puberty and hyperplasic and neoplastic endometrium and human cervical cancer (*3*). The toxic effects of zearalenone usually originate from its estrogenic properties (*4*). The U.S. Food and Drug Administration and the Korean Food and Drug Administration have no legal regulations for zearalenone, but several countries have set legal limits for zearalenone, ranging from 0.02 to 1000 μg/kg, that apply to both specific foodstuffs and all foods (*5*). Therefore, no international legal limit has been set for zearalenone.

For minimization of zearalenone risk to humans and animals, much research haa been conducted to develop sensitive and specific methods for zearalenone determination. Thin-layer chromatography (TLC) (*6*), gas chromatography−mass spectrometry (GC-MS) (*7*), and high-performance liquid chromatography (HPLC) with fluorescence or mass spectrometry (*8*, *9*) are accepted for the analysis of zearalenone in cereals, but most of these methods are laborious, expensive, and time-consuming and unsuitable for analysis of the routine screening of large sample numbers. On the other hand, several immunological methods such as enzyme-linked immunosorbent assay (ELISA) (*10*, *11*), fluorescence polarization immunoassay (FPIA) (*12*), dipstick immunoassay (*13*), and automatic flow though immunosensor (*14*) have been developed and found widespread application as rapid screening techniques for zearalenone because of their sensitivity, specificity, rapidity, and suitability for high sample throughput. However, they still require long reaction time, expensive instruments, and complicated steps, and their use is limited to laboratories equipped with special instruments and devices, so they are also unsuitable for on-site screening.

Recently, many kinds of immunochromatographic assays (ICG) using gold nanoparticle−antibody conjugates have been developed for the detection of low molecular weight materials, such as mycotoxins (*15*, *16*), pesticides (*17*, *18*), and antibiotics, (*19*, *20*) in food and agricultural products. Several papers have reported ICG methods for aflatoxin B_1 and ochratoxin A (*15*, *16*, *21*, *22*), but no paper with a detailed description for the optimization and development of ICG for zearalenone has been reported so far.

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In the present study we developed an ICG method for the detection of zearalenone. The ICG was optimized, validated, and applied to the analysis of zearalenone in corn samples, and its results are compared with those of direct competitive ELISA and HPLC.

MATERIALS AND METHODS

Chemicals and Reagents. Zearalenone, other related mycotoxins, bovine serum albumin (BSA), horseradish peroxidase (HRP), Tween 20, sucrose, dextran, tetrachloroauric acid, complete and incomplete Freund's adjuvant, sodium citrate, and goat anti-mouse−IgG (whole molecule) were purchased from Sigma Chemical Co. (St. Louis, MO). 3,3′,5,5′-Tetramethylbenzidine (TMB) was obtained from Zymed Laboratories (San Francisco, CA). The protein G agarose was purchased from Bioprogen (Daejeon, South Korea). The maxisorp polystyrene 96-microwell plates were obtained from Nunc (Roskilde, Denmark). All other chemicals and organic solvents were of analytical grade or higher.

Sample pad, nitrocellulose membrane, conjugate pad, and absorbent pad were provided from Millipore (Bedford, MA). Semirigid polyethylene sheets were purchased from a local market. Water used in all experiments was purified with a NANOpure system of Barnstead International (Dubuque, IA). Standard solutions of zearalenone and other mycotoxins were prepared by dilution of stock solutions of 1 mg/mL in methanol.

Zearalenone−BSA conjugate was synthesized by the active ester method described in a previous paper (*23*), and zearalenone−HRP conjugate was also prepared according to the active ester method with modification (*11*). Zearalenone−BSA conjugate was immobilized as a capture reagent at test line on a membrane of the ICG, and zearalenone−HRP conjugate was employed as a competitor to free zearalenone in direct competitive ELISA (DC-ELISA).

