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Analytical Methods

Development of a class-specific monoclonal antibody-based ELISA for aflatoxins in peanut

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ABSTRACT

Three class-specific monoclonal antibodies against aflatoxins were screened by a designed strategy in which aflatoxin G_2 was used as competitor in the screening ELISA system. With a high cross-reactivity (65%) to aflatoxin G_2, antibody 10C9 had the most similar sensitivity for five aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1), whose I_{50} values were in a range of 2.1–3.2 ng ml^{-1}. So, antibody 10C9 was selected to develop an ELISA for determination of aflatoxin B_1, B_2, G_1, G_2 and total of them in peanut samples. And spiked recoveries were from 87.5% to 102.0%. The results indicate that the ELISA developed can accurately determine total aflatoxins in samples of peanuts after the simple and rapid extraction procedure.

1. Introduction

Aflatoxins are highly toxic and carcinogenic compounds, which are a group of structurally related toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasticus* (Eaton & Groopman, 1994). The main kinds of aflatoxins include aflatoxin B_1, B_2, G_1, G_2 (Fig. 1) and M_1. These aflatoxins are consistently found contaminating food and feed supplies in many areas of the world (Aycicek, Aksoy, & Saygi, 2005; Ehrlich, Kobbeman, Montalbano, & Cotty, 2007; Magnoli et al., 2008; Nguyen, Tozlovanu, Tran, & Pfohl-Leszkowicz, 2007; O'Riordan & Wilkinson, 2008; Polychronaki et al., 2008; Zinedine et al., 2007). Groundnuts and maize are most frequently contaminated. Owing to their potential health hazards to humans, regulatory levels have recently been documented. In the European Union, the total aflatoxins level in human commodities is regulated with maximum residue levels (MRLs) that cannot be more than 4 μg/kg (European Economic Community Council, 1998). The Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme adopted a limit of 15 μg/kg for total aflatoxins (Codex Alimentarius Commission, 2001).

The analysis of aflatoxins is a challenging task. There are a variety of well established methodologies reported for analysing aflatoxins in many different food staffs, such as thin liquid chromatography (Var, Kabak, & Gok, 2007), high-performance liquid chromatography (Manetta et al., 2005), over pressured layer chromatography (Moricz, Fater, Otta, Tyihak, & Mincsovics, 2007), immunoaffinity chromatography-high-performance liquid chromatography (Calleri, Marrubini, Brusotti, Massolini, & Caccialanza, 2007), near infrared spectroscopy (Hernández-Hierro, García-Villanova, & González-Martín, 2008) and so on.

Immunoassay methods such as enzyme-linked immunosorbent assay (ELISA) are well suited for the rapid, routine diagnostic application of aflatoxin detection (Calleri et al., 2007). The availability of adequate and inexpensive antibodies against aflatoxins is important for wide adoption of immunochemical analytical procedures for aflatoxins, especially in the developing tropical countries where aflatoxin contamination is rampant (Gathumbi, Usleber, & MaErtlbauer, 2001). Therefore, several monoclonal antibodies were reported by Candlish, Smith, and Timson (1990); Cervino, Weber, Knopp, and Niessner (2008); Devi et al. (1999); Gathumbi et al. (2001); Groopman, Trudelt, Donahuet, Marshak-rothstein, and Wogant (1984) and so on. The previous results showed that those monoclonal antibodies reported had low cross-reactivity to aflatoxin G_2. As for total assay for a type of analytes, similar sensitivity to the isomers is very important (Mak et al., 2005). In this paper, a class-specific monoclonal antibody against aflatoxins screened using aflatoxin G_2 as competitor and development an ELISA for determination of aflatoxin B_1, B_2, G_1 and G_2 in peanut samples were reported.

