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Research Article

Performance of the Elabscience Lateral Flow Immuno Assay in Aflatoxin Detection in Uganda

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Abstract

Background: *Maize and groundnuts are staple foods in Uganda. These foods are prone to aflatoxin contamination during pre-harvest and post-harvest stages. However, there is a shortage of screening tests that can be used to routinely detect for presence or absence aflatoxins which are a hazard to human health. The available aflatoxin tests in the market have no established validity. It is, therefore, important to determine the extent to which these tests are able to identify the likely presence or absence of a condition of interest so that their findings encourage appropriate decision making. This study compared the diagnostic performance of the Elabscience Lateral Flow Immuno Assay in detecting aflatoxins between maize and ground nuts samples. We also determined discordances of aflatoxin contamination between maize and ground nut sample compositions using ELISA assay.*

Results: *Lateral Flow Immuno Assay showed aflatoxin diagnostic sensitivity of 11.1%, specificity of 85%, PPV of 25% and NPV of 68% in ground nut samples compared to diagnostic sensitivity of 0%, specificity of 85%, PPV; 0% and NPV of 51.5% in maize samples. Maize whole seeds and homogenized/ crushed foods were more contaminated with aflatoxins than ground whole seeds and homogenized/crushed foods. The difference in aflatoxin contamination between maize and groundnut foods was not statistically significant (p=0.23). Conclusion: Lateral Flow Immuno Assay has a low aflatoxin diagnostic power with sensitivity ranging from 0-11% and specificity of 85%.*

Keywords: Aflatoxins, AF, ELISA, LFIA, Rapid Test, Groundnuts, Maize, Diagnosis, Uganda, Elabscience

1. Introduction

Maize and ground nuts are a staple food in Uganda and constitute a significant proportion of Ugandan diets as the source of various nutrients for both animal and human benefit. Traditionally, these foods are consumed in different forms; as fresh nuts, dried, roasted, as paste or powder (flour). Unfortunately, recent studies have reported that some cereals and legumes on the Ugandan market pose a major health hazard due the Aflatoxin contamination, which may happen during pre-harvest or post-harvest due to poor drying and storage procedures. Studies reveal that aflatoxin concentrations in locally produced foods on Uganda markets are way above the recommended levels set by Uganda National Bureau of Standards (UNBS) and the World Health Organization (WHO) [1-3]. The highest population of aflatoxin producing fungi in Uganda was recorded in raw ground nuts and maize sampled from informal market outlets [4]. Aflatoxins were reported in 80% of ground nut and paste samples traded in metropolitan Kampala with 40% of these having aflatoxin content exceeding WHO compliance limit of 20 µg/kg [5].

1.1. Aflatoxins

Aflatoxins are mycotoxins that are biologically active, meaning that they can multiply, given the right conditions. About 18 different types of aflatoxins have been identified and the most commonly occurring ones are aflatoxin B1, B2, G1, G2, M1 and M2. The M aflatoxins (M1 and M2) are derivatives of B series and have been reported in milk products of animals fed on aflatoxin contaminated foods. Aflatoxins are an extremely toxic and a class 1 carcinogenic compound. Exposure to aflatoxins results in several health-related conditions including acute and chronic aflatoxicosis, aflatoxin-related immune suppression, liver cancer, liver cirrhosis and nutrition -related problems such as stunted growth in children [6-9].

1.2. Safe Aflatoxin Limits

The Uganda National Bureau of Standards in collaboration with other standards bureaus in the East African Community set an aflatoxin contamination limit of 10ppb for all foods and feeds. However, the international standards vary between 4 - 30ppb [10]. Unfortunately, testing for aflatoxins remains a preserve to certify products for the export markets. There is minimal oversight over the quality of food and food products sold in the local markets. Ordinary Ugandans are powerless against aflatoxin contaminated products circulating on the market because they lack affordable, accessible and easy to use aflatoxin detection tools to help them identify the healthy grains that they can buy or sell.

1.3. Testing for Aflatoxins

Aflatoxins are tested using several ways like thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), Immuno Assays like enzyme immunoassays and Lateral Flow Immuno Assays (LFIA) [10,11]. The easiest and cheapest testing methods of these are ELISA (enzyme linked immunosorbent assay) and LFIAs. ELISA is a quantitative test that returns numbers representing the level of contamination, meaning that the higher the number, the more contaminated the sample is. Commercial ELISA kits for detection of Aflatoxins are available in the International market like ELISA Agra Quant Total Aflatoxin, Romer. (Jalan Bukit Merah, Singapore), ELISA BIO SHIELD M1 ES, Pro Gnosis Biotech A.E. (Larissa, Greece), ELISA RIDASCREEN Total Aflatoxin, r-biopharma (Darmstadt, Germany) and Elabscience AF (Total Aflatoxin) ELISA Kit, (Texas, USA) [11,12].

