Wageningen Academic Publishers

# Results of a proficiency test for multi-mycotoxin determination in maize by using methods based on LC-MS/(MS)

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Received: 20 February 2012 / Accepted: 11 January 2013


## Abstract

Liquid chromatography coupled with single or tandem mass spectrometry (LC-MS/(MS)) is routinely used for the simultaneous determination of mycotoxins in food and feed although official methods using this technique have not yet been adopted by the European Committee for Standardization and the Association of Analytical Communities. A proficiency test (PT) was conducted for the simultaneous determination of up to 11 mycotoxins (aflatoxin $B_1$ ($AFB_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_1$ ($AFG_1$), aflatoxin $G_2$ ($AFG_2$), ochratoxin A (OTA), deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZEA), fumonisin $B_1$ ($FB_1$) and fumonisin $B_2$ ($FB_2$)) in maize using LC-MS/(MS) to benchmark laboratories currently using this technique and to obtain information on currently used methodologies and method-related performances. Each participant received the following: instructions; a comprehensive questionnaire; a mixed mycotoxins calibration solution; a spiking solution ($AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$, OTA, DON, T-2, HT-2, ZEA, $FB_1$ and $FB_2$); and two test materials, namely a contaminated maize sample and a blank maize sample to be spiked with a spiking solution containing 11 mycotoxins. Laboratory results were rated with *z*-scores. Of the 64 laboratories enrolled in the PT, 41 laboratories from 14 countries returned 43 sets of results for various combinations of analytes. The majority of laboratories (61%) reported results for all 11 mycotoxins, whereas the remaining laboratories reported results for a restricted combination (from 2 to 10 analytes). For contaminated maize and spiked maize the percentage of satisfactory *z*-score values ($|z| \leq 2$) were: DON 55% and 49%, $FB_1$ 50% and 30%, $FB_2$ 52% and 38%, ZEA 68% and 64%, T-2+HT-2 toxins 82% and 85%, OTA 58% and 60%, $AFB_1$ 56% and 62%, $AFG_1$ 73% and 84%, $AFB_2$ 40% and 78%, $AFG_2$ 64% and 78%, respectively. The poorest performance ($|z| > 3$) was obtained for $FB_1$ (31%), $FB_2$ (32%), $AFB_1$ (32%) and $AFB_2$ (32%) in contaminated maize and for DON (35%), $FB_1$ (63%) and $FB_2$ (52%) in spiked maize. Mean recovery results were acceptable for all mycotoxins (74% to 109%), except for fumonisins, where these were unacceptably high (159% for $FB_1$ and 163% for $FB_2$). A robust and reliable method for simultaneous determination of 11 mycotoxins in maize could not be identified from the results of this PT. Additional experimental work is necessary to set up a method suitable for inter-laboratory validation. The results of this PT and the relevant method's details can be useful to identify methodology strengths and weaknesses.

**Keywords:** maize, test-material, validation, z-score

## 1. Introduction

Most of the EU-regulated mycotoxins, including deoxynivalenol (DON), zearalenone (ZEA), fumonisin $B_1$ ($FB_1$), fumonisin $B_2$ ($FB_2$), aflatoxin $B_1$ ($AFB_1$), aflatoxin $G_1$ ($AFG_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_2$ ($AFG_2$), ochratoxin A (OTA), T-2 toxin (T-2) and HT-2 toxin (HT-2), can contaminate maize alone or in various combinations

(CAST, 2003; Commission of the European Communities, 2006; Miller, 2008). Several liquid chromatography coupled with single or tandem mass spectrometry (LC-MS/(MS)) based methods have been recently developed for multi-mycotoxin determination in maize, food and feed (reviewed in Shephard *et al.*, 2012, 2013). A survey on the use and application of methods for the determination of mycotoxins in food and feed revealed that 42% of participant laboratories routinely use LC-MS/(MS) methodology for their single or simultaneous determination (Solfrizzo *et al.*, 2009). However, within both the European Committee for Standardization (CEN) and the Association of Analytical Communities (AOAC International) contexts there are no LC-MS/(MS)-based official methods for the measurement of this group of contaminants. The Mycotoxins and Phycotoxins Working Group (MPWG) of the MoniQA Network of Excellence conducted an enquiry among their 22 members about their willingness to participate in a collaborative study for validation of an LC-MS/(MS)-based method for simultaneous determination of the 11 mycotoxins regulated in maize in the European Union i.e. DON, ZEA, $FB_1$, $FB_2$, $AFB_1$, $AFG_1$, $AFB_2$, $AFG_2$, OTA, T-2 and HT-2. Ten respondent members positively judged the initiative, but actually only 5 of them were interested to take part in the proposed validation study. It was therefore decided to conduct a proficiency test (PT) to benchmark laboratories currently using this technique and to obtain information on currently used methodologies. This would be the first step to select (and exclude) methods with the aim to organize an inter-laboratory validation trial (e.g. in CEN and/or AOAC International context) to derive performance characteristics of a method for simultaneous determination of EU-regulated mycotoxins in maize. Only laboratories having experience with LC-MS(MS) determinations of mycotoxins in food and feed were accepted to take part in the PT. Laboratories were not obliged to determine all the regulated mycotoxins and were free to report results only for those mycotoxins that can be simultaneously determined with their LC-MS(MS)-based method. In this paper we report the results of the PT, involving 41 laboratories from 14 countries.

## 2. Materials and methods

### Organization of the proficiency test

Invitation letters were sent to potential participants among MoniQA partners and associated partners, universities, research centres, control and private laboratories and members of the CEN working group Biotoxins (CEN/TC 275/WG 5). Sixty-four participants located in 18 countries registered to the PT. Each participant received: (a) one plastic bottle containing approximately 25 g of contaminated maize and one containing approximately 25 g of blank maize; (b) one ampoule containing the blind spiking solution and one containing the calibration solution; (c) an accompanying form with instruction on sample storage; and (d) instructions, spiking protocol, reporting sheets and a detailed questionnaire to describe the method used.

Forty-one laboratories located in 14 countries performed the exercise and reported results for contaminated maize and spiked maize. A laboratory (lab code 2) reported two sets of results for contaminated maize that were obtained by analysing the sample with two different methods. Another laboratory (lab code 60) reported two sets of results for both contaminated and spiked maize. These results were obtained by splitting each final extract in two aliquots that were analysed with two different LC-MS(MS) apparatuses. In total, 43 sets of results were obtained for contaminated maize and 42 for spiked maize. Thirteen laboratories reported no results and no explanation for this behaviour. One laboratory did not perform the analyses because the parcel was stuck in the customs for a long time and was therefore considered unsuitable. Three registered laboratories were from the same organization, therefore only one laboratory performed the analyses. The results of one laboratory could not be considered because they were received too late. Six laboratories from two countries did not receive the parcels because they were rejected at customs.

The PT aimed to assess the concentration of up to 11 mycotoxins in both spiked and contaminated test maize materials. The laboratories were free to choose and use the method (based on LC/MS technology) they believe was most appropriate for simultaneous determination of the target mycotoxins in maize. A request, however, was that they had to start from a test portion size of 20.0 g. The laboratories were asked to spike the blank maize material by using the provided blind spiking solution according to the provided spiking protocol. The results were to be reported in µg/kg by completing the provided reporting sheets. The laboratories were also asked to complete a questionnaire that was intended to provide detailed information on the method used and relevant LC-MS(MS) apparatus and conditions.

### Spiking and calibration solutions

A mixed DON, ZEA, $FB_1$, $FB_2$, $AFB_1$, $AFG_1$, $AFB_2$, $AFG_2$, OTA, T-2 and HT-2 spiking solution was prepared by mixing adequate volumes of each mycotoxin standard solution. Mycotoxins were sourced as follows: $FB_1$ and $FB_2$ (powder) were purchased from Sigma-Aldrich (Milan, Italy) and dissolved in acetonitrile:water (50:50); powder of DON was purchased from Romer Labs® Diagnostic GmbH (Tulln, Austria) and dissolved in acetonitrile. Commercial standard solutions of ZEA, $AFB_1$, $AFG_1$, $AFB_2$, $AFG_2$, OTA, T-2 and HT-2, in acetonitrile, were purchased from Romer Labs® Diagnostic GmbH. Adequate aliquots of these standard solutions were mixed to obtain a spiking solution containing

40.0 µg/ml DON, 8.4 µg/ml ZEA, 21.0 µg/ml $FB_1$, 9.0 µg/ml $FB_2$, 0.2 µg/ml $AFB_1$, 0.2 µg/ml $AFG_1$, 0.083 µg/ml $AFB_2$, 0.083 µg/ml $AFG_2$, 0.2 µg/ml OTA, 5.0 µg/ml T-2 and 5.0 µg/ml HT-2. Seventy-five amber glass vials were filled each with 0.6 ml of spiking solution, sealed and stored at -20 °C until dispatch. Fifteen ml of spiking solution were diluted to 150.0 ml with acetonitrile and used to prepare 75×2 ml calibration solutions in amber glass vials that were sealed and stored at -20 °C until dispatch. The calibration solutions contained the 11 mycotoxins at concentrations one-tenth of the spiking solutions and were suitable to determine the mycotoxin levels in spiked and contaminated test maize samples.

### Test materials

A maize sample naturally contaminated with $FB_1$ (580 µg/kg) and $FB_2$ (145 µg/kg) was ground and further fortified with culture extracts of mycotoxigenic species of *Fusarium sporotrichioides*, *F. graminearum*, *F. verticillioides*, *Aspergillus ochraceus* and *A. parasiticus* (deposited at the Institute of Sciences of Food Production collection, http://www.ispa.cnr.it/Collection), cultured on cereals. In particular, each fungal culture was dried, ground and extracted with methanol, water or mixtures of methanol:water. Aliquots of culture extracts were adequately diluted with mobile phase and analysed by HPLC to measure their mycotoxin concentrations. To reach the levels around the regulatory limits of each mycotoxin, adequate amounts of fungal culture extracts were added to ground maize naturally contaminated with $FB_1$ and $FB_2$. The contaminated maize was then slurry homogenized with water (1:1, w/w) for 5 min and freeze-dried for 48 h. The homogenized freeze-dried material was ground to a particle size <500 µm. To prepare the blank maize material, two different samples of maize were mixed, ground and slurry homogenized with water (1:1, w/w) for 5 min, freeze-dried for 48 h and ground to a particle size <500 µm. This material contained low levels of some mycotoxins (see Table 1). Two and ten kg of blank and contaminated maize materials, respectively, were prepared. The two test materials were then dispensed (about 25 g each) in plastic bottles that were labelled, sealed and stored at -20 °C until dispatch. For the homogeneity study bigger aliquots (3×150 g for blank maize, 10×300 g for contaminated maize) were sampled during the filling sequence.

### Homogeneity testing

Homogeneity of the contaminated maize material was evaluated according to chapter 3.11.2 of the international harmonized protocol for the proficiency testing of analytical chemistry laboratories (Thompson *et al.*, 2006). In particular, 10 aliquots of about 300 g of contaminated maize were taken at regular intervals from the filling sequence. Each of the 10×300 g samples was divided in 6×50 g aliquots that were analysed in duplicate. In this way 6 sets of 10 identical test portions were obtained. Each set of test portions was analysed by a different reference method specific for a mycotoxin or group of mycotoxins. These were: (a) CEN EN 15851:2010 for $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$; (b) Solfrizzo *et al.* (2011) for $FB_1$ and $FB_2$; (c) Visconti *et al.* (2005) for T-2 and HT-2 toxins; (d) MacDonald *et al.* (2005b) for DON; (e) Entwisle *et al.* (2000) for OTA;

**Table 1. Mean levels of mycotoxins in contaminated and blank maize test materials derived from the homogeneity study.**

| Analyte | Contaminated maize[1] (µg/kg±SD) | Blank maize[2] (µg/kg±SD) |
|---|---|---|
| DON | 652±59 | 94±3 |
| $FB_1$ | 2,150±101 | 29±2 |
| $FB_2$ | 729±63 | nd[3] |
| ZEA | 437±20 | 6±1 |
| T-2 | nd | nd |
| HT-2 | 189±3 | 25±4 |
| T-2+HT-2 | 189±3 | 25±4 |
| OTA | 7.1±0.2 | 0.4±0.01 |
| $AFB_1$ | 5.9±0.3 | nd |
| $AFG_1$ | 11.6±0.4 | nd |
| $AFB_2$ | 0.5±0.02 | nd |
| $AFG_2$ | 0.7±0.03 | nd |

[1] Overall mean from 10 samples analysed in duplicate.

[2] Mean from 3 samples.

[3] Not detected (limit of detection for $FB_2$ was 10 µg/kg, for T-2 8 µg/kg, for $AFB_1$ and $AFG_1$ 0.07 µg/kg, for $AFG_2$ 0.02 µg/kg, respectively).

and (f) MacDonald *et al.* (2005a) for ZEA. In Table 2 are the homogeneity test results reported for all mycotoxins in the contaminated maize. Since the between-sample variance ($S_{sam}^2$) was lower than the critical factor (c) for all mycotoxins, the test for homogeneity passed meaning that the contaminated maize was sufficiently homogeneous. Moreover, the visual appraisal of analytical results did not show any systematic effects such as discordant duplicated results, trends or discontinuities. The presence and levels of mycotoxins in the blank maize material were checked in 3 samples (150 g each). The 3×150 g samples were each divided in 6 aliquots that were analysed by using the 6 reference HPLC methods reported above. This homogeneity study for the blank material was limited in scope due to the limited availability of material. The levels of mycotoxins in contaminated and blank maize derived from the homogeneity study are reported in Table 1. No detectable levels of aflatoxins, $FB_2$ and T-2 were found in the blank maize material whereas DON, $FB_1$, ZEA, HT-2 and OTA were present and measured at low levels. A true blank maize material for 11 mycotoxins could not be found. The measured levels of DON, $FB_1$ ZEA, T-2+HT-2 and OTA were reasonably low (≤10% of spiking levels, see Tables 1 and 3) and assumed to be acceptable for the purpose of this study. As shown in Table 1 T-2 was not detected in contaminated maize whereas the level of HT-2 was higher than expected since the culture extract of *F. sporotrichioides*, used to fortify the contaminated maize material, contained both T-2 (66 µg/ml) and HT-2 (16 µg/ml). This is not surprising because T-2 was expected to convert to HT-2 during the homogenization of the test material (addition of water, slurry and freeze-drying) as a result of a selective enzymatic deacetylation of T-2 to give HT-2 (Lattanzio *et al.*, 2009). This was confirmed in the coordinating laboratory since the increase in HT-2 was similar as the decrease of T-2. Maize and other cereals contain inherently hydrolytic enzymes that are activated in the presence of water and are able to convert T-2 into HT-2 (Lattanzio *et al.*, 2009).

### Stability study

Eight vials of calibration solutions and 8 bottles of contaminated maize test material were stored for 1, 3 and 6 months at -18 °C, 4 °C and 25 °C. At the end of each storage period calibration solutions and test materials were transferred at -18 °C to be analysed in duplicate at the end of the stability study within the shortest time. The results of this study will be reported in a separate paper.

### Scores and evaluation criteria

Individual laboratory performance is expressed in terms of *z*-score in accordance with Thompson *et al.* (2006):

$$Z = \frac{X_{lab} - X_{ref}}{\sigma_p}$$

where:
$X_{lab}$ is the measurement result reported by a participant expressed as a dimensionless mass ratio, e.g. 1 µg/kg = $10^{-9}$;
$X_{ref}$ is the assigned (consensus) value expressed as a dimensionless mass ratio, e.g. 1 µg/kg = $10^{-9}$;
and $\sigma_p$ is the standard deviation for proficiency assessment (target standard deviation).

The assigned value for each mycotoxin in contaminated maize was the median of reported results. The assigned value for each mycotoxin in spiked maize was the spiking level.

The target standard deviation ($\sigma_p$) was calculated by the modified Horwitz equation (Thompson, 2000):

**Table 2. Homogeneity test results for all mycotoxins in the contaminated maize.**

| Mycotoxin | $s^2_{an}$ | $s_{sam}^2$ | $\sigma_p$ | $\sigma^2_{all}$ | c |
|---|---|---|---|---|---|
| DON | 1,115 | 2,458 | 111 | 1,113 | 3,218 |
| $FB_1$ | 3,018 | 7,535 | 306 | 8,454 | 18,941 |
| $FB_2$ | 1,603 | 2,440 | 122 | 1,345 | 4,148 |
| ZEA | 176 | 227 | 79 | 564 | 1,238 |
| HT-2[1] | 12 | 0 | 39 | 135 | 267 |
| OTA | 0.03 | 0.003 | 1.55 | 0.22 | 0.44 |
| $AFB_1$ | 0.04 | 0.034 | 1.30 | 0.15 | 0.33 |
| $AFB_2$ | 0.0001 | 0.0002 | 0.11 | 0.0010 | 0.0023 |
| $AFG_1$ | 0.135 | 0.037 | 2.54 | 0.58 | 1.23 |
| $AFG_2$ | 0.0002 | 0.0005 | 0.16 | 0.002 | 0.005 |

Abbreviations used: $s_{an}$ = experimental estimate of analytical standard deviation; $s_{sam}$ = experimental estimate of sampling standard deviation; $\sigma_p$ = standard deviation for proficiency testing; $\sigma_{all}$ = allowed standard deviation; c = critical value in a test for sufficient homogeneity ($c = F_1\sigma^2_{all} + F_2 s^2_{an}$).
[1] T-2 was not detected in contaminated maize.

- for analyte concentrations <120 µg/kg (OTA, $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$): $\sigma_p = 0.22 \times c$
- for analyte concentrations ≥120 µg/kg (DON, $FB_1$, $FB_2$, ZEA, HT-2, T-2+HT-2, Spiked T-2): $\sigma_p = 0.22 \times c^{0.8495}$

where:
c = concentration of the assigned value, expressed as a dimensionless mass ratio, e.g. 1 µg/kg = $10^{-9}$, 1 mg/kg = $10^{-6}$.

The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test ($\sigma_p$).