Preparation of Monoclonal Antibody. The monoclone hybridoma named 2C5 was developed by cell fusion using myeloma cells and spleen cells obtained from mice immunized with zearalenone−BSA conjugate and expended in cell culture medium and intraperitoneally injected into BALB/c mice that had been pretreated with an intraperitoneal injection of 0.5 mL of pristine. Ascites fluid was taken from the mice and purified by precipitation with saturated ammonium sulfate followed with affinity chromatography on a protein G agarose. Protein concentration of the purified monoclonal antibody (mAb) was determined with a protein assay kit (Bio-Rad Laboratories, Richmond, CA). In a previous paper by one of the current authors (*11*), the mAb has already been confirmed to be highly specific to zearalenone and other related mycotoxins (α-zearalenol, β-zearalenol, α-zearalanol, and β-zearalanol) by DC-ELISA.

Preparation of Gold Nanoparticle. Gold nanoparticles with a mean diameter of 40 nm were prepared according to the method of Frens (*24*) and were used as a marker in immunochromatographic assay. Procedures for the preparation of gold nanoparticle solution were as follows: 100 mL of 0.01% tetrachloroauric acid was heated to boiling point under stirring, and 1 mL of sodium citrate (1%) was rapidly added. The reaction solution was stirred and boiled constantly until the color change from purple to reddish-orange (approximately 5 min) and was cooled at room temperature. The average particle diameter was determined to be 35−40 nm by transmission electron microscope. The gold nanoparticle solution could be stored at 4 °C for several months.

Synthesis of mAb−Gold Nanoparticle Conjugate. The mAb−gold nanoparticle conjugate was prepared according to the method of Roth (*25*). The purified 2C5 mAb (0.1 mg) was dissolved in 1 mL of 2 mM borax ($Na_2B_4O_7 \cdot 10H_2O$), and the gold nanoparticle solution was adjusted to pH 8.5 with 0.1 M K_2CO_3. To determine a minimum amount of mAb to stabilize the gold nanoparticle solution, 500 μL of gold nanoparticle solution was added to series tubes and the mAb was added to each tube from 0 to 75 μL. All tubes were made up to 575 μL with 2 mM borax, mixed, and incubated at room temperature for 3 min. Fifty microliters of 10% NaCl was added to all tubes and agitated for 1 min, and then the solution was measured with a spectrophotometer at 540 nm. If a tube contained a minimum amount of the mAb for stabilization of gold nanoparticle solution, the color did not change to blue. Fifteen microliters of mAb was found to be the minimum amount to stabilize 500 μL of the gold nanoparticle solution. Thus, 10 mL of gold nanoparticle was added dropwise with 300 μL of mAb solution (0.1 mg/mL) and incubated at room temperature for 1 h under stirring. After 1 h, 1.4 mL of 10% BSA was added to block residual surfaces of the gold nanoparticles. The mixure was incubated for 1 h at room temperature and centrifuged for 15 min at 10000 rpm, and then the supernatant was discarded. The pellets were washed three times with 2 mM borate buffer (pH 7.2), and the final pellets were suspended in 1 mL of 2 mM borate buffer (pH 7.2) containing 1% BSA, 1% sucrose, and 0.05% sodium azide. The mAb−gold conjugates were stored at 4 °C and diluted in 20 mM borate buffer (pH 8.2) containing 1% BSA and 1% sucrose before use.

