Comparison of Visual, Enzyme-Linked Immunosorbent Assay Screening, and HPLC Methods in Detecting Aflatoxin in Farmers Stock Peanut Grade Samples

R. J. Cole*, J. W. Dorner, J. W. Kirksey, and F. E. Dowell

ABSTRACT

Grade samples from 152 lots of farmers stock peanuts were analyzed for aflatoxin by both an Enzyme-Linked Immunosorbent Assay (ELISA) rapid screening test and high performance liquid chromatography (HPLC). Results from HPLC and ELISA were compared to the results of the visual inspection method used by the Federal State Inspection Service (FSIS). The results showed 41% of the grade samples with visible *Aspergillus flavus* (Segregation 3) contained less than 20 ppb aflatoxin when analyzed by both ELISA and HPLC methods; 18.7% of Segregation 1 peanuts actually contained aflatoxin with a range of 26-2542 ppb. The results of ELISA and HPLC agreed in 98.6% of the composite lot analyses with the detection of 20 ppb or greater. However, the ELISA rapid screening test failed to give positive tests 12 of 13 times when the aflatoxin content was between 20-43 ppb in the component samples.

Key Words: ELISA, HPLC, visual inspection, rapid screening methods, *Aspergillus flavus*.

As part of the U.S. aflatoxin control program and the U.S. peanut grading system, peanuts are inspected for the presence of visible *Aspergillus flavus* on the various seed components of the grade sample (1,2,4). These components consist of loose-shelled kernels (LSK), small whole kernels (OK), sound splits (SS), sound mature kernels (SMK), and damaged kernels (DK). If *A. flavus* is found on any kernel of these grade components, the load is assumed to contain aflatoxin and is classified as Segregation 3. Segregation 3 peanuts must be used for oil stock and the meal cannot be used for animal feed. Any aflatoxin in the unprocessed oil is degraded during the oil refining process (Parker and Melnick, 1966).

This paper compares the effectiveness for determining the presence of aflatoxin in farmers stock grade samples of the official visual method and an ELISA rapid screening method to a high performance liquid chromatographic (HPLC) method. The samples used for comparison were official grade samples that the Federal State Inspection Service (FSIS) inspectors pulled from 152 loads of crop year (CY) 1986 farmers stock Florunner peanuts at two locations in the southwest Georgia peanut producing area.

Materials and Methods

Grading Procedure

The farmers stock peanut sample (≐48 lb) was subdivided by the FSIS inspector to about an 1800 g subsample with a riffle-type sample divider. The LSK's were separated from the rest of the subsample and visually examined for *A. flavus* (2). A 500 g subsample of the 1800 g sample was used to detemine the remainder of the grade components. The 500 g subsample was sized, shelled and screened over a 6.35 mm (16/64 in.) screen. The grade components ultimately collected were sound splits (SS), small whole kernels (which fell through the 6.35 mm screen [OK]), sound mature kernels (that rode the 6.35 screen) [SMK]), and damaged kernels (DK). All these components were visually examined for *A. flavus*. If *A. flavus* was found on any kernel of any of these grade components, the load was presumed to be contaminated with aflatoxin and classified as Segregation 3. If *A. flavus* was not detected, the lot was classified as Segregation 1 (edible category) and was presumed to be aflatoxin-free. The kernels that contained the mold were removed and held for confirmation by the supervisor. The remaining peanuts in all the grade components of each sample were placed in individual, labeled paper bags and returned to the laboratory for aflatoxin analyses.

Extraction and Aflatoxin Analyses

The SMK and SS were combined and analyzed as one component. The LSK, OK, and DK components were analyzed separately. The components of each sample were individually analyzed by an ELISA rapid screening card test (Environmental Diagnostics, Inc., Burlington, NC 27215) and HPLC (3) using the same extract for both analyses. Due to limited quantities the damaged kernels were analyzed by HPLC only.

Each component was extracted with methanol-water (80-20/;2 mL/g) by homogenization in a Waring Blender for one minute. The homogenate was filtered through Whatman No. 4 filter paper. Duplicate 2-3 mL aliquots were collected for the ELISA card test. Duplicate 15 mL aliquots were collected for HPLC analyses.