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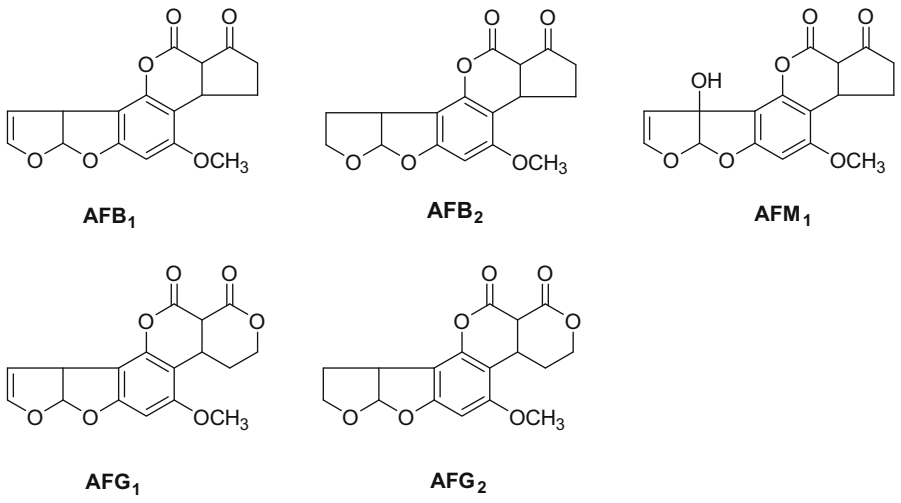

Fig. 1. Chemical structures of main aflatoxins.

2. Materials and methods

2.1. Chemicals and instruments

Standards of aflatoxin (AF) B_1 (solid), B_2, G_1, G_2 and M_1, *N*-hydroxysuccinimide (NHS), *N*,*N*-dicyclohexylcarbodiimide (DCC), tetramethylbenzidine (TMB), Peroxidase-labeled goat anti-mouse immunoglobulin (GAM-HRP), ovalbumin (OVA), bovine serum albumin (BSA), (aminooxy)acetic acid hemihydrochloride culture media RPMI-1640, hypoxanthine, aminopterin, and thymidine (HAT), poly(ethylene glycol) (PEG) 1500 and complete and incomplete Freund's adjuvant were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse monoclonal antibody isotyping kit was obtained from Roche Diagnostics Corporation (Indianapolis, USA). 96-well microtiter plates (Corning-Costar, 3590) and cell culture clusters (Corning-Costar, 3599/3524) were purchased from Corning Incorporated (NY, USA). Conjugates of AFB_1-BSA and AFB_1-OVA were prepared according to the method described by Sapsford et al. (2006). Selective hemi-solid media were prepared according to the patent (Li et al., 2008).

ELISA plates were washed with Wellwash Plus and well absorbances were measured with a microtiter plate reader, which was controlled by a personal computer containing the standard software package EasySoftware. They were both from Thermo Electron Co., (MA, USA). HPLC series (Agilent 1100) were consisted of a fluorescence detector, a C18-column (5 μm particle size, 150 mm × 4.6 mm I.D.) and a post-column derivative system, for which samples were cleaned up by immunoaffinity columns supplied by Beijing Chinainvent Instrument Tech. Co. Ltd. (Beijing, PRC).

2.2. Immunization

Six BALB/c female mice (8–10 weeks old) were immunized with AFB_1-BSA conjugates. First dose consisted of 50 μg of conjugate intraperitoneally injected as an emulsion of PBS and complete Freund's adjuvant. Two subsequent injections were given at 3-week intervals emulsified in incomplete Freund's adjuvant. One week after the last injection, mice were tail-bled and sera tested for anti-hapten antibody titer by indirect ELISA and for analyte recognition properties by competitive indirect ELISA. After a resting period of at least 3 weeks from the last injection in adjuvant, mice selected to be spleen donors for hybridoma production received a final soluble intraperitoneal injection of 80 μg of conjugate in PBS, 3 days prior to cell fusion.

2.3. Cell fusion and cloning

SP 2/0 murine myeloma cells were cultured in RPMI-1640 media supplemented with 20% fetal bovine serum. Cell fusion was carried out as described by Galfre and Milstein (1981). Mice splenocytes were mixed with the myeloma cells at the ratio of 5–10:1 and centrifuged. One milliliter of PEG 1500 at 37 °C was dropped onto the cell pellet over 1 min. After an addition of 30 ml of non-complete media (RPMI-1640) over 5 min, the cells were left aside for 5–10 min at 37 °C. Then the fused cells were spun down and removed the non-complete media. After that cells were resuspended with 20 ml of complete media (RPMI-1640 + 20% fetal bovine serum), they were cultured overnight. And then the cells were averaged removed into selective hemi-solid media containing HAT and feeding cells cultured over 2 weeks. Finally, many white tips of monoclonal hybridomas which could be seen by naked eyes were removed onto 96-well culture plates, respectively.

