



METHOD ARTICLE

REVISED Development of an IgY-based lateral flow immunoassay for detection of fumonisin B in maize [version 2; peer review: 1 approved, 2 approved with reservations]

Tien Viet Tran^{1*}, Binh Nhu Do ^{1*}, Thao Phuong Thi Nguyen², Tung Thanh Tran ², Son Cao Tran³, Ba Van Nguyen¹, Chuyen Van Nguyen¹, Hoa Quang Le²

¹Vietnam Military Medical University, Hanoi, 100000, Vietnam

²School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Hanoi, 100000, Vietnam

³Laboratory of Food Toxicology and Allergens Testing, National Institute for Food Control, Hanoi, Vietnam

* Equal contributors

v2 First published: 10 Jul 2019, 8:1042 (<https://doi.org/10.12688/f1000research.19643.1>)
 Latest published: 12 Dec 2019, 8:1042 (<https://doi.org/10.12688/f1000research.19643.2>)

Abstract

Fumonisin B is among the most prevalent mycotoxins in maize, causing substantial economic losses and potential health risks in humans and animals. In the present study, in-house polyclonal IgY antibody against fumonisin B1 (FB₁) and B2 (FB₂) was applied for the development of a competitive lateral flow immunoassay detecting these mycotoxins in maize grains with the limit of detection of 4000 µg/kg, which corresponds to the maximum residue limit adopted by the European Commission. To this end, factors affecting the test performance including nitrocellulose membrane type, dilution factor of maize homogenates in running buffer, amount of detection conjugate, and incubation time between detection conjugate and samples were optimized. Under the optimal condition (UniSart[®] CN140 nitrocellulose membrane, FB₁-BSA immobilized at 1 µg/cm, 1:10 dilution factor, 436 ng of gold nanoparticle conjugate, 30 minutes of incubation), the developed test could detect both FB₁ and FB₂ in maize with limit of detection of 4000 µg/kg, and showed no cross-reactivity to deoxynivalenol, ochratoxin A, aflatoxin B1 and zearalenone. When applied to detect FB₁ and FB₂ in naturally contaminated maize samples, results obtained from the developed assay were in good agreement with those from the high-performance liquid chromatography method. This lateral flow immunoassay is particularly suitable for screening of fumonisins in maize because of its simplicity and cost-effectiveness.

Keywords

fumonisin B, rapid methods, lateral flow immunoassay, IgY

Open Peer Review

Reviewer Status ? ✓ ?

	Invited Reviewers		
	1	2	3
REVISED			
version 2 published 12 Dec 2019		✓ report	
		↑	
version 1 published 10 Jul 2019	? report	✗ report	? report

- Venkataramana Mudili**, Lorven Biologics Pvt. Ltd., Hyderabad, India
- Zhanhui Wang** , China Agricultural University, Beijing, China
- Boris B. Dzantiev**, Russian Academy of Sciences (RAS), Moscow, Russian Federation

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Corresponding author: Hoa Quang Le (hoa.lequang@hust.edu.vn)

Author roles: **Tran TV:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Do BN:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Nguyen TPT:** Data Curation, Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation; **Tran TT:** Data Curation, Formal Analysis, Investigation, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Tran SC:** Investigation, Methodology, Writing – Review & Editing; **Nguyen BV:** Formal Analysis, Funding Acquisition, Investigation, Project Administration, Resources, Validation, Writing – Original Draft Preparation; **Nguyen CV:** Formal Analysis, Funding Acquisition, Investigation, Project Administration, Resources, Validation, Writing – Original Draft Preparation; **Le HQ:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Research reported in this publication was supported by the Hanoi Office of Science and Technology [01C-06/02-2015-2]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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How to cite this article: Tran TV, Do BN, Nguyen TPT *et al.* **Development of an IgY-based lateral flow immunoassay for detection of fumonisin B in maize [version 2; peer review: 1 approved, 2 approved with reservations]** F1000Research 2019, 8:1042 (<https://doi.org/10.12688/f1000research.19643.2>)

First published: 10 Jul 2019, 8:1042 (<https://doi.org/10.12688/f1000research.19643.1>)

REVISED Amendments from Version 1

The following major revisions were made to the manuscript based on the comments and suggestions of the three referees:

- Information on the toxicity of fumonisins in the introduction has been revised and updated with recent literature.
- The principle of conjugate preparation has been added to the section "Methods" and the conjugation protocol has been rewritten for better clarity.
- Decision number of the ethics approval for animal use in this study has been added to the section "Methods". The approval was also uploaded to figshare at <https://doi.org/10.6084/m9.figshare.8320775>.
- Details on flow rates of the membranes were added to the section "Methods".
- A discussion part was added to further explain the need for a new lateral flow immunoassay (LFIA) for total fumonisins (defined as the sum of FB₁ and FB₂) with the limit of detection (LOD) equal to the maximum residue limit (MRL) of this mycotoxin group in raw maize and to underline the advantages of IgY to produce LFIA.
- Details on the stability and affinities towards FB₁ and FB₂ of the polyclonal IgY antibody used in this study were added to the discussion.
- Conclusions of the manuscript were revised as some information had been moved to the discussion.

Any further responses from the reviewers can be found at the end of the article

Introduction

Fumonisins are a group of mycotoxins from *Fusarium* species, mostly *Fusarium proliferatum* and *Fusarium verticillioides* (Scott, 2012). To date, four groups of fumonisin have been identified (A, B, C and P-series), among which fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) are the most common mycotoxins found in corn, and were found to have various toxic and carcinogenic effects (Munkvold *et al.*, 2019; Scott, 2012). For instance, highly significant associations between intake of fumonisin-contaminated maize and oral cancer, pharyngeal cancer, and esophageal cancer have been observed (Alizadeh *et al.*, 2012; Franceschi *et al.*, 1990; Sun *et al.*, 2007). Equine leukoencephalomalacia and porcine pulmonary edema have also been revealed to be related to consumption of fumonisin-contaminated maize (Haliburton & Buck, 1986; Marasas, 2001). Due to its toxicity, the European Commission has adopted the maximum residue limit (MRL) for the presence of total fumonisins (as the sum of FB₁ and FB₂) in raw maize at 4000 µg/kg (EC, 2007).

Recent studies pointed out fumonisin contamination in corn represents a major public-health concern in diverse countries including China (Fu *et al.*, 2015; Guo *et al.*, 2016; Hu *et al.*, 2019; Liu *et al.*, 2017), Brazil (Scussel *et al.*, 2014), Kenya (Mutiga *et al.*, 2015), South Africa (Mngqawa *et al.*, 2016), Malawi (Mwalwayo & Thole, 2016), Tanzania (Kamala *et al.*, 2016), Nigeria (Chilaka *et al.*, 2016), Ethiopia (Getachew *et al.*, 2018), Somalia (Wielogorska *et al.*, 2019). In Vietnam, Hieu Phung *et al.* (2015) showed that FB₁ and FB₂ were the major mycotoxin that contaminated maize with 67% of incidence, a range

of positive samples for FB₁ and FB₂ at 102 to 10799 µg/kg and 102–5051 µg/kg respectively.

Conventionally, chromatography methods such as high performance liquid chromatography (HPLC) or liquid chromatography tandem mass spectrometry (LC-MS/MS) could be used to detect fumonisins in maize (Cigić & Prosen, 2009; Gruber-Dorninger *et al.*, 2018). However, they are laborious, time consuming and require specialized equipment. On the other hand, lateral flow immunoassays (LFIAs) are cost-effective, easy to use and suitable for on-site analysis. Several LFIAs have been developed for quick and simple screening of fumonisins in various types of sample (Anfossi *et al.*, 2010; Venkataramana *et al.*, 2014; Wang *et al.*, 2013; Wang *et al.*, 2014; Yu *et al.*, 2015). Nonetheless, most available LFIAs today are based on monoclonal or polyclonal IgG from mammals, which increases the cost of production and involves ethical issues of animal welfare

Polyclonal IgY antibodies from egg yolk of laying hens represent an attractive alternative to monoclonal and rodent polyclonal antibodies. With one course of immunization, IgY could be extracted non-invasively in a large quantity (up to 40–80 mg), with 2–10% of which being antigen specific (Kovacs-Nolan & Mine, 2004; Pauly *et al.*, 2011). As a result, IgY has been increasingly employed for the development of rapid tests. Its usefulness in LFIAs has been demonstrated for detection of morphine (Gandhi *et al.*, 2009), methicillin-resistant *Staphylococcus aureus* (Yamada *et al.*, 2013), staphylococcal enterotoxins (Jin *et al.*, 2013), and rhein (Zhang *et al.*, 2018b).

In the present study, we demonstrated the development of a IgY-based LFIA for simple and cost-effective screening of total fumonisins (as the sum of FB₁ and FB₂) in raw maize with LOD equal to the MRL of 4000 µg/kg.

Methods

Preparation of FB₁-BSA conjugate

Conjugation of FB₁ to BSA was performed following the protocol by Szurdoki *et al.* (1996) with some modifications. Glutaraldehyde (GA) solution 50 % (W/V) (Sigma-Aldrich, Cat N° 340855) was used as the cross-linker reagent while BSA (Sigma-Aldrich, Cat N° A9085) was used as the carrier protein. Specifically, BSA (5 mg/mL) was dialyzed in 20 mM sodium phosphate buffer pH 6.0. A total of 10 µl of GA 50% (W/V) were then incubated with 1 mL of the dialyzed BSA solution overnight at room temperature. After incubation, excess GA was removed by dialyzing in Phosphate-buffered saline (PBS), followed by addition of 1 mg of FB₁ (Santa Cruz Biotechnology, Cat N° sc-201395A) to achieve the molar ratio of 20:1 (FB₁:BSA-GA). The mixture was incubated at 4°C overnight on a Dynal Biotech rotary shaker (10 rpm) before the addition of 80 µl of glycine 1 M (Bio Basic, Cat N° GB0235) to block unreacted aldehyde groups. The reaction mixture was further incubated at room temperature for 4 hours. Subsequently, sodium borohydride powder (Sigma-Aldrich, Cat N° 452882) was added to the mixture (final concentration of 10 mg/mL) and incubated for 4 hours at room temperature. The obtained solution was then dialyzed and concentrated in 10 mM Borat buffer pH 8.5 using

a 10 kDa Amicon® Ultra-4 Centrifugal Filter Unit (Millipore, Cat N° UFC801024). Lastly, FB₁-BSA conjugate was quantified using Nano Drop 2000 (Thermo Fisher Scientific) and stored at 4 °C.

Production of IgY against FB₁-KLH

Animal procedures. All animal procedures were approved by the Research Ethics Committee of Vietnam Military Medical University (Decision No. 29/2015/HVQY-HD3). All efforts were made to ameliorate harm to the animals, by conforming to the Principles of animal care and use in research adopted by the Vietnam Military Medical University.

A total of two Fayoumi hens (aged 20 weeks) were sourced from Thuy Phuong Poultry Research Center, Vietnam for IgY production. Hens were housed individually in standard battery cages (800 cm²/hen) and received commercial rations (A55, Anova Feed) and water *ad libitum*. The temperature was kept between 25 and 35°C and the light cycle was 16 hours light/8 hours dark.