The z-score is interpreted as:

| | |
|---|---|
| $\|z\| \leq 2$ | satisfactory result |
| $2 < \|z\| \leq 3$ | questionable result |
| $\|z\| > 3$ | unsatisfactory result |

## 3. Results and discussion

This is the first PT for multi-mycotoxin determination by using LC-MS(MS) methodology. The results of the PT are summarised in the Tables 3 and 4 for spiked maize and contaminated maize, respectively. The statistical evaluations of the results for contaminated maize and spiked maize materials are reported in Tables 5 and 6, respectively. Individual results for each mycotoxin in contaminated maize and spiked maize materials are reported in Tables 7 and 8, respectively. Individual *z*-score results for each mycotoxin in contaminated maize and spiked maize materials are reported in Tables 9 and 10, respectively. Some details of the analytical methods used and the mycotoxins analysed by each participant are reported in Table 1 of the Supplementary Online Material. Graphical representation of *z*-scores and kernel density plot for each mycotoxin in contaminated maize and spiked maize materials are shown in Figures 1-10 and 11-21, respectively. Kernel density plots were computed (ProLab™ Software, Quodata, Dresden, Germany) from the analytical results by representing the individual numeric values each as a normalised Gaussian distribution centred on the respective analytical value. The sum of these normal distributions forms then the Kernel density distribution (Kunsagi *et al.*, 2010). The mean mycotoxin values of the PT results, for contaminated maize, matched quite well with mean values obtained from the homogeneity study with the exception of ZEA and $AFG_2$ and to a lesser extent of $FB_2$ and $AFG_1$ (Table 5). For contaminated maize a high percentage of satisfactory *z*-scores ($|z| \leq 2$) was obtained for HT-2 (82%), T-2+HT-2 (82%) and $AFG_1$ (73%) (Table 4). For spiked maize a high percentage of satisfactory *z*-scores ($|z| \leq 2$) was obtained for T-2 (74%), HT-2 (76%), T-2+HT-2 (85%), $AFG_1$ (84%), $AFB_2$ (78%) and $AFG_2$ (78%) (Table 3). The poorest performance ($|z| > 3$) for contaminated maize were obtained for $FB_1$ (31%), $FB_2$ (32%), $AFB_1$ (32%) and $AFB_2$ (32%) (Table 4). For spiked maize they occurred for DON (35%), $FB_1$ (63%), $FB_2$ (52%) (Table 3). As shown in Tables 9 and 10 only 2 laboratories (lab codes 32 and 41) scored acceptable values of *z*-score for all mycotoxins, both in contaminated and spiked maize. However, only the method used by laboratory 41 can be considered a multi-toxin method. Indeed, the method used by laboratory 32 could not be considered a multi-toxin method because the sample was divided in four aliquots that were separately extracted with 4 different extraction solvent mixtures. As reported in

**Table 3. Spiked maize test material: results of mycotoxin analysis and relevant scoring.**

| Analyte | Assigned value[1] (µg/kg) | Mean of reported results (µg/kg) | Mean recovery[2] (%) | No. of results[3] | % satisfactory z-scores ($\|z\| \leq 2$) | % questionable z-scores ($2 < \|z\| \leq 3$) | % unsatisfactory z-scores ($\|z\| \geq 3$) |
|---|---|---|---|---|---|---|---|
| DON | 1000.0 | 869.5 | 87 | 37 | 49 | 16 | 35 |
| $FB_1$ | 525.0 | 834.5 | 159 | 30 | 30 | 7 | 63 |
| $FB_2$ | 225.0 | 366.1 | 163 | 29 | 38 | 10 | 52 |
| ZEA | 210.0 | 155.0 | 74 | 36 | 64 | 17 | 19 |
| T-2 | 125.0 | 96.4 | 77 | 31 | 74 | 6 | 20 |
| HT-2 | 125.0 | 136.7 | 109 | 33 | 76 | 15 | 9 |
| T-2+HT-2 | 250.0 | 225.7 | 90 | 33 | 85 | 3 | 12 |
| OTA | 5.0 | 5.25 | 105 | 30 | 60 | 13 | 27 |
| $AFB_1$ | 5.0 | 3.89 | 78 | 32 | 62 | 19 | 19 |
| $AFG_1$ | 5.0 | 4.11 | 82 | 32 | 84 | 10 | 6 |
| $AFB_2$ | 2.0 | 1.68 | 84 | 31 | 78 | 16 | 6 |
| $AFG_2$ | 2.0 | 1.58 | 79 | 27 | 78 | 15 | 7 |

[1] Spiking level.
[2] Based on the spiked values, mycotoxin levels measured in blank maize were not considered.
[3] Out of 42 sets of data.

**Table 4. Contaminated maize test material: results of mycotoxin analysis and relevant scoring.**

| Analyte | Assigned value[1] (µg/kg) | No. of results[2] | % satisfactory z-scores (\|z\| ≤2) | % questionable z-scores (2< \|z\| ≤3) | % unsatisfactory z-scores (\|z\| ≥3) |
|---|---|---|---|---|---|
| DON | 567.2 | 38 | 55 | 19 | 26 |
| $FB_1$ | 2,085.0 | 32 | 50 | 19 | 31 |
| $FB_2$ | 726.0 | 31 | 52 | 16 | 32 |
| ZEA | 273.0 | 37 | 68 | 5 | 27 |
| T-2 | na | - | - | - | - |
| HT-2 | 184.7 | 34 | 82 | 6 | 12 |
| T-2+HT-2 | 184.7 | 34 | 82 | 6 | 12 |
| OTA | 6.7 | 31 | 58 | 16 | 26 |
| $AFB_1$ | 4.8 | 34 | 56 | 12 | 32 |
| $AFG_1$ | 8.7 | 34 | 73 | 9 | 18 |
| $AFB_2$ | 0.5 | 25 | 40 | 28 | 32 |
| $AFG_2$ | 1.0 | 25 | 64 | 8 | 28 |

[1] Median of reported results.
[2] Out of 43 sets of data.
[3] na = not applicable.

**Table 5. Contaminated maize material: statistical evaluation of results for each mycotoxin.**

| | | DON | $FB_1$ | $FB_2$ | ZEA | T-2 | HT-2 | T-2+HT-2 | OTA | $AFB_1$ | $AFG_1$ | $AFB_2$ | $AFG_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of results | | 38 | 32 | 31 | 37 | na | 34 | 34 | 31 | 34 | 34 | 25 | 25 |
| Range of results | from | 44.4 | 160.0 | 56.7 | 3.7 | na | 11.4 | 11.7 | 0.4 | 0.4 | 0.5 | nd | nd |
| (µg/kg) | to | 1,512.5 | 6,794.8 | 2,814.2 | 720.0 | na | 724.6 | 728.2 | 13.2 | 11.8 | 18.2 | 1.7 | 3.3 |
| Assigned value (median) (µg/kg) | | 567.2 | 2,085.0 | 726.0 | 273.0 | na | 184.7 | 184.7 | 6.7 | 4.8 | 8.7 | 0.5 | 1.0 |
| Mean (µg/kg) | | 562.6 | 2,258.0 | 942.6 | 269.9 | na | 184.6 | 193.7 | 7.1 | 4.9 | 8.8 | 0.5 | 1.1 |
| Target standard deviation (µg/kg) | | 98.8 | 298.6 | 121.9 | 53.1 | na | 38.1 | 38.1 | 1.5 | 1.1 | 1.9 | 0.1 | 0.2 |
| Overall standard deviation (µg/kg) | | 266.9 | 970.1 | 447.3 | 119.4 | na | 46.5 | 53.0 | 3.6 | 2.9 | 3.9 | 0.4 | 0.7 |
| Relative target standard deviation (%) | | 17.4 | 14.3 | 16.8 | 19.4 | na | 20.6 | 20.6 | 21.9 | 22.0 | 21.9 | 21.7 | 22.0 |
| Overall relative standard deviation (%) | | 47.0 | 46.5 | 61.6 | 43.7 | na | 25.2 | 28.7 | 53.6 | 59.6 | 45.3 | 84.1 | 66.6 |
| Lower limit of tolerance (z = -2) (µg/kg) | | 369.6 | 1,487.8 | 482.3 | 166.8 | na | 108.5 | 108.5 | 3.8 | 2.7 | 4.9 | 0.3 | 0.6 |
| Upper limit of tolerance (z = 2) (µg/kg) | | 764.9 | 2,682.2 | 969.7 | 379.2 | na | 260.9 | 261.0 | 9.6 | 6.9 | 12.5 | 0.7 | 1.4 |
| Number of results of \|z\| ≤2 (%) | | 20 (53) | 16 (50) | 16 (52) | 25 (68) | na | 28 (82) | 28 (82) | 18 (58) | 19 (56) | 25 (73) | 10 (40) | 16 (64) |
| Number of results of 2< \|z\| ≤3 (%) | | 7 (18) | 6 (19) | 5 (16) | 2 (5) | na | 2 (6) | 3 (9) | 5 (16) | 4 (12) | 3 (9) | 7 (28) | 2 (8) |
| Number of results of \|z\| >3 (%) | | 11 (29) | 10 (31) | 10 (32) | 10 (27) | na | 4 (12) | 3 (9) | 8 (26) | 11(32) | 6 (18) | 8 (32) | 7 (28) |
| Homogeneity study results: mean±standard deviation (µg/kg) | | 626±59 | 2,150±101 | 729±63 | 437±20 | nd | 189±3 | 189±3 | 7.1±0.2 | 5.9±0.3 | 11.6±0.4 | 0.5±0.02 | 0.7±0.03 |

Abbreviations used: na = not applicable; nd = not detected.

**Table 6. Spiked maize material: statistical evaluation of results for each mycotoxin.**

| | | DON | $FB_1$ | $FB_2$ | ZEA | T-2 | HT-2 | T-2+HT-2 | OTA | $AFB_1$ | $AFG_1$ | $AFB_2$ | $AFG_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of results | | 37 | 30 | 29 | 36 | 31 | 33 | 33 | 30 | 32 | 32 | 31 | 27 |
| Range of results | from | 8.4 | 38.8 | 7.0 | 0.9 | 0.02 | 13.6 | 13.6 | 0.3 | 0.4 | 0.3 | 0.1 | 0.04 |
| (µg/kg) | to | 4,800.0 | 2,000.4 | 965.5 | 290.0 | 168.8 | 319.5 | 429.0 | 11.0 | 9.7 | 6.9 | 2.7 | 3.3 |
| Assigned value (spiking level) (µg/kg) | | 1000.0 | 525.0 | 225.0 | 210.0 | 125.0 | 125.0 | 250.0 | 5.0 | 5.0 | 5.0 | 2.0 | 2.0 |
| Median (µg/kg) | | 915.5 | 820.0 | 313.0 | 179.2 | 109.5 | 136.2 | 250.9 | 5.1 | 3.9 | 4.3 | 1.7 | 1.6 |
| Mean (µg/kg) | | 869.5 | 834.5 | 366.1 | 155.0 | 96.4 | 136.7 | 225.7 | 5.2 | 3.9 | 4.1 | 1.7 | 1.6 |
| Target standard deviation (µg/kg) | | 160.0 | 92.5 | 45.1 | 42.5 | 27.3 | 27.3 | 49.3 | 1.1 | 1.1 | 1.1 | 0.4 | 0.4 |
| Overall standard deviation (µg/kg) | | 396.2 | 496.7 | 190.0 | 73.9 | 43.4 | 43.4 | 64.8 | 3.1 | 2.1 | 1.3 | 0.7 | 0.5 |
| Relative target standard deviation (%) | | 16.0 | 17.6 | 20.0 | 20.2 | 21.9 | 21.9 | 19.7 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| Overall relative standard deviation (%) | | 39.6 | 94.6 | 84.4 | 35.2 | 34.7 | 34.7 | 25.9 | 62.1 | 42.0 | 26.6 | 33.3 | 25.4 |
| Mean recovery (%) | | 87 | 159 | 163 | 74 | 77 | 109 | 90 | 105 | 78 | 82 | 84 | 79 |
| Lower limit of tolerance (z = -2) (µg/kg) | | 680.1 | 339.9 | 134.9 | 125.0 | 70.3 | 70.3 | 151.5 | 2.8 | 2.8 | 2.8 | 1.1 | 1.1 |
| Upper limit of tolerance (z = 2) (µg/kg) | | 1319.9 | 710.1 | 315.1 | 295.0 | 179.7 | 179.7 | 348.5 | 7.2 | 7.2 | 7.2 | 2.9 | 2.9 |
| Number of results of \|z\| ≤2 (%) | | 18 (49) | 9 (30) | 11 (38) | 23 (64) | 23 (74) | 26 (79) | 28 (85) | 18 (60) | 20 (62) | 27 (84) | 24 (78) | 21 (78) |
| Number of results of 2< \|z\| ≤3 (%) | | 6 (16) | 2 (7) | 3 (10) | 6 (17) | 2 (7) | 5 (15) | 1 (3) | 4 (13) | 6 (19) | 3 (9) | 5 (16) | 4 (15) |
| Number of results of \|z\| >3 (%) | | 13 (35) | 19 (63) | 15 (52) | 7 (19) | 6 (19) | 2 (6) | 4 (12) | 8 (27) | 6 (19) | 2 (7) | 2 (6) | 2 (7) |

Table 1 of the Supplementary Online Material, laboratory 41 used MeOH:$H_2O$ (80:20) as extraction solvent and no clean-up of crude extract that was directly analysed by ultra performance liquid chromatography-MS/MS (UPLC-MS/MS). The standard calibration curve was used (no matrix effect compensation) for all mycotoxins and a very small amount of matrix equivalent (0.083 mg in 0.5 µl of undiluted crude sample extract) was injected in the UPLC-MS/MS apparatus. Despite its simplicity in sample preparation this method seems to be very reliable if under control. It could be proposed for a possible inter-laboratory validation provided that the elements that need to be kept under control for reliable measurements can be identified and transferred to other laboratories. Further, the very small amount of matrix equivalent injected (0.083 mg) requires a very sensitive mass spectrometer apparatus to reach the limits of quantification required to allow the measurement of the 11 mycotoxins in maize at EU-limits. Indeed, laboratory 12 used a method similar to the method used by laboratory 41 but a UPLC column with a different selectivity, chromatographic conditions and mass spectrometer apparatus. This laboratory did not detect $AFB_2$ and $AFG_2$ in contaminated maize and $AFG_2$ in spiked maize (Tables 7 and 8). Moreover, this laboratory scored acceptable *z*-score for only 5/10 and 4/11 mycotoxins for contaminated maize and spiked maize, respectively (Tables 9 and 10).

Satisfactory mean recovery results (74 to 109%) were obtained for all mycotoxins with the exception of $FB_1$ and $FB_2$ which were unacceptably high (159-163%) (Table 3). Although the mean recovery for DON was acceptable (87%) a consistent group of laboratories reported low recovery values for this mycotoxin as shown in Figure 11 (*z*-score <-3 for 11/37 laboratories). The results of these 11 laboratories were also evident from the kernel density plot that showed a bimodal distribution of analytical results (Figure 11). In particular, these laboratories reported results of DON <530 µg/kg as compared to a spiking level of 1000 µg/kg (Table 8). The examination of the methods used by these laboratories showed that most of them (9 laboratories) used a standard calibration curve (no matrix effect compensation), 6 laboratories used the multi antibodies immunoaffinity column whereas 3 laboratories analysed the crude extract (Tables 8 and Table 1 of the Supplementary Online Material). The DON results of contaminated maize were more balanced, however the number of laboratories that scored values of *z*-score <-3 was higher (n=6) than the number of laboratories that scored values of *z*-score >3 (n=4) (Figure 1). Moreover, for contaminated maize, the 6 laboratories that scored values of z-score <-3 belong to the group of 11 laboratories that scored values of *z*-score <-3 for spiked maize (Figures 1 and 11).

A more complex set of results was obtained for fumonisins in spiked maize since the majority of laboratories (16/30) scored values of *z*-score >3 (Figures 12 and 13). Historically laboratories have often had problems with low recoveries of fumonisins whereas in this case a consistent overestimation was observed for the majority of laboratories. In particular, unacceptable high mean recoveries were obtained for spiked maize (159% for $FB_1$ and 163% for $FB_2$). When looking at the methods used by the 16 laboratories with *z*-score >3 no common factor could be identified that may explain these results (Supplementary Online Material Table 1). The calibration solution and the spiking solution provided to participants should not be blamed because they were

