First Colombian interlaboratory study for the determination of aflatoxin B1 in yellow corn

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Abstract

An interlaboratory study for the determination of aflatoxin B1 in yellow corn was conducted with 16 laboratories that analyze for aflatoxins in Colombia. Naturally contaminated ground yellow corn (Monsanto DK 4004) with an assigned reference value of 26.3 µg/kg aflatoxin B1 was distributed as double blind duplicates in sachets of 55 g. z-Scores were computed for each of the results; repeatability of the two replicate analysis was also calculated. Four of the participating laboratories used HPLC, seven used TLC, one used fluorometry, one ELISA and three a semi quantitative analytical technique (Aflacard®). Only 10 of the 26 quantitative results (39%) had satisfactory z-scores, two scores were questionable (8%) and 14 of the 26 results had unsatisfactory z-scores (54%). A total of 8 laboratories had satisfactory repeatability (62%), and 5 had unsatisfactory repeatability (39%). The present study indicates that only about one third of the results for aflatoxin reported by Colombian laboratories have good accuracy (as measured by the z-score of the result), although satisfactory precision (measured as repeatability) is achieved by about two thirds of the laboratories. These results indicate that an improvement in quality assurance is needed in Colombian laboratories. The routine use of reference standards and reference materials is highly recommended.

Introduction

Aflatoxins are a group of highly toxic fungal metabolites synthesized mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (1). Exposure to aflatoxins may result in hepatotoxicity, mutagenesis, immunosuppression, teratogenesis or carcinogenesis (1). Studies conducted in Colombia have revealed the presence of aflatoxins, both in human foods and animal feeds, at levels above the widely accepted international level of 10-20 µg/kg total aflatoxins (2,3). This contamination suggests that it is necessary to continuously monitor aflatoxin levels in Colombia by using reliable analytical methods. In order to obtain reliable results, closest to the true values as possible, a laboratory must implement appropriate programs of quality assurance procedures. This issue is even more important when foods and feeds are analyzed for potentially harmful substances such as mycotoxins (4).
An important part of the analytical quality assurance is the use of control materials. These are characterized substances that are inserted into the run alongside the test materials and subjected to exactly the same treatment. A control material must contain an appropriate concentration of the analyte, and a value of that concentration must be assigned to the material (5). Control materials can also be used to conduct laboratory-performance studies. An interlaboratory study consists of one or more analysis or measurements by a group of laboratories on one or more homogeneous, stable test items, by the method selected or used by each laboratory. The reported results are compared with those of other laboratories or with the known or assigned reference value, generally with the objective of evaluating or improving laboratory performance (6).

The Toxicology Laboratory of the College of Veterinary Medicine, National University of Colombia, the leading Colombian laboratory in mycotoxin testing, organized the first interlaboratory study for the determination of aflatoxin B1 in yellow corn. A reference material was prepared, found to be sufficiently homogeneous, and then distributed in the form of duplicate bind samples to 16 laboratories. The objective of this study was to determine the performance of the laboratories that analyze aflatoxins in human foods and animal feeds in Colombia.

Materials and methods

Preparation and distribution

Naturally contaminated yellow corn (Monsanto DK 4004) was first ground with a grinding-subsampling mill (Romer Series II mill, Romer Labs, Union, USA) to pass a 20 mesh sieve and then ground again so that >90% of the particles passed through a 0.6 mm sieve. The material was then homogenized by mixing in a twin-shell blender for 6 hours in 30 min cycles. After each cycle the sample was mixed by hand. Eight randomly selected samples of the test material were analyzed in duplicate for aflatoxin B1 using the method of Trucksess et al. (7). Briefly, 50 g samples were extracted with 100 ml acetonitrile-water (84:16, v/v) for 1 hour using a wrist-action shaker at high speed. The extract was filtered and 5 ml were transferred into a 10 ml culture tube. A multifunctional cleanup column (Micotox M2002 column, Micotox Ltda., Bogotá, Colombia) was used for the purification of the extract; 200 µl of purified extract were derivatized with 700 µl of trifluoroacetic acid-glacial acetic