Polyclonal IgY antibody against FB₁-KLH was obtained as described previously (Do *et al.*, 2016). Briefly, FB₁-KLH was prepared according to the procedure described by Szurdoki *et al.* (1996). Glutaraldehyde (GA) solution 50 % (W/V) (Sigma-Aldrich, Cat N° 340855) was used as the cross-linker reagent. A total of 10 mg of KLH (Thermo Fisher Scientific, Cat N° 77600) was dissolved in 12 mL of water and dialyzed against 2 L of 0.2% GA in 0.01 M PBS (pH 7.5) for 20 hours. Excess GA was removed by dialyzing in PBS, followed by dropwise addition of 2 mg of FB₁ (Santa Cruz Biotechnology, Cat N° sc-201395A). The mixture was incubated at 4°C overnight on a Dynal Biotech rotary shaker (10 rpm) before the addition of 80 µl of glycine 1 M (Bio Basic, Cat N° GB0235) to block unreacted aldehyde groups. The reaction mixture was further incubated at room temperature for 4 hours. The obtained solution was then dialyzed and concentrated in PBS pH 7.5 using a 100 kDa Amicon® Ultra-4 Centrifugal Filter Unit (Millipore, Cat N° UFC810024). Lastly, FB₁-KLH conjugate was quantified using Nano Drop 2000 (Thermo Fisher Scientific) and stored at 4 °C.

The chickens were intramuscularly immunized three times in 10 days intervals to elicit strong immune response. For the first immunization, an injection dose of 1.0 mL was prepared by mixing 0.2 mg of FB₁-KLH with an equal volume of complete Freund's adjuvant (Sigma-Aldrich, Cat N° F5881). For the two subsequent booster immunizations, the amount of immunogen was decreased to 0.1 mg of FB₁-KLH and incomplete Freund's adjuvant (Sigma-Aldrich, Cat N° F5506) was used. Eggs were collected two weeks after the last immunization and stored at 4°C. The extraction of IgY was performed by polyethylene glycol (PEG) (Sigma-Aldrich, Cat N° 81255) precipitation as described by Pauly *et al.* (2011). The eggshell was carefully cracked, and the yolk was transferred to a "yolk spoon" and filter paper to remove egg white. The egg yolk skin membrane was cut before the yolk was poured into a 50 ml tube. Twice the egg yolk volume of PBS was added to the tube

and mixed by vortexing. PEG 6000 was added to achieve the final concentration of 3.5 % (w/v) and the tube was vortexed and rolled for 10 minutes on a Dynal Biotech rotary shaker (30 rpm) before being centrifuged at 8000 × g, 4°C for 10 minutes. The supernatant was subjected to filtration and then to precipitation of IgY by adding PEG 6000 (final concentration 12 % (w/v)). The tube was vortexed and centrifuged at 8000 × g, 4°C for 30 minutes and the supernatant was discarded. The pellet was dissolved in 10 mL PBS and PEG 6000 was added to achieve the final concentration of 12 % (w/v). Subsequently, the tube was centrifuged at 8000 × g, 4°C for 30 minutes. The pellet was dissolved in 5 mL of PBS and IgY was further purified by microfiltration via a 0.45 µm membrane and ultrafiltration using 100 kDa Amicon® Ultra-4 Centrifugal Filter Units (Millipore, Cat N° UFC810008). Finally, IgY was stored at -80°C in small aliquots.

Preparation of IgY-conjugated gold nanoparticles

IgY was conjugated to gold nanoparticles via covalent immobilization, following instructions of BioReady 40 nm Carboxyl Gold (Nanocomposix, Cat N° AUXR40-5M). The procedure involves linking the primary amine groups of the antibody to the carboxylic groups on the surface of the particles by the use of EDC/Sulfo-NHS coupling chemistry.

Specifically, before conjugation, 10 mg/mL EDC (Sigma-Aldrich, Cat N° 03449) and 10 mg/mL Sulfo-NHS (Sigma Aldrich, Cat N° 56485) were freshly prepared in H₂O; and the polyclonal IgY antibody was dialyzed in 10 mM potassium phosphate (pH 7.4) using Amicon Ultra-0.5 Centrifugal Filter Unit (Millipore, Cat N° UFC501096). One milliliter (0.83 mg) of BioReady 40 nm Carboxyl Gold (NanoComposix, Cat N° AUXR40-5M) was mixed with 20 µl and 40 µl of the prepared EDC and Sulfo-NHS respectively. The mixture was then incubated on a Dynal Biotech rotary shaker (15 rpm) at room temperature for 30 minutes then centrifuged at 3600 × g for 10 minutes. The supernatant was then removed completely, and the gold nanoparticles were resuspended in 1 mL of Reaction Buffer (5 mM potassium phosphate, 0.5 % 20K MW PEG, pH 7.4). The reaction tube was then incubated with 50 µg of IgY on a Dynal Biotech rotary shaker (15 rpm) at room temperature for 2 hours. Subsequently, blocking of remaining NHS-esters was performed using 10 µl of 50% (w/v) hydroxylamine. IgY-conjugated gold nanoparticles were then washed three times with 1 mL of Reaction Buffer and resuspended in 10 mL of Conjugate Diluent (0.1X PBS, 0.5% BSA, 0.05% Sodium Azide) and stored at 4°C.

Preparation of LFIA test strips

Test strips were prepared following Posthuma-Trumpie *et al.* (2008) with some modifications. Briefly, a Linomat V (Camag, Cat N° 022.7808) was used to dispense FB₁-BSA and Mouse monoclonal 0.8C Anti-Chicken IgY H&L (Abcam, Cat N° ab82229) at the test line and control line positions of a nitrocellulose membrane respectively. For the control line, immunoglobulins were dispensed at a dose of 0.5 µg/cm, at the position of 2 cm away from the dipping point. For the test line, FB₁-BSA was dispensed at a dose of 1 µg/cm at the position of 1.5 cm away from the dipping point. The membrane was then

dried for 2 hours at 37°C. A second plastic backing and an absorption pad (Extra Thick Blot Paper, BIO-RAD, Cat N° 1703969) were applied; and the membranes were cut into 4 mm-wide test strips using an Autokun cutter (Hangzhou Autokun Technology). Test strips were sealed in aluminum packages with a desiccation pad and stored at 4°C until use. Three different membranes were tested, namely *CNPC-SS12*, 10 µm with wicking time of 140 ± 28s/40 mm (MDI technologies, Cat N° CNPC-SS12-10µm-25mm), UniSart® CN140 with wicking time of 95-155s/40 mm (Sartorius, Cat N° 1UN14ER100025NTB), and UniSart® CN 95 with wicking time of 65-115s/40 mm (Sartorius, Cat N° 1UN95ER100040WS).

Sample preparation and assay procedures

Blank and naturally contaminated maize grains were collected from local markets in Hanoi, Vietnam during the year of 2017. The samples were finely ground using an A 11 basic Analytical mill (IKA) and a 500 µm sieve.

Spiking of FBs into maize was performed on a blank sample. Briefly, 5 g of ground maize were spiked with 10–40 µl of FB₁ or FB₂ stock solution of 1 mg/mL to achieve final content of 2000 – 8000 µg/kg. Spiked samples were left 24 hours at 4°C. Extraction of FBs and LFIA analysis were performed as described below.

The protocol for FB extraction from naturally contaminated or spiked samples (Figure 1) was based on the work of Pietri & Bertuzzi (2011) and Lattanzio *et al.* (2012). Instead of using organic solvents, FB was extracted with 0.4 M phosphate

buffer (PB) at pH 7.5 (Pietri & Bertuzzi, 2011). Specifically, 5 g of maize flour were mixed with 45 mL of PB and blended using a T10 basic ULTRA-TURRAX® (IKA) at the highest speed for 3 minutes. The blended samples were then allowed to settle for 3 minutes to recover the supernatant, which was further diluted 1:3, 1:5, 1:10 or 1:20 in Running Buffer (100 mM Borat Buffer, 0.5 % BSA, 0.05% Tween®-20, 0.02 % NaN₃, pH 8.5). For LFIA analysis, 100 µl of the diluted extracts were dispensed into a 2-mL lyophilization glass vials and incubated with 174 ng, 436 ng or 697 ng (corresponding to 2, 5, 8 µl) of detection conjugate for 0 to 60 minutes before being flowed vertically onto LFIA test strips. After 25 minutes, results could be read with the naked eye or captured by a Perfection V600 scanner (Epson). Optical densities of test lines and control lines were digitalized to obtain signal values using ImageJ software (ver.1.47) (Schneider *et al.*, 2012). GraphPad Prism 6.0 (GraphPad Software Inc.) was used to statistically analyze and graph the data. Unpaired, two-tailed *t*-tests were performed to determine statistical significance.

Specificity test

To test the specificity of the developed assay, four following mycotoxins at 100-fold and 1000-fold of the maximum residue limit (MRL) (deoxynivalenol at 1750 ng/mL and 17500 ng/mL; ochratoxin A at 5 ng/mL and 50 ng/mL; aflatoxin B₁ at 10 ng/mL and 100 ng/mL; and zearalenone at 350 ng/mL and 3500 ng/mL) were spiked into diluted extracts of blank samples. All of these mycotoxins were purchased from FERMENTEK Ltd (Cat N°: 51481-10-8, 303-47-9, 1162-65-8, 17924-92-4). The subsequent LFIA analyses were performed as mentioned above.

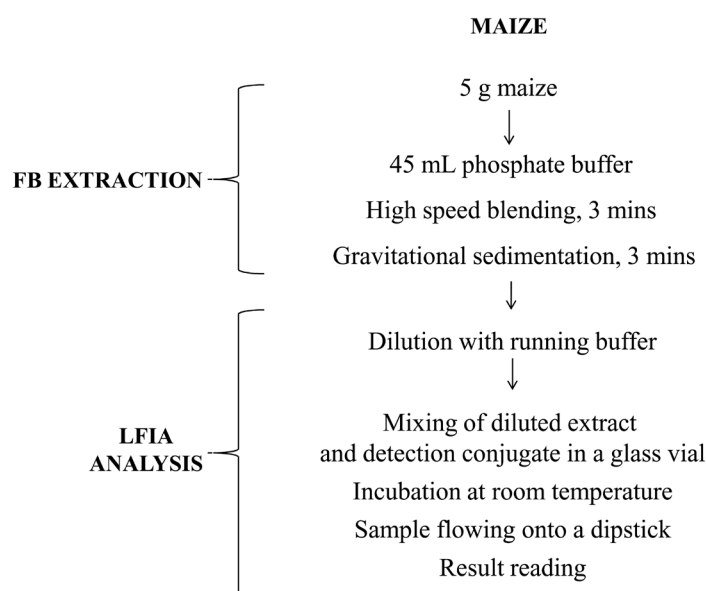


Figure 1. Schematic representation of fumonisin extraction and lateral flow analysis for maize samples. A total of 5 g of maize were homogenized in 45 mL of phosphate buffer for 3 minutes. The mixture was allowed to settle for 3 minutes before collection of the supernatant, which was further diluted in running buffer. A hundred microliters of the diluted extract were used for lateral flow immunoassays (LFIA) analysis. After being incubated with detection conjugate at room temperature, samples were flowed onto LFIA strips. Results were read with the naked eye after the strips absorbed fluid completely.