ELISA was conducted according to recommendations of the supplier. This assay reportedly detects aflatoxins B_1, B_2, and G_1. A 100 µL aliquot of the sample extract was mixed with 200 µL of buffer, and a 50 µL aliquot was added to the sample port on the card. After the extract was completely absorbed, one drop of enzyme solution was added to the sample port. After the enzyme drop had completely absorbed, one drop of wash solution was added to the sample port. After absorption of the wash, the excess liquid was carefully wiped from around the sample port with a clean tissue. Two drops of substrate were added to the sample port, one drop at a time, allowing the first drop to absorb before applying the second drop. The results were read immediately. If the port turned gray-blue, the sample contained <20 ppb; if the port reamined white, the sample contained >20 ppb.

HPLC analyses were done according to the method of Dorner and Cole (3). The HPLC system included two M6000A pumps, Model 712 WISP automatic injector, Model 730 data module with Intelink (Waters Chromatography Div., Millipore Corp., Milford, MA 01757). The method employed a 10 cm x 8 mm Nova-PAK Phenyl Radial-PAK cartridge used with a Model RCM-100 radial compression module (Waters-Millipore). The technique used post-column derivatization with aqueous iodine to enhance fluorescence of aflatoxins B_1 and G_1 with a Model PCRS 510 heater system for HPLC post-column reactions (Kratos Analytical, Ramsey, NJ 07446). The aflatoxins were detected with a Model LS-1 fluorescence detector with excitation wavelength of 365 nm and emission cutoff at 418 nm (Perkin Elmer, Norwalk, CT 06856). The mobile phase was water-tetrahydrofuran (80:20 v/v) with a flow rate of 1.5 mL/min and a iodine reagent flow rate of 0.4 mL/min. The column temperature was maintained at 40 C and the post-column reaction temperature was 75 C. Aflatoxin standards (commercial mixed standards B_1 and G_1, 5 µg/mL/ B_2 and G_2, 1.5 µg/mL) were obtained from Applied Science Laboratories (State College, PA 16901). The detection limits were 0.1 ng B_1 and G_1/g and 0.06 ng B_2 and G_2/g.

[1]Research Microbiologist, Microbiologist, Plant Pathologist, and Agricultural Engineer, USDA, ARS, National Peanut Research Laboratory, 1011 Forrester Drive, S.E., Dawson, Georgia 31742.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available.

Results and Discussion

Table 1 presents a comparison of the visual method, ELISA card test and HPLC analyses of 44 component and composite farmers stock grade samples determined to be positive (>20 ppb) by HPLC. Twenty positive samples ranging in contamination levels from 27-2542 ppb (composite values) were classified as Segregation 1 (47%), while 23 positive samples ranging from 61-2540 ppb were classified as Segregation 3 by the visual method. These results showed that the visual method was only 53% effective in correctly identifying aflatoxin contaminated samples of peanuts as Segregation 3. However, it should be pointed out that if one 0.5 g damaged kernel was removed from the grade sample for confirmation by the inspector's supervisor, it would only need to be contaminated at a level of 16,000 ppb to make the composite grade sample average 20 ppb assuming a 400 g shelled sample. There is no way of knowing if any damaged kernels were removed for confirmation by the inspector's supervisor and, if so, what level of contamination, if any, it or they contained.

The ELISA card test classified 42 samples as greater than 20 ppb (97.6% effective) and one sample (27 ppb) as less than 20 ppb indicating that the lower limit of ELISA detection may be ~50 ppb. Only four samples of the SMK+SS were positive (29-111 ppb) by HPLC, and these components constituted 79.5% of the total kernel weight of the grade samples. The LSK fractions (7.9% of total kernel weight of grade sample) had 34 samples positive (20-14833 ppb) by HPLC, while the OK samples (11.2% of total kernel weight of grade samples) had 24 samples positive (31-7109 ppb). The damaged component contained 13 positive samples (172-298,173 ppb), but constituted an average of only 1.4% of the total kernel weight of the grade samples.

Table 1. Visual, ELISA card test and HPLC analyses of 44 component and composite farmers stock grade samples determined to be positive by HPLC.

