Efficacy of the Visual, Minicolumn, and Thin Layer Chromatography Methods to Test Farmers Stock Peanuts for Aflatoxin[1]

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ABSTRACT

This study estimated the efficacy of the visual *A. flavus* (VAF), minicolumn (MCL), and thin layer chromatography (TLC) methods to detect farmers stock peanuts which contain aflatoxin. Aflatoxin tests on grade samples from each of 2300 lots of farmers stock peanuts was used to estimate the distribution of farmers stock lots according to their aflatoxin concentration (lot distribution). This lot distribution (with an average aflatoxin concentration of 59.5 parts per billion) was incorporated into each of the 3 computer models that simulate the testing of farmers stock peanuts for aflatoxin when the VAF, MCL, and TLC methods are used. The number of lots accepted and the average aflatoxin concentration (AA) in the accepted lots was predicted. Results indicate that when a given percentage of the lots are accepted, lots accepted by the VAF method have less aflatoxin than those lots accepted by either the MCL or TLC methods. When the present visual method was used to test the above lot distribution, 75.8% of the lots tested were accepted and the AA in the accepted and rejected lots were 4.1 and 232.8 parts per billion, respectively.

Key Words: Peanuts, aflatoxin, sampling, farmers stock, visual method, thin layer chromatography, minicolumn method.

A visual examination technique developed by Dickens *et al.* (2) is used to detect lots of farmer stock peanuts which contain aflatoxin. Approximately 465 g of kernels from the official grade sample are examined for the presence of the fungus, *Aspergillus flavus*. The 465-g sample of kernels includes about 82 g of loose shelled kernels (LSK) that are present in the approximately 2-kg official grade sample taken from the lot and about 383 g of kernels that are shelled from a 500-g subsample of pods during the grading operation. If one or more kernels in the 465-g sample are found to contain *A. flavus* growth, the lot is classified segregation 3 and diverted from food use.

Quantitative techniques to measure aflatoxin in samples of farmers stock peanuts have not been implemented at buying points due to cost and length of time required to extract and quantify the aflatoxin. With improvement of the minicolumn assay method (3), there has been an interest in comparing the efficacy of the visible *A. flavus* (VAF) method and the minicolumn assay (MCL) method. Two studies have been made to compare the VAF, MCL, and thin layer chromatography (TLC) methods. In the first study, the variability associated with testing farmers stock peanuts with each of the 3 methods was determined experimentally (1), and in the second study computer models were developed to simulate each of the above testing methods (6). The computer models were used to determine the probability of accepting lots with given aflatoxin concentrations when 465-g samples of kernels from official grade samples are used. Plots of the probability of accepting lots verus lot concentration (operating characteristic or OC curves) for the VAF, MCL, and TLC methods are shown in Figs. 1, 2, and 3, respectively. Each of the four OC curves shown in each figure represents a different critical level (the maximum number of VAF kernels or the maximum aflatoxin concentration allowed in the sample for lot acceptance). In order to predict the number of lots accepted, the number of lots rejected, and the average aflatoxin concentration (AA) in the lots accepted by a given testing method, the distribu-

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tion of all lots to be tested according to the lot aflatoxin concentration (lot distribution) must be estimated (4,5).

The objectives of this study were (a) to develop a distribution of farmers stock lots according to lot concentration that would be typical of lots marketed at buying points, (b) to couple the lot distribution to the OC curves for the VAF, MCL, and TLC methods and compute the number of lots accepted, the AA in the accepted lots, and the number of lots rejected by each method, and (c) to determine the efficacy of the VAF, MCL, and TLC methods to test those lots of farmers stock peanuts for aflatoxin.