**Table 7. Contaminated maize material: individual results for each mycotoxin reported by participating laboratories.**

| Lab code | DON (µg/kg) | $FB_1$ (µg/kg) | $FB_2$ (µg/kg) | ZEA (µg/kg) | T-2 (µg/kg) | HT-2 (µg/kg) | T-2+HT-2 (µg/kg) | OTA (µg/kg) | $AFB_1$ (µg/kg) | $AFG_1$ (µg/kg) | $AFB_2$ (µg/kg) | $AFG_2$ (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1,512.5 | 2,294.5 | 1,019.0 | 231.0 | nd | 183.5 | 183.5 | 8.8 | 4.0 | 7.6 | nd | nd |
| 2 | 730.3 | 6,794.8 | 2,814.2 | 379.0 | 1.4 | 217.5 | 218.9 | 13.2 | 9.5 | 13.3 | 0.7 | 3.3 |
| 2A | 728.4 | 1,291.0 | 695.7 | 313.7 | nd | 185.9 | 185.9 | 6.2 | 11.7 | 13.7 | na | 3.2 |
| 3 | na | na | na | na | na | na | na | na | 3.2 | 7.1 | 0.2 | 0.4 |
| 4 | 777.0 | na | na | 286.0 | 91.0 | 196.0 | 287.0 | na | na | na | na | na |
| 5 | 765.9 | 3,335.9 | 2,566.4 | 70.0 | 2.2 | 91.7 | 93.9 | 12.7 | 2.1 | 5.1 | nd | nd |
| 6 | 620.0 | 1,712.0 | 755.0 | 481.0 | 16.0 | 208.0 | 224.0 | 12.4 | nd | 7.9 | nd | nd |
| 8 | 144.2 | 2,926.9 | 1,930.6 | 327.1 | nd | 231.5 | 231.5 | 6.5 | 0.9 | 0.5 | 0.7 | nq |
| 11 | 462.0 | na | na | na | 59.8 | na | na | na | na | na | na | na |
| 12 | 482.0 | 2,500.0 | 1,310.0 | 313.0 | nd | 195.0 | 195.0 | 2.9 | 1.9 | 7.8 | nd | nd |
| 13 | 430.0 | na | na | 284.0 | 3.7 | 175.2 | 178.9 | na | na | na | na | na |
| 14 | 720.9 | 3,356.4 | 1,737.9 | 166.7 | nd | 219.2 | 219.2 | 13.2 | 5.1 | 14.9 | nd | nd |
| 18 | 153.0 | 1,724.0 | 726.0 | 68.0 | nd | 38.0 | 38.0 | 2.7 | 1.1 | 1.2 | 0.1 | 0.2 |
| 21 | 329.8 | 4,271.4 | nd | 261.2 | 5.0 | 112.8 | 117.8 | 10.4 | 6.0 | 11.0 | nd | nd |
| 22 | 44.4 | 160.0 | 56.7 | 21.0 | 0.3 | 11.4 | 11.7 | 0.4 | 0.4 | 0.6 | 0.0 | 0.0 |
| 24 | 558.2 | 1,003.9 | 436.2 | 61.5 | 3.4 | 196.5 | 199.9 | 5.3 | 2.1 | 5.0 | 0.7 | 1.4 |
| 25 | 850.7 | 1,354.8 | 531.3 | nr | 31.3 | 193.6 | 224.9 | nr | 9.6 | 12.3 | 0.7 | 1.2 |
| 26 | 480.3 | 884.0 | 392.4 | 415.2 | na | na | na | na | 4.8 | 10.2 | 0.5 | 0.8 |
| 27 | 873.8 | 3,979.1 | 1,904.6 | 375.9 | 2.9 | 204.9 | 207.8 | 11.7 | 6.8 | 10.8 | 1.0 | 1.2 |
| 28 | 540.4 | 1,321.3 | 565.9 | 111.5 | 106.9 | 42.8 | 149.7 | 8.0 | 2.7 | 10.2 | 0.1 | 1.1 |
| 29 | 613.5 | na | na | 364.4 | 6.6 | 167.3 | 173.9 | na | na | na | na | na |
| 30 | 784.2 | 1,623.2 | 724.6 | 360.1 | 3.6 | 724.6 | 728.2 | 8.0 | 5.5 | 11.2 | 1.0 | 1.4 |
| 32 | 692.0 | 1,838.0 | 907.0 | 273.0 | nd | 142.0 | 142.0 | 7.1 | 4.8 | 10.4 | 0.5 | 1.1 |
| 33 | na | 2,490.0 | 934.0 | na | na | na | na | na | na | na | na | na |
| 34 | 649.2 | 2,520.0 | 1,079.0 | 280.1 | nd | 216.0 | 216.0 | 8.6 | 6.0 | 8.9 | nd | nd |
| 35 | 232.0 | 2,615.0 | 697.0 | 265.0 | na | na | na | 8.6 | 9.4 | 18.2 | 1.7 | 1.8 |
| 36 | na | na | na | na | 2.5 | 155.3 | 157.8 | na | na | na | na | na |
| 38 | 529.0 | na | na | 272.0 | nd | 149.0 | 149.0 | 3.5 | 5.0 | >10 | 0.5 | 0.7 |
| 39 | 45.9 | 1,040.0 | 594.0 | 190.0 | nd | 154.0 | 154.0 | 6.7 | 1.2 | 6.3 | 0.2 | 0.4 |
| 40 | 680.8 | na | na | 351.3 | 3.0 | 198.8 | 201.8 | na | na | na | na | na |
| 41 | 545.1 | 2,130.0 | 903.0 | 265.9 | 2.6 | 161.5 | 164.1 | 5.8 | 5.4 | 8.9 | 0.5 | 0.7 |
| 43 | na | na | na | 248.3 | na | na | na | 5.7 | 4.8 | 8.5 | 0.4 | 0.8 |
| 44 | 476.0 | 2,900.0 | 240.0 | 3.7 | 1.1 | na | na | 1.6 | 5.1 | 8.9 | 0.4 | 1.0 |
| 45 | 463.6 | 1,241.6 | 637.5 | 47.2 | 3.0 | 196.6 | 199.6 | 2.6 | 1.8 | 3.9 | 0.2 | 0.6 |
| 47 | 638.2 | 1,970.3 | 832.2 | 372.0 | 3.6 | 180.0 | 183.6 | 7.6 | 6.3 | 12.1 | 0.8 | 1.1 |
| 48 | 331.2 | 3,017.2 | 1,520.9 | 232.2 | nq | 161.8 | 161.8 | 7.5 | 4.6 | 7.4 | 0.4 | 0.8 |
| 51 | 972.0 | 2,040.0 | 807.0 | 447.0 | 9.4 | 214.0 | 223.4 | na | na | na | na | na |
| 56 | 900.0 | 2,400.0 | 700.0 | 720.0 | nd | na | na | 13.0 | 11.8 | 7.9 | nd | nd |
| 57 | 46.2 | 1,683.8 | 93.4 | 200.0 | 0.7 | 191.8 | 192.5 | 5.6 | 3.8 | 7.3 | 0.4 | 0.8 |
| 58 | na | 2,134.5 | 696.5 | na | na | na | na | na | 9.2 | 15.4 | 0.6 | 1.1 |
| 60 | 687.5 | na | na | 238.0 | nd | 130.6 | 130.6 | 4.3 | 1.2 | 8.5 | nd | nd |
| 60A | 576.3 | na | na | 283.5 | 11.5 | 274.6 | 286.1 | 6.1 | 4.0 | 5.8 | nd | nd |
| 61 | 315.2 | 1,703.3 | 412.9 | 405.9 | nd | 155.2 | 155.2 | 5.0 | 6.9 | 9.9 | 0.3 | 1.8 |

Abbreviations used: na = not analysed; nd = not detected (below limit of detection); nr = not reported because of problems with internal standard of ZEA and OTA; nq = not quantifiable (below limit of quantification).

**Table 8. Spiked maize material: individual results for each mycotoxin reported by participating laboratories.**

| Lab code | DON (µg/kg) | $FB_1$ (µg/kg) | $FB_2$ (µg/kg) | ZEA (µg/kg) | T-2 (µg/kg) | HT-2 (µg/kg) | T-2+HT-2 (µg/kg) | OTA (µg/kg) | $AFB_1$ (µg/kg) | $AFG_1$ (µg/kg) | $AFB_2$ (µg/kg) | $AFG_2$ (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1,801.0 | 1,343.0 | 683.7 | 180.4 | 148.2 | 111.3 | 259.5 | 6.3 | 3.5 | 3.2 | 1.1 | 1.5 |
| 2 | 1,113.2 | 2,000.4 | 965.5 | 227.9 | 134.4 | 136.2 | 270.6 | 9.6 | 3.9 | 3.6 | 2.2 | 1.8 |
| 3 | na | na | na | na | na | na | na | na | 2.6 | 2.9 | 1.0 | 0.9 |
| 4 | 946.0 | na | na | 178.0 | 2.8 | 123.0 | 125.8 | na | na | na | na | na |
| 5 | 917.6 | 1,081.3 | 378.7 | 92.4 | 100.1 | 91.6 | 191.7 | 9.3 | 5.2 | 4.8 | 2.1 | 1.6 |
| 6 | 933.0 | 501.0 | 226.0 | 201.0 | 130.0 | 124.0 | 254.0 | 9.0 | nd | nd | 2.1 | nd |
| 8 | 220.3 | 1,064.4 | 744.4 | 214.2 | nd | 171.6 | 171.6 | 4.7 | nq | nq | nq | nq |
| 11 | 302.0 | na | na | na | 1.6 | na | na | na | na | na | na | na |
| 12 | 901.0 | 848.0 | 496.0 | 115.0 | 88.5 | 205.0 | 293.5 | 2.1 | 1.9 | 3.7 | 1.9 | nd |
| 13 | 658.0 | na | na | 192.2 | 124.6 | 124.4 | 249.0 | na | na | na | na | na |
| 14 | 1,028.6 | 1,381.7 | 778.1 | 93.6 | 52.6 | 188.9 | 241.5 | 11.0 | 2.8 | 6.4 | 1.4 | nd |
| 18 | 243.0 | 889.0 | 449.0 | 49.0 | 14.0 | 32.0 | 46.0 | 2.3 | 1.0 | 0.8 | 0.4 | 0.4 |
| 21 | 613.0 | 1476.8 | nd | 206.8 | 71.8 | 97.8 | 169.6 | 5.8 | 5.7 | 4.9 | 1.5 | nd |
| 22 | 50.0 | 38.8 | 13.4 | 0.9 | 0.0 | 13.6 | 13.6 | 0.3 | 0.4 | 0.3 | 0.1 | 0.0 |
| 24 | 658.0 | 370.9 | 254.7 | 46.6 | 128.7 | 149.7 | 278.4 | 4.4 | 1.3 | 3.1 | 1.0 | 1.0 |
| 25 | 1,426.5 | 505.9 | 242.5 | nr | 141.0 | 144.4 | 285.4 | nr | 6.9 | 4.8 | 2.3 | 1.5 |
| 26 | 390.3 | 365.9 | 201.9 | 231.8 | na | na | na | na | 4.1 | 4.1 | 1.3 | 1.1 |
| 27 | 1,064.2 | 1,539.4 | 775.3 | 240.4 | 144.4 | 138.2 | 282.6 | 7.2 | 5.3 | 5.3 | 2.6 | 2.1 |
| 28 | 1,107.9 | 441.2 | 255.6 | 102.1 | 101.8 | 187.2 | 289.0 | 7.0 | 4.0 | 6.9 | 2.4 | 3.3 |
| 29 | 1,079.1 | na | na | 221.8 | 131.8 | 127.9 | 259.7 | na | na | na | na | na |
| 30 | 1,049.6 | 694.0 | 319.5 | 212.0 | 109.5 | 319.5 | 429.0 | 5.3 | 4.9 | 4.3 | 1.7 | 1.8 |
| 32 | 1,207.0 | 552.0 | 313.0 | 214.0 | 138.0 | 127.0 | 265.0 | 5.6 | 5.3 | 5.3 | 2.3 | 2.2 |
| 33 | na | 939.0 | 410.0 | na | na | na | na | na | na | na | na | na |
| 34 | 1,094.0 | 1,058.0 | 543.0 | 190.6 | 129.7 | 122.9 | 252.6 | 5.4 | 5.1 | 4.8 | 2.0 | 2.4 |
| 35 | 269.0 | 730.0 | 216.0 | 187.0 | na | na | na | 8.6 | 3.8 | 3.8 | 2.1 | 1.9 |
| 36 | na | na | na | na | 131.2 | 146.3 | 277.5 | na | na | na | na | na |
| 38 | 609.0 | na | na | 135.0 | 98.0 | 79.0 | 177.0 | 2.0 | 3.0 | 4.4 | 1.6 | 1.3 |
| 39 | 8.4 | 816.0 | 294.0 | 103.0 | nq | 157.0 | 157.0 | 4.2 | 2.1 | 4.1 | 1.1 | 1.3 |
| 40 | 1,071.8 | na | na | 209.2 | 158.4 | 159.2 | 317.6 | na | na | na | na | na |
| 41 | 869.8 | 594.6 | 287.5 | 152.4 | 102.7 | 100.2 | 202.9 | 4.1 | 4.5 | 4.3 | 1.7 | 1.8 |
| 43 | na | na | na | 166.0 | na | na | na | 3.8 | 3.8 | 3.9 | 1.7 | 1.7 |
| 44 | 510.0 | 1,186.0 | 7.0 | 55.6 | 0.6 | nd | 0.6 | 1.5 | 4.3 | 4.2 | 1.9 | 1.6 |
| 45 | 915.5 | 749.5 | 318.7 | 35.6 | 168.8 | 143.5 | 312.3 | 3.9 | 1.9 | 2.3 | 0.9 | 1.4 |
| 47 | 1,086.8 | 884.0 | 391.4 | 226.3 | 124.9 | 126.0 | 250.9 | 5.2 | 4.8 | 5.1 | 2.2 | 1.7 |
| 48 | 289.2 | 582.4 | 359.2 | 121.8 | nq | 182.0 | 182.0 | 4.9 | 4.1 | 4.3 | 2.0 | 1.7 |
| 51 | 1,148.0 | na | na | 268.0 | 137.0 | 144.0 | 281.0 | na | na | na | na | na |
| 56 | 4,800.0 | 1,300.0 | 300.0 | 290.0 | 50.0 | nd | nd | 7.0 | 7.7 | 6.7 | 2.7 | 1.6 |
| 57 | 39.2 | 165.4 | 38.0 | 129.8 | nd | 171.6 | 171.6 | 3.3 | 2.6 | 2.6 | 1.5 | 1.2 |
| 58 | na | 824.0 | 288.0 | na | na | na | na | na | 9.7 | 6.2 | 1.5 | 1.8 |
| 60 | 548.9 | na | na | 62.5 | 39.4 | 57.8 | 97.2 | 2.8 | 0.8 | 2.3 | nd | nd |
| 60A | 1,061.9 | na | na | 47.8 | 84.4 | 130.0 | 214.4 | 2.4 | 1.5 | 3.5 | nd | nd |
| 61 | 142.0 | 1,14.9 | 67.0 | 180.9 | nd | 177.9 | 177.9 | 8.5 | 5.9 | 4.6 | 1.7 | 2.1 |

Abbreviations used: na = not analysed; nd = not detected (below limit of detection); nr = not reported because of problems with internal standard of ZEA and OTA; nq = not quantifiable (below limit of quantification).

**Table 9. Contaminated maize material: individual *z*-score results for each mycotoxin.**

| Lab code | DON | $FB_1$ | $FB_2$ | ZEA | T-2 | HT-2 | T-2+HT-2 | OTA | $AFB_1$ | $AFG_1$ | $AFB_2$ | $AFG_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.57 | 0.70 | 2.40 | -0.79 | nd | -0.03 | -0.03 | 1.43 | -0.76 | -0.58 | nd | nd |
| 2 | 1.65 | 15.77 | 17.13 | 2.00 | nc | 0.86 | 0.90 | 4.42 | 4.42 | 2.41 | 2.40 | 10.45 |
| 2A | 1.63 | -2.66 | -0.25 | 0.77 | nd | 0.03 | 0.03 | -0.34 | 6.50 | 2.62 | na | 10.00 |
| 3 | na | na | na | na | na | na | na | na | -1.52 | -0.84 | -2.60 | -2.73 |
| 4 | 2.12 | na | na | 0.24 | nc | 0.30 | 2.68 | na | na | na | na | na |
| 5 | 2.01 | 4.19 | 15.10 | -3.82 | nc | -2.44 | -2.38 | 4.08 | -2.56 | -1.88 | nd | nd |
| 6 | 0.53 | -1.25 | 0.24 | 3.92 | nc | 0.61 | 1.03 | 3.88 | nd | -0.42 | nd | nd |
| 8 | -4.28 | 2.82 | 9.88 | 1.02 | nd | 1.23 | 1.23 | -0.14 | -3.69 | -4.29 | 2.40 | nq |
| 11 | -1.07 | na | na | na | nc | na | na | na | na | na | na | na |
| 12 | -0.86 | 1.39 | 4.79 | 0.75 | nd | 0.27 | 0.27 | -2.59 | -2.75 | -0.47 | nd | nd |
| 13 | -1.39 | na | na | 0.21 | nc | -0.25 | -0.15 | na | na | na | na | na |
| 14 | 1.55 | 4.26 | 8.30 | -2.00 | nd | 0.91 | 0.90 | 4.42 | 0.27 | 3.25 | nd | nd |
| 18 | -4.19 | -1.21 | 0.00 | -3.86 | nd | -3.85 | -3.85 | -2.72 | -3.50 | -3.93 | -3.60 | -3.64 |
| 21 | -2.40 | 7.32 | nd | -0.22 | nc | -1.89 | -1.76 | 2.52 | 1.12 | 1.20 | nd | nd |
| 22 | -5.29 | -6.45 | -5.49 | -4.75 | nc | -4.55 | -4.54 | -4.29 | -4.16 | -4.24 | -4.20 | -4.41 |
| 24 | -0.09 | -3.62 | -2.38 | -3.98 | nc | 0.31 | 0.40 | -0.95 | -2.56 | -1.94 | 2.40 | 1.82 |
| 25 | 2.87 | -2.45 | -1.60 | nr | nc | 0.23 | 1.05 | nr | 4.52 | 1.88 | 2.40 | 0.91 |
| 26 | -0.88 | -4.02 | -2.74 | 2.68 | na | na | na | na | -0.01 | 0.79 | 0.40 | -0.91 |
| 27 | 3.10 | 6.34 | 9.67 | 1.94 | nc | 0.53 | 0.60 | 3.40 | 1.88 | 1.10 | 5.40 | 0.91 |
| 28 | -0.27 | -2.56 | -1.31 | -3.04 | nc | -3.72 | -0.92 | 0.88 | -1.99 | 0.79 | -3.60 | 0.45 |
| 29 | 0.47 | na | na | 1.72 | nc | -0.46 | -0.28 | na | na | na | na | na |
| 30 | 2.20 | -1.55 | -0.01 | 1.64 | nc | 14.17 | 14.26 | 0.88 | 0.65 | 1.31 | 5.40 | 1.82 |
| 32 | 1.26 | -0.83 | 1.49 | 0.00 | nd | -1.12 | -1.12 | 0.27 | -0.01 | 0.89 | 0.40 | 0.45 |
| 33 | na | 1.36 | 1.71 | na | na | na | na | na | na | na | na | na |
| 34 | 0.83 | 1.46 | 2.90 | 0.13 | nd | 0.82 | 0.82 | 1.29 | 1.12 | 0.10 | nd | nd |
| 35 | -3.39 | 1.77 | -0.24 | -0.15 | na | na | na | 1.29 | 4.33 | 4.97 | 12.40 | 3.64 |
| 36 | na | na | na | na | nc | -0.77 | -0.71 | na | na | na | na | na |
| 38 | -0.39 | na | na | -0.02 | nd | -0.94 | -0.94 | -2.18 | 0.18 | >0.68 | 0.40 | -1.36 |
| 39 | -5.28 | -3.50 | -1.08 | -1.56 | nd | -0.81 | -0.81 | 0.00 | -3.41 | -1.26 | -2.60 | -2.73 |
| 40 | 1.15 | na | na | 1.47 | nc | 0.37 | 0.45 | na | na | na | na | na |
| 41 | -0.22 | 0.15 | 1.45 | -0.13 | nc | -0.61 | -0.54 | -0.61 | 0.56 | 0.10 | 0.40 | -1.36 |
| 43 | na | na | na | -0.47 | na | na | na | -0.68 | -0.01 | -0.10 | -0.60 | -0.91 |
| 44 | -0.94 | 2.73 | -3.99 | -5.07 | nc | nd | nd | -3.47 | 0.27 | 0.10 | -0.60 | 0.00 |
| 45 | -1.05 | -2.82 | -0.73 | -4.25 | nc | 0.31 | 0.39 | -2.79 | -2.84 | -2.51 | -2.60 | -1.82 |
| 47 | 0.72 | -0.38 | 0.87 | 1.86 | nc | -0.12 | -0.03 | 0.61 | 1.41 | 1.78 | 3.40 | 0.45 |
| 48 | -2.39 | 3.12 | 6.52 | -0.77 | nq | -0.60 | -0.60 | 0.54 | -0.20 | -0.68 | -0.60 | -0.91 |
| 51 | 4.10 | -0.15 | 0.66 | 3.28 | nc | 0.77 | 1.01 | na | na | na | na | na |
| 56 | 3.37 | 1.05 | -0.21 | 8.42 | nc | na | na | 4.29 | 6.59 | -0.42 | -4.60 | -4.55 |
| 57 | -5.27 | -1.34 | -5.19 | -1.38 | nc | 0.19 | 0.20 | -0.75 | -0.95 | -0.73 | -0.60 | -0.91 |
| 58 | na | 0.17 | -0.24 | na | na | na | na | na | 4.14 | 3.51 | 1.40 | 0.45 |
| 60 | 1.22 | na | na | -0.66 | nd | -1.42 | -1.42 | -1.63 | -3.41 | -0.10 | nd | nd |
| 60A | 0.09 | na | na | 0.20 | nc | 2.36 | 2.66 | -0.41 | -0.76 | -1.52 | nd | nd |
| 61 | -2.55 | -1.28 | -2.57 | 2.50 | nd | -0.77 | -0.78 | -1.16 | 1.97 | 0.63 | -1.60 | 3.64 |

Abbreviations used: na = not analysed; nd = not detected (below limit of detection); nr = not reported because of problems with internal standard of ZEA and OTA; nq = not quantifiable (below limit of quantification); nc = not calculated.