2.4. Screening ELISA by using aflatoxin G_2 as competitor

When the hybridoma cells had grown to approximately 30–40% confluent in the well (4–10 days), culture supernatants were collected and screened for the presence of anti-AF antibodies. During the first hybridoma screening, non-competitive indirect ELISA using AFB_1-OVA as the coating antigen was used. During the second screening from positive culture supernatants, competitive indirect ELISA using AFG_2 as competitors was used to search antibodies with high assay inhibition for AFG_2. Then stable antibody-producing clones were expanded for preparation of antibodies. Selected clones were also cryopreserved in liquid nitrogen.

2.5. Preparation and characterisation of antibodies

Antibody ascites from BALB/c female mice were prepared according to the method described by Groopman et al. (1984). AF antibodies in ascites were purified by ammonium sulfate precipitation followed by affinity column of protein A. Most ascites were able to provide enough monoclonal antibody (over 6 mg/ml) for characterisation studies and further work. Purified antibodies were stored in refrigerator at −20 °C.

Subclasses of antibodies were determined by an antibody isotyping kit.

The affinities of the antibodies were determined by indirect non-competitive ELISA, as described by Beatty, Barbara, and William (1987).

Sensitivity and specificity were determined by a competitive indirect ELISA. Microplates were coated overnight at 4 °C with 50 μl per well of the appropriate coating antigen concentration in 0.05 M carbonate–bicarbonate buffer (pH 9.6). After washing with PBST (PBS with Tween-20: 8 g l^{-1} NaCl, 1.15 g l^{-1} Na_2HPO_4, 0.2 g l^{-1} KH_2PO_4, 0.2 g l^{-1} KCl, and 0.05% Tween-20, v/v), the surface of the wells was blocked with 100 μl of a 1% gelatin in PBS (or 1% OVA, 3% skimmed milk powder) for 1 h at 37 °C. After another washing step, 25 μl per well of antiserum diluted in PBS and 25 μl per well of analyte solution were added, and incubated for 1 h. Following a washing step, GAM-HRP conjugate (1:2000 in PBST, 50 μl per well) was added and incubated for 1 h at 37 °C. The plates were washed again, and 50 μl per well of TMB solution (3.3 μl of 30% H_2O_2, 400 μl of 0.6% TMB in DMSO per 25 ml of acetate buffer, pH 5.5) was added. The color development was stopped after 10–15 min with 2 M H_2SO_4 (25 μl per well). The absorbance was measured at 450 nm. Sigmoidal curves were fitted to a logistic equation (Raab, 1983) from which I_{50} values (concentration at which binding of the antibody to the coating antigen is inhibited by 50%) were determined. Specificities of the antibodies against five aflatoxins were measured and calculated as follows: CR (%) = [I_{50} (AFB_1)/I_{50} (test compound)] × 100.

2.6. Analysis of samples

The accuracy was evaluated by spiked samples experiment. To study spike recovery, peanuts without AF contamination were spiked with different concentrations of AFB_1, B_2, G_1 and G_2 and analysed in a blind fashion by the ELISA protocol. These samples were purchased from a local supermarket in Wuhan. For the spike-and-recovery test, two final concentrations (1 or 2, 2 or 4 ng/ml) of every AFs for each of the above samples were prepared. Peanut samples first were finely chopped, and then 75% methanol was used to extract AFs. The extracted solutions were finally analysed by the ELISA without any other purification procedure (the samples were diluted with PBS-methanol buffer for 10 times).

The above spiked samples were also analysed by HPLC method with a fluorescence detector (wavelength of excitation 360 nm and emission 420 nm). The experiments were referred to a Chinese detection standard method (Wang, Zhang, Zhang, & Shao, 2003).

3. Results and discussion

3.1. Screening strategies for class-specific monoclonal antibody

Lots of reported AF antibodies from immunization with AFB_1 conjugates showed some cross-reactivity with AFB_2, AFG_1 and AFG_2 (Candlish et al., 1990; Devi et al., 1999; Gathumbi et al., 2001; Groopman et al., 1984). It is interesting that almost all of those antibodies have the lowest cross-reactivity with AFG_2. So it was postulated that antibody with high-affinity to AFG_2 had the most possibility to be a class-specific antibody for AFs.

On one hand, we tried to obtain monoclonal hybridomas as much as possible to increase positive probability. On the other hand, 10 ng ml^{-1} of AFG_2 as competitor was used to screen antibody with high-affinity to AFG_2 to increase probability of obtainment class-specific antibody for AFs because aflatoxin B_1, B_2, G_1 and G_2 have only the same moiety as the common of aflatoxin G_2 and the immunizing hapten. Finally three class-specific monoclonal antibodies: 8E11, 10C9 and 8F6 were obtained, and ELISA of which showed high inhibition for AFG_2.