Materials and Methods

Lot Distribution - The distribution of farmers stock peanut lots according to their aflatoxin concentration may be estimated from aflatoxin test results for those lots. The average of the aflatoxin test results and the average of the lot concentrations should be the same. However, there is a significant difference between the distribution of aflatoxin test results and the distribution of lot concentrations. This difference is mostly due to sampling error and is more pronounced when small samples are taken from each lot than when large samples are used. A procedure has been developed by Whitaker and Dickens (5) to convert a distribution of aflatoxin test results to a distribution of lot concentrations. The procedure employs the system of equations given below:

$$\begin{aligned}
Y_1 &= a_1^1X_1 + a_1^2X_2 + \ldots + a_1^jX_j + \ldots + a_1^nX_n \\
Y_2 &= a_2^1X_1 + a_2^2X_2 + \ldots + a_2^jX_j + \ldots + a_2^nX_n \\
&\vdots \\
Y_i &= a_i^1X_1 + a_i^2X_2 + \ldots + a_i^jX_j + \ldots + a_i^nX_n \\
&\vdots \\
Y_m &= a_m^1X_1 + a_m^2X_2 + \ldots + a_m^jX_j + \ldots + a_m^nX_n
\end{aligned} \qquad (1)$$

where Y_i is the number of test results with i or less ppb aflatoxin, X_j is the number of lots with j ppb aflatoxin, and a_i^j is the probability of obtaining a test result i or less from a lot with concentration j. The distribution of lot concentrations $X_1,\ldots, X_j$ can be determined given the distribution of test results $Y_1,\ldots, Y_i$ and the probability coefficients a_i^j.

Equation 1 was used to develop a distribution for lots of farmers stock peanuts. The distribution of test results $Y_1,\ldots, Y_i$ was obtained by analyzing 2300 samples of kernels from 2300 farmers stock lots marketed in the southeast in 1980. The samples were collected as part of a collaborative study with the National Peanut Research Laboratory, Agricultural Research Service, USDA, Dawson, GA, to evaluate the minicolumn, visual, and TLC method for detecting aflatoxin in farmers stock lots. The samples weighed about 465 g each and were analyzed for aflatoxin by the MCL method. The probability coefficients a_i^j were determined from the negative binomial probability function which was used to develop OC curves for the MCL method (4). Using trial and error techniques, different trial lot distributions $X_1,\ldots, X_j$ were substituted into Equation 1. The predicted distributions of test results $\hat{Y}_1,\ldots, \hat{Y}_m$ were computed and compared to the observed distribution of test results $Y_1,\ldots, Y_m$. The lot distribution was chosen when the sum of the deviations between the computed distribution of test results and the observed distribution of test results was minimized and the AA for the lot distribution was equal to the AA of the test results.

The maximum value of j used in Equation 1 for the lot distribution was limited by the average of all test results M or the probability coefficients a_i^j. The value of n was determined by trial and error such that

$$M = \sum_{j=0}^{n} (X_j * j) / \sum_{j=0}^{n} X_j. \qquad (2)$$

However, fewer than n terms could be used if the probability coefficient $a_i^j = 0$ when $j < n$. The maximum value of i in the distribution of test results, Y_i, was determined by the largest observed test result.

Coupled Equations - The number of lots accepted $\ell a(m)$ with a specific lot concentration m, is the product of the acceptance probability P(m) for that lot concentration m, the number of lots tested TL and the fraction of total lots F(m) with concentration m.

$$\ell a(m) = P(m)*TL*F(m). \qquad (3)$$

Values of F(m) and P(m) are obtained from the lot distribution and the OC curve, respectively. The total number of lots accepted LA, is given by Equation 4.

$$LA = \sum_{m=0}^{max} \ell a(m) = TL \sum_{m=0}^{max} P(m)*F(m), \qquad (4)$$

where max is the maximum observable lot concentration. The number of lots rejected $\ell r(m)$ with a specific lot concentration m is given by Equation 5.

$$\ell r(m) = TL*F(m)*(1-P(m)), \qquad (5)$$

where (1-P(m)) is the probability of rejecting a lot. The total number of lots rejected LR is

$$LR = \sum_{m=0}^{max} \ell r(m) = TL \sum_{m=0}^{max} F(m)*(1-P(m)). \qquad (6)$$

The AA in the lots accepted by the testing program is given by Equation 7.

$$AA = (TL/LA) \sum_{m=0}^{max} P(m)*F(m)*m. \qquad (7)$$

Efficacy - The computed lot distribution, and the OC curves for the specific critical levels shown in Figs. 1, 2, and 3 were used in conjunction with Equations 4 and 6 to compute the total number of lots accepted and rejected by each method. Equation 7 was used to compute AA. The test method that is the most effective in discriminating among lots according to their aflatoxin concentration is the method that has the lowest AA for a given percent of lots accepted. Efficacy of a method can be defined with the help of equations 4 and 7.