Development of Immunochromatographic Assay for Zearalenone. Generally, an immunochromatographic assay (ICG) consisted of three pads (sample, conjugate, and absorbent pad) and a nitrocellulose (NC) membrane. The sample pad was treated with 50 mM borate buffer (pH 7.4) containing 1% BSA, 0.5% Tween 20, 5% sucrose, 5% dextran, and 0.05% sodium azide and then dried at 60 °C for 2 h. The conjugate pad was saturated with 5 μL of mAb−gold conjugate (absorbance at 540 nm) and dried at 37 °C for 30 min. The NC membrane was treated with the zearalenone−BSA conjugate [0.7 μL, 0.007 mg/mL in phosphate buffer saline (PBS, pH 7.4)] and anti-mouse IgG (0.7 μL, 0.05 mg/mL in PBS) at the test and control lines by a manual dispenser fabricated in our laboratory. The treated conjugate pad and NC membrane were dried at 37 °C for 30 min. All pads and the NC membrane were placed on semirigid polyethylene sheets. The NC membrane was pasted at the center of the semirigid polyethylene sheet, and the conjugate pad was attached by overlapping 2 mm with the NC membrane. The sample pad was also pasted by overcrossing 4 mm with the conjugate pad, and the absorbent pad was placed to the other side of the NC membrane with an overlap of 3 mm. The standard solutions or sample extracts (200 μL) were placed into wells of microtiter plate. The sample pad of the ICG was immersed in the standard or extract solution, and then the liquid migrated up the ICG into the absorbent pad. After 15 min, test results were evaluated visually. The negative samples resulted in color development at the test and control zones, whereas the positive samples gave only one red line at the control region. If no color development was observed at the control zone, the performance of the ICG strip was invalid.

Validation of ICG. Validation of the ICG strip was carried out by the determination of cross-reactivity for zearalenone-related compounds and matrix effect from samples and by analyzing corn samples spiked with various concentrations of zearalenone. For determination of cross-reactivity, other mycotoxin standards at different concentrations (0, 1, and 5 ng/mL) were prepared in PBS containing 10% methanol and 0.05% Tween 20 (10% MeOH/PBST), and they were applied to the ICG strip.

Zearalenone-free corn samples confirmed by HPLC were ground, and 1 g of NaCl was added to 4 g of the ground samples. The mixture were extracted with 20 mL of methanol/water (60:40, v/v) for 20 min at room temperature and then centrifuged at 3000 rpm at 4 °C for 10 min. The supernatants were diluted 2-fold with PBS for minimization of matrix and methanol influence, and the diluents were directly applied to ICG. Zearalenone-positive corn samples were prepared by spiking with a known amount of zearalenone at final concentrations of 10, 20, 30, 50, and 100 μg/kg to zearalenone-free corn, and the samples were dried at 60 °C for 1 h. The spiked samples were extracted and diluted as described above. The positive samples for zearalenone were prepared five times on different days and analyzed by the ICG.

DC-ELISA Procedures. Anti-mouse IgG (100 μL, 2.5 μg/mL in PBS) was immobilized on wells of 96-well microtiter plate overnight at 4 °C and then washed with PBS containing 0.05% Tween 20 (PBST) by using an 8-channel microtiter plate washer. The wells were coated with 100 μL of zearalenone 2C5 mAb (1.5 μg/mL in

PBS) for 1.5 h at 37 °C and then washed with PBST. Zearalenone standards in 10% MeOH/PBS (50 μL) or samples (50 μL) and 50 μL of zearalenone−HRP solution (1:10000 in 1% BSA) were added to the wells. After incubation for 30 min with shaking at 37 °C, the plates were washed, and 100 μL of TMB substrate solution was added. After incubation for 30 min at room temperature, color development was stopped with 1 M HCl (50 μL per well), and the absorbance was measured at 450 nm using a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Richmond, CA).

Analysis of Zearalenone in Corn Samples. Thirty-eight corn samples were obtained from the Department of Crop Life Safety, National Institute of Agricultural Science and Technology, Korea. Zearalenone extraction was performed as described above, and the extracts were analyzed by the ICG. The results were compared with those obtained by DC-ELISA and HPLC. Sample preparation for ELISA analysis was similar to that for ICG strip analysis with the exception that the extracts were diluted 6-fold with PBS before analysis. The pretreatment of sample and HPLC performance were performed according to that described previously (*11*).