Table 1
Sensitivity (I_{50} [a,b]) and specificity (CR[c]) of three antibodies against aflatoxins.

Aflatoxin	Antibody of 8E11		Antibody of 8F6		Antibody of 10C9	
	I_{50}	CR	I_{50}	CR	I_{50}	CR
AFB_1	1.25	100	1.70	100	2.09	100
AFB_2	5.99	20.9	1.63	103.8	2.23	93.6
AFG_1	1.42	88.4	1.69	100.1	2.19	95.4
AFG_2	1.60	78.1	3.60	47.0	3.21	65.2
AFM_1	1.43	87.6	2.61	65.0	2.95	70.9

[a] Unit is ng ml^{-1}.
[b] The average coefficient of variation (CV) was 7.4%, and all were below 10%.
[c] CR means cross-reactivity, and its unit is percent.

3.2. Characterisation of antibodies

The above antibodies 8E11, 10C9 and 8F6 had the same isotype determined as IgG1. From the highest to the lowest, the sequence of these antibody affinities to AFB_1-OVA were 8E11, 8F6 and 10C9 (data were not shown).

Sensitivity and specificity were the most important parameters for antibodies and for their assay method. Generally, I_{50} values were usually used to evaluate sensitivity of ELISA (Zhang et al., 2007). I_{50} and cross-reactivity of the above antibodies were shown in Table 1.

Results of sensitivity showed that I_{50} values of antibody 8E11 was from 1.4 ng ml^{-1} to 6.0 ng ml^{-1}, antibody 8F6 from 1.6 ng ml^{-1} to 3.6 ng ml^{-1}, and antibody 10C9 from 2.1 ng ml^{-1} to 3.2 ng ml^{-1}. These results suggest that sensitivities of the three antibodies were very close to each other, and that antibody 10C9 had the most similar sensitivity for five aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1).

It can easily be seen from the cross-reactivity data that the three antibodies screened using AFG_2 as competitor have all high cross-reactivity with AFG_2. However, antibody 8E11 had only 20.9% of cross-reactivity with AFB_2, and cross-reactivity of antibody 8F6 with AFG_2 was 47.0% lower than that of antibody 10C9. These may result from the total of electronegative and geometrical features of analytes (Wang et al., 2007). Cross-reactivity with AFG_2 of AF antibodies in references (Candlish et al., 1990; Devi et al., 1999; Gathumbi et al., 2001; Groopman et al., 1984) are also less than 50%. In this paper, antibody 10C9 had not only a high cross-reactivity with AFG_2 but also an interesting range of cross-reactivity with five aflatoxins from 65% to 100%.

Therefore, 10C9 here was the best class-specific antibody against aflatoxins, and were selected for further sample analysis experiments.

3.3. Analysis of peanut samples

The spiked recoveries were used to represent the accuracy of the ELISA (Zhang et al., 2008) with limit of detection of 0.06–0.09 ng ml^{-1}. Therefore, aflatoxins in peanut samples were detected using the selected ELISA, after the simple sample preparation procedure as described above. It was found that the average recoveries of spiked peanuts were from 87.5% to 102.0% (Table 2), and that those coefficients of deviation were less than 11%. We also detected the spiked samples by a standard HPLC method, whose recoveries were from 87.7% to 97.6%. Comparatively, it can be seen that there is only few difference for the recoveries of total aflatoxins between the ELISA and HPLC methods. Overall, the ELISA developed in this study can accurately determine total aflatoxins in samples of peanuts after the simple and rapid extraction procedure.

Table 2
Recovery test of aflatoxins in peanuts with a competitive ELISA[a] and a HPLC method.