Results and Discussion

The cumulative distributions of farmers stock lots, observed test results, and predicted test results are shown in Fig. 4. The figure shows the agreement between the observed and predicted distributions of test results. The average aflatoxin concentration for the lot distribution is 59.5 ppb which is the same as for the observed distribution of test results.

The percentage of the total number of lots that was accepted by each method for each critical level versus the AA in the accepted lots for each critical level is plotted in Fig. 5. The AA is lower for the VAF method than either the MCL or TLC method for a given percentage of lots accepted. For example, if each of the 3 testing methods accept 84% of the lots tested, the lots accepted by the VAF, MCL, and TLC methods would have an AA of 7.8, 9.5, and 9.5 ppb, respectively. Figure 5 indicates that the VAF method is more effective than the MCL method with a critical level of 10 ppb or greater and is more effective than the TLC method with a critical level of 7 ppb or greater. For the sample size and lot distribution used in this study, the only way to achieve

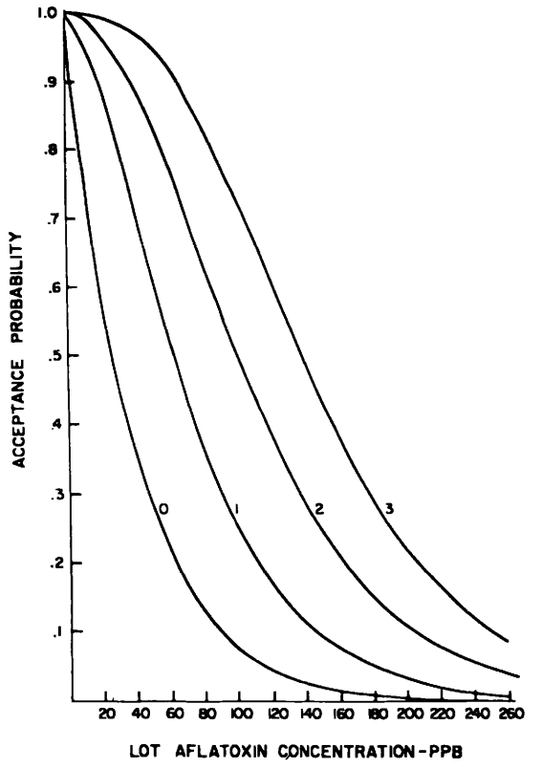

Fig. 1. Acceptance probabilities associated with the visible *A. flavus* method (VAF), 465-g sample, and critical levels of 0, 1, 2, and 3 VAF kernels.

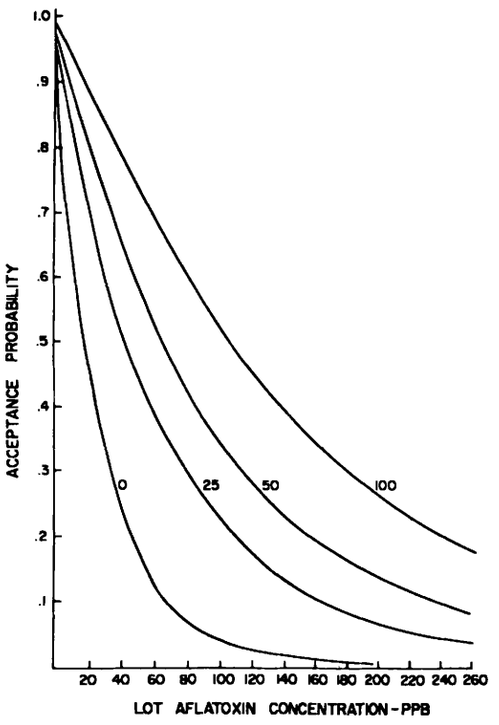

Fig. 3. Acceptance probabilities associated with the thin layer chromatography method (TLC), 465-g sample, and critical levels of 0, 25, 50, and 100 ppb.