RESULTS AND DISSCUSSION

Fitness of Gold Nanoparticle−mAb Probe in Development of ICG. Gold nanoparticle particles (35−40 nm diameter), obtained by chemical reduction of chloroauric acid ($HAuCl_4$) using sodium citrate, were coupled with 2C5 mAb for use as a marker in the ICG. The mAb was directly adsorbed on the surfaces of gold nanoparticles by van der Waals force and hydrophobic interaction. To attain a strong adsorption between the gold nanoparticles and mAb, a preliminary titration was performed by comparing the absorption at 540 nm after the addition of sodium chloride to gold nanoparticle solution containing different amounts of antibody. Coating the gold particle with the optimal amount of antibody can prevent degradation of the gold surface and keep the color of the gold nanoparticle solution (red wine color). According to the results of the preliminary titration, the minimal amount of zearalenone−mAb to stabilize the gold nanoparticle was approximately 3 μg per 1 mL of gold nanoparticle. However, we performed the synthesis of gold nanoparticle−antibody probe with 4 μg/mL of mAb because 3 μg/mL of mAb often caused coagulation of gold particles during the synthesis.

After the synthesis of the gold nanoparticle−antibody probe, the suitability of the probe was tested on the strip and was compared with that of BSA−gold conjugate used as a control test (**Figure 1**). In the case of the control test, there is no red line on the membrane after testing with 10% MeOH/PBST. This result means that BSA used as a blocking reagent for gold particles does not cause a nonspecific binding to coating antigen (zearalenone−BSA conjugate) and anti-mouse IgG immobilized on test and control lines. Color development was observed at test and control lines, when the ICG strip was assembled with the conjugate pad containing gold−2C5 mAb probe and performed with zearalenone negative (0 ng/mL in 10% MeOH/PBST). However, only one red line at the control line appeared in the application of zearalenone positive (10 ng/mL in 10% MeOH/PBST). This result indicates that the gold−2C5 mAb probe is specific to zearalenone and can be used as a marker in the development of the ICG.

Optimization of ICG. In the inhibition assay for mycotoxins, decreasing concentration of immunoreagents (antibody and coating antigen) increases the sensitivity of the assay. To develop a sensitive ICG, checkerboard assays were performed to determine the optimum amount of gold−2C5 mAb probe (3, 5, and 7 μL) and zearalenone−BSA conjugate

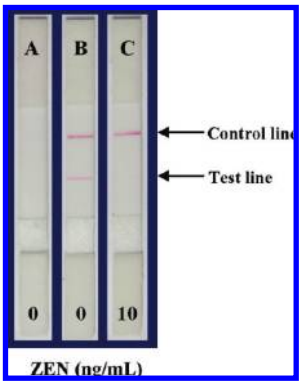

Figure 1. Confirmation of zearalenone mAb−gold conjugate: (**A**) the conjugate pad contained BSA−gold conjugate; (**B**, **C**) the conjugate pads contained 2C5 mAb−gold conjugate.

(0.005−0.01 mg/mL in PBS), and the results are represented in **Figure 2**. The ICG treated with zearalenone−BSA conjugate of 0.007 mg/mL showed the clear, stable, and high intensity of the red band at the test, and its detection limit was the lowest (2.5 ng/mL) (**Figure 2A**). In addition, 5 μL of gold−2C5 mAb probe (absorbance at 540 nm was 1.5) was confirmed to be the minimum amount for developing a sensitive ICG because 3 μL of the probe often showed a faint red color, which was difficult to judge as a result, at the test line, and 7 μL of the probe represented lower sensitivity than 5 μL of the probe (**Figure 2B**). Thus, the gold−2C5 mAb probe at 5 μL was treated to the conjugate pad in further experiments.

Because different organic solvents (methanol, ethanol, and acetonitrile) used for the extraction of zearalenone from food samples generally extract various substances existing in complex matrices and it affects antigen−antibody interaction in immunoassays, the appropriate kind and concentration of organic solvent were examined by preparing standard solutions in PBST containing 10% of each organic solvent and various concentrations (0−60% in PBST) of organic solvent. The red bands at the control and test lines were developed more quickly and with higher intensity using methanol, whereas the presence of ethanol and acetonitrile gave a higher effect to decrease the intensity of the test line and to increase the assay times. The effect was lowest at 10% MeOH/PBST with an assay time of only 15 min (data not shown). Thus, we selected 10% methanol in PBST as a working solution for the ICG strip.