Control number	Card test[2]	HPLC[3] Component				
		SMK/SS	LSK	OK	Damage	Composite[4]
Segregation 1[1]						
1	+	*	20	872	6470	210
12	+	0	0	460	1	48
18	+	0	243	0	*	64
31	+	0	3449	1149	1	800
32	+	0	240	0	199	38
34	+	0	1	859	6	56
64	-	29	*	10	0	27
65	+	3	5374	3	172	2542
71	+	0	450	0	0	123
86	+	6	1547	0	1	479
97	+	0	398	0	*	122
114	+	0	192	0	*	27
119	+	0	1378	414	0	282
123	+	0	0	1500	0	96
130	+	0	114	0	0	27
132	+	0	767	8	0	133
136	+	0	10	735	0	70
139	+	9	2158	4056	298173	2397
140	+	0	0	756	2	102
152	+	0	2338	0	19	632
Segregation 3[1]						
4	+	0	212	9	948	62
6	+	0	198	112	118636	574
8	+	2	1879	3525	*	957
10	-	0	20	9	5242	104
15	+	0	62	7109	0	626
19	+	0	2469	704	1935	827
22	+	7	3283	0	4767	601
25	+	0	14833	5	0	2540
40	+	111	53	209	19630	414
43	+	0	0	982	0	79
54	+	0	363	31	1002	124
59	+	0	2210	6900	956	858
60	+	0	13	1665	0	77
62	+	0	408	18	3	96
70	+	0	7697	181	16	2533
82	+	111	320	597	3	188
87	+	1	403	0	2	121
93	+	0	228	296	1	63
103	+	0	403	0	0	61
107	+	0	303	144	0	61
117	+	0	2733	1	1234	1077
120	+	0	1998	325	5	341
121	+	0	1540	4493	3	544
135	+	101	0	0	55900	496

*No analysis of component.

[1]Visually: Seg 1 = No A. flavus; Seg 3 = A. flavus detected.

[2]Card test: Positive = >20; negative = <20.

[3]HPLC = ppb with limits of detection at 1 ppb or greater.

[4]Derived from the weighted average of all components.

Eighty-seven samples classified as Segregation 1 were negative (81.3%), while 20 were positive (18.7%) for aflatoxin by HPLC analysis. Sixteen Segregation 3 samples were negative (41%), while 23 (59.0%) were positive. Therefore, the visual method correctly identified 75% of all the samples when considering both contaminated and non-contaminated samples.

Although the ELISA card test was very effective at identifying aflatoxin contaminated samples overall (98.6%), it had the greatest difficulty in identifying samples as positive when the sample was between 20-43 ppb (Table 2). It identified only one sample component out of thirteen that contained between 20-43 ppb as determined by HPLC.

In conclusion, the direct ELISA method was considerably more effective (97.6% effective) than the visual *A. flavus* method (51%) at identifying farmers stock grade samples that were contaminated with aflatoxin at levels >20 ppb. The implementation of a direct analyt-

Table 2. Comparison between ELISA and HPLC test results for samples with 10-50 ppb contamination level.

		Card		HPLC	
Sample	Component	Rep 1	Rep 2	Rep 1	Rep 2
1	LSK	-	*	19.6	*
10	LSK	-	-	19.2	20.8
28	SMK/SPL	-	-	22.9	23.7
39	LSK	-	-	38.3	43.2
54	OK	-	-	29.7	32.1
60	LSK	-	-	12.5	13.2
62	OK	-	-	17.6	18.6
64	SMK/SPL	-	-	29.2	29.6
67	LSK	-	-	16.0	18.1
77	LSK	-	-	35.0	36.6
128	LSK	-	-	11.0	11.2
141	SMK/SPL	-	-	19.0	18.7
146	OK	-	-	16.7	16.2
149	LSK	+	-	21.5	22.0