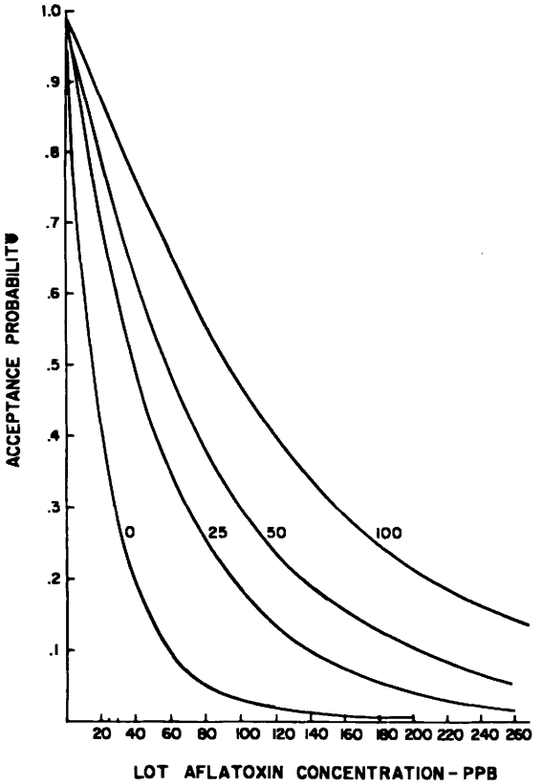

Fig. 2. Acceptance probabilities associated with the minicolumn (MCL) method, 465-g sample, and critical levels of 0, 25, 50, and 100 ppb.

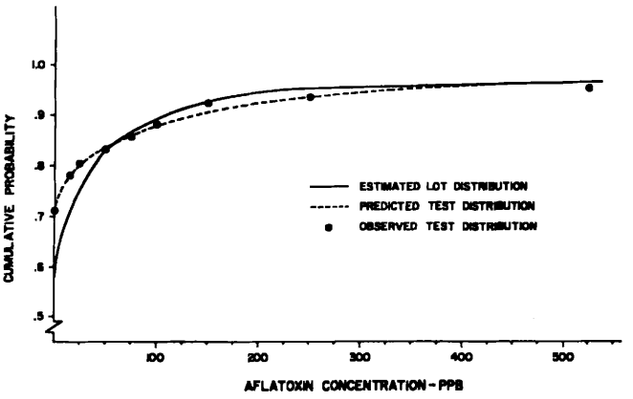

Fig. 4. A cumulative distribution of observed tests on 2,300 lots of farmers stock peanuts, an estimated cumulative distribution of the 2,300 lots, and a predicted cumulative distribution of test results on the 2,300 lots. The average aflatoxin concentration for each distribution is 59.5 parts per billion (ppb). The complete distributions are not shown. The maximum observed test result was 15,000 ppb and the maximum estimated lot concentration was 1450 ppb.

ppb aflatoxin in the accepted lots is to use the MCL method with a critical level < 10 ppb or the TLC method with a critical level < 7 ppb. An AA of 2.5 ppb can be achieved with the MCL method if a critical level of 0 ppb is used, but only 72% of the lots would be accepted.

The cricital level required by each method to obtain a specific AA can be estimated from Fig. 5. For example, critical levels of 0 VAF kernels, 7 and 10 ppb for the VAF, TLC, and MCL methods, respectively, would be