Characterization and Validation of ICG. After optimal conditions of the ICG for zearalenone were obtained, the detection limit and cross-reactivity of the method were investigated with zearalenone standard solutions (200 μL) at 0, 1, 2.5, 5, and 10 ng/mL and other mycotoxins (200 μL) at 1 and 5 ng/mL in 10% MeOH/PBST. The existence of control and test lines on the NC membrane means that the zearalenone concentration is below the detection limit, and the result is concluded to be negative, whereas the emergence of only the control line indicates that the test is positive, and zearalenone concentration was above the detection limit. As shown in **Figure 3**, the sensitivity of the ICG was confirmed to be 2.5 ng/mL, and qualitative results were obtained within 15 min. To ensure the cutoff level of the ICG, 10 measurements with the ICG were tested by using the zearalenone standard solutions. According to the cross-reactivity test, the ICG developed was confirmed to possess cross-reaction to α-zearalenol and β-zearalenol.

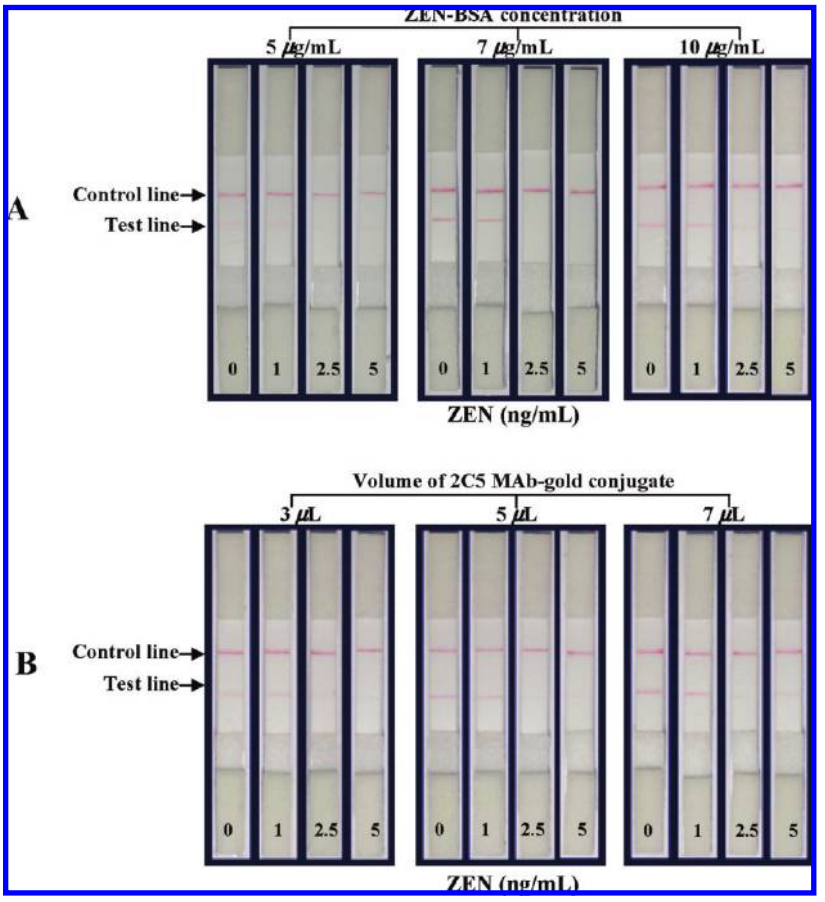

Figure 2. Determination of optimal zearalenone−BSA conjugate concentration (**A**) and 2C5 mAb−gold conjugate volume (**B**) for test line on nitrocellulose membrane and conjugate pad, respectively. Nitrocellulose membrane and conjugate pad were dried for 30 min at 37 °C after treatment.