Aflatoxin	Spiked (ng ml^{-1})	ELISA			HPLC		
		Theoretical (ng ml^{-1})	Found (ng ml^{-}1)	Average recovery ± SD (%)	Theoretical (ng ml^{-1})	Found[c] (ng ml^{-}1)	Average recovery ± SD (%)
AFB_1	1.0	0.1	0.102	102.0 ± 10.3	1.67	1.49	89.2 ± 8.8
	2.0	0.2	0.194	97.0 ± 9.8	3.33	3.03	91.0 ± 7.6
AFB_2	2.0	0.2	0.177	88.5 ± 8.0	3.33	3.16	94.9 ± 6.8
	4.0	0.4	0.376	94.0 ± 6.9	6.67	6.44	96.6 ± 7.0
AFG_1	2.0	0.2	0.182	91.0 ± 9.2	3.33	2.92	87.7 ± 10.9
	4.0	0.4	0.370	92.5 ± 8.3	6.67	6.11	91.6 ± 7.3
AFG_2	2.0	0.2	0.201	100.5 + 11.2	3.33	3.25	97.6 + 8.2
	4.0	0.4	0.383	95.8 ± 7.4	6.67	6.30	94.5 ± 6.6
$AFB_1 + B_2 + G_1 + G_2$	1.0 + 1.0 + 1.0 + 1.0	0.4	0.350[b]	87.5 ± 6.8	6.67	6.43	96.4 ± 9.8

[a] Data were determined in the respective ELISA systems of antibody 10C9.
[b] Data were determined in an average ELISA system of antibody 10C9.
[c] Data were averaged from two repeats.

4. Conclusions

Class-specific antibodies against AFs are very important for their total assay. According to most of the AF antibodies in reference have low cross-reactivity with AFG_2, we designed a strategy for screening class-specific monoclonal antibodies against aflatoxins using aflatoxin G_2 as competitor, and successfully obtained three such antibodies 8E11, 8F6 and 10C9. Because of the most similar sensitivity and cross-reactivity with AFB_1, B_2, G_1 and G_2, antibody 10C9 was selected to develop an ELISA for determination aflatoxin B_1, B_2, G_1 and G_2 in peanut samples. Results of spiked recovery indicated that the ELISA developed could accurately determine total aflatoxins in samples of peanuts after the simple and rapid extraction procedure.

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References

Aycicek, H., Aksoy, A., & Saygi, S. (2005). Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. *Food Control, 16*(3), 263–266.

Beatty, J. D., Barbara, G. B., & William, G. V. (1987). Measurement of monoclonal antibody affinity by noncompetitive enzyme immunoassay. *Journal of Immunological Methods, 100*, 173–179.

Calleri, E., Marrubini, G., Brusotti, G., Massolini, G., & Caccialanza, G. (2007). Development and integration of an immunoaffinity monolithic disk for the on-line solid-phase extraction and HPLC determination with fluorescence detection of aflatoxin B_1 in aqueous solutions. *Journal of Pharmaceutical and Biomedical Analysis, 44*(2), 396–403.

Candlish, A. A. G., Smith, E., & Timson, W. H. S. (1990). Aflatoxin monoclonals: Academic development to commercial production. *Letters in Applied Microbiology, 10*, 167–169.

Cervino, C., Weber, E., Knopp, D., & Niessner, R. (2008). Comparison of hybridoma screening methods for the efficient detection of high-affinity hapten-specific monoclonal antibodies. *Journal of Immunological Methods, 329*(1–2), 184–193.

Codex Alimentarius Commission (2001). Joint FAO/WHO food standards programme, codex committee on food additives and contaminants. Thirty-third session CODEX, Hague, Netherlands.

Devi, K. T., Mayo, M. A., Reddy, K. L. N., Delfosse, P., Reddy, G., Reddy, S. V., et al. (1999). Production and characterization of monoclonal antibodies for aflatoxin B_1. *Letters in Applied Microbiology, 29*, 284–288.

Eaton, D. L., & Groopman, J. D. (1994). *The toxicology of aflatoxins: Human health, veterinary and agricultural significance*. New York: Academic Press.

Ehrlich, K. C., Kobbeman, K., Montalbano, B. G., & Cotty, P. J. (2007). Aflatoxin-producing *Aspergillus* species from Thailand. *International Journal of Food Microbiology, 114*(2), 153–159.

European Economic Community Council (1998). Commission regulation (EC) No.1525/98. *Official Journal of European Communities, L201*, 43–46.

Galfre, G., & Miltstein, C. (1981). Preparation of monoclonal antibodies: Strategies and procedures. *Methods in Enzymology, 73*, 3–46.

Gathumbi, J. K., Usleber, E., & MaErtlbauer, E. (2001). Production of ultrasensitive antibodies against aflatoxin B_1. *Letters in Applied Microbiology, 32*, 349–351.

Groopman, J. D., Trudelt, L. J., Donahuet, P. R., Marshak-rothstein, A., & Wogant, G. N. (1984). High-affinity monoclonal antibodies for aflatoxins and their application to solid-phase immunoassays. *Proceedings of the National Academy of Sciences USA, 81*, 7728–7731.