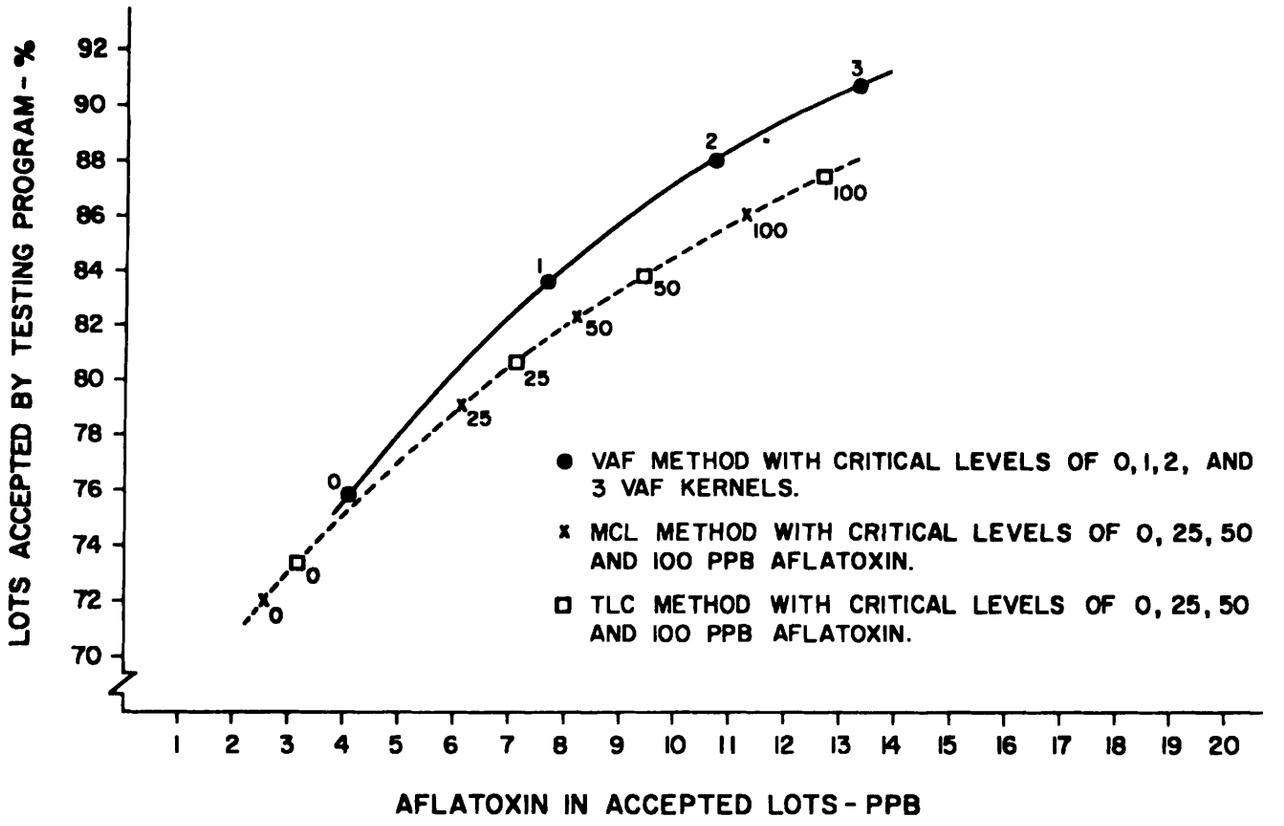

Fig. 5. The percent lot accepted versus the average aflatoxin concentration in the accepted lots when 2,300 lots of farmers stock peanuts were tested with the visual (VAF), minicolumn (MCL), and thin layer chromatography (TLC) methods.

required to achieve an AA of 4 ppb. The critical level required by each method to accept the same number of lots can also be estimated from Fig. 5. For example, critical levels of 0 VAF kernels, 10 and 15 ppb for the VAF, TLC, and MCL methods, respectively, accept about 76% of the lots tested.

With the presently used VAF testing method (0 VAF kernels in Fig. 5), 75.8% of the lots would be accepted, and the AA in the accepted lots would be about 4.1 ppb. There would be an AA of 232.9 ppb in the rejected (segregation 3) lots. These data are in reasonable agreement with a previous study by Dickens *et al.* (2) where the average aflatoxin concentrations of accepted and rejected lots were 14 and 281 ppb, respectively, when the percent of accepted lots was 74%.

Summary

This study predicts the performance of the VAF, MCL, and TLC method when there is a high incidence of aflatoxin-contaminated lots and when the analytical sample consists of only 465 g of kernels. Results indicate that the VAF method is more effective than the MCL method with a critical level of 10 ppb or greater and more effective than the TLC method with a critical level of 7 ppb or greater. The AA in the lots accepted by the MCL and TLC methods when using critical levels below 10 and 7 ppb, respectively, would be less than using the VAF method with a critical level of 0 VAF kernels.

The AA in the lots accepted by all 3 methods probably can be reduced by increasing the size of the analytical sample. The computer models developed for this study can be used to estimate the effects of sample size on the efficacy of each of the 3 test methods.

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