**Table 10. Spiked maize material: individual *z*-score results for each mycotoxin.**

| Lab code | DON | $FB_1$ | $FB_2$ | ZEA | T-2 | HT-2 | T-2+HT-2 | OTA | $AFB_1$ | $AFG_1$ | $AFB_2$ | $AFG_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.01 | 8.84 | 10.18 | -0.70 | 0.85 | -0.50 | 0.19 | 1.18 | -1.36 | -1.64 | -2.05 | -1.14 |
| 2 | 0.71 | 15.95 | 16.44 | 0.42 | 0.34 | 0.41 | 0.42 | 4.18 | -1.00 | -1.27 | 0.45 | -0.45 |
| 3 | na | na | na | na | na | na | na | na | -2.18 | -1.91 | -2.27 | -2.50 |
| 4 | -0.34 | na | na | -0.75 | -4.47 | -0.07 | -2.52 | na | na | na | na | na |
| 5 | -0.52 | 6.01 | 3.41 | -2.77 | -0.91 | -1.22 | -1.18 | 3.91 | 0.18 | -0.18 | 0.23 | -0.91 |
| 6 | -0.42 | -0.26 | 0.02 | -0.21 | 0.18 | -0.04 | 0.08 | 3.64 | nd | nd | 0.23 | nd |
| 8 | -4.87 | 5.83 | 11.53 | 0.10 | nd | 1.70 | -1.59 | -0.27 | nq | nq | nq | nq |
| 11 | -4.36 | na | na | na | -4.51 | na | na | na | na | na | na | na |
| 12 | -0.62 | 3.49 | 6.02 | -2.24 | -1.34 | 2.93 | 0.88 | -2.64 | -2.82 | -1.18 | -0.23 | nd |
| 13 | -2.14 | na | na | -0.42 | -0.01 | -0.02 | -0.02 | na | na | na | na | na |
| 14 | 0.18 | 9.26 | 12.28 | -2.74 | -2.65 | 2.34 | -0.17 | 5.45 | -2.00 | 1.27 | -1.36 | nd |
| 18 | -4.73 | 3.93 | 4.97 | -3.79 | -4.06 | -3.40 | -4.14 | -2.45 | -3.64 | -3.82 | -3.64 | -3.64 |
| 21 | -2.42 | 10.29 | nd | -0.08 | -1.95 | -0.99 | -1.63 | 0.73 | 0.64 | -0.09 | -1.14 | nd |
| 22 | -5.94 | -5.25 | -4.70 | -4.92 | -4.57 | -4.07 | -4.80 | -4.27 | -4.18 | -4.27 | -4.32 | -4.45 |
| 24 | -2.14 | -1.67 | 0.66 | -3.85 | 0.14 | 0.90 | 0.58 | -0.55 | -3.36 | -1.73 | -2.27 | -2.27 |
| 25 | 2.67 | -0.21 | 0.39 | nr | 0.59 | 0.71 | 0.72 | nr | 1.73 | -0.18 | 0.68 | -1.14 |
| 26 | -3.81 | -1.72 | -0.51 | 0.51 | na | na | na | na | -0.82 | -0.82 | -1.59 | -2.05 |
| 27 | 0.40 | 10.96 | 12.22 | 0.72 | 0.71 | 0.48 | 0.66 | 2.00 | 0.27 | 0.27 | 1.36 | 0.23 |
| 28 | 0.67 | -0.91 | 0.68 | -2.54 | -0.85 | 2.28 | 0.79 | 1.82 | -0.91 | 1.73 | 0.91 | 2.95 |
| 29 | 0.49 | na | na | 0.28 | 0.25 | 0.11 | 0.20 | na | na | na | na | na |
| 30 | 0.31 | 1.83 | 2.10 | 0.05 | -0.57 | 7.11 | 3.63 | 0.27 | -0.09 | -0.64 | -0.68 | -0.45 |
| 32 | 1.29 | 0.29 | 1.95 | 0.09 | 0.48 | 0.07 | 0.30 | 0.55 | 0.27 | 0.27 | 0.68 | 0.45 |
| 33 | na | 4.47 | 4.11 | na | na | na | na | na | na | na | na | na |
| 34 | 0.59 | 5.76 | 7.06 | -0.46 | 0.17 | -0.08 | 0.05 | 0.36 | 0.09 | -0.18 | 0.00 | 0.91 |
| 35 | -4.57 | 2.22 | -0.20 | -0.54 | na | na | na | 3.27 | -1.09 | -1.09 | 0.23 | -0.23 |
| 36 | na | na | na | na | 0.23 | 0.78 | 0.56 | na | na | na | na | na |
| 38 | -2.44 | na | na | -1.77 | -0.99 | -1.68 | -1.48 | -2.73 | -1.82 | -0.55 | -0.91 | -1.59 |
| 39 | -6.20 | 3.14 | 1.53 | -2.52 | nq | 1.17 | -1.89 | -0.73 | -2.64 | -0.82 | -2.05 | -1.59 |
| 40 | 0.45 | na | na | -0.02 | 1.22 | 1.25 | 1.37 | na | na | na | na | na |
| 41 | -0.81 | 0.75 | 1.39 | -1.36 | -0.82 | -0.91 | -0.96 | -0.82 | -0.45 | -0.64 | -0.68 | -0.45 |
| 43 | na | na | na | -1.04 | na | na | na | -1.09 | -1.09 | -1.00 | -0.68 | -0.68 |
| 44 | -3.06 | 7.12 | -4.84 | -3.64 | -4.55 | na | na | -3.18 | -0.64 | -0.73 | -0.23 | -1.36 |
| 45 | -0.53 | 2.43 | 2.08 | -4.10 | 1.60 | 0.68 | 1.26 | -1.00 | -2.82 | -2.45 | -2.50 | -1.36 |
| 47 | 0.54 | 3.88 | 3.69 | 0.38 | 0.00 | 0.04 | 0.02 | 0.18 | -0.18 | 0.09 | 0.45 | -0.68 |
| 48 | -4.44 | 0.62 | 2.98 | -2.08 | nq | 2.08 | -1.38 | -0.09 | -0.82 | -0.64 | 0.00 | -0.68 |
| 51 | 0.93 | na | na | 1.37 | 0.44 | 0.69 | 0.63 | na | na | na | na | na |
| 56 | 23.75 | 8.38 | 1.66 | 1.88 | -2.74 | nd | nd | 1.82 | 2.45 | 1.55 | 1.59 | -0.91 |
| 57 | -6.01 | -3.89 | -4.15 | -1.89 | nd | 1.70 | -1.59 | -1.55 | -2.18 | -2.18 | -1.14 | -1.82 |
| 58 | na | 3.23 | 1.40 | na | na | na | na | na | 4.27 | 1.09 | -1.14 | -0.45 |
| 60 | -2.82 | na | na | -3.47 | -3.13 | -2.46 | -3.10 | -2.00 | -3.82 | -2.45 | nd | nd |
| 60A | 0.39 | na | na | -3.82 | -1.49 | 0.18 | -0.72 | -2.36 | -3.18 | -1.36 | nd | nd |
| 61 | -5.36 | -4.43 | -3.51 | -0.68 | nd | 1.93 | -1.46 | 3.18 | 0.82 | -0.36 | -0.68 | 0.23 |

Abbreviations used: na = not analysed; nd = not detected (below limit of detection); nr = not reported because of problems with internal standard of ZEA and OTA; nq = not quantifiable (below limit of quantification).

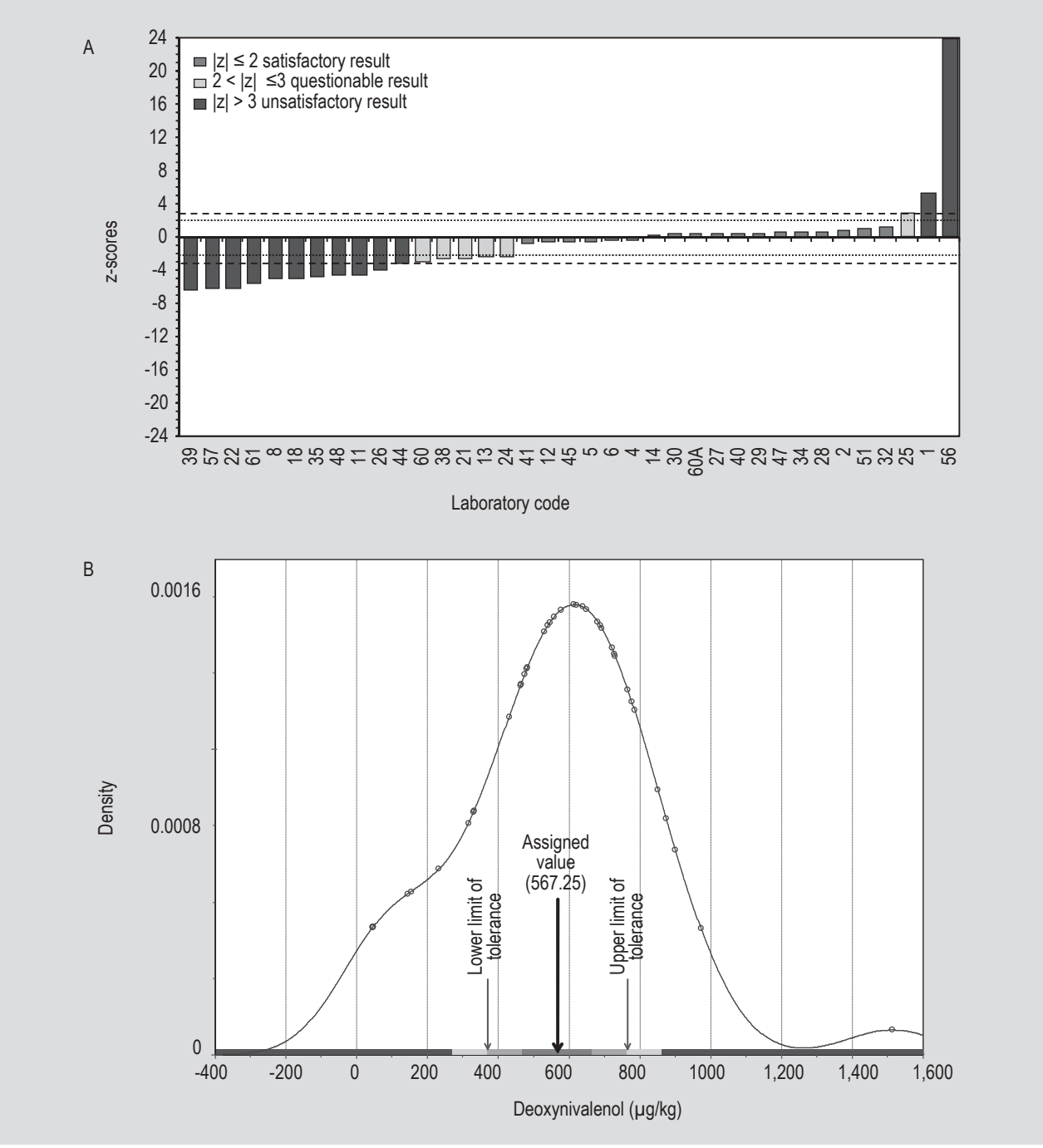


**Figure 1. Deoxynivalenol in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

prepared from the same stock solution. As observed for DON the fumonisin results of contaminated maize were more balanced, however the number of laboratories that scored values of *z*-score >3 was higher than the number of laboratories that scored values of *z*-score <-3 (Figures 2 and 3). Moreover, for contaminated maize, 5 of the 6 laboratories with *z*-score >3 belong to the group of the 16 laboratories that scored values of *z*-score >3 for spiked maize. A clear bimodal distribution of analytical results was also observed in the kernel density plot of ZEA in contaminated maize (Figure 4). In particular, 6 laboratories reported results of ZEA <115 µg/kg as compared to an assigned value of 273 µg/kg (Table 7). The examination of the methods used by these laboratories showed that all of them used a standard calibration curve (no matrix effect compensation), 2 laboratories analysed the crude extract, 2 laboratories purified the sample extract with a multi antibodies immunoaffinity column whereas 1 laboratory purified the sample extract with a liquid-liquid partitioning with *n*-hexane (Tables 7 and Table 1 of the Supplementary Online Material).