Quantification of FB₁ and FB₂ in maize using HPLC-MS method

FBs in maize were quantified by the EN 13585:2001 standard method (CEN, 2001) with some modifications. Briefly, 5g of maize were mixed with 5 mL of methanol-water and blended for 5 minutes using a T10 basic ULTRA-TURRAX® (IKA). Maize extracts were then collected by centrifugation (500 × g, 10 minutes) and filtering (Whatman) and 1 mL aliquot of filtrates was loaded into a preconditioned Bond-Elut strong-anion-exchanging cartridge (Agilent, Cat N° 14102017). After washing with methanol, elution was performed using 10 mL of methanol-acetic acid (99:1 v/v). The eluate was then evaporated under a stream of nitrogen, washed with 1 mL of methanol and evaporated again. Dried samples were reconstituted in 1 mL of methanol before HPLC-MS/MS analysis. HPLC injection (10 µl) was performed on a system consisting of a Shimadzu LC-20ADVP pump; a Symmetry HPLC column (150 mm × 3.0 mm i.d. × 3.5 µm) maintained at 30°C (Waters, 186000695); and a SCIEX Triple Quad™ 5500 mass spectrometer. The analytical separation was performed with water-acid formic (99.9-0.1, v/v) and acetonitrile as mobile phases A and B respectively. The gradient elution program began with an isocratic step of 80:20 A:B for 2 minutes and then increased linearly to 10:90 A:B over 5 minutes, which was maintained for 3 minutes, and returned to the starting condition. The condition was then held constant for 3 minutes. The flow rate was kept at 0.5 mL/min. The HPLC column effluent was pumped to the MS/MS system, with the electrospray ionization (ESI) probe operating in positive mode. The following parameters were used: capillary voltage, 5000 V; desolvation gas temperature, 450°C; ion source gas 1 and gas 2 pressure, 40 and 30 psi, respectively. Detection was carried out in multiple reaction monitoring (MRM) mode with two transitions for each compound. Nitrogen was used as the collision gas, and the collision cell pressure was 7 psi. The reference standards of FBs were purchased from LGC Standards (Cat N° B-MYC0400-C and B-MYC0420-1).

Results

Development and optimization of LFIA

The LFIA developed in the present study is based on the competitive format in which polyclonal IgY antibody, showing recognition specificity toward both FB1 and FB2 (Do *et al.*, 2016), is conjugated to gold nanoparticle (see underlying data (Tran *et al.*, 2019)). The labeled antibody was mixed with the sample extract in a glass vial, and the mixture was incubated to allow antigen-antibody complexes to form before flowing onto the nitrocellulose membrane which contains a test line and a control line. In our assay, FB₁-BSA conjugate was immobilized on the test line while a secondary antibody against chicken IgY was coated on the control line. In a negative sample, the free detection antibody binds to the FB₁-BSA conjugate immobilized on the test line, forming a visible line. An excess of the labeled antibody migrates to the control line and binds to the secondary antibody. As a result, a negative sample will form two visible lines on the nitrocellulose membrane. In a positive sample, FBs in the sample extract will react with all of the available binding sites of the antibody, thus preventing

attachment of the detection antibody to the FB₁-BSA conjugate on the test line. All of the detection conjugate will migrate to the control line and will form a visible line. Consequently, a positive sample will form only one line at the control zone.

Optimization has been performed with FB₁, the most common mycotoxin in maize, so that the samples with FB₁ concentration equal to or beyond the maximum residue limit of 4000 µg/kg, will result in no visible line at the test zone. To this end, the effects of nitrocellulose membrane type, dilution factor of maize homogenates in running buffer, amount of detection conjugate, and the incubation time between sample extract and detection conjugate, on the test performance were evaluated.

Selection of nitrocellulose membrane. Flow rate and protein-binding capacity of nitrocellulose membranes directly affect sensitivity and run time of a LFIA (O'Farrell, 2008). Generally, nitrocellulose membranes with a low flow rate will facilitate the formation of immunocomplexes at the test and control lines. However, it could lead to extended run times and false positive results (O'Farrell, 2008). In the present study, selection of nitrocellulose membrane was carried out by analyzing running buffer mixed with detection conjugate (negative controls) on three different nitrocellulose membranes. Figure 2 indicated that UniSart® CN140 (Sartorius) and CNPC-SS12, 10 µm (MDI technologies) produced higher signal intensities than UniSart® CN95 (Sartorius). Although the difference in signal intensity between UniSart® CN140 and CNPC-SS12, 10 µm was not statistically significant ($p = 0.9209$), sample uptake time was significantly lower on UniSart® CN140 (Figure 2B). Therefore, UniSart® CN140 from Sartorius was chosen for subsequent experiments.

Optimization of dilution factor of maize extract. Food sample extracts are commonly diluted before analysis by LFIA to minimize the negative effects of sample matrix on antibody-antigen reactions (Anfossi *et al.*, 2011; Lattanzio *et al.*, 2012). To determine the optimal dilution factor, blank maize grains were subjected to extraction using phosphate buffer (PB) and dilution in running buffer with ratios ranging from 1:3 to 1:20. According to Pietri & Bertuzzi (2011), average recovery percentages were 95.5±1.9% and 96.7±2.1% for FB₁ and FB₂ when extracted in PB. Furthermore, this extraction method does not require the use of toxic solvents and it may prevent possible inhibiting effects of organic solvents on antibody-antigen reaction (Rehan & Younus, 2006; Russell *et al.*, 1989). Test line intensities generated by the diluted extract samples were compared with those from negative controls. Figure 3 revealed that signal intensities were decreased at dilution factors of 1:3 and 1:5, comparing to those from negative controls. However, starting from 1:10 dilution, test line signals were similar to negative controls (Figure 3B). As a result, an optimal dilution factor of 1:10 was set for subsequent experiments.

Optimization of amount of detection conjugate. Quantity of labeled antibody directly affects the limit of detection of a competitive LFIA. In fact, if a low amount of detection conjugate is

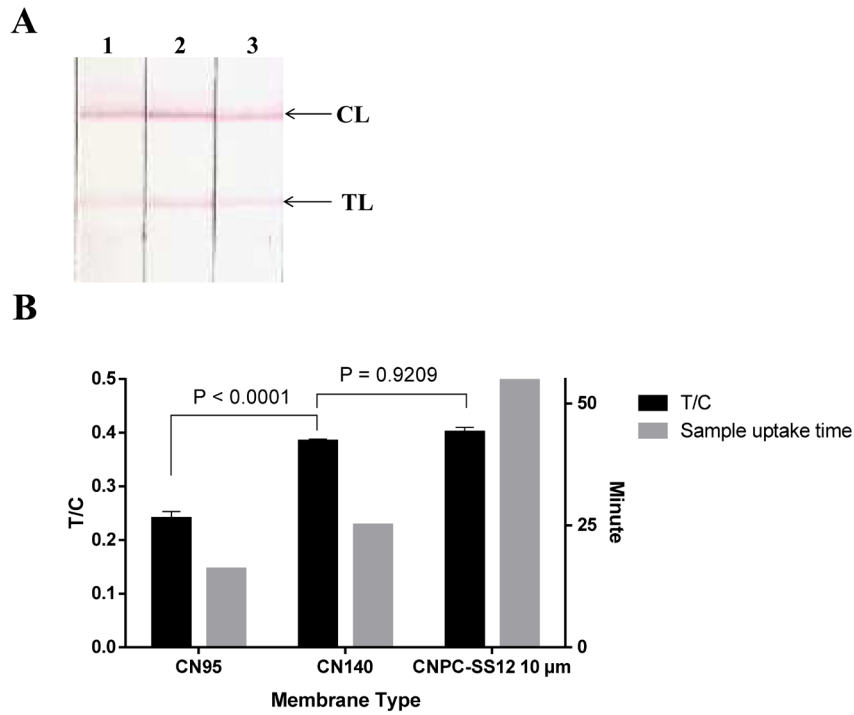


Figure 2. Selection of nitrocellulose membrane. (A) Images of negative controls (0 μg/mL fumonisin group B (FB)) on three different nitrocellulose membranes; 1, UniSart® CN95 (Sartorius), 2, UniSart® CN140 (Sartorius); 3, CNPC-SS12 10 μm (MDI technologies); CL, control line; TL, test line. (B) Quantification of signal intensities and sample uptake time for each type of membrane. Sample uptake time is defined as the total time required for membranes to absorb fluid completely. T, test line signal; C, control line signal.

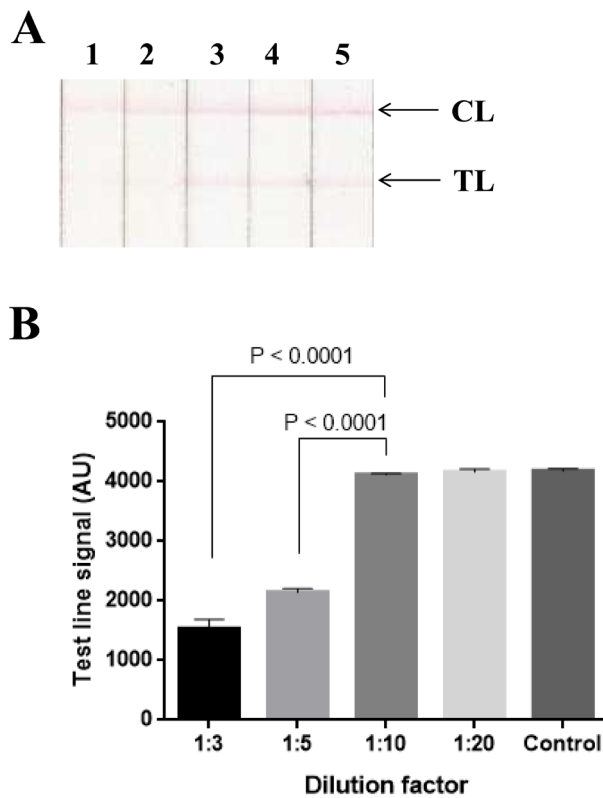


Figure 3. Optimization of dilution factor of maize extract. (A) Images of diluted extracts of blank maize samples (negative with fumonisin group B (FB)) by high performance liquid chromatography (HPLC) with different dilution factors on representative lateral flow immunoassay strips; 1, 2, 3, 4, maize homogenates diluted 3-, 5-, 10-, 20-fold respectively in running buffer; 5, negative controls. CL, control line; TL, test line. (B) Quantification of test line signals. Control, negative control; AU, arbitrary unit.

used, no visible test lines will be formed even low levels of FB₁ (less than 4000 µg/kg) are present in the samples. Furthermore, using a low amount of detection conjugate will decrease the signal intensities at both test and control lines, causing difficulties in result interpretation. On the other hand, using an excessive amount of detection conjugate will negatively affect the analytical sensitivity of the assay as more toxins are required to saturate all the binding sites of the detection antibody.

In the present study, various amounts (174 ng, 436 ng, and 697 ng corresponding to 2, 5, and 8 µl) of detection conjugate were used to react with FB₁ extracted from blank samples spiked with this toxin at 2000 µg/kg, 4000 µg/kg or 8000 µg/kg. For samples spiked with 2000 µg/kg FB₁, test lines were observed on all test strips regardless of the amounts of detection conjugate used (Figure 4). Conversely, no test line was observed when FB₁ is present at 8000 µg/kg (Figure 4). At the cut-off level of 4000 µg/kg, test line signal was still present when 697 ng of detection conjugate were used while no test line was visible when 174 ng or 436 ng of detection conjugate were used. However, using 174 ng of detection conjugate resulted in low signals of test line on negative controls (Figure 4). Therefore, 436 ng of IgY-conjugated gold nanoparticles were used for further studies.

Optimization of incubation time between samples and detection conjugate. Effects of incubation step between detection conjugate and FB₁ at the cut-off level (extracted from blank samples spiked with FB₁ at 4000 µg/kg) on the test performance were assessed by varying the incubation time from 0 to 60 minutes. Results (Figure 5) indicated that test lines were still visible when the incubation time was 0 or 15 minutes, while no test lines were observed when the incubation time was 30 or 60 minutes. To shorten the analytical procedure, an incubation time of 30 minutes was chosen.