A LFIA is essentially a strip that is infused with reagents. Once a liquid sample is applied, it flows along the membrane, encounters the reagents, and immuno reactions take place which results in a change of color [12]. Usually, the strips have three lines, one being the control line, and the other lines signify positive or negative reactions. In Uganda, one of the commonest ELISA kits on the market, is the Elabscience AF (Total Aflatoxin) ELISA Kit [12]. This is popular among the suppliers because it is cheap, effective and is very sensitive (low detection limits). However, ELISA method still needs expensive equipment, specialized skills to operate and samples need to be in the detection process for a long time [10]. Therefore, it is not available to ordinary people like farmers and retailers, who do not possess the skills to operate the ELISA equipment. On the other hand, strip-based LFIA are easy to use by even a lay man who does not have sophisticated knowledge of operating machines. Elabscience developed an Aflatoxin LFIA which is simple, fast, and said to be sensitive to the detection of aflatoxins. The kit is easy for use by an ordinary person requiring only 2-3 drops of the sample and the observation of line development. Moreover, its storage does not need refrigeration or any special considerations.

The Elabscience LFIA is not popular in Uganda, despite being developed by the same company that produces the popular ELISA test kit. Yet, if the strip is promoted, it provides a faster, more affordable way of testing for aflatoxins on the local Ugandan market. Secondly, LFIA diagnostic performance is not established. It is important to determine the extent to which this test is able to identify the likely presence or absence of a flatoxins so that the findings can encourage appropriate decision-makings. Therefore, this research compared the diagnostic performance of the Elabscience LFIA in detecting aflatoxins between Ugandan variety of maize and ground nuts samples. The study also determined discordances of aflatoxin contamination between maize and ground nut sample compositions using ELISA assay [11]. Compared three ELISA kits with HPLC and the performance was varied in only 10% of the samples. In LFIA was compared to HPLC for the detection of cyromazine and melamine and found to be of comparable performance. In a dual lateral flow immunoassay for detection of aflatoxin B1 was developed and the results show that its specificity and sensitivity matched that of liquid chromatography-tandem mass spectrometry (LC-MS) [13-15]. ELISA was compared with the strip test immuno assay for the detection of aflatoxins in milk from Italian cows and goats; and the results showed both methods passed the fitness for purpose test [16]. However, there is no study in the literature that presents a comparison of the ELISA and LFIA performance on Ugandan variety maize and groundnuts. Such a study is important to inform the circumstances which favor the use of the LFIAs, so as to provide confidence to consumers to adopt the LFIAs in their usual operations in order to reduce their economic or health related losses.

2. Materials and Methods

This study compares diagnostic performance of the Elabscience LFIA and the two grains, which are: Zea mayis L and Arachis hypogaea L, the groundnut and maize varieties respectively.

2.1. Study Design and Population Set Up

The study was conducted in two Agro-ecological zones of Uganda, each represented by a district. We collected maize samples from Masindi district, which is the second highest maize producing district in Western Uganda. It is located in the mid-Western part of Uganda, with its headquarters 216 Kms away from Kampala, the capital city of Uganda. The district is at an average altitude of 1,295 meters above sea level, situated between 10 22’ and 20 20 North of the Equator, longitude 310 22’ and 320 23’ East of the Greenwich Meridian. Groundnuts were collected from Soroti district, which is the highest groundnut producing district in Uganda. Soroti is located in the Eastern part of Uganda, with its headquarters 352 Kms away from Kampala, the capital city of Uganda. The district is at an average altitude of 1,131 meters above sea level, and 1° 42' 52.70" North of the Equator and 33° 36' 40.07" East of the Greenwich Meridian.

2.2. Sample Collection

In January 2022, we bought 40 maize samples (Figure 1a) and 40 groundnut samples (Figure 1b) from food chain dealers in Masindi and Soroti districts respectively. We picked both processed and unprocessed samples in form of seeds, flour or paste, and either raw, roasted or boiled. We bought the samples from farms, transporters, whole sellers,

retailers, processors or restaurants, collected them from street vendors, hotels, super markets, small retail shops, local markets and homesteads. For each sample, 500 grams were packed in sterile zip lock bags, which were sealed, labeled and placed in iced cool boxes at temperature ranges of 2 oC to 6oC and transported to the Uganda Industrial Research Institute (UIRI) laboratory for aflatoxin testing by LFIA and ELISA aflatoxin assay.