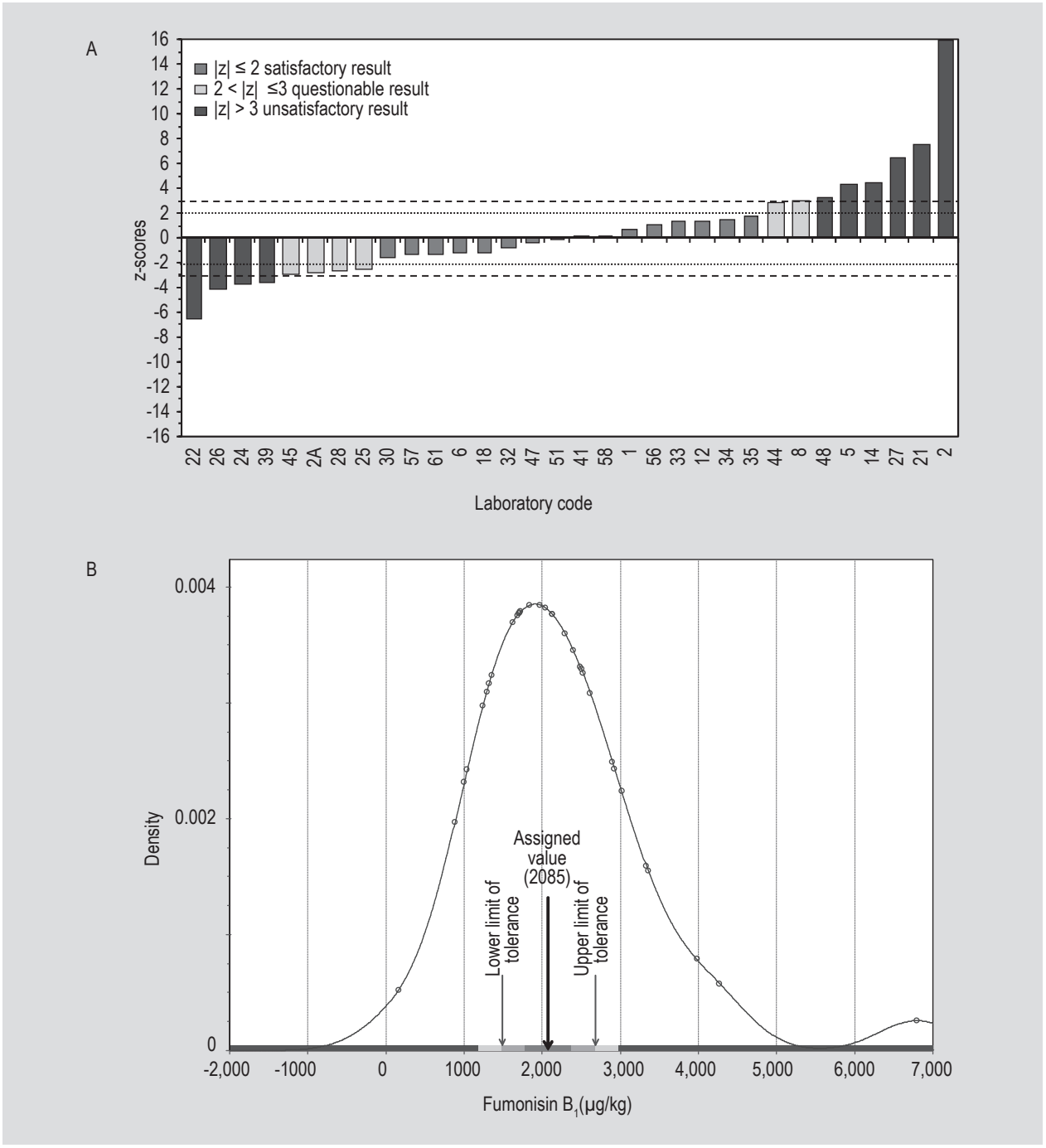


**Figure 2. Fumonisin $B_1$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

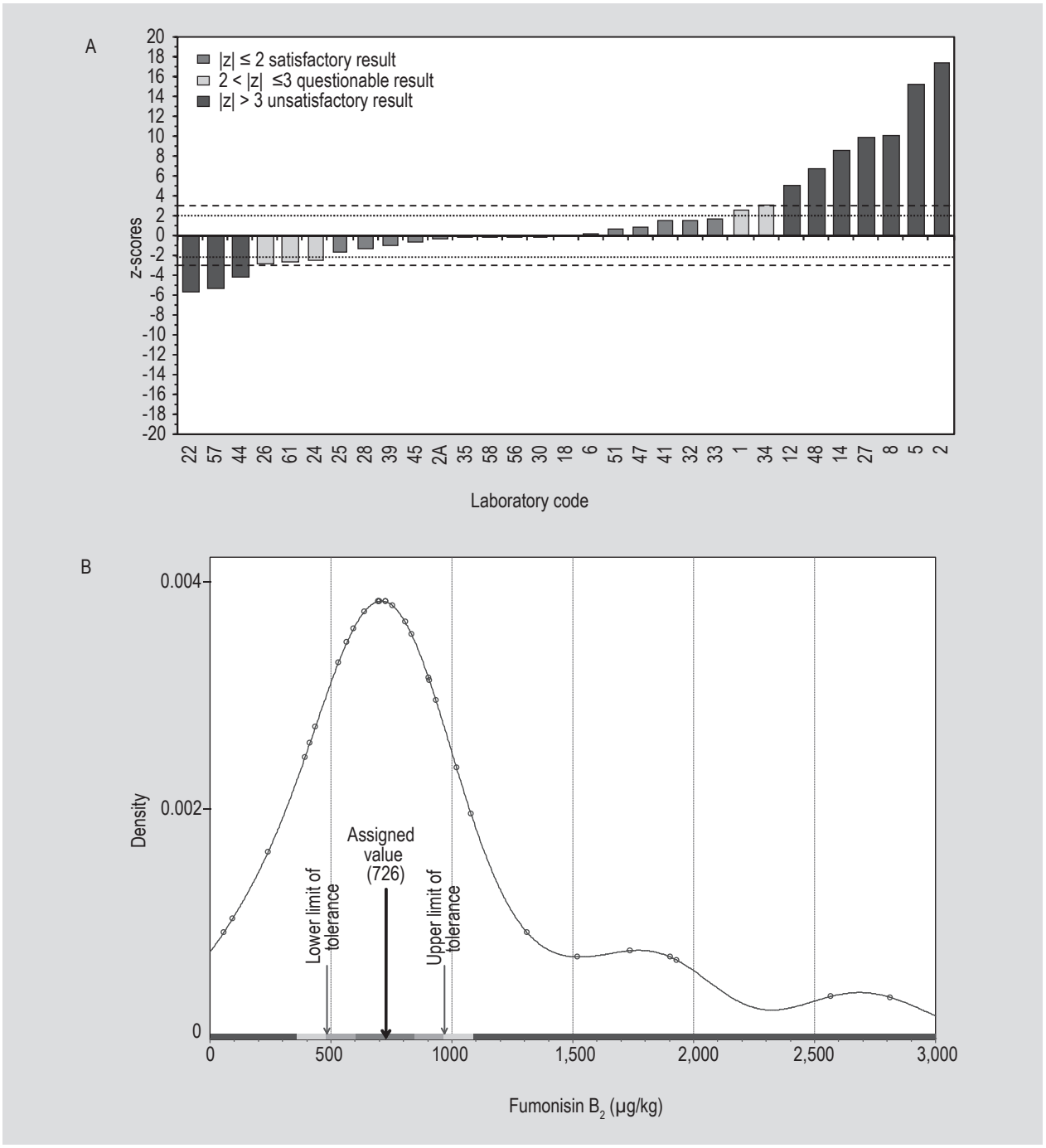


**Figure 3. Fumonisin $B_2$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

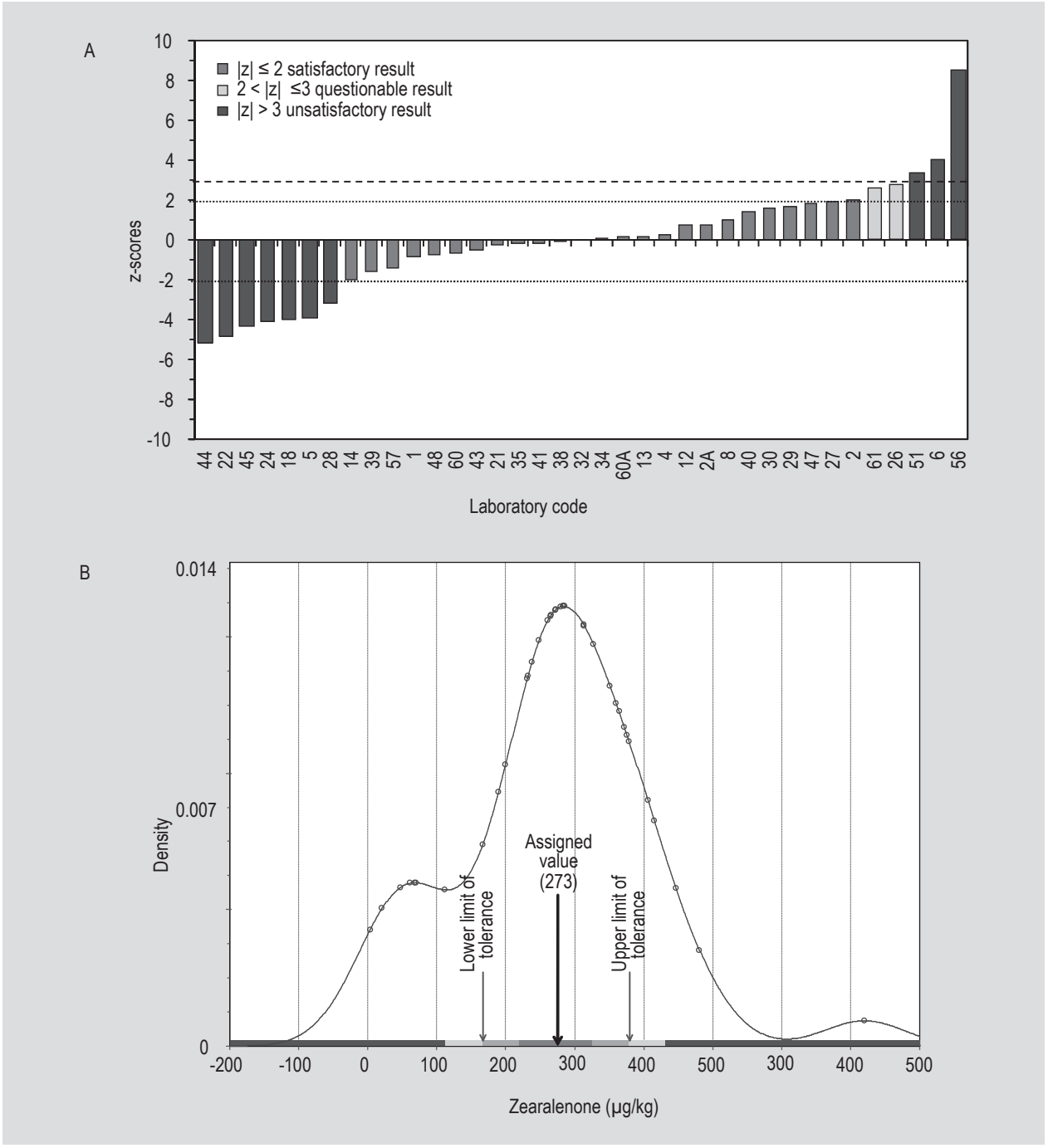


**Figure 4. Zearalenone in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

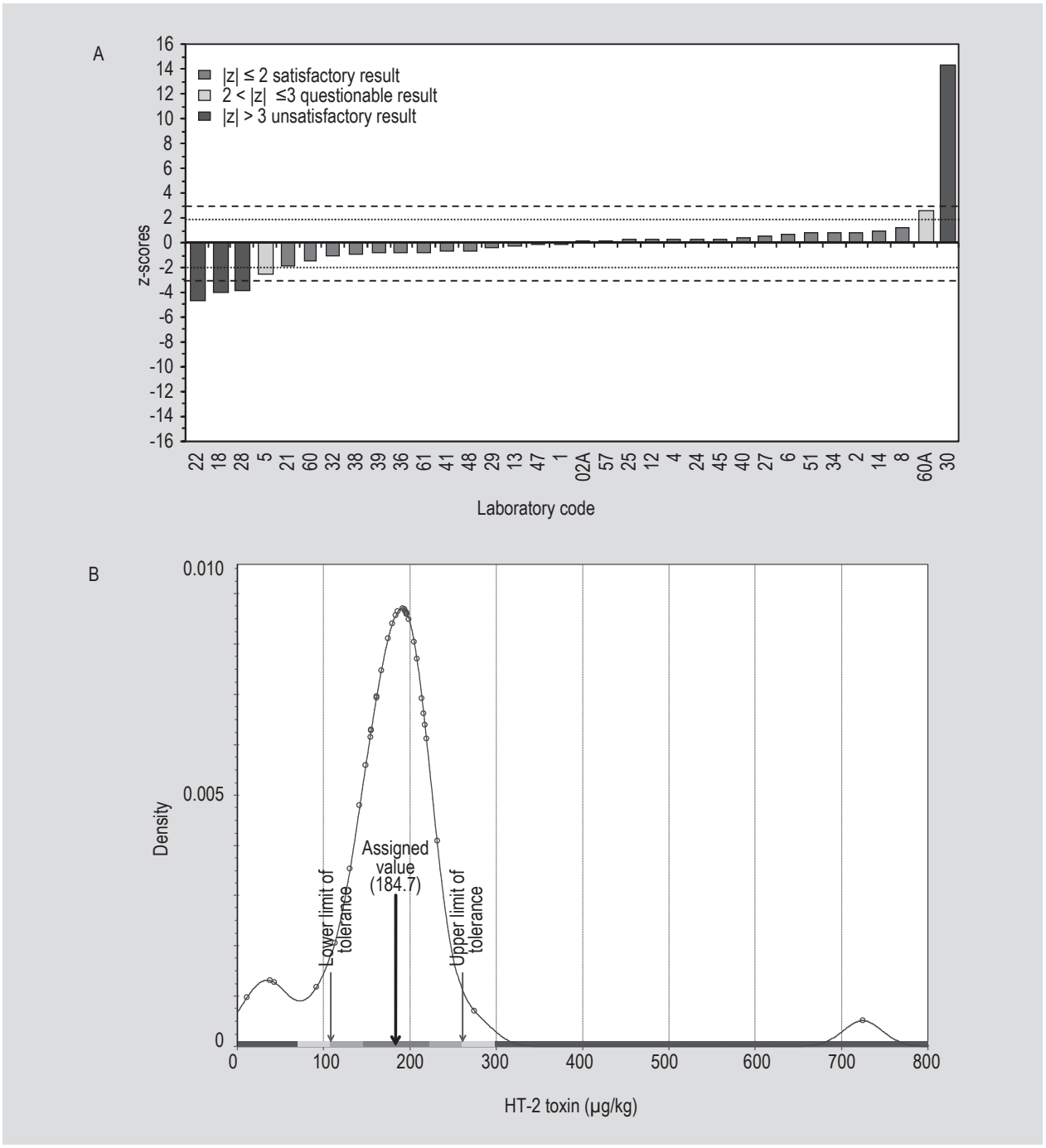


**Figure 5. HT-2 toxin in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

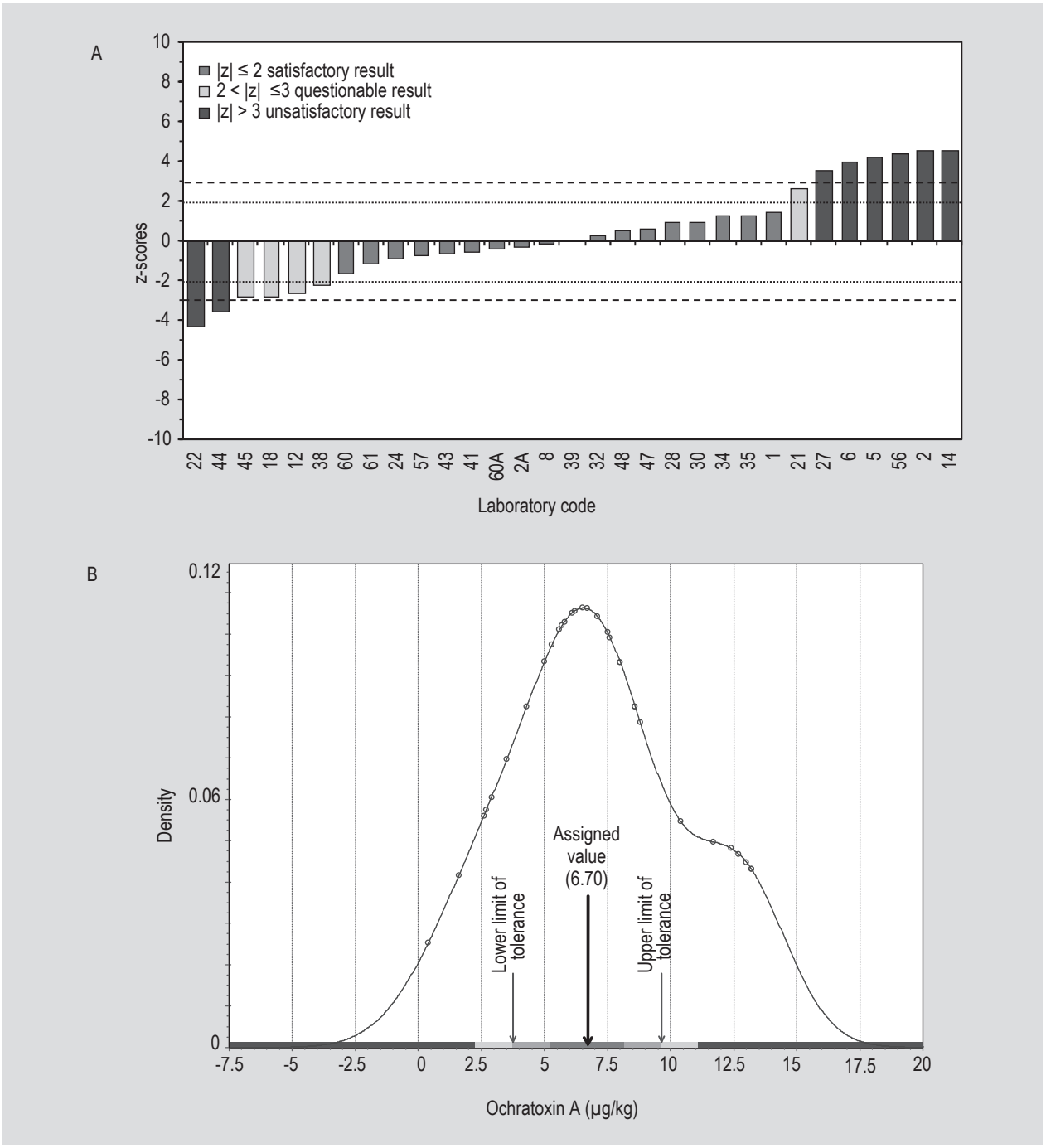


**Figure 6. Ochratoxin A in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

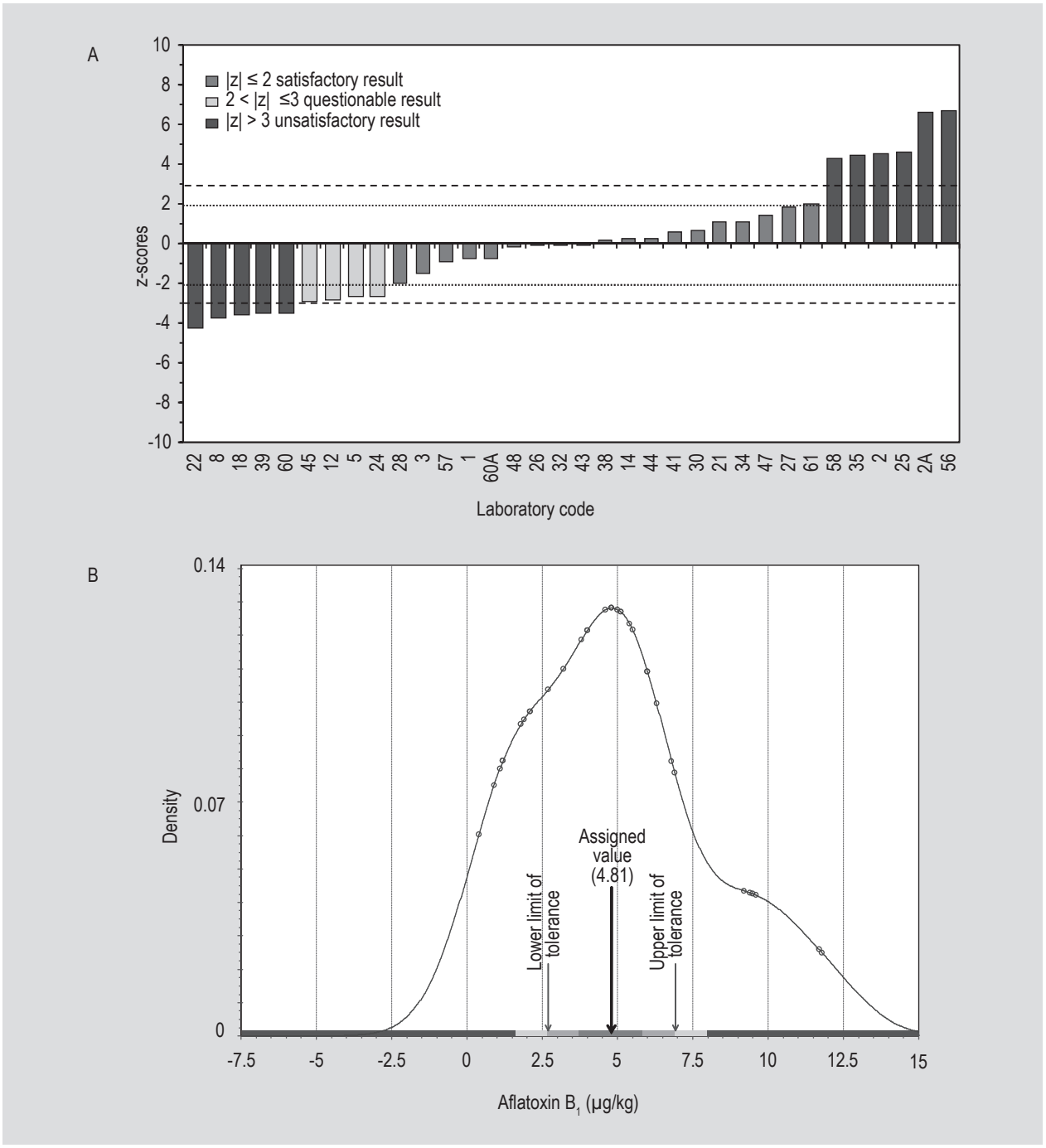


**Figure 7. Aflatoxin $B_1$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

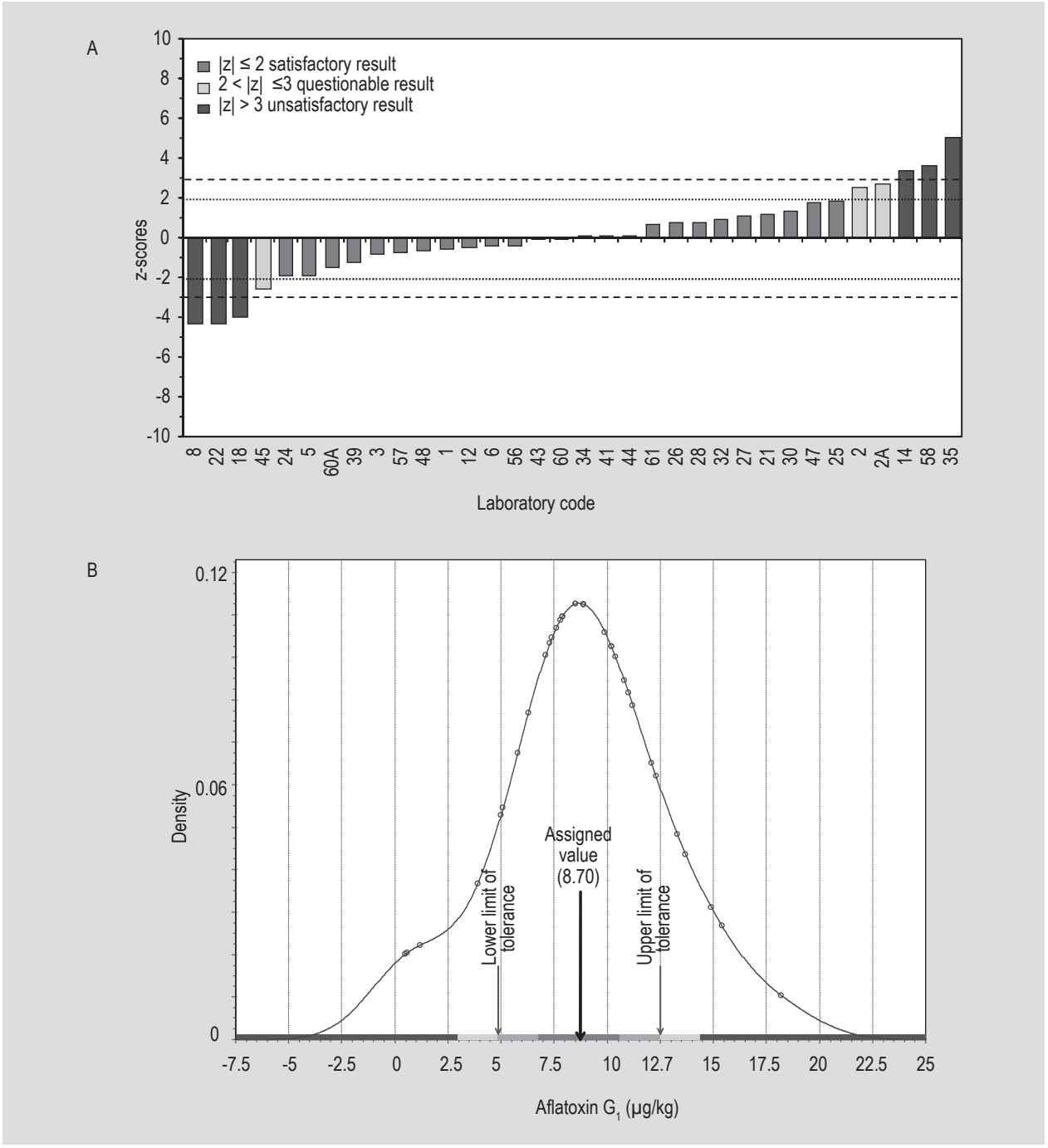


**Figure 8. Aflatoxin $G_1$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

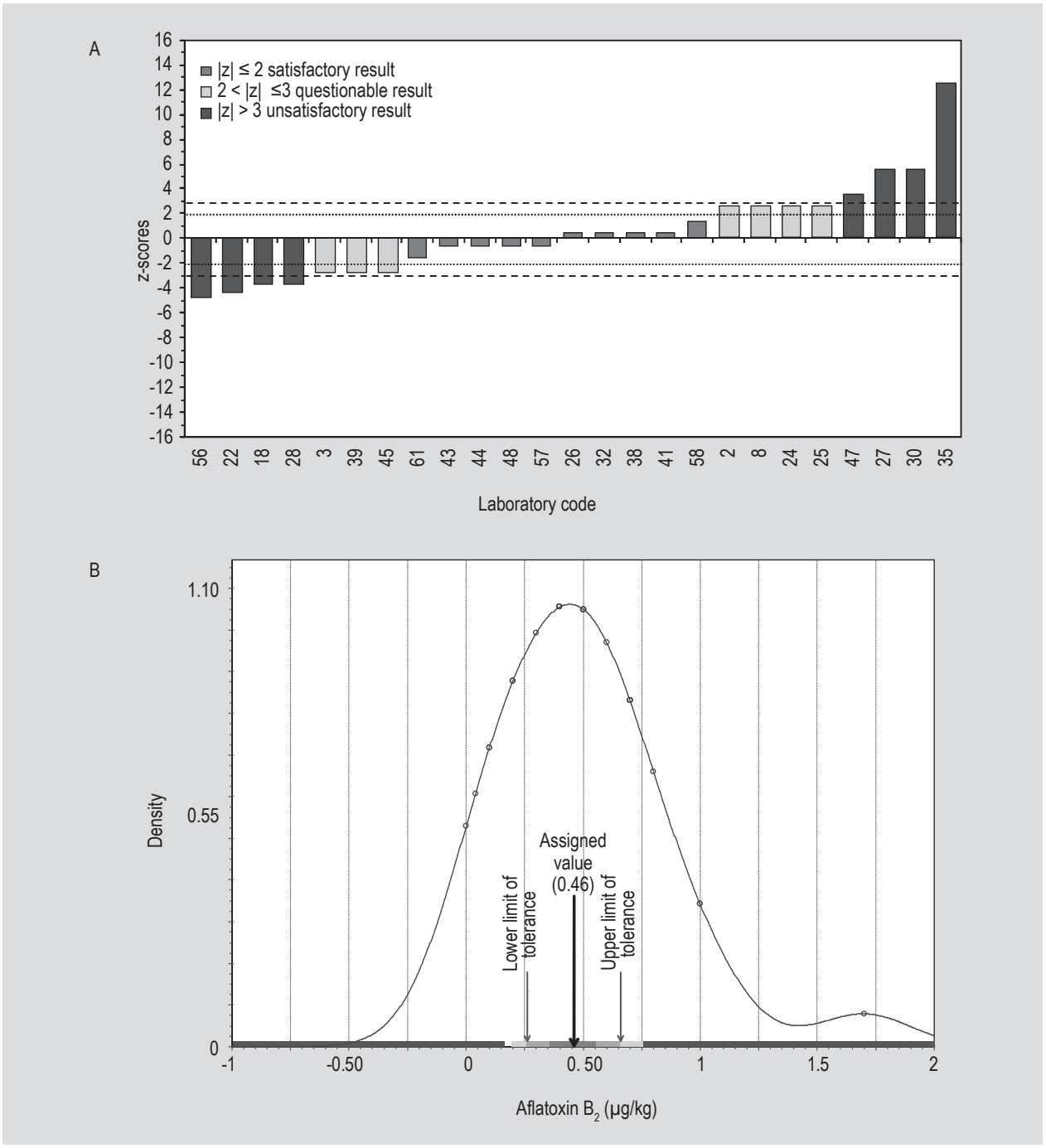


**Figure 9. Aflatoxin $B_2$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

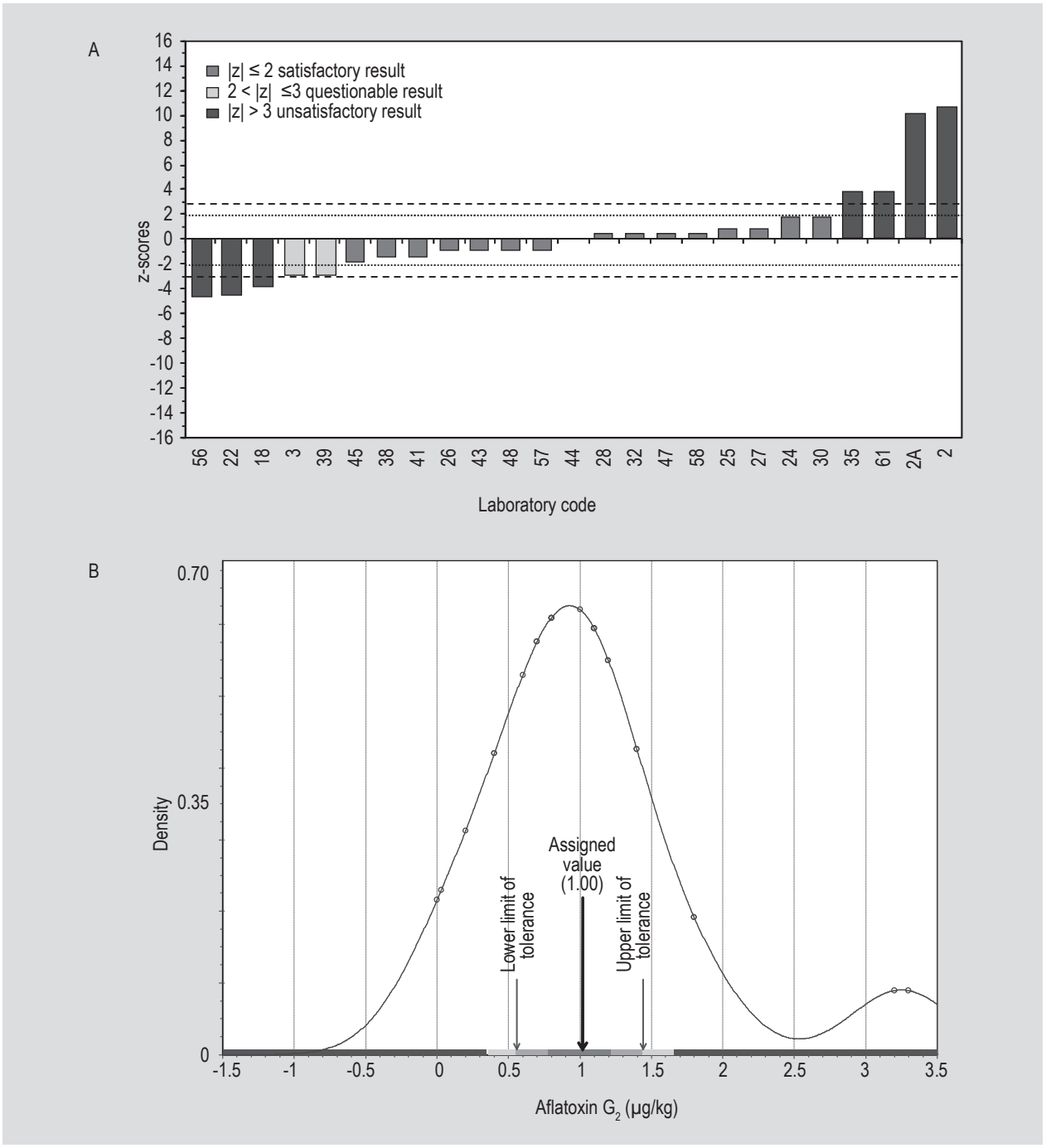


**Figure 10. Aflatoxin $G_2$ in contaminated maize: (A) individual *z*-score results and (B) kernel density plot.**

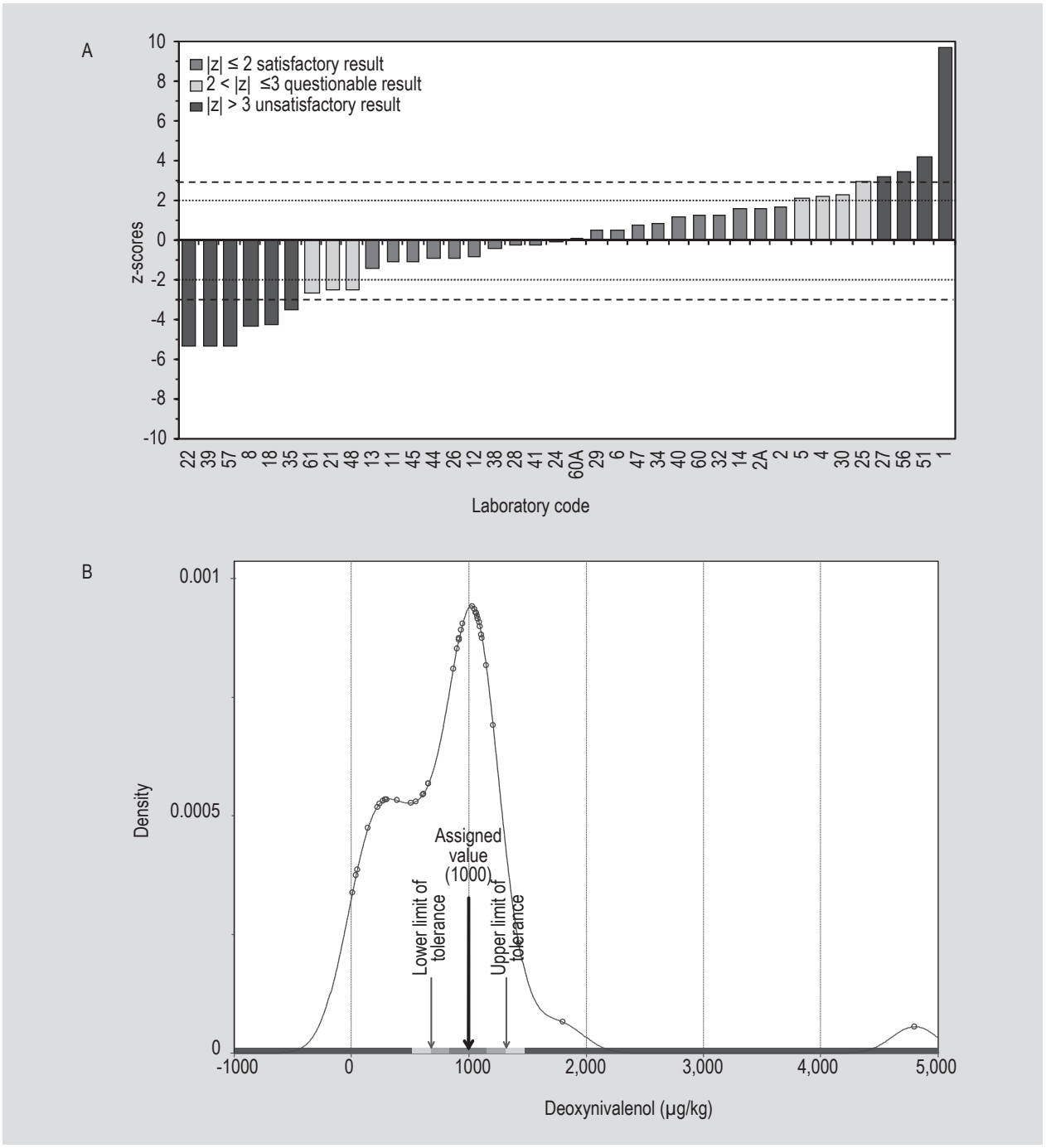


**Figure 11. Deoxynivalenol in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

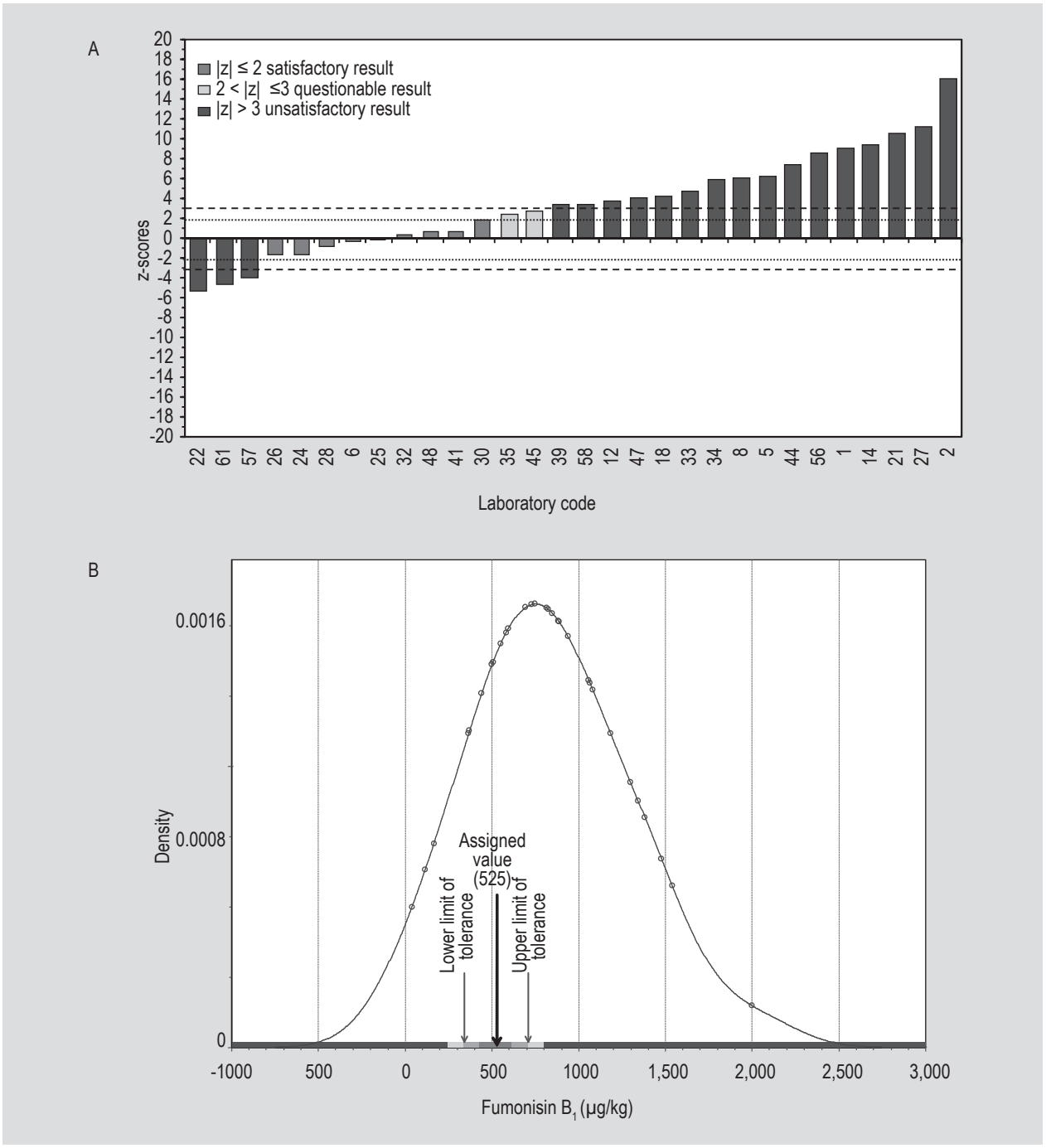


**Figure 12. Fumonisin $B_1$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

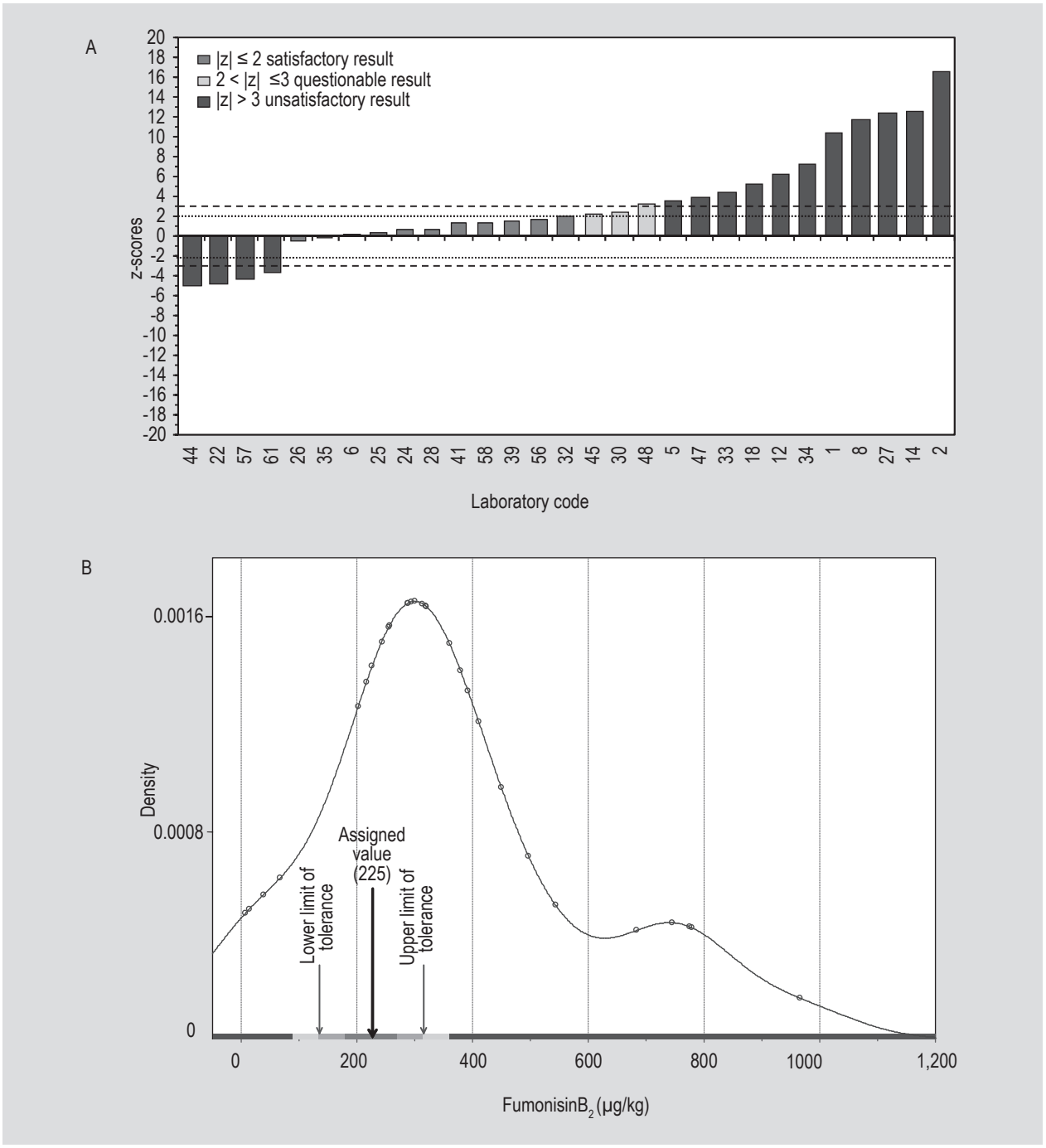


**Figure 13. Fumonisin $B_2$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

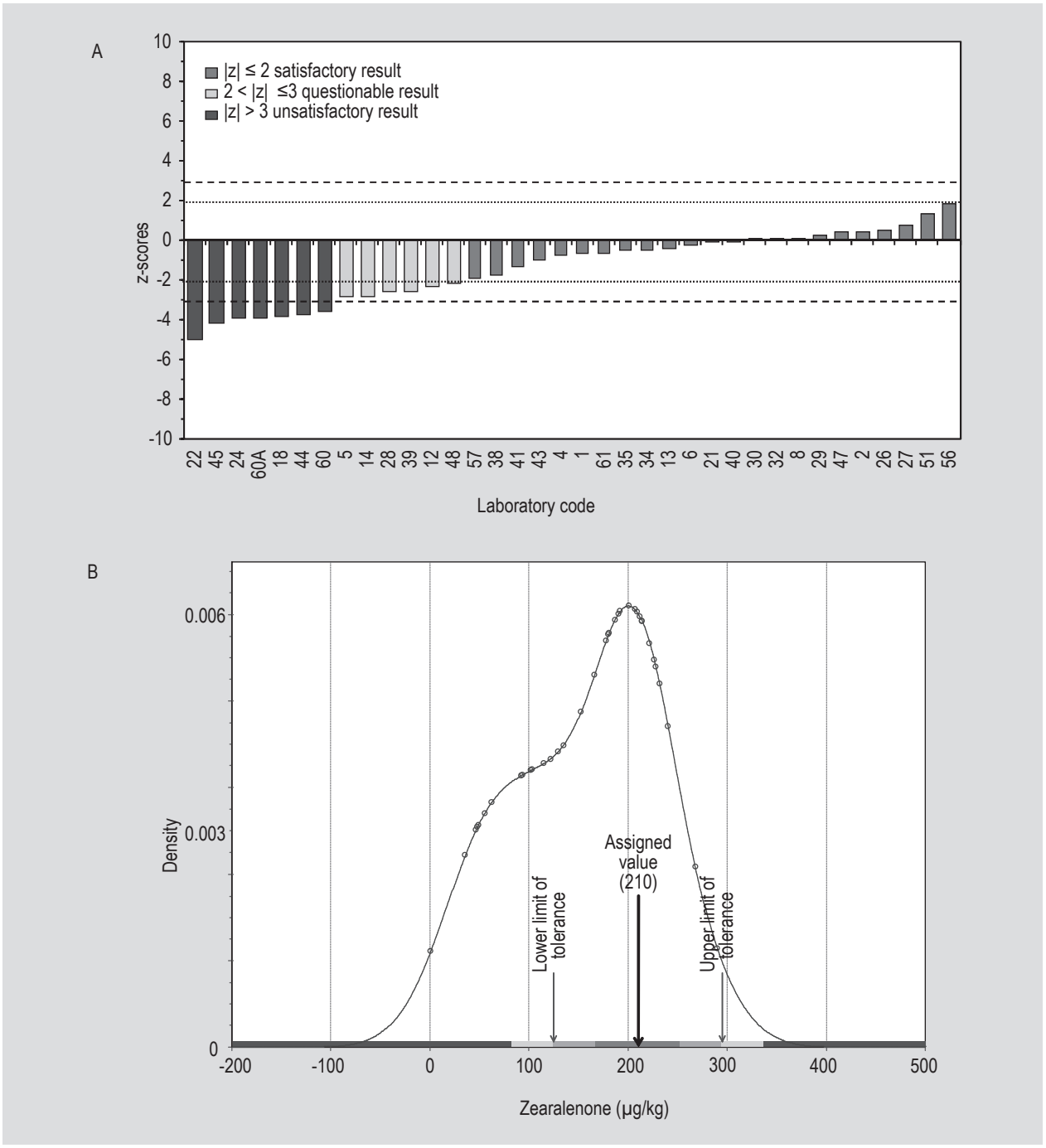


**Figure 14. Zearalenone in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

**4.**

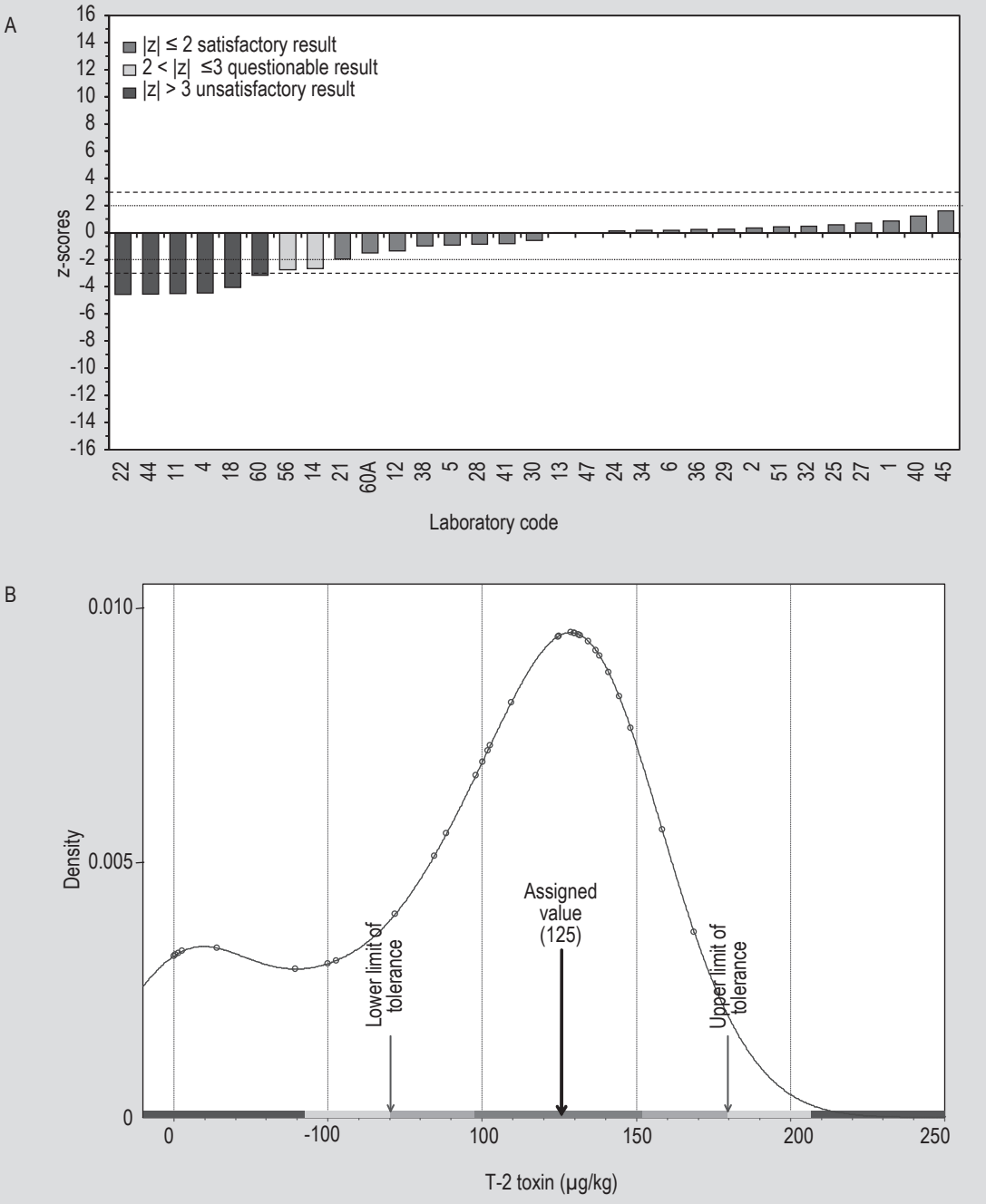