Determination of limit of detection of the developed LFIA for FB₂ in maize

Previously, we have shown that the polyclonal IgY antibody used in the present study, recognized FB₁ and FB₂ with different affinities (IC₅₀ = 10 and 49 ng/ml for FB₁ and FB₂ respectively) (Do *et al.*, 2016). To determine if the developed LFIA could detect FB₂ in maize at the cut-off level of 4000 µg/kg, this toxin was spiked into a blank sample at 2000 µg/kg, 4000 µg/kg, and 8000 µg/kg. Results (Figure 6) showed that no visible line was formed at test zone for samples spiked with 4000 µg/kg and 8000 µg/kg of FB₂. On the contrary, faint signals were still observed at the test line for samples spiked with 2000 µg/kg of FB₂. Therefore, the limit of detection of our

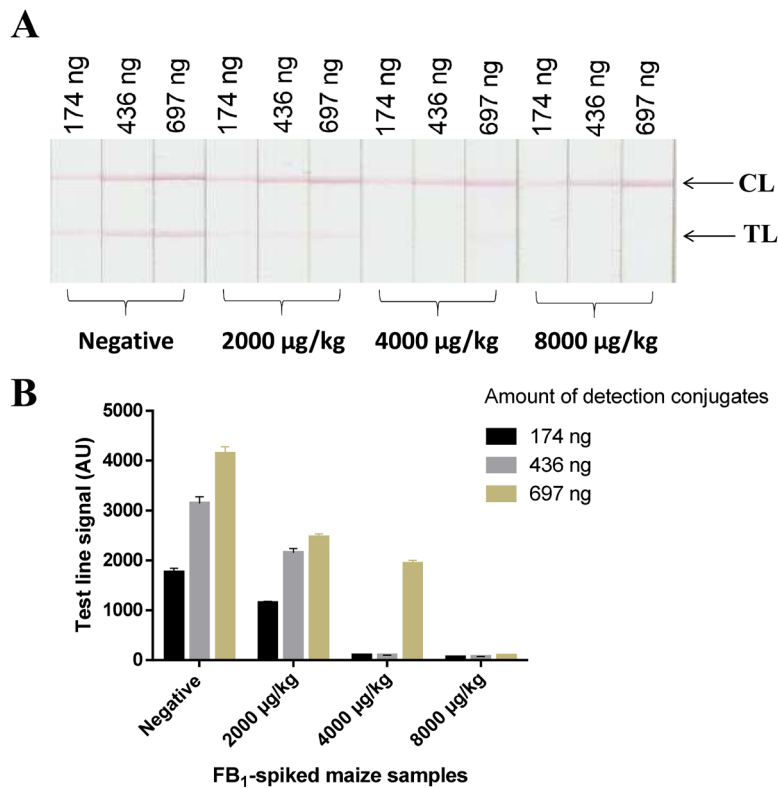


Figure 4. Optimization of amount of detection conjugate to achieve the detection limit of 4000 µg/kg of fumonisin B₁ (FB₁). (A) Images of negative controls and diluted extracts of blank maize samples spiked with FB₁ at 2000 µg/kg, 4000 µg/kg, and 8000 µg/kg using 174 ng, 436 ng or 697 ng of detection conjugate on representative lateral flow immunoassay (LFIA) strips. Maize extracts were incubated with detection conjugate for one hour at room temperature before being analyzed on LFIA strips. Experiment was performed with 8 replicates. CL, control line; TL, test line. (B) Quantification of test line signals. AU, arbitrary unit.

LFIA for FB₂ was also 4000 µg/kg, meaning that the developed test could be used for screening of total FBs in maize. The extended incubation time between detection conjugate and the toxins (30 minutes) and the optimal amount of labeled antibody are likely able to compensate for the difference in affinity of IgY antibody for FB₁ and FB₂.

Cross-reactivity tests

Cross-reactivity tests were performed using deoxynivalenol, ochratoxin A, aflatoxin B1 and zearalenone spiked into blank maize extracts at two different concentration levels (100-fold and 1000-fold of MRL). Figure 7 showed that there was no difference in signal intensities at test line position between

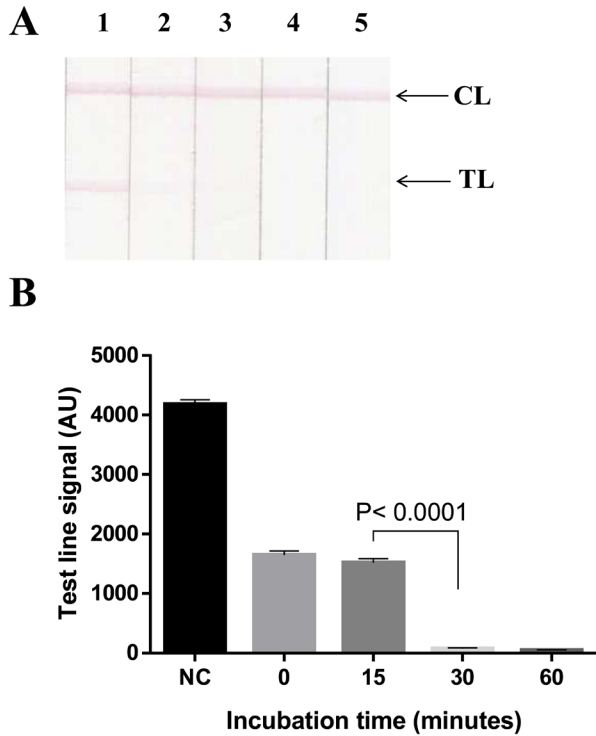


Figure 5. Optimization of incubation time between detection conjugate and samples to be analyzed. (A) Images of diluted extract (from blank sample spiked with fumonisin B₁ (FB₁) at 4000 µg/kg) incubated with 436 ng of detection conjugate for 0, 15, 30 and 60 minutes on representative lateral flow immunoassay strips. 1, negative control; 2, 3, 4, 5, incubation time of 0, 15, 30, 60 minutes respectively; CL, control line; TL, test line. (B) Quantification of signal intensities. NC, negative control; AU, arbitrary unit.

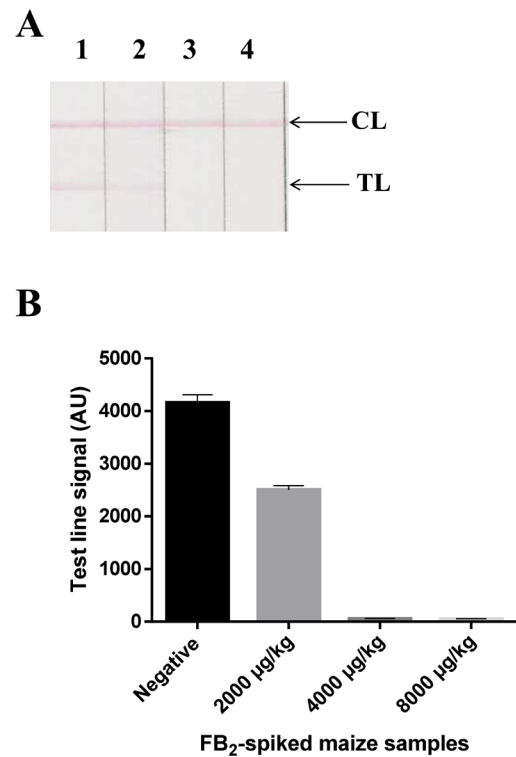


Figure 6. Limit of detection of fumonisin B₂ (FB₂) in maize by the developed lateral flow immunoassay. (A) Images of negative controls (1) and diluted extracts of blank maize sample spiked with FB₂ at 2000 µg/kg (2), 4000 µg/kg (3), and 8000 µg/kg (4) on representative lateral flow immunoassay strips. CL, control line; TL, test line. Experiment was performed with 8 replicates. (B) Quantification of test line signals. AU, arbitrary unit.

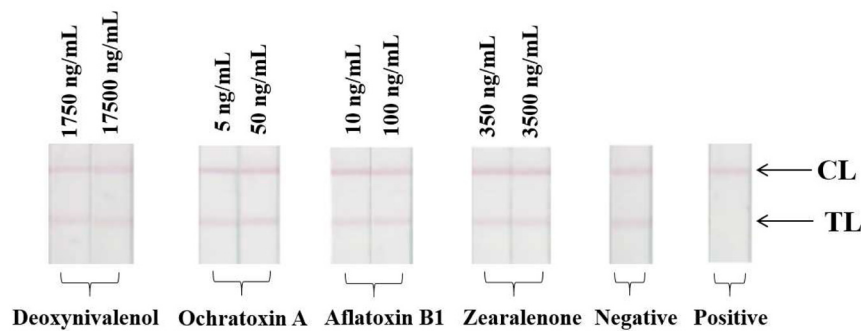


Figure 7. Cross-reactivity of the developed lateral flow immunoassay against deoxynivalenol, ochratoxin A, aflatoxin B1, and zearalenone. Deoxynivalenol (1750 and 17500 ng/mL), ochratoxin A (5 and 50 ng/mL), aflatoxin B1 (10 and 100 ng/mL), zearalenone (350 and 3500 ng/mL) were spiked into diluted extracts of blank maize samples and incubated with detection conjugate for 30 minutes before being analyzed on lateral flow immunoassay (LFIA) test strips. Negative, negative controls; Positive, 40 ng/mL fumonisin B₁ (FB₁), CL, control line; TL, test line.

negative control and samples spiked with low and high concentrations of the tested mycotoxins. Therefore, the developed LFIA did not cross-react with deoxynivalenol, ochratoxin A, aflatoxin B1 and zearalenone.

Analysis of naturally contaminated maize samples by IgY LFIA

A total of 19 maize samples were analyzed by HPLC-MS/MS and the developed assay. By HPLC-MS/MS, all samples were negative with FB₂, while 11 samples were positive with FB₁, with concentrations ranging from 27 µg/kg to 7850 µg/kg (Table 1). One sample (No 16: 7850 µg/kg of FB₁) exceeded the maximum legislative limits (EU) of 4000 µg/kg (EC, 2007). Notably, only this sample was positive by IgY LFIA. Although the sample size was relatively low, these findings indicated that results by the IgY LFIA were in good agreement with those from the standard method as no false positive or false negative results were found.

Discussion

Fumonisin contamination in maize is now a widely recognized problem and it has been shown to be relevant in many regions of the World, including Vietnam (Hieu Phuong *et al.*, 2015; Hu *et al.*, 2019; Mngqawa *et al.*, 2016; Scussel *et al.*, 2014). Among fumonisins, the most prevalent that contaminates maize is FB₁, followed by FB₂ (Anfossi *et al.*, 2010; Marasas, 2001). In

term of toxicity, fumonisins have been associated with adverse health conditions such as pulmonary edema (Harrison *et al.*, 1990); liver and nephron damage (Gelderblom *et al.*, 1988); or liver and esophagus cancers (Marasas *et al.*, 1988). As a result, international regulations have been adopted to restrict the maximum residue limit of total fumonisins (as the sum of FB₁ and FB₂) in raw maize at 4000 µg/kg (EC, 2007). Conventional analytical methods for fumonisins consist of liquid chromatography coupled with detectors such as UV-Vis spectrophotometry, fluorescence, and mass spectrometry (Kamle *et al.*, 2019). Although these methods allow quantification of individual fumonisin in food samples, they require complex equipment and need several hours to complete. Consequently, rapid methods for fumonisin analysis such as LFIAs have become increasingly important as they are less expensive, easier to use, very rapid and suitable for on-site analysis. However, LFIAs can only provide qualitative or semi-quantitative results and are therefore recommended for screening purpose (Zheng *et al.*, 2006). For any positive samples detected by LFIAs, the exact mycotoxin concentration needs to be determined by a confirmatory reference method such as HPLC (Zheng *et al.*, 2006).