However, the cross-reactivities of α-zearalanol and β-zearalanol, T-2 toxin, deoxynivalenol, nivalenol, aflatoxin B_1, ochratoxin A, patulin, and citrinin were negligible by the presence of the red band at the test line (**Figure 4**). Although Kolosova et al. (*26*) recently reported a lateral-flow immunoassay based on immunochromatographic assay for the simultaneous determination of zearalenone and deoxynivalenol, the detection limit of that method for zearalenone was 100 ng/mL, which is not enough to be applied in real samples. Meanwhile, we consider that the ICG for zearalenone developed in this study possesses a higher sensitivity than that developed by Kolosova et al. and is suitable for on-site determination of zearalenone.

The validation of the ICG was performed by analyzing the spiked samples with zearalenone at five levels (10, 20, 30, 50, and 100 μg/kg). The zearalenone-positive samples were prepared as described above and analyzed on different days (5 days) by the ICG and DC-ELISA. As presented in **Table 1**, clear red lines on the test zone appeared for corn spiked with 0, 10, and 20 μg/kg zearalenone, but no red lines on the test zone were obtained from application of corn spiked with 30, 50, and 100 μg/kg zearalenone. We decided on 30 μg/kg zearalenone as the cutoff value of the ICG for corn. After sample preparation, the concentration of zearalenone in the corn spiked with 30 μg/kg zearalenone will be 3 ng/mL. Fortunately, this level could be detected by the ICG. Therefore, we are certain that the ICG developed in our study possesses sufficient sensitivity for the rapid detection of zearalenone in corn without special instrumentation within maximum levels established by several countries (*5*). The spiked corn samples were further tested to determine

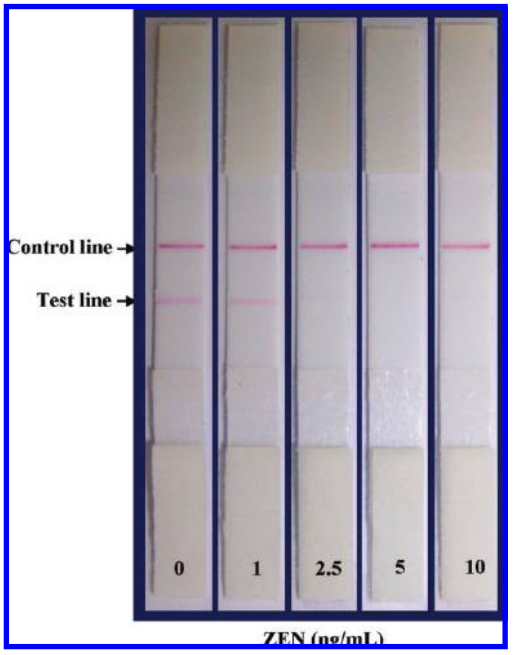

Figure 3. Detection limit of the ICG for the detection of zearalenone. The tests were run four times using various concentrations of zearalenone in 10% methanol/PBST.

recovery by DC-ELISA, and the recoveries averaged from 90.65 to 116.54% (**Table 1**).

Screening of Zearalenone in Corn. We used DC-ELISA and the ICG strip developed in this study and HPLC to