acid-water (2:1:7, v/v/v) at 65°C for 10 minutes and 50 µl were injected into the chromatograph. The results were subjected to appropriate statistical evaluation (8) to check the homogeneity of the material (Table 1). Once homogeneity was demonstrated, samples of 55 g of the material were packed in small sachets, labeled and coded with a two digit number. Two blind duplicates (9) of the material were sent to each of the 16 participating laboratories along with a proforma. Each laboratory was required to report the results within a specified timeframe, stating the analytical method used.

Table 1. Homogeneity data for yellow corn test material

Sample identity	*Analyte*	
	Aflatoxin B1 (µg/kg)	
	Replicate 1	**Replicate 2**
1	22.7	26.1
2	25.8	27.7
3	22.8	26.8
4	28.1	30.6
5	20.9	25.3
6	26.2	27.6
7	23.9	28.1
8	30.3	27.4
mean, n	26.3	16
Origin of target std deviation (σ_p)	Collaborative trial*	
Absolute target std deviation (σ_p) and as RSD_R (%)	4.05	15.4%
Analytical variance: s^2_{an}	2.30	
Sampling variance: s^2_{sam}	2.00	
Allowable variance: σ^2_{all}	1.48	
Critical	9.59	
s^2_{sam}<critical?	ACCEPT	

*Trucksess et al., 1994.

Statistical evaluation of results

Calculation of the assigned value. The assigned value, that is, the best estimate of the true concentration of aflatoxin B1 in the yellow corn batch, was set as the mean value of the 8 duplicate analysis conducted using the collaboratively evaluated method of Trucksess et al. (7), as shown in Table 1.

Target standard deviation (σ_p). The value of σ_p sets the limits of satisfactory performance. The standard deviation of reproducibility determined in collaborative trials is usually considered to be an appropriate estimate of the best agreement that can be obtained between laboratories (10). For the present study, σ_p was derived from an appropriate collaborative trial (7) as shown in Table 1.

Individual z-scores. The value of σ_p used to calculate z-scores was 4.05 and was derived as explained before. The z-scores of the participants were calculated as follows:

$$z = (x-X)/\sigma_p,$$

where x is the participant's reported result, X is the assigned value, and σ_p is the target value for standard deviation. z-Scores were interpreted according to Thompson and Wood (10), as follows:

$\|z\| \leq 2$	Satisfactory
$2 < \|z\| < 3$	Questionable
$\|z\| \geq 3$	Unsatisfactory

Repeatability. Intralaboratory repeatability can be easily calculated by determining the absolute difference between the results of the analysis of two portions of the same material (9). The absolute difference between the two test results should be less than or equal to the repeatability limit (r), which corresponds to 2.8 x σ_p (6).

Results and Discussion

The material used for the present study was shown to be sufficiently homogeneous by using the statistical analysis of Fearn and Thompson (8), as shown in Table 1. Once the material was found to be homogeneous it was distributed to the participating laboratories in the form of bind duplicates. Each participant was given a laboratory number assigned according to the analytical technique used and the order of receipt of results. Four of the participating laboratories reported the use of HPLC, seven used TLC, one used fluorometry, one ELISA, and three a semi quantitative screening method (Aflacard®, R-Biopharm Rhone, Glasgow, Scotland). This analytical technique indicates whether the sample contains more or less analyte than the specified limit of detection of the card. One of the three laboratories that used this technique reported only one result.