Several lateral flow immunoassays for fumonisin detection in maize have been reported, with limits of detection (LODs) ranging from 12-1200 µg/kg (Anfossi *et al.*, 2010; Molinelli *et al.*, 2009; Urusov *et al.*, 2017; Wang *et al.*, 2013). Nevertheless,

Table 1. Analysis of naturally contaminated maize samples by high performance liquid chromatography–mass spectrometry (HPLC-MS/MS) method and the IgY Lateral Flow Immunoassay.

Sample	FB ₁ concentration by HPLC-MS/MS (µg/kg)	FB ₂ concentration by HPLC-MS/MS (µg/kg)	Analysis by the developed LFIA
1	1760	Not detected	Negative
2	Not detected	Not detected	Negative
3	183	Not detected	Negative
4	95	Not detected	Negative
5	Not detected	Not detected	Negative
6	Not detected	Not detected	Negative
7	Not detected	Not detected	Negative
8	27	Not detected	Negative
9	129	Not detected	Negative
10	1150	Not detected	Negative
11	Not detected	Not detected	Negative
12	840	Not detected	Negative
13	Not detected	Not detected	Negative
14	Not detected	Not detected	Negative
15	148	Not detected	Negative
16	7850	Not detected	Positive
17	Not detected	Not detected	Negative
18	35	Not detected	Negative
19	322	Not detected	Negative

the low LODs of these assays are not relevant in regard to the adopted MRL of 4000 µg/kg, as they would generate an increased number of false-positive results (according to the adopted MRL) that will be revealed by costly confirmatory methods. This will increase the cost of the whole analytical procedure. To mitigate this problem, some commercial LFIA require a reader, which allows semi-quantitative analysis of fumonisins in the samples (Reveal® Q+ from Neogen, Fumo-V from Vicam, AgraStrip® WATEX® from Romer Labs). However, the applicability of these assays is limited in low-resourced environments because of the high cost of the reader. In the present study, we have developed a novel lateral flow immunoassay for total fumonisin (as the sum of FB₁ and FB₂) with LOD equal to the MRL of 4000 µg/kg. In the developed assay, a positive sample with a total fumonisin concentration greater than or equal to 4000 µg/kg will result in no visible signal at the test line, while a negative sample with a fumonisin concentration less than or equal to 2000 µg/kg will generate a visible signal at the test line. This would reduce the false positive rate comparing to other LFIAs reported in the literature, while maintaining the simplicity of the assay as the results could be read with the naked eye.

Another novelty of this study is the use of polyclonal IgY antibody for the preparation of detection conjugate in LFIA. To the best of our knowledge, this is the first immunochromatographic test for fumonisins that employs IgY as the principal component. Most LFIAs for fumonisins reported to date are based on monoclonal antibodies that are costly to produce and involves ethical issues of animal welfare (Dias da Silva & Tambourgi, 2010). We have previously shown that the IC₅₀ values of the IgY used in this study were 10 and 49 ng/mL for FB1 and FB2 respectively (Do *et al.*, 2016), meaning that the affinities of our IgY towards these mycotoxins are comparable to or even higher than monoclonal antibodies reported in the literature (Ling *et al.*, 2014; Maragos *et al.*, 2018; Yu & Chu, 1999; Zhang *et al.*, 2018a). Therefore, even though there is no difference in reactant consumption between IgY-based LFIA and IgG-based tests for fumonisin detection, IgY-based LFIA is significantly cheaper due to the abundance of IgY in the egg yolk. We obtained approximately 8 mg of purified IgY from a single egg, which is enough to produce 320000 tests. Of note, IgY in this study was produced in 2016, stored at -80°C, and used for the development of the assay during the period of 2018–2019, meaning that polyclonal IgY

antibody was quite stable over extended periods of time under preservation at -80°C.

One limitation of the LFIA developed in this study is that the analysis time is longer than those of commercial assays as it requires an incubation step of 30 minutes to allow FB₁ and FB₂ to saturate the binding sites of detection antibodies. However, as mentioned above, this additional step was likely able to compensate for the difference in affinity of IgY antibody for FB₁ and FB₂, enabling the new assay to meet the cut-off level of 4000 µg/kg for both these toxins.

Conclusions

In conclusion, this is the first study to use polyclonal IgY antibody in LFIA for simple detection of total fumonisins (as the sum of FB1 and FB2) in maize with the limit of detection equal to the MRL of 4000 µg/kg. The main advantage of this assay is its cost-effectiveness and ability to accurately detect total fumonisins at MRL in maize.

Data availability

Underlying data

Figshare: Raw data for “Development of an IgY-based lateral flow immunoassay for detection of fumonisin B in maize”. <https://doi.org/10.6084/m9.figshare.8320775> (Tran *et al.*, 2019)

This project contains the following underlying data:

- Dataset 1_Raw scans of lateral flow test strips.rar (Folder includes raw images of lateral flow strips)
- Dataset 2.pptx (PowerPoint file includes HPLC-MS chromatograms of naturally contaminated samples for quantification of FB1 and FB2.)
- Dataset 3.xlsx (Excel file includes quantification of signal intensities of lateral flow test strips and quantification of FB1 and FB2 from HPLC-MS output.)

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

We would like to thank Trang Huyen Thi Tran and Trang Mai Ta for their support during the production of IgY.

References

Alizadeh AM, Rohandel G, Roudbarmohammadi S, *et al.*: **Fumonisin B1 contamination of cereals and risk of esophageal cancer in a high risk area in northeastern Iran.** *Asian Pac J Cancer Prev.* 2012; 13(6): 2625–2628.
[PubMed Abstract](#) | [Publisher Full Text](#)

Anfossi L, Calderara M, Baggiani C, *et al.*: **Development and application of a quantitative lateral flow immunoassay for fumonisins in maize.** *Anal Chim Acta.* 2010; 682(1–2): 104–109.
[PubMed Abstract](#) | [Publisher Full Text](#)

Anfossi L, D'Arco G, Calderara M, *et al.*: **Development of a quantitative lateral flow immunoassay for the detection of aflatoxins in maize.** *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2011; 28(2): 226–234.
[PubMed Abstract](#) | [Publisher Full Text](#)

Chilaka CA, De Boevre M, Atanda OO, *et al.*: **Occurrence of *Fusarium* Mycotoxins in Cereal Crops and Processed Products (Ogi) from Nigeria.** *Toxins (Basel).* 2016; 8(11): pii: E342.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

- Cigić IK, Prosen H: **An overview of conventional and emerging analytical methods for the determination of mycotoxins.** *Int J Mol Sci.* 2009; **10**(1): 62–115. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dias da Silva W, Tambourgi DV: **IgY: a promising antibody for use in immunodiagnostic and in immunotherapy.** *Vet Immunol Immunopathol.* 2010; **135**(3–4): 173–180. [PubMed Abstract](#) | [Publisher Full Text](#)
- Do NB, Tran THT, Ta MT, *et al.*: **Obtaining high affinity IgY against fumonisin with potential use in immunodiagnostic.** *Vietnamese Journal of Military Pharmacology.* 2016; **41**(5): 29–34.
- EC, European Commission: **Commission Regulation (EC) no 1126/2007 of 28 September 2007 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products.** *Official Journal of the European Union.* 2007; **255**: 14–17. [Reference Source](#)
- European Committee for Standardization (CEN): **CEN EN 13585 – Foodstuffs – Determination of fumonisins B1 and B2 in maize – HPLC method with solid phase extraction clean-up.** European Committee for Standardization, Brussels, Belgium, 2001. [Reference Source](#)
- Franceschi S, Bidoli E, Barón AE, *et al.*: **Maize and risk of cancers of the oral cavity, pharynx, and esophagus in northeastern Italy.** *J Natl Cancer Inst.* 1990; **82**(17): 1407–1411. [PubMed Abstract](#) | [Publisher Full Text](#)
- Fu M, Li R, Guo C, *et al.*: **Natural incidence of Fusarium species and fumonisins B₁ and B₂ associated with maize kernels from nine provinces in China in 2012.** *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2015; **32**(4): 503–511. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gandhi S, Caplash N, Sharma P, *et al.*: **Strip-based immunochromatographic assay using specific egg yolk antibodies for rapid detection of morphine in urine samples.** *Biosens Bioelectron.* 2009; **25**(2): 502–505. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gelderblom WC, Jaskiewicz K, Marasas WF, *et al.*: **Fumonisin--novel mycotoxins with cancer-promoting activity produced by Fusarium moniliforme.** *Appl Environ Microbiol.* 1988; **54**(7): 1806–1811. [PubMed Abstract](#) | [Free Full Text](#)
- Getachew A, Chala A, Hofgaard IS, *et al.*: **Multimycotoxin and fungal analysis of maize grains from south and southwestern Ethiopia.** *Food Addit Contam Part B Surveill.* 2018; **11**(1): 64–74. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gruber-Dorminger C, Jenkins T, Schatzmayr G: **Multi-mycotoxin screening of feed and feed raw materials from Africa.** *World Mycotoxin Journal.* 2018; **11**(3): 369–383. [Publisher Full Text](#)
- Guo C, Liu Y, Jiang Y, *et al.*: **Fusarium species identification and fumonisin production in maize kernels from Shandong Province, China, from 2012 to 2014.** *Food Addit Contam Part B Surveill.* 2016; **9**(3): 203–209. [PubMed Abstract](#) | [Publisher Full Text](#)
- Haliburton JC, Buck WB: **Equine leucoencephalomalacia: an historical review.** In: *Diagnosis of Mycotoxicoses.* Springer. 1986; **33**: 75–79. [Publisher Full Text](#)
- Harrison LR, Colvin BM, Greene JT, *et al.*: **Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of Fusarium moniliforme.** *J Vet Diagn Invest.* 1990; **2**(3): 217–221. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hieu Phuong N, Quang Thieu N, Ogle B, *et al.*: **Aflatoxins, Fumonisin and Zearalenone Contamination of Maize in the Southeastern and Central Highlands Provinces of Vietnam.** 2015; **5**(4): 1195–1203. [Publisher Full Text](#)
- Hu L, Liu H, Yang J, *et al.*: **Free and hidden fumonisins in raw maize and maize-based products from China.** *Food Addit Contam Part B Surveill.* 2019; **12**(2): 90–96. [PubMed Abstract](#) | [Publisher Full Text](#)
- Jin W, Yamada K, Ikami M, *et al.*: **Application of IgY to sandwich enzyme-linked immunosorbent assays, lateral flow devices, and immunopillar chips for detecting staphylococcal enterotoxins in milk and dairy products.** *J Microbiol Methods.* 2013; **92**(3): 323–331. [PubMed Abstract](#) | [Publisher Full Text](#)
- Kamala A, Kimanya M, Haesaert G, *et al.*: **Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro ecological zones of Tanzania.** *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016; **33**(3): 551–559. [PubMed Abstract](#) | [Publisher Full Text](#)
- Kamle M, Mahato DK, Devi S, *et al.*: **Fumonisin: Impact on Agriculture, Food, and Human Health and their Management Strategies.** *Toxins (Basel).* 2019; **11**(6): pii: E328. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kovacs-Nolan J, Mine Y: **Avian egg antibodies: basic and potential applications.** *Avian Poult Biol Rev.* 2004; **15**(1): 25–46. [Reference Source](#)
- Lattanzio VM, Nivarlet N, Lippolis V, *et al.*: **Multiplex dipstick immunoassay for semi-quantitative determination of Fusarium mycotoxins in cereals.** *Anal Chim Acta.* 2012; **718**: 99–108. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ling S, Pang J, Yu J, *et al.*: **Preparation and identification of monoclonal antibody against fumonisin B₁ and development of detection by Ic-ELISA.** *Toxicol.* 2014; **80**: 64–72. [PubMed Abstract](#) | [Publisher Full Text](#)
- Liu Y, Jiang Y, Li R, *et al.*: **Natural occurrence of fumonisins B₁ and B₂ in maize from eight provinces of China in 2014.** *Food Addit Contam Part B Surveill.* 2017; **10**(2): 113–117. [PubMed Abstract](#) | [Publisher Full Text](#)
- Maragos CM, Sieve KK, Busman M: **Development of antibodies for N-(1-deoxy-D-fructos-1-yl) fumonisin B₁ and cross-reaction with modified fumonisins.** *World Mycotoxin J.* 2018; **11**(4): 493–502. [Publisher Full Text](#)
- Marasas WF: **Discovery and occurrence of the fumonisins: a historical perspective.** *Environ Health Perspect.* 2001; **109** Suppl 2: 239–243. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Marasas WF, Jaskiewicz K, Venter FS, *et al.*: **Fusarium moniliforme contamination of maize in oesophageal cancer areas in Transkei.** *S Afr Med J.* 1988; **74**(3): 110–114. [PubMed Abstract](#)
- Mngqawa P, Shephard GS, Green IR, *et al.*: **Mycotoxin contamination of home-grown maize in rural northern South Africa (Limpopo and Mpumalanga Provinces).** *Food Addit Contam Part B Surveill.* 2016; **9**(1): 38–45. [PubMed Abstract](#) | [Publisher Full Text](#)
- Molinelli A, Grossalber K, Krska R: **A rapid lateral flow test for the determination of total type B fumonisins in maize.** *Anal Bioanal Chem.* 2009; **395**(5): 1309–1316. [PubMed Abstract](#) | [Publisher Full Text](#)
- Munkvold GP, Arias S, Taschl I, *et al.*: **Chapter 9 - Mycotoxins in Corn: Occurrence, Impacts, and Management.** In S. O. Serna-Saldivar (Ed.), *Corn (Third Edition)*. Oxford: AACC International Press. 2019; 235–287. [Publisher Full Text](#)
- Mutiga SK, Hoffmann V, Harvey JW, *et al.*: **Assessment of Aflatoxin and Fumonisin Contamination of Maize in Western Kenya.** *Phytopathology.* 2015; **105**(9): 1250–1261. [PubMed Abstract](#) | [Publisher Full Text](#)
- Mwalwayo DS, Thole B: **Prevalence of aflatoxin and fumonisins (B₁ + B₂) in maize consumed in rural Malawi.** *Toxicol Rep.* 2016; **3**: 173–179. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- O'Farrell B: **Evolution in Lateral Flow-Based Immunoassay Systems.** *Lateral Flow Immunoassay.* 2008; 1–33. [Publisher Full Text](#)
- Pauly D, Chacana PA, Calzado EG, *et al.*: **IgY technology: extraction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation.** *J Vis Exp.* 2011; (51): pii: 3084. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pietri A, Bertuzzi T: **Simple Phosphate Buffer Extraction for the Determination of Fumonisin in Masa, Maize, and Derived Products.** *Food Anal Methods.* 2011; **5**(5): 1088–1096. [Publisher Full Text](#)
- Posthuma-Trumpie GA, Korf J, van Amerongen A: **Development of a competitive lateral flow immunoassay for progesterone: influence of coating conjugates and buffer components.** *Anal Bioanal Chem.* 2008; **392**(6): 1215–1223. [PubMed Abstract](#) | [Publisher Full Text](#)
- Rehan M, Younus H: **Effect of organic solvents on the conformation and interaction of catalase and anticatalase antibodies.** *Int J Biol Macromol.* 2006; **38**(3–5): 289–295. [PubMed Abstract](#) | [Publisher Full Text](#)
- Russell AJ, Trudel LJ, Skipper PL, *et al.*: **Antibody-antigen binding in organic solvents.** *Biochem Biophys Res Commun.* 1989; **158**(1): 80–85. [PubMed Abstract](#) | [Publisher Full Text](#)
- Schneider CA, Rasband WS, Eliceiri KW: **NIH Image to ImageJ: 25 years of image analysis.** *Nat Methods.* 2012; **9**(7): 671–675. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Scott PM: **Recent research on fumonisins: a review.** *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2012; **29**(2): 242–248. [PubMed Abstract](#) | [Publisher Full Text](#)
- Scussell VM, Savi GD, Costas LL, *et al.*: **Fumonisin in corn (Zea mays L.) from Southern Brazil.** *Food Addit Contam Part B Surveill.* 2014; **7**(2): 151–155. [PubMed Abstract](#) | [Publisher Full Text](#)
- Sun G, Wang S, Hu X, *et al.*: **Fumonisin B₁ contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China.** *Food Addit Contam.* 2007; **24**(2): 181–185. [PubMed Abstract](#) | [Publisher Full Text](#)
- Szurdoki F, Trousdale E, Ward B, *et al.*: **Synthesis of Protein Conjugates and Development of Immunoassays for AAL Toxins.** *J Agric Food Chem.* 1996; **44**(7): 1796–1803. [Publisher Full Text](#)
- Tran VT, Do NB, Nguyen TPT, *et al.*: **Raw data for "Development of an IgY-based lateral flow immunoassay for detection of fumonisin B in maize".** *figshare.* Dataset. 2019. <http://www.doi.org/10.6084/m9.figshare.8320775.v4>