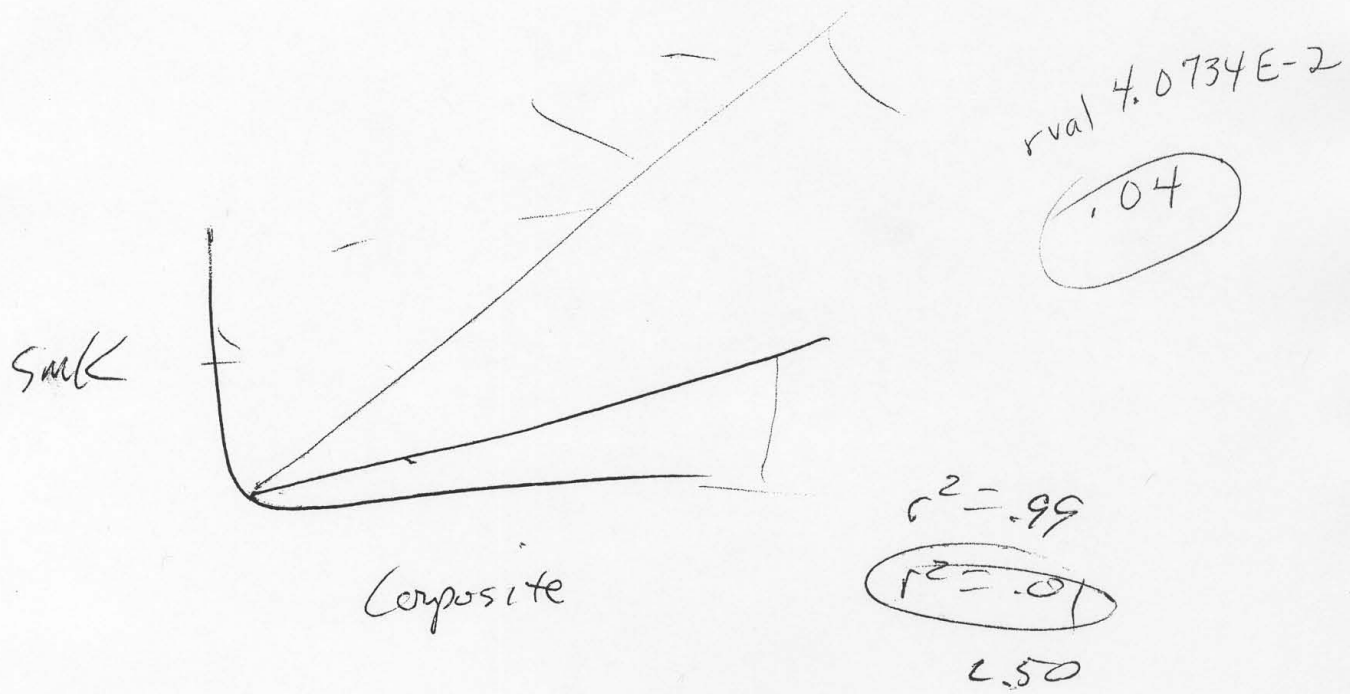
*Sample REP not analyzed.

ical method such as an ELISA test into the peanut grading procedure should improve the reliability of identifying aflatoxin contaminated lots of farmers stock peanuts.

Acknowledgment

We thank Milbra Schweikert for her excellent technical assistance. These studies were funded in part by a grant from the Georgia Agricultural Commodity Commission for Peanuts.

Literature Cited

1. Dickens, J. W. and L. W. Johnson. 1987. Peanut grading and quality evaluation. *In* E. M. Ahmed and H. E. Pattee (eds.), Peanut Quality - Its Assurance and Maintenance From Farm to End-Product. Florida Agric. Exp. Sta. Techn. Bull. 874.
2. Dickens, J. W. and J. B. Satterwhite. 1971. Diversion program for farmers stock peanuts with high concentrations of aflatoxin. Oleagineux 26:321-328.
3. Dorner, J. W. and R. J. Cole. 1988. Rapid determination of aflatoxins in raw peanuts by liquid chromatography with post-column iodination and modified minicolumn cleanup. J. Assoc. Off. Anal. Chem. 71:43-47.
4. Farmers Stock Peanuts: Inspection Instructions, USDA, AMS, Fruit and Vegetable Div., Fresh Products Branch, Washington, DC. Revised July 1986.
5. Parker, W. A. and D. Melnick. 1966. Absence of aflatoxin from refined vegetable oils. J. Assoc. Off. Chem. 43:635-638.

Accepted October 17, 1988