(a) (b)

Figure 1: (a) Groundnut Samples from Soroti (b) Maize Samples from Masindi

2.2.1. The Rapid Aflatoxin Lateral Flow Assay

The rapid aflatoxin lateral flow assay involved 2 steps; sample pretreatment and processing and was conducted following the manufacturers' instructions (Elabscience) [17,18]. The samples were crushed using a homogenizer and 2 grams of each crushed sample were added to a 15ml centrifuge tube. 10 ml s of 70% Methanol were added into the centrifuge tube and the mixture centrifuged at 4000 revolutions per minute for 5 minutes at room temperature. Diluted 0.1mls of the supernatant with 0.15mls of deionized water to form the working test sample. Tore the aluminum foil bag of the detection card, took out the detection card, and put it on a smooth, clean table. Pipetted 60µL (3 drops) of the working test sample to the sample well (S) vertically and slowly (while avoiding foaming). Incubated the detection card for 10 minutes, and recorded results within 30minutes of incubation. Appearance of only the control line in the observation well was interpreted as a positive test result while presence of both the test and control lines in the observation window was interpreted as a negative test result (Figure 2).

2.2.2. The ELISA Aflatoxin Assay

The ELISA aflatoxin assay also involved 2 steps; sample pretreatment and analysis [19]. Sample treatment Homogenized the samples (maize and groundnuts) with a homogenizer and mixed them thoroughly. Weighed 2grams of homogenate sample into the 50mls centrifuge tube and added 5mls of 70% Methanol. Vortexed the mixture for 5 min, centrifuged at 4000 r/min for 10 min at room temperature. Transferred 0.5mls of supernatant to another centrifuge tube, added 0.5mls of deionized water, mixed fully.

Sample Analysis

All reagents and samples were restored to room temperature (25°C) before use. All the reagents were mixed thoroughly by gently swirling before pipetting. Labeled multiple wells with sample and standard numbers in the ELISA plate. Added 50µL of standard or sample per well. Added 50µL of HRP Conjugate to each well, then added 50µL of Antibody Working Solution. Covered the plate with plate sealer, oscillated the mixture for 5 seconds, incubated for 30 min at 25°C in shading light. Uncovered the sealer carefully and removed the liquid. Immediately added 300µL of wash buffer to each well and washed. Repeated the wash procedure 5 times, at 30seconds intervals /time. Inverted the plate and pat it against thick clean absorbent paper. Added 50µL of substrate reagent A to each well, and then added 50µL of Substrate Reagent B. Gently oscillated for 5 seconds to thoroughly mix the preparation at incubated at 25°C for 15 min in shading light. Added 50µL of stop solution to each well, oscillated gently to mix thoroughly. Then, determined the optical density (OD value) of each well at 450 nm using a microplate reader. Created a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot [19]. Added average absorbance value of sample to standard curve to get corresponding concentration. A sample was declared positive once its aflatoxin concentration was ≥ 0.1 ng/ml and declared negative if no aflatoxins were detected or aflatoxin detection levels were below < 0.1 ng/ml.

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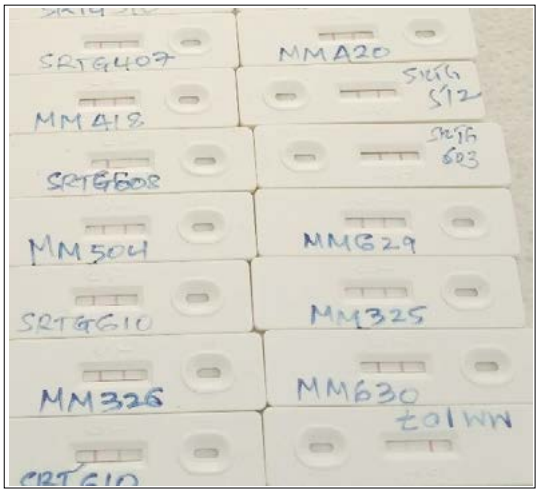

Figure 2: Strips Showing positive and Negative Test Results

2.3. Statistical Analysis

Data was analyzed and expressed in terms of performance parameters of ratios, percentages, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The parameters were computed as follows.