Hernández-Hierro, J. M., García-Villanova, R. J., & González-Martín, I. (2008). Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market. *Analytica Chimica Acta, 622*(1–2), 189–194.

Li, P., Zhang, W., Zhang, Q., Xie, L., Ding, X., Chen, X., et al. (2008). A method for screening monoclonal hybridoma. Patent in China, 200810047640.2.

Magnoli, A. P., Tallone, L., Rosa, C. A. R., Dalcero, A. M., Chiacchiera, S. M., & Sanchez, R. M. T. (2008). Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. *Applied Clay Science, 40*(1–4), 63–71.

Mak, S. K., Shan, G., Lee, H.-J., Watanabe, T., Stoutamire, D. W., Gee, S. J., et al. (2005). Development of a class selective immunoassay for the type II pyrethroid insecticides. *Analytica Chimica Acta, 534*, 109–120.

Manetta, A. C., Di Giuseppe, L., Giammarco, M., Fusaro, I., Simonella, A., Gramenzi, A., et al. (2005). High-performance liquid chromatography with post-column derivatisation and fluorescence detection for sensitive determination of aflatoxin M_1 in milk and cheese. *Journal of Chromatography A, 1083*(1–2), 219–222.

Moricz, A. M., Fater, Z., Otta, K. H., Tyihak, E., & Mincsovics, E. (2007). Over pressured layer chromatographic determination of aflatoxin B_1, B_2, G_1 and G_2 in red paprika. *Microchemical Journal, 85*(1), 140–144.

Nguyen, M. T., Tozlovanu, M., Tran, T. L., & Pfohl-Leszkowicz, A. (2007). Occurrence of aflatoxin B_1, citrinin and ochratoxin A in rice in five provinces of the central region of Vietnam. *Food Chemistry, 105*(1), 42–47.

O' Riordan, M. J., & Wilkinson, M. G. (2008). A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market. *Food Chemistry, 107*(4), 1429–1435.

Polychronaki, N., Wild, C. P., Mykkänen, H., Amra, H., Abdel-Wahhab, M., Sylla, A., et al. (2008). Urinary biomarkers of aflatoxin exposure in young children from Egypt and Guinea. *Food and Chemical Toxicology, 46*(2), 519–526.

Raab, G. M. (1983). Comparison of a logistic and a mass action curve for radioimmunoassay data. *Clinical Chemistry, 29*, 1757–1761.

Sapsford, K. E., Taitt, C. R., Fertig, S., Moore, M. H., Lassman, M. E., Maragos, C. M., et al. (2006). Indirect competitive immunoassay for detection of aflatoxin B_1 in corn and nut products using the array biosensor. *Biosensors and Bioelectronics, 21*, 2298–2305.

Var, I., Kabak, B., & Gok, F. (2007). Survey of aflatoxin B_1 in helva, a traditional Turkish food, by TLC. *Food Control, 18*(1), 59–62.

Wang, J., Zhang, P., Zhang, Y., Shao, M. (2003). Determination aflatoxins content in food-cleanup by immunoaffinity chromatography and determination by high-performance liquid chromatography and fluorometer. Standard of PR China, GB/T 18979-2003.

Wang, Z., Zhu, Y., Ding, S., He, F., Beier, R. C., Li, J., et al. (2007). Development of a monoclonal antibody-based broad-specificity ELISA for fluoroquinolone antibiotics in foods and molecular modeling studies of cross-reactive compounds. *Analytical Chemistry, 79*(12), 4471–4483.

Zhang, Q., Sun, Q., Hu, B., Shen, Q., Yang, G., Liang, X., et al. (2008). Development of a sensitive ELISA for the analysis of the organophosphorous insecticide fenthion in fruit samples. *Food Chemistry, 106*, 1278–1284.

Zhang, Q., Wang, L., Ahn, K. C., Sun, Q., Hu, B., Wang, J., et al. (2007). Hapten heterology for a specific and sensitive indirect ELISA for organophosphorus insecticide fenthion. *Analytica Chimica Acta, 596*, 303–311.

Zinedine, A., González-Osnaya, L., Soriano, J. M., Moltó, J. C., Idrissi, L., & Mañes, J. (2007). Presence of aflatoxin M_1 in pasteurized milk from Morocco. *International Journal of Food Microbiology, 114*(1), 25–29.