Evaluation of Florisil Tubes in Detection of Aflatoxin

JAMES VELASCO and **MARION E. WHITTEN**, Agricultural Marketing Research Institute, ARS, USDA, Beltsville, Maryland 20705

ABSTRACT

A study was made to determine the reliability and sensitivity of detecting aflatoxin in florisil tubes. Six panelists examined 40 samples with 222 responses recorded. At levels of 5 ppb and higher, panel members were able to detect aflatoxin with an accuracy of better than 99%. At levels above 10 ppb, accuracy was 100%.

INTRODUCTION

We have reported the use of small diameter florisil columns or tubes to detect aflatoxins in cottonseed products (1). Principal advantages of these tubes over thin layer chromatographic (TLC) plates are: shorter development time, simpler equipment and operation, and lower cost per sample. Probable disadvantages would be lower sensitivity and reliability in detection of aflatoxin.

The present study was conducted to determine the reliability and sensitivity with which aflatoxin can be detected in these tubes.

EXPERIMENTAL PROCEDURES

This was a two part study designed, first, to establish the reliability of detection with the florisil tubes at the critical aflatoxin levels between 0 and 25 ppb and, second, if results warranted, to determine the sensitivity or limits of detection using these tubes.

A panel of six members was formed to view and judge the tubes. Only two of the members (authors) had any previous experience with aflatoxin methodology.

Cottonseed samples used for this study were field samples, from a current sampling study of the distribution of aflatoxin in cottonseed lots, that were being analyzed routinely.

In order to present a complete outline of the study, a brief detail of the procedure is included.

Fifty grams of ground cottonseed meats were extracted with 250 ml acetone-water 85:15 for 30 min on a shaker. The extract solution was filtered, and 90 ml filtrate was added to a beaker containing 100 ml water and 10 ml 10% $FeCl_3$ solution. The solution was stirred on a magnetic stirrer for ca. 1 min before raising the initial pH of ca. 1.8 to 4.6 (±0.2) by the addition of 13.4 ml 4% NaOH. Stirring continued for several minutes after the addition of NaOH to allow complete adsorption of the pigment on the formed ferric gel. The solution was filtered, and the total filtrate (180 ± 5 ml) was transferred to a 500 ml separatory funnel. The solution was diluted with 200 ml water and extracted twice with 25 ml chloroform. The chloroform layers were combined in a stainless steel beaker and evaporated on a steam bath. The beaker was allowed to cool before quantitatively transferring the residue to a vial with three 2 ml portions of chloroform-acetone 9:1.

One milliliter of solution was withdrawn from the vial with a pipette and added to the prepared florisil tube (pyrex glass tube, 3 mm ID, layered with 6 mm sand, 6 mm florisil, 15 mm silica gel and 15 mm neutral alumina). The tube was allowed to drain before rinsing with 1 ml chloroform-acetone 9:1. Upon draining, the tubes were placed in a viewing cabinet and viewed under longwave UV light. Light source was a single 15 watt fluorescent tube 18 in. long positioned ca. 4 in. from the florisil tubes.

Because fluorescence in the tubes has been found to be remarkably stable, the tubes can be viewed while wet or dry. Intensities are slightly higher with wet than with dry tubes. A set of standard tubes prepared 18 months ago still shows good fluorescence at levels of 10 ppb and above.

For comparison, TLC plates were prepared. The vial with the remaining 5 ml sample solution was evaporated to dryness. An appropriate volume of chloroform, as determined by the screening with the florisil tube, was added to the vial and solution spotted on a TLC plate. The plate was scored in half to allow two developments per plate, and was developed for 30 min using diethyl ether-methanol-water solvent 96:3:1. The fluorescent intensities of the spots were measured with a Photovolt densitometer after allowing the plate to equilibrate to room condition for ca. 2 hr.

After completion of the above, the following procedure was undertaken to determine the limits of detection in the tubes.

Standard aflatoxin B_1 was added in increasing amounts to a series of tubes (Fig. 1). The amounts added were 0, 2.5, 5, 10, 20, 40 ng, which are equivalent to 0, 1, 2, 4, 8, 16 ppb in a 2.5 g sample called for in the procedure. Two and a half grams of uncontaminated residue was also added to each tube to provide normal background encountered in tubes. Three separate sets of tubes were prepared and viewed by the panel on different days. The panel was instructed to remove the brightest tube from the cabinet first and to continue this process until all tubes had been removed. The tubes would thus be removed in order of decreasing strength, 16, 8, 4 ppb, etc.

TABLE I

Panel[a] Detection of Aflatoxin in Florisil Tubes Compared with Thin Layer Chromatography Findings

Aflatoxin range,[b] ppb	Thin layer chromatography, no. samples	Panel responses	
		Positive	Negative
50+	8	47	0
35-50	2	12	0
25-35	3	17	0
20-25	2	11	0
15-20	1	6	0
10-15	3	16	0
5-10	7	36	1
0-5	3	6	11
0	11	7	52

[a]Members of panel varied from five to six.

[b]Aflatoxin B_1 and B_2 as determined by thin layer chromatography.

TABLE II

Sensitivity of Florisil Tubes in Detection of Aflatoxin

B_1,[a] ppb	Incorrect responses[b] by judge					
	A	B	C	D	E	F
16	0	0	0	0	0	0
8	0	0	0	0	0	0
4	0	0	0	0	0	1
2	1	0	0	0	1	2
1	1	1	0	1	1	2
0	0	1	0	1	0	1

[a]Actual amounts of aflatoxin B_1 added: 40, 20, 10, 5, 2.5 and 0 ng per tube, respectively.

