Validation and adoption of a novel method of aflatoxin detection in peanut using a tablet

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FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

FEED THE FUTURE PEANUT & MYCOTOXIN INNOVATION LAB

Validating the accuracy of a Mobile Assay mReader for aflatoxin testing

Accurate quantitation of aflatoxin contamination generally requires sophisticated, expensive laboratory equipment, potentially dangerous solvents, and skilled laboratory technicians.

These limitations create barriers to improved aflatoxin detection and control for markets, as well as research in developing countries.

Beginning in 2014, PMIL began collaboration with Mobile Assay, the producer of a lab-on-mobile platform software application, to validate and beta test their technology for use with peanuts in target countries.

Using spiked samples in a wide array of concentrations and mediums, one study proved that the Mobile Assay system was consistently accurate when compared to a uHPLC standard (Table 1) and comparable or better than widely accepted methods, such as Immunoaffinity Column (IAF) or Mycosep/ELISA (Table 2).

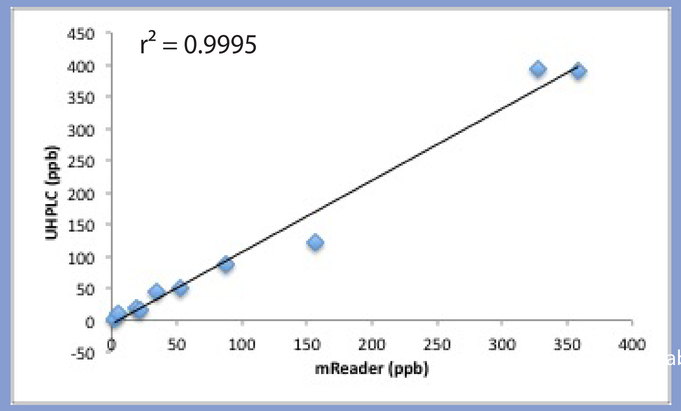
Some advantages include:

- Comparative analysis using spiked and naturally contaminated samples have shown the method to be adequately sensitive and accurate.
- Use of non-reagent grade ethanol for extraction is less toxic than other solvents and cost and durability have proven to be favorable to other methods.
- Data storage is cloud-based, with GPS location per assay.
- Uses commercially available test strips that have a one-year shelf life and do not require refrigeration (<30C)
- The system allows tests to be conducted closer to the source of the samples, reducing the risk of contamination.

In conclusion, initial results indicate that the system is highly accurate and would provide a good alternative to current systems relying on more complicated, costly and toxic procedures.

TABLE 1

Correlation between mReader and uHPLC aflatoxin detection



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Peanut flour samples spiked with aflatoxin standards were prepared and analyzed in JS Wang's laboratory using the ultra-HPLC and Mobile Assay mReader. The very high correlation ($r^2 = 0.99$) across a wide range of aflatoxin concentrations indicate that the Mobile Assay mReader method is highly comparable to the uHPLC method. Note that samples must be diluted when using the mReader for aflatoxin concentrations greater than 50 ppb.

TABLE 2

Comparison of aflatoxin testing methods of spiked samples

	uHPLC	mReader1	mReader2	**Average**	IAC method	Mycosep method
Spiked Peanut paste 1	0.2	1.68	2.1	**1.89**	1.04	0
Spiked Peanut paste 2	19.65	18.48	13.83	**16.155**	14.82	8.44
Spiked Peanut paste 3	121.15	156.7	47.08	**101.89**	70.16	36.73
RUTF 1	10.96	4.4	4.7	**4.55**	4.9	2.98
RUTF 2	44.1	34.59	30.88	**32.735**	15.51	5.33
RUTF 3	391.95	327.15	398.8	**362.975**	171.35	0.59
Peanut Flour 1	390.14	358.75	419.01	**388.88**	311.55	27.59
Peanut Flour 2	2241.67	2483.5	2213.18	**2348.34**	1629.23	147.53
Infected oil	50.75	52.38	57.46	**54.92**	47.57	11.18
Spiked Oil 1	88.55	87.35	83.71	**85.53**	91.7	24.35
Spiked Oil 2	16.72	22.1	11.59	**16.845**	13.97	2
Spiked Oil 3	3.03	5.16	5.79	**5.475**	1.02	0.09

Aflatoxin-spiked samples (prepared by K Mallikarjunan) were analyzed in JS Wang's laboratory using three different analytical methods (IAC and Mycosep) and HPLC detection, Sep-Pak preparation-ultra-HPLC, and the Mobile Assay mReader. Results presented in the table indicated that the mReader found alfatoxin concentrations similar to those obtained using the uHPLC.

EXTRACTION

500g sample of peanuts ground to fine particle size → **10g sub-sample of ground peanut taken** → **30mL ethanol added** → **100µL of extraction taken** → **500µL diluent added** → **Test strip processed**

Large sample size increases likelihood of capturing any mycotoxin- tainted nuts or kernels.

Manual grinder, such as ones used in food preparation, can be used, as long as a fine particle size is achieved.

Ethanol is less toxic than methanol.

Test currently gives results ranging from 0-50 ppb, but range is easily extended through sample dilutions.

Result processes in 5 minutes.

DEPLOYMENT

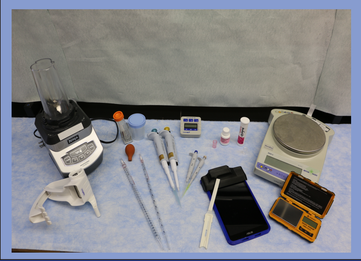
The Peanut & Mycotoxin Innovation Lab has equipped and trained researchers around the globe, on the mobile system. Here are some of the projects they are working on:

A. Grad student Alidu Abdul-Hafiz (in the lab at the University for Development Studies in Tamale, Ghana in March 2016) is evaluating the level of aflatoxin in groundnuts sorted at a local market. Abdul-Hafiz bought nuts that were culled from the higher quality crop; many of these poor-quality nuts show signs of mold, but will remain in the food supply.

B. Chancy Sibakwe, a master's student at Lilongwe University of Agriculture and Natural Resources, is studying whether different drought scenarios impact the development of mold in groundnuts, leading to aflatoxin contamination. Some of his work was done at Chitedze Agricultural Research Station in Malawi (seen here in February 2016).

C. Ethiopian PhD candidate Abdi Hassen, who trained others at Haramaya University in Dire Dawa, Ethiopia, on Mobile Assay, uses the system to continue to analyze isolates of Aspergillus. Hassen began evaluating isolates at the USDA National Peanut Research Lab in Dawson, Ga.

D. Emmanuel Zuza, who is working with the Institute of Agriculture Investigation in Mozambique (IIAM) and Eduardo Mondlane University (UEM), is evaluating the effect of harvest timing on yield and aflatoxin contamination, as well as the effect of drying methods in management of aflatoxin.



A simple kit of low-cost and easily replaced equipment can allow aflatoxin assays in the lab or in the field. Since the system does not require lab infrastructure or stable electricity, it can reduce the possibility of contamination due to transport and storage.

- Supplies,
- Grinder (electric or manual)
- Scale
- Timer
- Container to shake
- Pipetters and tips
- Graduated cylinders
- Sample cups
- Diluent
- Lateral-flow test strips
- Tablet
- Specialized fitting for reader

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