Urusov AE, Petrakova AV, Gubaydullina MK, *et al.*: **High-sensitivity immunochromatographic assay for fumonisin B, based on indirect antibody labeling.** *Biotechnol Lett.* 2017; **39**(5): 751–758.

[PubMed Abstract](#) | [Publisher Full Text](#)

Venkataramana M, Navya K, Chandranayaka S, *et al.*: **Development and validation of an immunochromatographic assay for rapid detection of fumonisin B1 from cereal samples.** *J Food Sci Technol.* 2014; **51**(9): 1920–1928.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Wang YK, Shi YB, Zou Q, *et al.*: **Development of a rapid and simultaneous immunochromatographic assay for the determination of zearalenone and fumonisin B1 in corn, wheat and feedstuff samples.** *Food Control.* 2013; **31**(1): 180–188.

[Publisher Full Text](#)

Wang Z, Li H, Li C, *et al.*: **Development and application of a quantitative fluorescence-based immunochromatographic assay for fumonisin b1 in maize.** *J Agric Food Chem.* 2014; **62**(27): 6294–6298.

[PubMed Abstract](#) | [Publisher Full Text](#)

Wielogorska E, Mooney M, Eskola M, *et al.*: **Occurrence and Human-Health Impacts of Mycotoxins in Somalia.** *J Agric Food Chem.* 2019; **67**(7): 2052–2060.

[PubMed Abstract](#) | [Publisher Full Text](#)

Yamada K, Wanchun J, Ohkura T, *et al.*: **Detection of methicillin-resistant**

***Staphylococcus aureus* using a specific anti-PBP2a chicken IgY antibody.** *Jpn J Infect Dis.* 2013; **66**(2): 103–108.

[PubMed Abstract](#) | [Publisher Full Text](#)

Yu FY, Chu FS: **Production and Characterization of Monoclonal Antibodies against Fumonisin B1.** *Food Agr Immunol.* 1999; **11**(4): 297–306.

[Publisher Full Text](#)

Yu Q, Li H, Li C, *et al.*: **Gold nanoparticles-based lateral flow immunoassay with silver staining for simultaneous detection of fumonisin B1 and deoxynivalenol.** *Food Control.* 2015; **54**: 347–352.

[Publisher Full Text](#)

Zhang X, Wang Z, Fang Y, *et al.*: **Antibody Microarray Immunoassay for Simultaneous Quantification of Multiple Mycotoxins in Corn Samples.** *Toxins (Basel).* 2018a; **10**(10): pii: E415.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Zhang Y, Kong H, Liu X, *et al.*: **Quantum dot-based lateral-flow immunoassay for rapid detection of rhein using specific egg yolk antibodies.** *Artif Cells Nanomed Biotechnol.* 2018b; **46**(8): 1685–1693.

[PubMed Abstract](#) | [Publisher Full Text](#)

Zheng MZ, Richard JL, Binder J: **A review of rapid methods for the analysis of mycotoxins.** *Mycopathologia.* 2006; **161**(5): 261–273.

[PubMed Abstract](#) | [Publisher Full Text](#)

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Version 2

Reviewer Report 08 January 2020

<https://doi.org/10.5256/f1000research.23755.r57741>

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Zhanhui Wang 

Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, China Agricultural University, Beijing, China

I generally agree the author's response to my review and suggest the paper should be accepted after minor revision. I suggest the authors should cited recent and typical references to support their study, for example, from my group. We have tested the cross-reactivity of antibody for FB3 and we have extensively study immunoassay for FB1 detection.

1. Chenglong Li, Tiejun Mi, Gea Oliveri Conti, Qing Yu, Kai Wen, Jianzhong Shen, Margherita Ferrante, **Zhanhui Wang***. Development of a screening fluorescence polarization immunoassay for the simultaneous detection of fumonisins B₁ and B₂ in maize. *Journal of Agricultural and Food Chemistry*, 2015, 63, 4940–4946¹.
2. Qing Yu, Heng Li, Chenglong Li, Suxia Zhang, Jianzhong Shen, **Zhanhui Wang***. Gold nanoparticles-based lateral flow immunoassay with silver staining for simultaneous detection of fumonisin B1 and deoxynivalenol in maize. *Food Control*, 2015, 54, 347–352².
3. **Zhanhui Wang**, Heng Li, Chenglong Li, Qing Yu, Jianzhong Shen*, Sarah De Saeger. Development and application of a quantitative fluorescence-based immunochromatographic assay for fumonisin B₁ in maize. *Journal of Agricultural Food and Chemistry*, 2014, 62, 6294–6298³.
4. Yajie Sheng, Wenxiao Jiang, Sarah De Saeger, Jianzhong Shen, Suxia Zhang, **Zhanhui Wang***. Development of a sensitive enzyme-linked immunosorbent assay for the detection of fumonisin B₁ in maize. *Toxicon*, 2012, 60, 1245–1250⁴.
5. Chenglong Li, Kai Wen, Tiejun Mi, Xiya Zhang, Huiyan Zhang, Suxia Zhang, Jianzhong Shen, **Zhanhui Wang***. A universal multi-wavelength fluorescence polarization immunoassay for multiplexed detection of mycotoxins in maize. *Biosensors & Bioelectronics*, 2016, 79, 258–265⁵.