Figure 1. z-Scores for aflatoxin B1 (26.3 µg/kg) in yellow corn test material

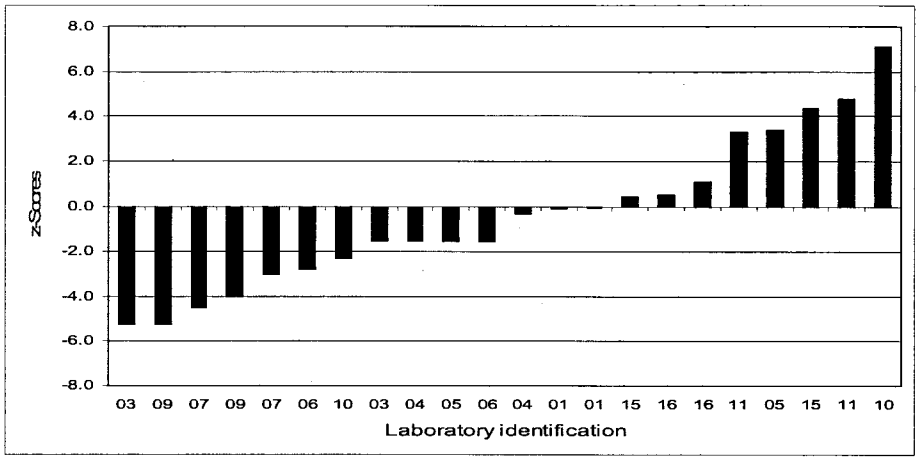

Table 2. Analytical techniques, results reported, z-scores and interpretation of the z-score in yellow corn test material

		Analyte				
		Aflatoxin B1			z-score	
Lab. number	Analytical technique	Sample number	Assigned value	Reported value	Value	Interpretation
01	HPLC	1	26.3	25.9	-0.1	Satisfactory
		2	26.3	26.0	-0.1	Satisfactory
02	HPLC	1	26.3	208.9	45.4	Unsatisfactory
		2	26.3	217.2	47.1	Unsatisfactory
15	HPLC	1	26.3	44	4.4	Unsatisfactory
		2	26.3	28	0.4	Satisfactory
16	HPLC	1	26.3	28.3	0.5	Satisfactory
		2	26.3	30.8	1.1	Satisfactory
03	TLC	1	26.3	5	-5.3	Unsatisfactory
		2	26.3	20	-1.6	Satisfactory
04	TLC	1	26.3	20	-1.6	Satisfactory
		2	26.3	25	-0.3	Satisfactory
05	TLC	1	26.3	20	-1.6	Satisfactory
		2	26.3	40	3.4	Unsatisfactory
06	TLC	1	26.3	15	-2.8	Questionable
		2	26.3	20	-1.6	Satisfactory
07	TLC	1	26.3	14	-3.0	Unsatisfactory
		2	26.3	8	-4.5	Unsatisfactory
08	TLC	1*	26.3	N.D.	∞	Unsatisfactory
		2*	26.3	N.D.	∞	Unsatisfactory
09	TLC	1	26.3	10	-4.0	Unsatisfactory
		2	26.3	5	-5.3	Unsatisfactory
10	Fluorometry	1	26.3	55	7.1	Unsatisfactory
		2	26.3	17	-2.3	Questionable
11	ELISA	1	26.3	45.7	4.8	Unsatisfactory
		2	26.3	39.7	3.3	Unsatisfactory

*z-score undetermined because of the reported results of non detectable.

Table 2 summarizes the z-scores calculated for each of the two results obtained on the samples analyzed by each laboratory reporting quantitative results. Figure 1 shows the same results in the form of a histogram, excluding extreme values

(z-scores >|10|). Only 10 of the 26 quantitative results (39%) had satisfactory z-scores, two z-scores were questionable (8%) and 14 results had unsatisfactory z-scores (54%). The closest agreement to the assigned reference value of 26.3 µg/kg was obtained by laboratories using HPLC followed by those using TLC. None of the results obtained by fluorometry and ELISA analysis had satisfactory z-scores. The mean value of the HPLC results with a satisfactory z-score was 27.8 µg/kg, whereas the mean value of those TLC results with a satisfactory z-score was 21 µg/kg. The percentage of laboratories that obtained satisfactory z-scores in the present study was found to be well below international standards. A proficiency study for aflatoxin determination in corn was recently organized by the Central Science Laboratory (Sand Hutton, York, UK) under the food analysis performance assessment scheme (FAPAS®) program (11). In this international study, test material was distributed to laboratories in 33 countries, and 57 of a total of 65 scores for aflatoxin B1 (88%) were satisfactory. This percentage is 2.3 times higher than the one found in the present study and indicates the need to improve the performance of the Colombian laboratories that determine aflatoxins.