**Figure 15. T-2 toxin in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

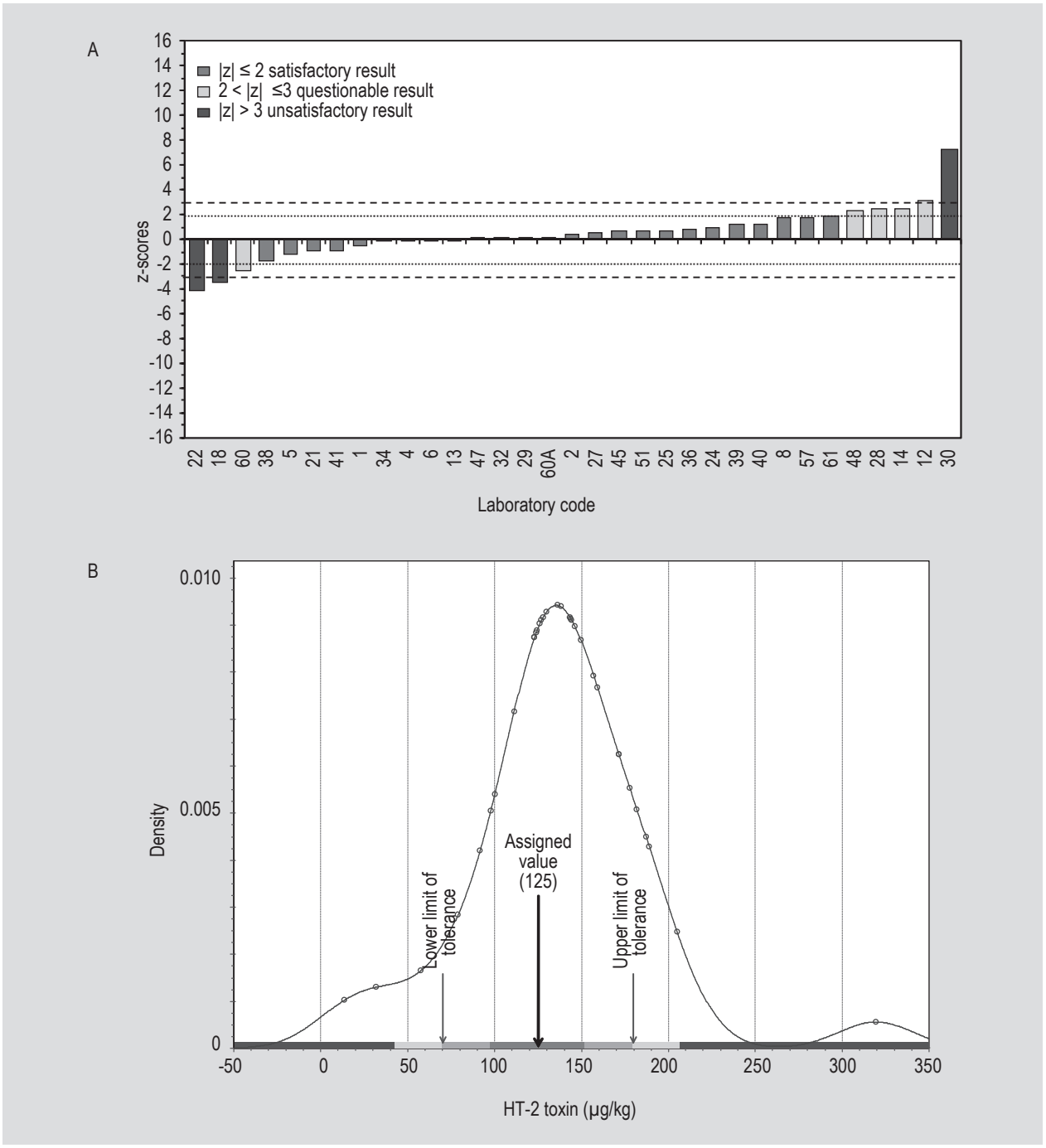


**Figure 16. HT-2 toxin in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

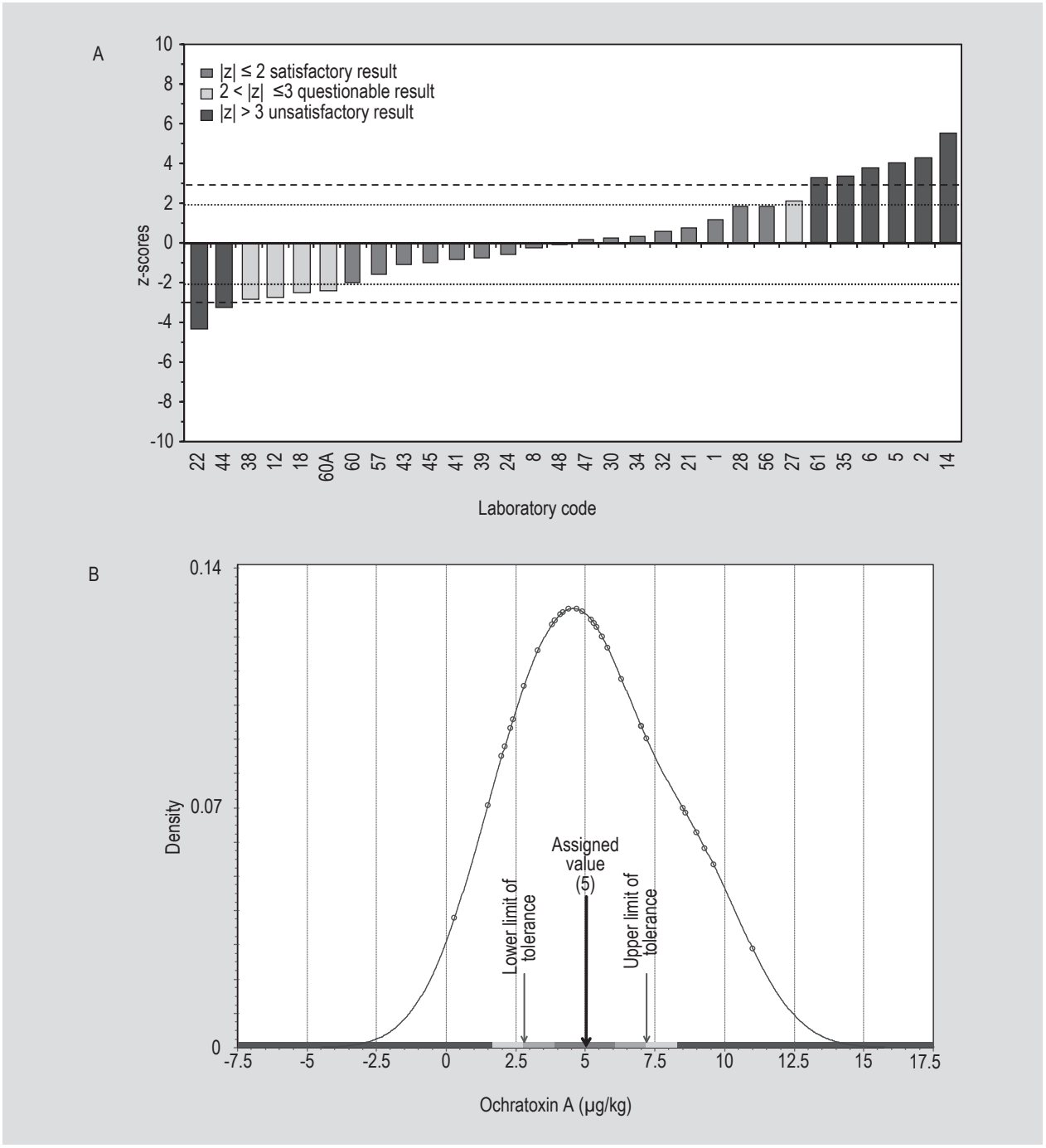


**Figure 17. Ochratoxin A in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

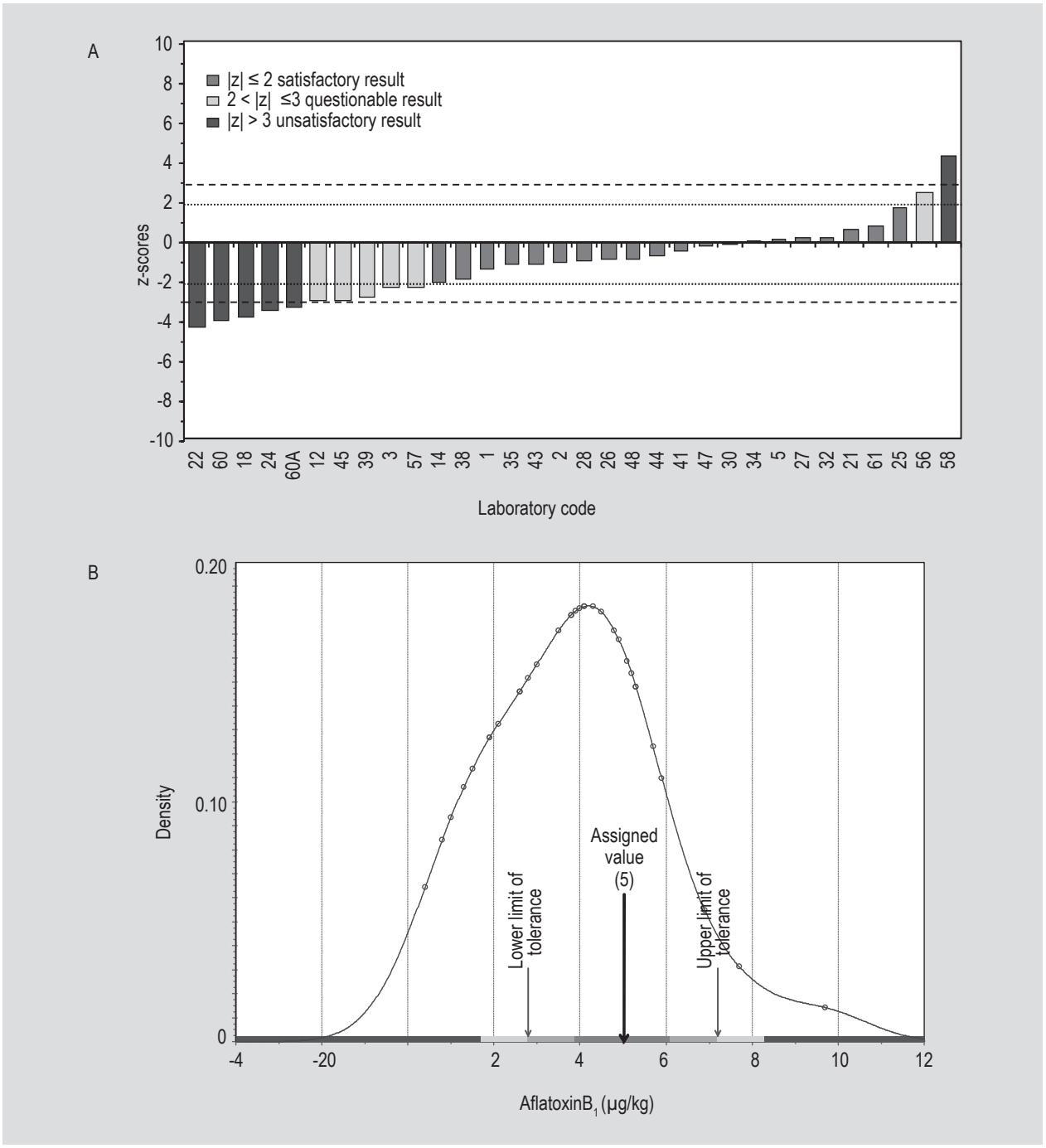


**Figure 18 Aflatoxin $B_1$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

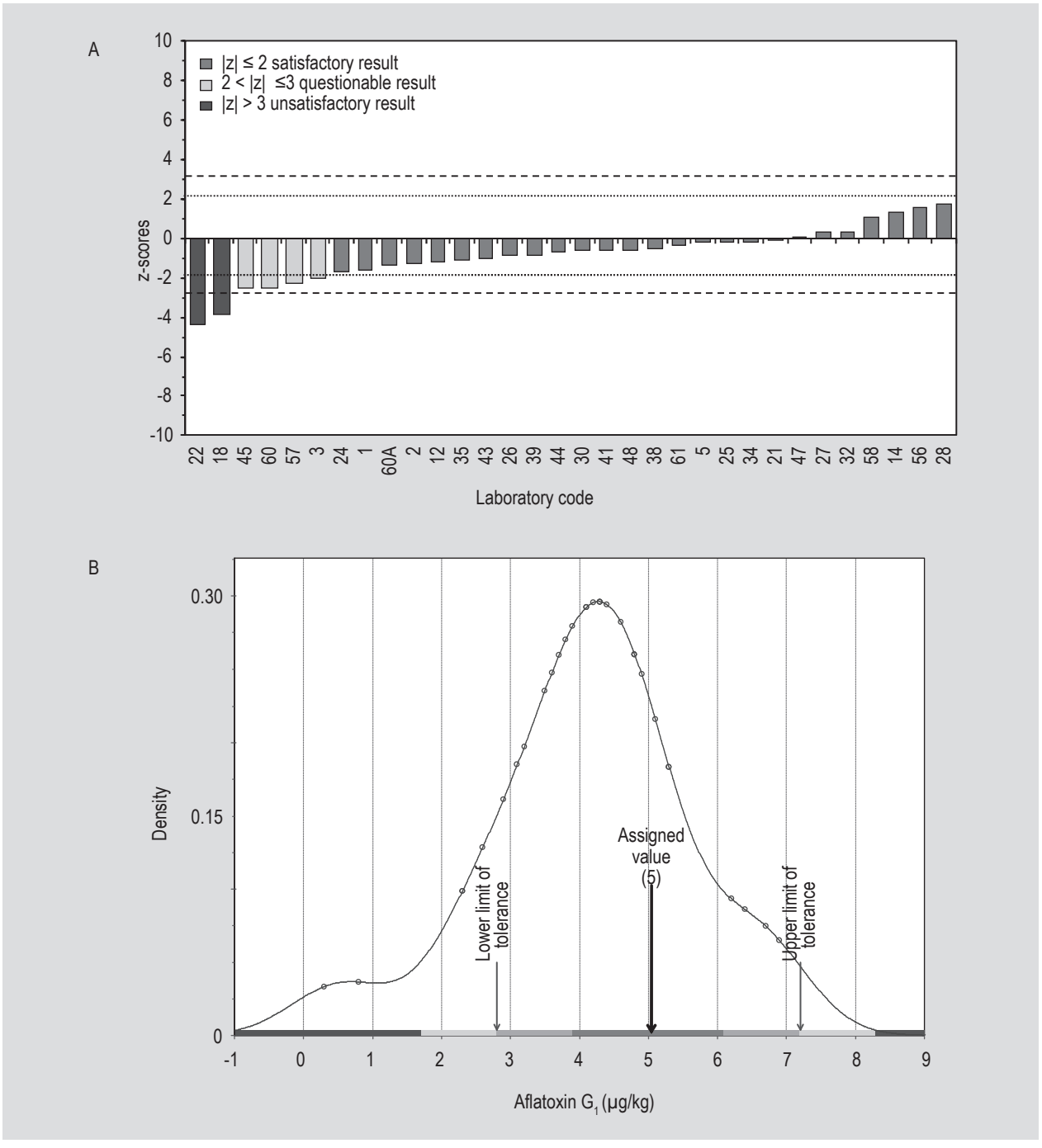


**Figure 19. Aflatoxin $G_1$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

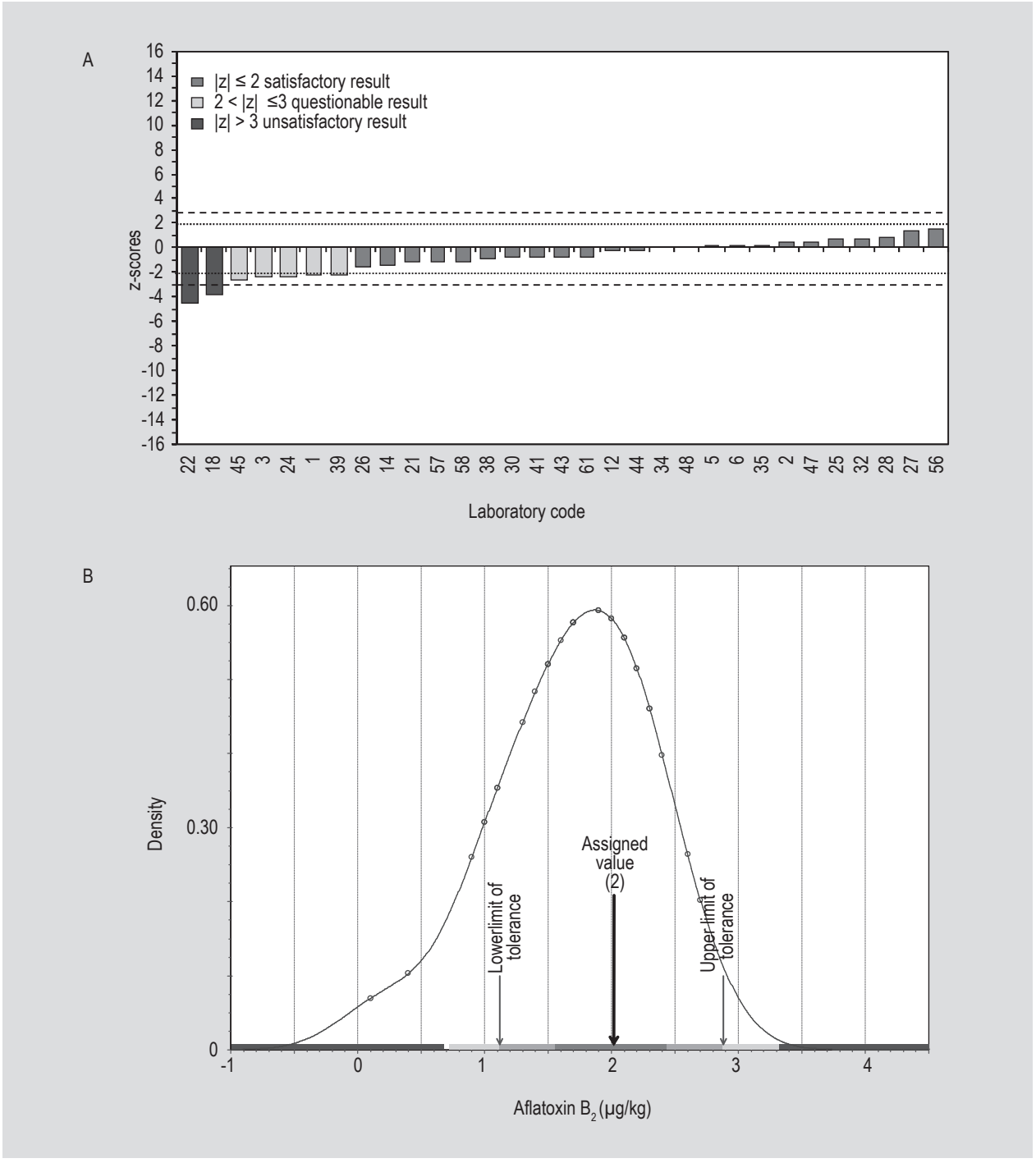


**Figure 20. Aflatoxin $B_2$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

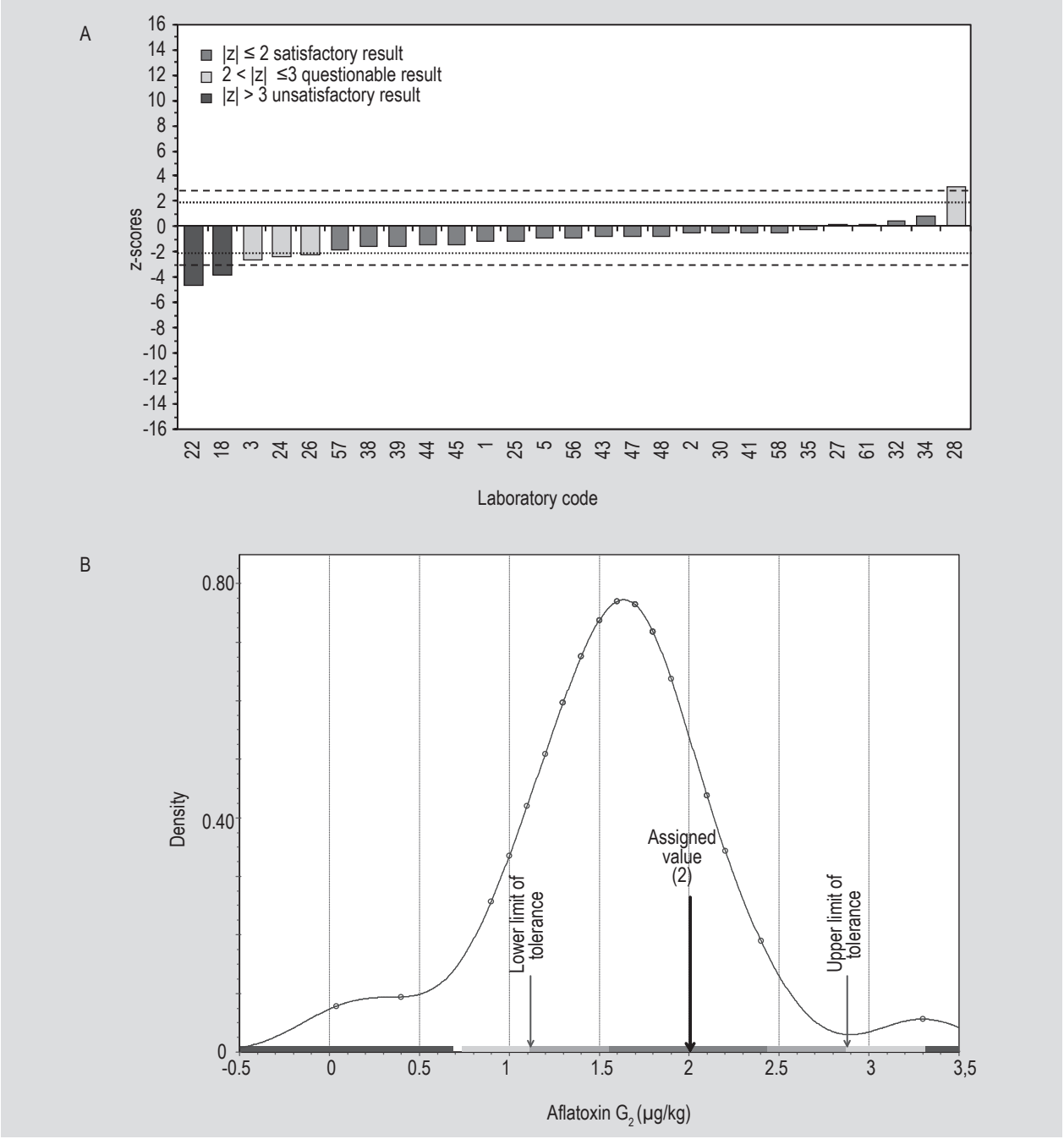


**Figure 21. Aflatoxin $G_2$ in spiked maize: (A) individual *z*-score results and (B) kernel density plot.**

## Conclusion

In conclusion, a robust and reliable method for simultaneous determination of 11 mycotoxins in maize could not be identified from the results of this proficiency test. A more detailed evaluation of methods and conditions used by participant laboratories in relation to the results obtained as well as further conclusions concerning the exclusion of certain methods used by some laboratories will be reported in a separate manuscript (A. De Girolamo, unpublished data). The results of this PT and the relevant method's details will be used to identify methodology strengths and weaknesses. Additional experimental work is necessary to set up a method suitable for inter-laboratory validation.

## Acknowledgements

We are grateful to the European Commission for financial support provided through the 'MoniQA' Network of Excellence (food-CT-2006-036337) to enable this proficiency test. We thank the laboratories listed in Table 11 for participation in this proficiency test, Zoltan Kunsagi