References

1. Li C, Mi T, Conti GO, Yu Q, Wen K, Shen J, Ferrante M, Wang Z: Development of a screening fluorescence polarization immunoassay for the simultaneous detection of fumonisins B₁ and B₂ in maize. *J Agric Food Chem*. 2015; **63** (20): 4940-6 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Yu Q, Li H, Li C, Zhang S, Shen J, Wang Z: Gold nanoparticles-based lateral flow immunoassay with silver staining for simultaneous detection of fumonisin B1 and deoxynivalenol. *Food Control*. 2015; **54**: 347-352 [Publisher Full Text](#)
3. Wang Z, Li H, Li C, Yu Q, Shen J, De Saeger S: Development and application of a quantitative fluorescence-based immunochromatographic assay for fumonisin b1 in maize. *J Agric Food Chem*. 2014; **62** (27): 6294-8 [PubMed Abstract](#) | [Publisher Full Text](#)
4. Sheng Y, Jiang W, De Saeger S, Shen J, Zhang S, Wang Z: Development of a sensitive enzyme-linked immunosorbent assay for the detection of fumonisin B₁ in maize. *Toxicon*. 2012; **60** (7): 1245-50 [PubMed Abstract](#) | [Publisher Full Text](#)
5. Li C, Wen K, Mi T, Zhang X, Zhang H, Zhang S, Shen J, Wang Z: A universal multi-wavelength fluorescence polarization immunoassay for multiplexed detection of mycotoxins in maize. *Biosens Bioelectron*. 2016; **79**: 258-65 [PubMed Abstract](#) | [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 02 September 2019

<https://doi.org/10.5256/f1000research.21541.r52435>

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Boris B. Dzantiev

A.N. Bach Institute of Biochemistry, Research Centre of Biotechnology, Russian Academy of Sciences (RAS), Moscow, Russian Federation

The first IgY-based lateral flow immunoassay of fumonisin B is presented. The earlier known techniques were based on the IgG use. At whole, the study is well-described and contains all necessary stages of the assay optimization, characterization and validation. However, the necessity of new test is not clear from the manuscript. The common advantages of IgY are widely known, but were not demonstrated for the proposed test system. To clarify this question, the data about reached advantages (lower reactants consumption, higher stability, etc.) of the proposed test in comparison with the known IgG-based test strips for fumonisin B should be added with their experimental / quantitative confirmation.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: immunoassays, immunosensors, immunochromatography, nanoparticles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2019

Tung Tran, Hanoi University of Science and Technology, Hanoi, Vietnam

We thank the reviewer for comments and detailed suggestions. We herein clarify issues shown by the reviewer. The manuscript has been rewritten according to the comments, and a discussion part has been added to further explain the need for a new lateral flow immunoassay (LFIA) for total fumonisins (defined as the sum of FB₁ and FB₂) with the limit of detection (LOD) equal to the maximum residue limit (MRL) of this mycotoxin group in raw maize and to underline the advantages of IgY for the production of LFIA.

- The first IgY-based lateral flow immunoassay of fumonisin B is presented. The earlier known techniques were based on the IgG use. At whole, the study is well-described and contains all necessary stages of the assay optimization, characterization and validation. However, the necessity of new test is not clear from the manuscript. The common advantages of IgY are widely known, but were not demonstrated for the proposed test system. To clarify this question, the data about reached advantages (lower reactants consumption, higher stability, etc.) of the proposed test in comparison with the known IgG-based test strips for fumonisin B should be added with their experimental / quantitative confirmation.
- The affinities of polyclonal IgY used in this study (IC₅₀ = 10 and 49 ng/mL for FB₁ and FB₂) towards FB₁ and FB₂ are comparable to monoclonal antibodies reported in the literature (Ling et al., 2014; Maragos et al., 2018; Yu & Chu, 1999; Zhang et al., 2018). Therefore, there is likely no difference in reactant consumption between producing IgY-based and IgG-based LFIAs for fumonisin detection. However, IgY can be produced in large quantity at a much lower cost. We obtained approximately 8 mg of purified IgY from one egg, which is enough to produce 320000 tests.
- IgG has been shown to be more stable than IgY under acid, heat-treatment, and Guanidine-HCl denaturation (Shimizu et al., 1992). However, from our experiences, the IgY polyclonal antibody in

this study was produced in 2016, stored at -80 °C, and used for the development of the assay during the period of 2018-2019. This shows that polyclonal IgY antibody was quite stable over extended periods of time under preservation at -80°C.

- On the stability of the IgY-gold nanoparticle conjugates, our unpublished data showed that the conjugate remained stable beyond 6 months when kept at 4 °C in liquid form.

- In available IgG-based LFIA for fumonisin detection, detection conjugates are dried on conjugate pad, therefore, it is difficult to compare the stability of the developed test with known IgG-based LFIA.

References

Ling, S., Pang, J., Yu, J., Wang, R., Liu, L., Ma, Y., et al. (2014). Preparation and identification of monoclonal antibody against fumonisin B(1) and development of detection by Ic-ELISA. *Toxicon*, 80, 64-72. doi:10.1016/j.toxicon.2013.12.008

Maragos, C. M., Sieve, K. K., & Busman, M. (2018). Development of antibodies for N-(1-deoxy-D-fructos-1-yl) fumonisin B1 and cross-reaction with modified fumonisins. *World Mycotoxin Journal*, 11(4), 493-502. doi:10.3920/MMJ2018.2308

Shimizu, M., Nagashima, H., Sano, K., Hashimoto, K., Ozeki, M., Tsuda, K., et al. (1992). Molecular Stability of Chicken and Rabbit Immunoglobulin G. *Bioscience, Biotechnology, and Biochemistry*, 56(2), 270-274. doi:10.1271/bbb.56.270

Yu, F.-Y., & Chu, F. S. (1999). Production and Characterization of Monoclonal Antibodies against Fumonisin B1. *Food and Agricultural Immunology*, 11(4), 297-306. doi:10.1080/095401099999681

Zhang, X., Wang, Z., Fang, Y., Sun, R., Cao, T., Paudyal, N., et al. (2018). Antibody Microarray Immunoassay for Simultaneous Quantification of Multiple Mycotoxins in Corn Samples. *Toxins*, 10 (10), 415. doi:10.3390/toxins10100415

Competing Interests: None to be disclosed

Reviewer Report 23 August 2019

<https://doi.org/10.5256/f1000research.21541.r52437>

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 **Zhanhui Wang** 

Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, China Agricultural University, Beijing, China

The paper described a an IgY-based lateral flow immunoassay for detection of fumonisin B in maize. In general, the paper is more like an experimental report than scientific study in my opinion. The paper provided little new information for readers and the commercial products of LFIA for FBs are already available in the market with high performances. All techniques used in the study are conventional and no any improvement was achieved.

1. The LFIA for the rapid detection of FBs are reported by using many probes not only gold. The performance of the LFIA developed by the authors in term of sensitivity, specificity and accuracy

are not comparable with those of reports.

2. The novelty of the study relied on the first report of usage of IgY in LFIA for FB in my opinion, however, the production of IgY to FB already reported by the authors. The paper should show the advantages and disadvantages of usage of IgY in LFIA. And there are no comparative data with other antibodies like antibody from mouse or rabbit. Exactly, the production of IgY to FB is also a well established techniques.
3. The main body of the manuscript is the optimization of LFIA conditions, the procedure of optimization is necessary for any analytical methods and not the point of the study.
4. I do not think the IgY used in the study is the first choice since the affinity and specificity of IgY is inferior in comparison with reported antibodies, which may result in inaccuracy determination.
5. The authors should provide the confirmation data for protein conjugates, gold conjugates if they are firstly reported or just cited the reference if these data already reported. For LFIAs, the gold-antibody is important, why the authors have not optimized the preparation of gold-protein conjugates? The low sensitivity of the LFIAs maybe derived from the coating antigens and suggest the authors should evaluate different coating antigens differentiating with ratio, hapten and conjugation methods. In addition, the authors should test the cross-reactivity with FB3 since the analog is available and a potential interferent.

In conclusion, the paper did not describe a new experimental, observational, or computational method, test or procedure. I have to be against indexing and hope the suggestions could help the authors improve the study.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Analytical Chemistry, Food Safety, Antibody production and immunoassay.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 01 Dec 2019

Tung Tran, Hanoi University of Science and Technology, Hanoi, Vietnam

General comments:

We thank the reviewer for comments and detailed suggestions. Please find below our answers for the points raised by the reviewer. The manuscript has been rewritten according to the comments, and a discussion part has been added to further explain the need for a new lateral flow immunoassay (LFIA) for total fumonisins (defined as the sum of FB₁ and FB₂) with the limit of detection (LOD) equal to the maximum residue limit (MRL) of this mycotoxin group in raw maize and to underline the advantages of IgY for the production of LFIA.

- The paper described an IgY-based lateral flow immunoassay for detection of fumonisin B in maize. In general, the paper is more like an experimental report than scientific study in my opinion. The paper provided little new information for readers and the commercial products of LFIA for FBs are already available in the market with high performances. All techniques used in the study are conventional and not any improvement was achieved.
 - We agree that commercial Lateral flow immunoassays (LFIAs) for fumonisins B are available. However, most of which are based on monoclonal antibodies that are costly to produce. Several commercial LFIAs for fumonisins require a reader to obtain accurate results, which limits their applicability in low-resourced environments.
 - In this study, we developed a new polyclonal IgY-based LFIA, which significantly reduces the cost of production. Furthermore, result interpretation is based on complete test line disappearance, instead of a decreased intensity of test line color. Thus, results could be read with the naked eye.
 - Another novelty of this study is that the developed LFIA was optimized to have the limit of detection for total fumonisins equal to the maximum residue limit adopted by the Commission of the European Communities, which would reduce the false positive rate comparing to other LFIAs reported in the literature, while maintaining the simplicity of the assay as the results could be read with the naked eye
- The LFIAs for the rapid detection of FBs are reported by using many probes not only gold. The performance of the LFIA developed by the authors in term of sensitivity, specificity, and accuracy are not comparable with those of reports.
 - We agree that other research groups have successfully applied probes other than gold nanoparticles, which increased the sensitivity of their LFIAs. However, in this case, the developed test is intended for screening of raw maize with LOD equal to the MRL (4000 µg/kg for the sum of FB₁ and FB₂) adopted by the Commission of the European communities. Of note, this maximum level is particularly high compared to MRLs of other mycotoxins in cereals (5 µg/kg for ochratoxin A, 4 µg/kg for total aflatoxins...) (EC, 2006). For that reason, we chose gold nanoparticles, which have high stability, as probes (Guerrini et al., 2018; Sajid et al., 2015).
 - In terms of accuracy, we have performed analyses of naturally contaminated maize samples by both the developed IgY LFIA and HPLC-MS/MS method and found perfect agreement between the two assays. One positive sample by LFIA was confirmed, by HPLC-MS/MS, to be contaminated with FB₁ at the level higher than 4000 µg/kg.

- The novelty of the study relied on the first report of usage of IgY in LFIA for FB in my opinion, however, the production of IgY to FB already reported by the authors. The paper should show the advantages and disadvantages of usage of IgY in LFIA. And there are no comparative data with other antibodies like antibody from mouse or rabbit. Exactly, the production of IgY to FB is also a well established techniques.
 - Indeed, this is the first report of application of IgY in development of LFIA for total fumonisins. In our previous study, IC_{50} values of the obtained IgY were 10 and 49 ng/mL for FB_1 and FB_2 respectively (Do et al., 2016). These results show that the affinities of our IgY towards these mycotoxins are comparable to or even higher than some monoclonal antibodies reported in the literature (Ling et al., 2014; Maragos et al., 2018; Yu & Chu, 1999; Zhang et al., 2018).
 - As mentioned in the manuscript, the main advantage of using IgY in LFIA for total fumonisins is cost-effectiveness. From one egg of the immunized hen, we obtained approximately 8 mg of purified IgY, which is enough to produce 320000 tests.

- The main body of the manuscript is the optimization of LFIA conditions, the procedure of optimization is necessary for any analytical methods and not the point of the study.
 - The aim of the present study is to develop a new LFIA for total fumonisins with LOD equal to MRL of these toxins in raw maize, whose performance, LOD and cross-reactivity pattern rely on selection and optimization of test components. Therefore, it is necessary to perform and report optimization experiments.
 - Other researchers also demonstrated optimization results in their reports of development of LFIA for detection of fumonisins (Anfossi et al., 2010; Hao et al., 2018; Wang et al., 2013)

- I do not think the IgY used in the study is the first choice since the affinity and specificity of IgY is inferior in comparison with reported antibodies, which may result in inaccuracy determination.
 - Once again, with the MRL at 4000 μ g/kg, we do not need a test with very low LOD.
 - In term of test's accuracy, mycotoxins are low-molecular weight molecules, thus, they need to be conjugated with carrier proteins to act as immunogens. Therefore, cross-reactivity with other mycotoxins is not possible, even though certain levels of mycotoxins may be present in the chicken feeds.
 - Our unpublished data also showed an absence of cross-reactivity of IgY against BSA as KLH- FB_1 was used for antibody production while BSA- FB_1 was immobilized on LFIA membranes.