$$Ratio = \frac{Positives}{Positives + Negatives}$$

$$Percentage = \frac{Positives}{Positives + Negatives} * 100$$

$$Accuracy = \frac{True\ Positives + True\ Negatives}{All} * 100$$

$$Sensitivity = \frac{True\ positives}{True\ positives + False\ negatives} * 100$$

$$Specificity = \frac{True\ negatives}{True\ negatives\ + True\ positives} * 100$$

$$PPV = \frac{True\ positives}{True\ positives\ + False\ positives} * 100$$

$$NPV = \frac{True\ negatives}{True\ negatives\ + False\ negatives} * 100$$

3. Results

3.1. Accuracy of the LFIA using ELISA Assay as Gold Standard

Both maize and ground samples were tested using LFIA and ELISA assay. Out of the forty maize samples analyzed, thirty-five (87.5%) yielded valid results, while the five (12.5%) samples yielded invalid results. Based on ELISA assay, sixteen (45.7%) of the maize samples were found contaminated with aflatoxins. The screening LFIA yielded 0 True positives, 16 False negatives, 17 True negatives and 2 False positives. Table 1 provides details of this.

Results	Contaminated		Not contaminated		Total (n)	AF %
Samples	True Positives	*False negatives*	*True negatives*	*False positives*		
Maize	0	16	17	2	**35**	**45.7**
Ground nuts	1	8	17	3	**29**	**31**

Table 1: Shows Aflatoxin Contaminated (True Positive and False Negative) and Non-Aflatoxin Contaminated (True Negative and False Positive) Results of Maize and Ground Nut Samples Based on LFIA and ELISA Assay

For the Forty ground nut samples tested, twenty-nine (72.5%) yielded valid results while eleven (27.5%) samples produced invalid results. According to ELISA assay, nine (31%) of ground nut samples were confirmed to be contaminated with aflatoxins. The LFIA screened 1 True positive, 8 False negatives, 17 True negatives and 3 False positives. See Table 1 for details of this. Generally, the strip is 67% accurate with 71% accuracy on maize and 62% accuracy on groundnuts.

3.2. Performance Characteristics of the LFIA

Diagnostic performance of LFIA in testing for aflatoxins between maize and groundnuts was compared. LFIA showed a aflatoxin diagnostic sensitivity of 11.1%, PPV of 25% and NPV of 68% in ground nut samples compared to diagnostic sensitivity of 0%, PPV of 0% and NPV of 51.5%. On the other hand, LFIA had a slightly higher aflatoxin diagnostic specificity of 89% in maize samples compared to the specificity of 85% in ground nuts. Table 2 shows the comparison of the two tests.

Performance characteristic	Maize	Ground nuts
Sensitivity	0%	11.1%
Specificity	89%	85%
PPV	0%	25%
NPV	51.5%	68%

Table 2: Comparison of Diagnostic Performance of LFIA in Testing for Aflatoxins Between Maize and Ground Nut Samples Using ELISA Assay as Gold Standard

3.3. ROC Curve and AUC

The results are visualized using the Receiver Operating Characteristics (ROC) curves drawn using the Med Calc software [17]. The results revealed that the strip performs 9% better on Maize than on groundnuts, with AUC of 0.555 compared to 0.507 for groundnuts. Figure 3 displays the ROC curves of the two grains, which shows that performance on groundnuts is as good as that of a random test.

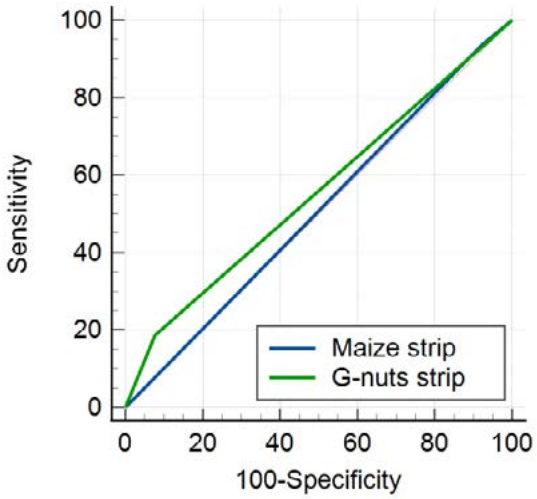

Figure 3: ROC Curves of Both Grains

3.4. Discordances of Aflatoxin Contamination Between Maize and Ground Nut Sample Compositions Using ELISA Assay as Gold Standard