**Table 11. Participating laboratories.**

| Laboratory | Country |
|---|---|
| Landesuntersuchungsamt Rheinland-Pfalz, Institut für Lebensmittelchemie Trier | Germany |
| Barilla G.R. F.lli Spa | Italy |
| Canadian Grain Commission, Grain Research Laboratory | Canada |
| Chelab s.r.l. | Italy |
| Chemisches und Veterinäruntersuchungsamt Sigmaringen | Germany |
| Chemisches und Veterinäruntersuchungsamt Stuttgart | Germany |
| Central Institute for Supervising and Testing in Agriculture (UKZUZ) | Czech Republic |
| Centre d'Economie Rurale, C.E.R. Groupe | Belgium |
| CNTA - Centro Nacional de Tecnología y Seguridad Alimentaria | Spain |
| Coop Italia | Italy |
| Covance Laboratories Inc. | USA |
| EC - Joint Research Centre - IRMM | Belgium |
| EMSL Analytical, Inc. | USA |
| ERSA - Regione Autonoma Friuli - Venezia Giulia | Italy |
| Eurofins Central Analytical Laboratories | USA |
| Food & Environment Research Agency | UK |
| Ghent University | Belgium |
| Hacettepe University | Turkey |
| Health Canada, Bureau of Chemical Safety | Canada |
| Institute of Environmental Science and Research Limited (ESR) | New Zealand |
| INZO Analytical development | France |
| Italian National Institute of Health (ISS) | Italy |
| Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna 'Bruno Ubertini' | Italy |
| Istituto Zooprofilattico Sperimentale Umbria e Marche (IZSUM) | Italy |
| LGC Limited | UK |
| Midwest Laboratories | USA |
| National Institute of Public Health, Brno | Czech Republic |
| National Reference Laboratory (NRL) Pesticides in Foods | the Netherlands |