It is interesting to note that chromatography (both HPLC and TLC), is the analytical technique preferred by the laboratories that determine aflatoxins. Probably this trend is due to the fact that the official analytical technique for aflatoxins in Colombia (Norma Técnica Colombiana ICONTEC NTC 1232) is based on the chromatographic determination of aflatoxins.

Table 3 summarizes the results obtained using the screening technique Aflacard®. The five results reported were satisfactory.

Table 3. Results and interpretation of the result in yellow corn test material analyzed by Aflacard®.

Laboratory number	Sample number	Assigned value	Reported result	Limit of detection	Interpretation
12	1	26.3	Positive	15 ppb	Satisfactory
	2	26.3	Positive	15 ppb	Satisfactory
13	1	26.3	Positive	10 ppb	Satisfactory
	2	26.3	Positive	10 ppb	Satisfactory
14	1	26.3	Positive	20 ppb	Satisfactory
	2*	26.3	-	-	-

*Laboratory 14 did not report the result for sample 2.

Blind duplicate results can be used to calculate the repeatability of the analysis of aflatoxin B1 in test material for each laboratory. Table 4 summarizes the repeatability obtained for the 13 laboratories that reported quantitative results. Repeatability limit corresponds to 2.8 x σ_p. Repeatability is interpreted as satisfactory or unsatisfactory depending on whether it falls below or above the repeatability limit (11.3 in the present study). Three of the four laboratories that used HPLC (75%) and 5 of the 7 laboratories using TLC (71%) were within the repeatability limit. The repeatability of the only laboratory that used ELISA was satisfactory but the repeatability of the laboratory reporting fluorometry was unsatisfactory.

Table 4. Repeatability for aflatoxin B1 (26.3 µg/kg) in yellow corn test material

		Analyte			
		Aflatoxin B1 (µg/kg)		Repeatability	
Lab. number	Analytical technique	Value 1	Value 2	Value[a]	Interpretation[b]
01	HPLC	25.9	26.0	0.1	Satisfactory
02	HPLC	208.9	217.2	8.3	Satisfactory
15	HPLC	44	28	16	Unsatisfactory
16	HPLC	28.3	30.8	2.5	Satisfactory
03	TLC	5	20	15	Unsatisfactory
04	TLC	20	25	5	Satisfactory
05	TLC	20	40	20	Unsatisfactory
06	TLC	15	20	5	Satisfactory
07	TLC	14	8	6	Satisfactory
08[c]	TLC	N.D.	N.D.	-	Unsatisfactory
09	TLC	10	5	5	Satisfactory
10	Fluorometry	55.0	17.0	38.0	Unsatisfactory
11	ELISA	45.7	39.7	6.0	Satisfactory

[a]Absolute difference between value 1 and value 2
[b]Repeatability limit = $2.8 \times \sigma_p = 11.3$
[c]Participant reported non detectable levels

The results of the present interlaboratory study indicate that the accuracy of the analysis performed for aflatoxin determination in Colombian laboratories is low, as determined by the low proportion of results that had satisfactory z-scores (39%). Precision, determined as repeatability, was found to be better that accuracy, with 62% of laboratories having satisfactory repeatability. These results indicate that an improvement in quality assurance is needed in Colombian laboratories. The routine use of reference standards and reference materials is strongly recommended as well as the routine participation in proficiency testing studies.

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