Discordances between maize and ground nut sample compositions were determined by comparing aflatoxin contamination rates. Aflatoxin contamination was present in 75% (6 out of 8) of the maize whole seed samples compared to 21.4% (3 out of 14) of the groundnut whole seed samples. In addition, 8 out of 18 maize homogenized/ crushed samples were contaminated representing a contamination rate of 44.4% while 4 out of 12 groundnut homogenized/crushed samples were contaminated representing a contamination rate of 33.3%. Coincidentally, 2 out of 3 of both the maize and groundnut cooked/ heated samples were contaminated with aflatoxins representing a dual contamination rate of 66.6%. Table 3 provides more details on this discordancy on the 29 samples.

Aflatoxin contamination	Maize		Ground nuts	
	Ratio	Percentage	Ratio	Percentage
Whole seeds	6/8	75%	3/14	21.4%
Homogenised/crushed	8/18	44.4%	4/12	33.3%
Cooked/heat-treated	2/3	66.6%	2/3	66.6%

Table 3: Discordances of Aflatoxin Contamination Between Maize and Ground Nut Sample Compositions Using ELISA Assay as Gold Standard

A chi-square test was carried out at 0.05 significant levels to determine whether there was a difference in aflatoxin contamination between maize and ground nuts. P-value was 0.23, indicating no significant difference in aflatoxin contamination between maize and ground nut sample compositions.

4. Discussion

The weakness of the strip is that it may not be as easy to use as expected, since as many as 20% of the samples were invalid. We actually had to further dilute the extracts in order for the strip to work. This means that an ordinary person would not simply squeeze out a liquid from the grain and

pour it into the LFIA but would need to know how to dilute it appropriately. This is an area that can be improved in future strip production. The strip is 67% accurate, meaning that on average, 67 out of 100 results will be true reflection of the state of aflatoxin contamination. The strip has a specificity ranging between 85-89%, meaning that when it displays a negative result, this is likely correct and can be believed. However, it has a low sensitivity that ranges between 0 – 11%, meaning that if it displays a positive result, then another test needs to be conducted to confirm the positive result. This is generally a fair performance of the LFIA. Furthermore, the strip performs slightly better on maize (71% accuracy, 9% more AUC) than on groundnuts (62% accuracy). These results are not all that good. HIV tests in Uganda were recommended if the minimum specificity and sensitivity is 98% and 99% respectively [18]. LFIA tests for malaria in Kampala district were recommended for sensitivities of atleast 85% and specificities of atleast 93% [19,20]. In the results from comparing malaria diagnostic RDTs with microscopy from patients in Uganda and Myanmar resulted in sensitives of 84% and 62% respectively. Rapid tests performed poorly for brucellosis in health facilities in Kenya [21]. Rapid tests for Covid-19 return a sensitivity of only 30% and for Tuberculosis, they return a sensitivity ranging from 0.97% to 59.7% and a specificity ranging from 53% to 98.7% [22,23]. Our results are in tandem with these results which show that in general, tests with high specificity had very low sensitivity.

5. Conclusion

This research assessed the accuracy of the Elabscience AF lateral flow Immuno Assay against the popular Elabscience ELISA test. The LFIA has been found to exhibit a fairly high specificity meaning it has good power to detect foods without aflatoxin contamination. On other hand, sensitivity of the LFIA is poor, implying that the assay has poor ability to detect samples contaminated with aflatoxins. This study recommends that the current Elabscience LFIA be improved to increase the diagnostic power on Zea mayis L and Arachis hypogaea L, the groundnut and maize varieties commonly produced and consumed in Uganda, to a sensitivity and specificity of atleast 90%. We also recommend that if the LFIA kit is used and returns a positive result, then the test needs to be repeated with another gold standard test, to confirm the aflatoxin contamination.

Declarations

Ethics Approval and Consent to Participate

The Mbale Regional Referral Hospital Research and Ethics Committee reviewed and approved all study documents including the study protocols, Standard Operating Procedures, data collection tools and consent forms. Written permission was also obtained from Masindi district headquarters.

Consent for Publication

Informed written consent was obtained from all respondents who participated in the study. To simplify communication with the non-English speaking participants, the consent forms were translated into three local languages which are Luganda, Runyoro and Ateso. All their information was kept confidential.

Availability of Data and Materials

The datasets used and analysed during the current study was gathered by the authors and are available from the corresponding author on reasonable request.

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