| National Research Council, Institute of Sciences of Food Production (CNR-ISPA) | Italy |
| NofaLab Laboratories | the Netherlands |
| RIKILT - Institute of Food Safety | the Netherlands |
| Spanish Food Safety and Nutrition Agency | Spain |
| Swiss Quality Testing Services (SQTS) | Switzerland |
| Staatlichen Veterinäruntersuchungsamtes Arnsberg | Germany |
| Texas A&M University, Office of the Texas State Chemist Veterinary Pathobiology | USA |
| TNO Quality of Life | the Netherlands |
| Università Cattolica del Sacro Cuore | Italy |
| University of Bari Aldo Moro | Italy |
| Università di Napoli Federico II | Italy |
| University of Natural Resources and Applied Life Sciences (IFA-Tulln) | Austria |
| Veterinary and Agrochemical Research Centre, CODA-CERVA | Belgium |

of the Institute for Reference Materials and Measurements for statistical evaluation of the results, the secretariat of CEN/TC 275/WG 5 for circulating the invitation letter within the members of WG 5, Matteo Luppi of Safe Food/Eurolab (Collecchio, Parma, Italy) and Stephen Powers of Vicam (Milford, MA, USA) for the help in distributing the parcels. The valuable technical assistance of Roberto Schena (CNR-ISPA) for the preparation of maize materials and the analyses for the homogeneity study is also acknowledged.

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MacDonald, S.J., Anderson, S., Brereton, P., Wood, R. and Damant, A., 2005a. Determination of zearalenone in barley, maize and wheat flour, polenta, and maize-based baby food by immunoaffinity column cleanup with liquid chromatography: interlaboratory study. Journal of AOAC International 88: 1733-1740.

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