- The authors should provide the confirmation data for protein conjugates, gold conjugates if they are firstly reported or just cited the reference if these data already reported. For LFIA, the gold-antibody is important, why the authors have not optimized the preparation of gold-protein conjugates? The low sensitivity of the LFIA maybe derived from the coating antigens and suggest the authors should evaluate different coating antigens differentiating with ratio, hapten and conjugation methods. In addition, the authors should test the cross-reactivity with FB_3 since the analog is available and a potential interferent.
 - We agree with the reviewer that optimization of gold nanoparticle-antibody is a crucial step for increasing the sensitivity of LFIA, which is relevant in developing an assay that detect contaminants with low MRL, such as Aflatoxin. However, in this study, we aimed to develop a LFIA that detect fumonisins with LOD of 4000 μ g/kg, therefore, gold nanoparticle-antibody conjugation was performed as recommended by the manufacturer to ensure the stability of the conjugate.
 - We agree that cross-reactivity with FB_3 should be tested. However, we currently do not have access to standards of FB_3 . Additionally, all LFIA reported in the literature did not evaluate the

cross-reactivity with FB₃. Furthermore, according to the Commission of the European communities, the maximum residue limit of fumonisins in raw maize accounts for the sum of FB₁ and FB₂ only.

Anfossi, L., Calderara, M., Baggiani, C., Giovannoli, C., Arletti, E., & Giraudi, G. (2010). Development and application of a quantitative lateral flow immunoassay for fumonisins in maize. *Anal Chim Acta*, 682(1-2), 104-109. doi:10.1016/j.aca.2010.09.045

Guerrini, L., Alvarez-Puebla, R. A., & Pazos-Perez, N. (2018). Surface Modifications of Nanoparticles for Stability in Biological Fluids. *Materials (Basel, Switzerland)*, 11(7), 1154. doi:10.3390/ma11071154

Hao, K., Suryoprabowo, S., Hong, T., Song, S., Liu, L., Zheng, Q., et al. (2018). Immunochromatographic strip for ultrasensitive detection of fumonisin B1. *Food and Agricultural Immunology*, 29(1), 699-710. doi:10.1080/09540105.2018.1439455

Ling, S., Pang, J., Yu, J., Wang, R., Liu, L., Ma, Y., et al. (2014). Preparation and identification of monoclonal antibody against fumonisin B(1) and development of detection by lc-ELISA. *Toxicon*, 80, 64-72. doi:10.1016/j.toxicon.2013.12.008

Maragos, C. M., Sieve, K. K., & Busman, M. (2018). Development of antibodies for N-(1-deoxy-D-fructos-1-yl) fumonisin B1 and cross-reaction with modified fumonisins. *World Mycotoxin Journal*, 11(4), 493-502. doi:10.3920/WMJ2018.2308

Sajid, M., Kawde, A.-N., & Daud, M. (2015). Designs, formats and applications of lateral flow assay: A literature review. *Journal of Saudi Chemical Society*, 19(6), 689-705. doi:https://doi.org/10.1016/j.jscs.2014.09.001

Wang, Y., Shi, Y.-B., Zou, Q., Sun, J.-H., Chen, Z.-F., Wang, H.-a., et al. (2013). Development of a rapid and simultaneous immunochromatographic assay for the determination of zearalenone and fumonisin B1 in corn, wheat and feedstuff samples. *Food Control*, 31, 180-188. doi:10.1016/j.foodcont.2012.09.048

Yu, F.-Y., & Chu, F. S. (1999). Production and Characterization of Monoclonal Antibodies against Fumonisin B1. *Food and Agricultural Immunology*, 11(4), 297-306. doi:10.1080/09540109999681

Zhang, X., Wang, Z., Fang, Y., Sun, R., Cao, T., Paudyal, N., et al. (2018). Antibody Microarray Immunoassay for Simultaneous Quantification of Multiple Mycotoxins in Corn Samples. *Toxins*, 10(10), 415. doi:10.3390/toxins10100415

Competing Interests: None to be disclosed

Reviewer Report 14 August 2019

<https://doi.org/10.5256/f1000research.21541.r51089>

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Venkataramana Mudili

Lorven Biologics Pvt. Ltd., Hyderabad, India

The present manuscript submitted by *Tien et al.* is a very interesting study in current food contaminants aspect. However, my main criticism goes to the detection limits of assay and the target selection in the study.

As Fumonisin are a group of toxins, out of which fumonisin was target in the present study was not clear, for example among the fumonisin FB1 is more potent and frequently reported one in many kind of food grains mostly in wheat and maize and the cross reactivity patterns with group of Fusarium toxins need to be studied for example DON, T-2 toxin, Nivalenol etc.

- Introduction part is too vague, need to be improved with recent and relevant references.
- Preparation of IgY-conjugated gold nano particles is not clear, it's totally confusing, authors should clear draft the protocol in a supplementary section or main text.
- Ethical committee guidelines and permission to carry out the study need be given the manuscript.
- ***Selection of nitrocellulose membrane: its very crucial in developing the LFA assay, in the present study sufficient information was not presented by the authors in this section. please be give the clear protocol how its been chosen.***
- OD 4000ug/kg is very high, many studies reported that the LD is under nano gram levels, this need to be further improved for their sensitivity.
- Over study looks good and can be indexed in this journal after substantial revision of the manuscript.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Toxicology and Immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2019

Tung Tran, Hanoi University of Science and Technology, Hanoi, Vietnam

General comments:

We thank the reviewer for comments and detailed suggestions. We herein clarify issues shown by the reviewer. The manuscript has been rewritten according to the comments, and a discussion part has been added to further explain the need for a new lateral flow immunoassay (LFIA) for total fumonisins (defined as the sum of FB₁ and FB₂) with the limit of detection (LOD) equal to the maximum residue limit (MRL) of this mycotoxin group in raw maize and to underline the advantages of IgY for the production of LFIA.

- As Fumonisins are a group of toxins, which fumonisin was target in the present study was not clear, for example, among the fumonisin, FB₁ is more potent and frequently reported one in many kinds of food grains, mostly in wheat and maize.
 - In the present study, the targets are Fumonisin B₁ and Fumonisin B₂ (FB₁ and FB₂) as they are the most frequently reported fumonisins in foods in general, and in raw maize in particular (Abbas, 2006; D'Arco et al., 2009; Magro et al., 2011; Yazar & Omurtag, 2008)
 - Besides, according to the Regulation No 1126/2007 of the Commission of the European communities, the maximum residue limit of fumonisins in raw maize (defined as sum of FB₁ and FB₂) is fixed at 4000 µg/kg (EC, 2007).

- The cross reactivity patterns with group of Fusarium toxins need to be studied for example DON, T-2 toxin, Nivalenol etc.
 - In the manuscript, we have showed an absence of cross-reactivity of the developed test with deoxynivalenol, ochratoxin A, aflatoxin B₁ and zearalenone.
 - DON, T-2 toxin, Nivalenol, which were suggested by the reviewer, belong to the same group of trichothecenes, and have similar structure (McCormick et al., 2011). Therefore, we performed only the cross-reactivity test using DON, which has been shown to be the most prevalent trichothecenes in cereals (Foroud et al., 2019; Payros et al., 2016).

- Introduction part is too vague, need to be improved with recent and relevant references.
 - We thank the reviewer for raising the point, the introduction has been modified and updated with recent literatures.

- Preparation of IgY-conjugated gold nano particles is not clear, it's totally confusing, authors should clear draft the protocol in a supplementary section or main text.
 - The principle of conjugate preparation has been added to the manuscript and the protocol has been rewritten for better clarity.

- Ethical committee guidelines and permission to carry out the study need to be given the manuscript.

- We have provided, in this revision of the manuscript, an ethics approval for animal use for this study.

- Selection of nitrocellulose membrane: it's very crucial in developing the LFA assay, in the present study, sufficient information was not presented by the authors in this section. please be give the clear protocol how it's been chosen.

- We thank the reviewer for raising the point. Details on flow rates of the membranes have been added as follows:

“CNPC-SS12, 10 μ m with wicking time of 140 ± 28 s/40 mm (MDI technologies, Cat N^o CNPC-SS12-10 μ m-25mm), UniSart® CN140 with wicking time of 95-155s/ 40 mm (Sartorius, Cat N^o 1UN14ER100025NTB), and UniSart® CN 95 with wicking time of 65-115s/ 40 mm (Sartorius, Cat N^o 1UN95ER100040WS)”.

- Furthermore, according to the manufacturers, CNPC-SS12-10 μ m is recommended for environmental and agriculture analytes; CN95 is recommended when a quick response is desirable; whereas CN140 is recommended when high sensitivity is required.

- LOD 4000ug/kg is very high, many studies reported that the LOD is under nano gram levels, this need to be further improved for their sensitivity.

- We thank the reviewer for raising this point. It is certainly feasible to optimize the assay to achieve a lower limit of detection. However, the LOD was set at 4000 μ g/kg as it is the maximum residue limit (MRL) adopted by the Commission of the European communities. Similar to other lateral flow immunoassays for detection of mycotoxins in foods, this assay is intended for quick screening, and positive results need to be confirmed by instrumental analytical techniques, such as HPLC, LC/MS. Consequently, if the sensitivity of the assay is in the range of nanograms, we will obtain a lot of false-positive results (according to the adopted MRL) that will be revealed by costly confirmatory methods. This will increase the cost of the whole analytical procedure.

References

Abbas, H. K. (2006). Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop protection*, v. 25(no. 1), pp. 1-9-2006 v.2025 no.2001.

D'Arco, G., Fernández-Franzón, M., Font, G., Damiani, P., & Mañes, J. (2009). Survey of fumonisins B1, B2 and B3 in conventional and organic retail corn products in Spain and Italy and estimated dietary exposure. *Food Additives & Contaminants: Part B*, 2(2), 146-153.
doi:10.1080/02652030903148314

EC, European Commission (2007). Commission Regulation (EC) no 1126/2007 of 28 September 2007 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of the European Union*, L, 255,14–17.

Foroud, N. A., Baines, D., Gagkaeva, T. Y., Thakor, N., Badea, A., Steiner, B., et al. (2019). Trichothecenes in Cereal Grains – An Update. *11*(11), 634.

Magro, S. L., Campaniello, M., Nardiello, D., & Muscarella, M. (2011). Assessment of Fumonisin B1 and B2 Levels in Commercial Maize-Based Food Products by Liquid Chromatography with Fluorimetric Detection and Postcolumn Chemical Derivatization. *J Food Sci*, 76(1), T1-T4.
doi:10.1111/j.1750-3841.2010.01948.x

McCormick, S. P., Stanley, A. M., Stover, N. A., & Alexander, N. J. (2011). Trichothecenes: from

simple to complex mycotoxins. *Toxins*, 3(7), 802-814. doi:10.3390/toxins3070802

Payros, D., Alassane-Kpembé, I., Pierron, A., Loiseau, N., Pinton, P., & Oswald, I. P. (2016). Toxicology of deoxynivalenol and its acetylated and modified forms. *Arch Toxicol*, 90(12), 2931-2957. doi:10.1007/s00204-016-1826-4

Yazar, S., & Omurtag, G. Z. (2008). Fumonisin, trichothecenes and zearalenone in cereals. *International journal of molecular sciences*, 9(11), 2062-2090. doi:10.3390/ijms9112062

Competing Interests: None to be disclosed

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