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Immunoassays for Trace Chemical Analysis

Monitoring Toxic Chemicals in Humans, Food, and the Environment

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Chapter 13

Aflatoxin Immunoassays for Peanut Grading

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Recent studies conducted to determine the accuracy, speed, and expense of Enzyme-Linked Immunosorbent Assay (ELISA) screening methods for aflatoxin are reviewed in light of proposals that such a method be implemented as part of the official grading system for farmers stock peanuts. The studies have shown several of the ELISA methods to be reliable with regard to screening accuracy. This combined with the speed of the analyses and the relatively low costs could make a practical change in the way incoming loads of farmers stock peanuts are evaluated for aflatoxin contamination.

The U.S. peanut industry has developed a system for aflatoxin management during the production and processing of peanuts. This system includes diversion of aflatoxin-suspect lots at the initial point of sale of farmers stock peanuts, analysis of shelled peanuts prior to sale, and removal of aflatoxin-contaminated kernels during the shelling, blanching, and manufacturing processes (1). Removal of contaminated kernels during these processes is achieved by screening out small kernels and removal of damaged kernels by electronic color sorting and hand-picking.

Sale of farmers stock peanuts starts at the grading point where a value is placed on the peanuts based on several grade factors. The official U.S. peanut grading system currently relies on an indirect visual method for detecting loads of peanuts suspected of being contaminated with aflatoxin and diverting them from edible channels to oil production. This procedure involves a visual examination of loose-shelled kernels from approximately 1800 g of farmers stock peanuts and of kernels from a 500 g subsample of pods for the presence of the aflatoxin-producing fungi. If any kernel shows evidence of *Aspergillus flavus* growth, the entire lot of approximately 4000 kg of farmers stock peanuts is diverted to oil stock without further evaluation. The refined oil is free of any aflatoxin, which resides primarily in the meal, and residual aflatoxin contained in the crude oil is degraded during the refining process. The meal cannot be used for feed purposes and must be destroyed, exported, or used for fertilizer purposes only.

The U.S. peanut industry is currently considering the feasibility of implementing a new approach for aflatoxin management at the grading point. This involves different sampling and detection methods for more effective aflatoxin control. This paper discusses

the feasibility of using Enzyme-Linked Immunosorbent Assay (ELISA) screening methods as part of the grading of farmers stock peanuts.

In order to implement use of a direct analytical method for detecting aflatoxin-contaminated lots at peanut grading points, the method should meet several criteria. First, it should be relatively inexpensive, because of limited available resources. Second, the method should be relatively accurate, particularly around the current FDA action level of 20 ppb. Third, it must be relatively simple to conduct. The peanut grading season lasts only 2-3 months; therefore, employees must be trained each year. In addition, employees may work 10-15 hours per day, which dictates that the analytical method be simple in order to minimize physical and mental fatigue. Finally, and, perhaps, most important criterion is speed, since any analytical method cannot impede the flow and sale of peanuts through the grading process, particularly during peak processing periods. Since the peanut harvest season can be very short and grading points are extremely variable in size (capacities for processing range from 20-200 samples daily), each test needs to be completed within five minutes.

Recently, the effectiveness of the official visual method was compared with a rapid immunoassay (EZ-Screen Quick Card [Environmental Diagnostics, Inc., Burlington, NC]) in determining the presence or absence of aflatoxin in farmers stock grade samples (2). The 152 samples used for comparison were official grade samples obtained for analysis after the grading inspectors had completed their inspection. The comparative analyses were conducted on common methanol-water (80:20; 2 ml/g) extracts. The ELISA test was conducted according to recommendations of the suppliers. HPLC analyses were done according to the method of Dorner and Cole (3).

The results showed 41% of the grade samples determined to be contaminated by visual inspection contained less than 20 ppb aflatoxin when analyzed by both ELISA and HPLC methods; 18.7% of peanuts determined to be uncontaminated by visual inspection actually contained aflatoxin with a range of 26-2542 ppb. The results of ELISA and HPLC agreed in 98.6% of the composite sample analyses with the detection of 20 ppb or greater. However, the ELISA screening method failed to give positive tests 12 of 13 times when the aflatoxin content was between 20-43 ppb in the component samples. Samples were analyzed at the rate of one every two minutes when duplicate analyses of ten samples (20 analyses) were performed.