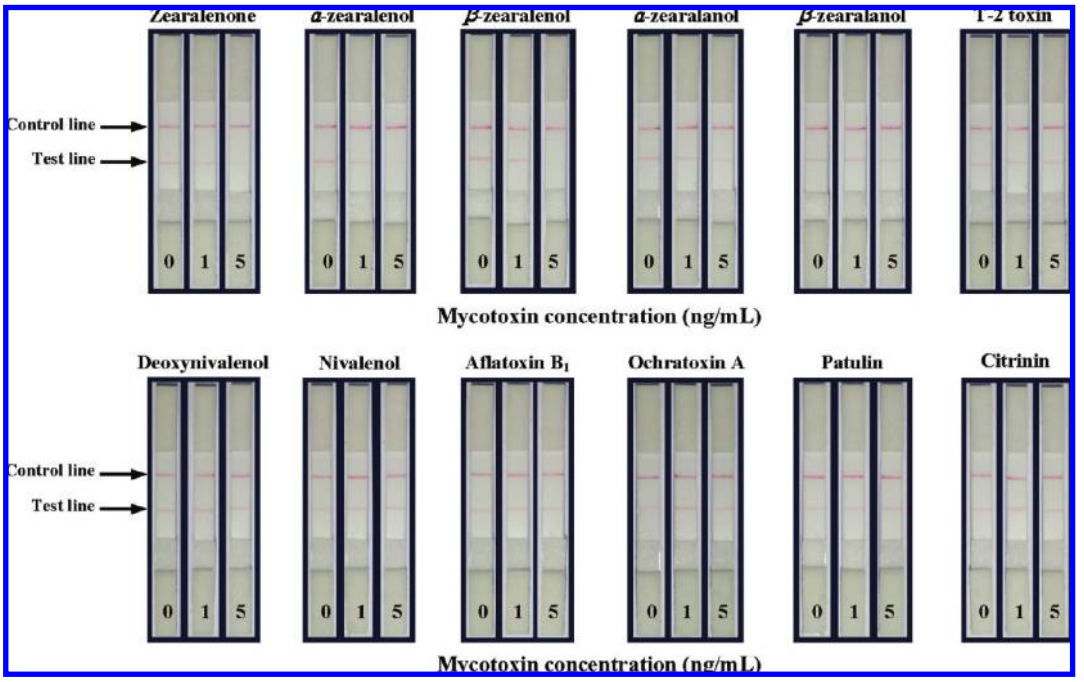

Figure 4. Cross-reactivity of zearalenone-related compounds and other mycotoxins by ICG.

Table 1. Analysis of Artificially Spiked Corn Samples[a] by the ICG and DC-ELISA Methods

	DC-ELISA		ICG	
zearalenone spike level (μg/kg)	detected zearalenone (μg/kg), mean ± SD	recovery (%), mean ± SD	test line	control line
0	ND	NC[b]	+,[c] +, +, +, +	+, +, +, +, +
10	11.7 ± 0.7	116.5 ± 7.4	+, +, +, +, +	+, +, +, +, +
20	20.5 ± 0.1	102.5 ± 0.5	+, +, +, -, +	+, +, +, +, +
30	34.4 ± 0.4	114.7 ± 1.3	−,[d] −, −, −, −	+, +, +, +, +
50	45.3 ± 2.5	90.7 ± 5.0	−, −, −, −, −	+, +, +, +, +
100	94.1 ± 5.5	92.3 ± 4.6	−, −, −, −, −	+, +, +, +, +

[a] All samples were prepared five times on different days and extracted as detailed under Materials and Methods. [b] Not calculated. [c] An obvious red band was observed. [d] No band was observed.

screen zearalenone occurrence in corn samples. Thirty-eight corn samples obtained were analyzed by the optimized DC-ELISA and ICG, and the results were compared with those of HPLC. Among 38 corn samples, zearalenone was detected in 9 and 7 samples by DC-ELISA and ICG, respectively (**Table 2**). DC-ELISA (detection limit = 0.35 ng/mL) has a higher sensitivity than the ICG strip (detection limit = 2.5 ng/mL) for the detection of zearalenone in corn samples, and the IC_{50} value of the DC-ELISA was 2.3 ± 0.3 ng/mL. Therefore, DC-ELISA could detect a low concentration of zearalenone in corn and showed more zearalenone positive samples than did ICG. Even if the ICG strip showed three false negatives in 35 tests of 7 positive samples, the error (8.5%) was negligible. To get an exact result, analysis by ICG strip should be tested more than two times. Because the assay time and testing procedures of the ICG are shorter and simpler than those of DC-ELISA and the final results are assessed visually by naked eyes without special devices, the ICG strip could be a useful tool for on-site and rapid screening of zearalenone in corn samples.