[b]Each figure represents a total of three observations by each judge.

RESULTS AND DISCUSSION

Preliminary trials were made to familiarize the panel with fluorescent intensities normally encountered in routine sample tubes. Two observations were noted during these trials: (a) Differences in fluorescent intensities were easier to judge in the range of 2 to 20 ppb (μg/kg), and (b) there was a tendency by panel members to judge the tube with the lowest fluorescence in the group as being at the zero level of contamination.

The first observation would enhance detection at the low critical levels. The second, however, would pose some problems since some positive tubes would be judged negative. For this reason it was necessary to include a known negative tube with every group of tubes observed by the panel.

To determine the reliability of detection it was decided to judge the tubes as either containing aflatoxin or not containing aflatoxin (positive or negative). Table I shows the results of panel responses to tubes as compared to results obtained by TLC. A total of 40 samples was viewed by the panel with 222 responses recorded. The response totals, per sample, are not an exact multiple of six since occasionally a panel member was absent. No panel observations were made, however, with less than five members present and only one group of samples was viewed each day. The number of samples in each group varied from four to six.

No incorrect judgments were made by panel members in 109 responses at levels above 10 ppb. In the 5-10 ppb range there was one incorrect judgment out of 37. This is a judgment error rate of ca. 3%. However, if this lone error is calculated to the total number of responses above the 5 ppb level, the chance of error is reduced to less than 1%.

As expected, detection with the tubes suffers most in the 0-5 ppb range. In this range there were more incorrect responses than correct—11 out of 17 incorrect. Aflatoxin present in the range of 0-5 ppb would seem to stand about a 65% chance of not being detected.

At the negative or zero level of contamination, there were seven incorrect responses out of 57. Negative samples would seem to have about a 12% chance of being erroneously called positive.

This first part of the study showed that panel members were able to detect aflatoxin with an accuracy of better than 99% at levels of 5 ppb and higher and with an accuracy of 100% at levels above 10 ppb.

The results of the second part of the study to determine sensitivity or limits of detection are shown in Table II. A total of 18 judgments were made at each level. The number of incorrect judgments by each judge is shown. There were no errors in selecting tubes containing 16 ppb over 8 ppb, or 8 ppb over 4 ppb. At the 4 ppb level there was one error out of 18 for a 6% chance of error; five errors at the 2 ppb level for a 28% error; six errors at the 1 ppb level for a 33% chance of error.

Judge F had about as many errors as the rest of the combined panel. Judge C, who made no errors, was a panel member with no previous experience in aflatoxin methodology. This indicates that detection of aflatoxin in the tubes is a matter of visual acuity rather than one of experience.

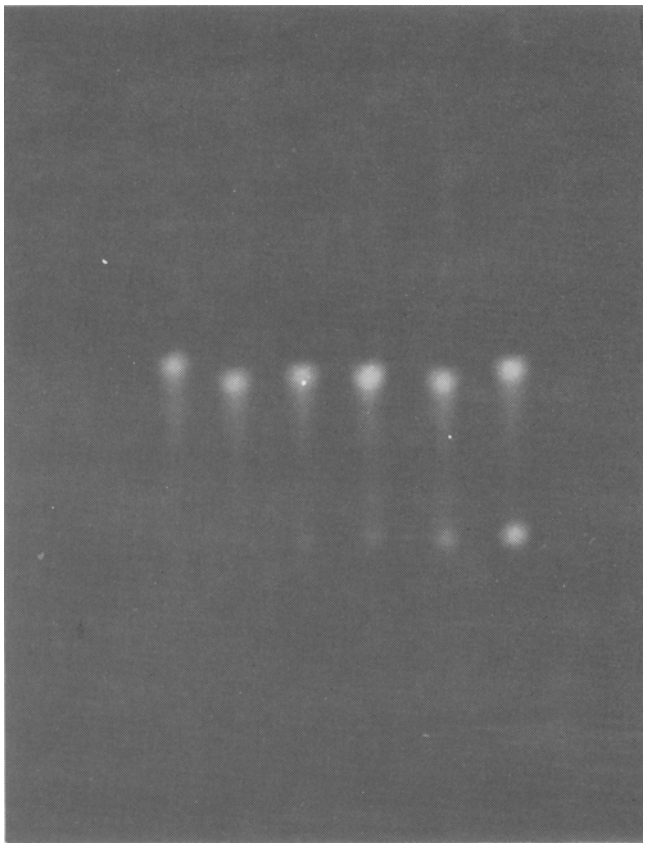

FIG. 1. Florisil tubes with increasing amounts of aflatoxin B_1 added, from left to right, are equivalent to 0, 1, 2, 4, 8 and 16 ppb (μg/kg). Upper fluorescent spots are interfering compounds trapped by alumina layer. Lower fluorescent spots are standard aflatoxin B_1 trapped by florisil layer.

These results bear out those of the first study and justify the following statements: (a) The tube method apparently is capable of detecting aflatoxin levels at and above 5 ppb. (b) The method enables differentiation of double amounts of aflatoxin between 4 and 16 ppb. (c) There is some uncertainty in judging presence of aflatoxin at levels lower than 5 ppb, with the error increasing as aflatoxin level decreases. (d) There is some chance of saying clean samples are contaminated with aflatoxin.

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