It was concluded that the direct ELISA method was considerably more effective (97.6% effective) than the visual method (51% effective) in identifying farmers stock grade samples that were contaminated with aflatoxin at levels >20 ppb.

This prompted a subsequent study comparing duplicate analyses of common extracts using the EZ-Screen Quick Card and Afla-10 Cup Test (International Diagnostics Systems Corp., St. Joseph, MI) with HPLC (4). However, in this case a large number of samples in the critical range of 0-50 ppb range were selected from an unrelated study by HPLC for the comparison. Both ELISA methods were performed according to manufacturers' instructions; the HPLC method was conducted according to the method presented previously. One hundred common extracts (methanol-water, 80:20; 2 ml/g) in the critical range of 0-50 ppb were analyzed in duplicate by all methods.

Each ELISA assay properly identified 95% of samples containing no detectable aflatoxin as negative and >97% of samples containing >10 ppb aflatoxin as positive. The EZ-Screen Quick Card, which had a 20 ppb detection threshold, identified as positive 32 of 34 samples in the 11-20 ppb range. This indicated that the card test might actually have a detection threshold closer to 10 ppb. Most of the errors associated with both assays occurred on samples containing <10 ppb aflatoxin. The cup and card tests identified 76 and 67% of samples, respectively, as negative in the range of 4-10 ppb.

The objective of an aflatoxin testing program is to identify

positive loads of farmers stock peanuts and to divert these to oil stock or possible cleanup. Results from this study indicated that either the card test or the cup test would reliably (>95%) identify samples of peanuts containing >10 ppb of aflatoxins. Likewise, both methods were reliable (95%) in properly identifying samples that were negative for aflatoxin. Because of this degree of accuracy, both methods were deemed well-suited for use as rapid screening tools at peanut grading points.

The USDA/Federal Grain Inspection Service recently completed a thorough study which compared six commercially available aflatoxin test kits to the currently used Holaday-Velasco minicolumn method for determining aflatoxin in corn (5). The study included five ELISA test kits. These were the Afla-20 Cup, Aflatest (VICAM, Somerville, MA), Agriscreen (Neogen, East Lansing, MI), EZ-Screen Quick Card and Oxoid (not an ELISA test). Criteria used for evaluation included accuracy, safety, user performance, speed, and variable and fixed costs. The objective of the FGIS study was to determine if a single alternate screening test could be used to replace the two currently used screening tests, the blacklight test and Holaday-Velasco minicolumn method (HV). The study was designed to compare the effectiveness of the alternate tests with the HV method. Three sample sets of corn containing spiked, naturally contaminated and negative samples were used in this study. The results showed that, with one exception (Agriscreen test), all tests evaluated were capable of providing the same reliability as the HV method. The performance of the Afla-20 Cup Test was the only test rated as better than the HV minicolumn. The Afla-20 Cup also scored highest on user preference where each analyst ranked the tests according to ease of use (Table 1). The average length of time required per assay, excluding sample preparation and extraction, was 5.71 min compared to 7.42 min for EZ-Screen, 9.09 min for AgriScreen and 10.33 min for Aflatest (Table 2). The Afla-Cup Test had the lowest variable costs at \$4.68, while the EZ-Screen had the lowest fixed costs/site at \$121.36 (Table 3).

Table I. Ranking based on user preference scores

Ranking	Test
First	Afla-Cup
Second	EZ-Screen
Third	Aflatest
Fourth	Agriscreen
Fifth	Oxoid

Taken from FGIS study

Table 2. Average length of time required per assay

Test	Average time per test (min)
Afla-Cup	5.71
EZ-Screen	7.42
Agriscreen	9.09
Aflatest	10.33
Oxoid	15.27

Taken from FGIS study

Table 3. Variable and fixed costs for each test.

<u>Test</u>	<u>Variable costs/test</u>	<u>Fixed costs/site</u>
Afla-Cup	\$4.68	\$184.50
EZ-Screen	6.02	121.36
Aflatest	6.05	2,681.56
Agriscreen	7.00	495.00
Oxoid	18.04	901.36

Taken from FGIS study

The results of this study further substantiated previous studies that the new ELISA screening tests provide an excellent opportunity to implement a direct analytical method at peanut grading points.

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