The positive samples analyzed by DC-ELISA and ICG were confirmed by HPLC to get more accuracy and quantitative results. As shown in **Table 2**, seven samples (C8, C9, JH3, JH4, YH1, YH2, and YH4) were found to be zearalenone positive. The results of zearalenone determination in naturally contaminated corn samples by the DC-ELISA and the ICG showed very good agreement with HPLC analysis. Especially, the samples found to be zearalenone positive by ICG and HPLC were identical.

Correlation between DC-ELISA, ICG, and HPLC was quite good, although there are some variations. However, there was very good correlation between ICG and HPLC for naturally contaminated corn samples. This study demonstrated that the determination of the concentration of calibrator solutions is still a serious problem for zearalenone, and extensive investigations on the standartization of the methods are needed.

There are several studies from Korea about the occurrence of zearalenone in different commodities. Of 164 samples (corn, corn-based foods, barley, barley-based foods, and dried fruit), zearalenone was detected in 35 samples (21%) with a mean level of 30 μg/kg (*27*). It should be noted that barley and barley-based foods had a higher incidence of zearalenone than corn and corn-based foods. Shon et al. (*28*) found that zearalenone was a minor contaminant in Korean corn. According to a recent investigation in Korea (*29*), only 3 of 88 rice samples were positive relative to zearalenone, and the mean zearalenone level was 38.5 μg/kg (21.7−47 μg/kg). In our work, zearalenone was found at a maximum level of 686 μg/kg by HPLC in corn and the zearalenone levels are

Table 2. Comparison of DC-ELISA, ICG, and HPLC Methods for Detecting Zearalenone from Naturally Contaminated Corn Samples

	zearalenone positive samples by		
corn	DC-ELISA (μg/kg)	ICG	HPLC (μg/kg)
C1	109.1 ± 14.8	−,[a] −, −, −, −	−
C6	41.0 ± 2.6	−, −, −, −, −	−
C8	909.8 ± 49.5	+,[b] +, +, +, +	686
C9	115.3 ± 9.3	+, +, +, +, −	192
JH3	645.1 ± 48.2	+, +, +, +, +	330
JH4	113.4 ± 13.6	+, −, +, +, +	218
YH1	343.5 ± 19.3	+, +, +, +, +	494
YH2	218.4 ± 24.7	+, −, +, +, +	474
YH4	554.8 ± 31.1	+, +, +, +, +	609
total	9	7	7

[a] zearalenone negative sample. [b] zearalenone positive sample.

significantly different from those before. Similar levels were found in previous papers by Scott (*30*) and Silva and Vargas (*8*) on the determination of zearalenone in a Canadian grain survey from 1986 to 1993 and in Brazilian corn, respectively. In this study, we confirmed the occurrence of zearalenone in corn from Korea. Therefore, although there is no regulation for zearalenone in food in Korea, continuous monitoring for zearalenone is necessary. The ICG method developed in this study could be applied to on-site monitoring of zearalenone in food and agricultural products because the method does not require complicated assay steps and instruments for judgment of assay results.

In conclusion, the ICG was developed, optimized, and validated for the detection of zearalenone in corn samples. The ICG strip was sufficiently sensitive and accurate to be useful as a rapid and on-site screening tool for the detection of zearalenone in food and agricultural products. The detection limits of the method were 2.5 ng/mL and 30 μg/kg for the standard and corn samples, respectively. The results obtained from the spiked corn and naturally contaminated corn samples by the ICG were in good agreement with those obtained by DC-ELISA and HPLC (**Tables 1** and **2**). The assay is simple and easy to perform, and results can be obtained within 15 min without the need for expensive equipment and washing or separation steps. With respect to on-site detection, the assay is better than immunoassays, such as ELISA, FPIA, and immunosensor.

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