Proficiency test for mycotoxins aflatoxins B1, B2, G1, G2 and ochratoxin A in maize flour and cocoa powder

EURLPT-M10 (2023)

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Distribution list:
- Participating laboratories
- Mr. F. Verstraete, European Commission, DG SANTE

Contents

Summary

A proficiency test (PT) for the quantitative determination of aflatoxins B1, B2, G1, G2 (AFB1, AFB2, AFG1, AFG2) and ochratoxin A (OTA) in maize flour and cocoa powder was organised by the European Union Reference Laboratory for Mycotoxins & Plant toxins in food and feed (EURL MP) between April and June 2023. This PT was carried out by Wageningen Food Safety Research (WFSR) under accreditation (R013, Dutch Accreditation Council RvA, ISO/IEC 17043:2010).

A maximum level for aflatoxin B1 in feed is laid down in Directive 2002/32/EC, and for ochratoxin A, a guidance value has been set in Recommendation 2006/576/EC. The maximum levels for aflatoxins and OTA in selected foodstuffs is laid down in EU Regulation (EC) No 2023/915. This document defines maximum levels for OTA in cocoa, while no recommended or guidance levels are given for aflatoxins in this matrix. The primary goal of this PT was to assess the capabilities of the National Reference Laboratories (NRLs) for mycotoxins in food and feed and Official Laboratories (OLs) that participated for this particular analysis.

The participants were asked to quantify aflatoxins and OTA in maize flour and cocoa powder and to report for each material six results, comprised of the individual aflatoxins, the sum of aflatoxins and OTA. The participants' performance was assessed as z-score in both materials for the individual mycotoxins.

Fifty-three laboratories, of which 37 National Reference Laboratories (NRLs) for mycotoxins and/or plant toxins in food and feed (from 23 EU Member States, Serbia, Iceland, Norway and Switzerland) and 16 OLs (from four EU Member States and Switzerland) participated in this PT.

The maize flour sample (material A) was naturally contaminated with aflatoxins and OTA. Material B, the cocoa powder, was spiked with a solution containing a mix of these mycotoxins. Each participant received both of the test materials. The participants were requested to report their results within six weeks after the dispatch of the samples.

From the provided information on the identification and quantification of the mycotoxins half of the participants used LC-MS/MS and the other half used LC-fluorescence detection. One participant applied LC-HRMS (High Resolution Mass Spectrometry). For the aflatoxins, the limit of quantification (LOQ) reported by the participants varied from 0.006 to 5 µg/kg, with the majority being in the range 0.1-1 µg/kg. For OTA, the LOQs ranged from 0.05 to 15 µg/kg, with the majority reporting in the range 0.5-1.5 µg/kg. One laboratory did not report LOQs.

In this PT the robust mean was used as consensus value. The consensus value based on the participants' results was used as the assigned value. The proficiency of the participants was assessed as z-scores in both materials, calculated using the assigned values and a relative target standard deviation for proficiency tests of 25%. Characteristics of the PT materials and the outcome of this PT are summarised in Table 1a and 1b. Results were calculated for the five individual mycotoxins and the sum of the aflatoxins in both materials.

For material A, the reported values ranged from 0.203 to 9.32 µg/kg for the individual mycotoxins. For material B, the reported values ranged from 0.459 to 5.85 µg/kg. For material A, the RSD_R of the reported results were below the target standard deviation (25%), except for aflatoxin G2. This concerned a low concentration (A= 0.203 µg/kg) which was close or below the LOQ for most of the participants. For material B, the RSD_R results for all analytes ranged between 26–56%, which were above the target standard deviation (25%). The RSD_R values for the total sum of the aflatoxins were 19% and 57% for material A and B, respectively.

For the results of both materials (A and B) combined, 81% of the results for the individual mycotoxins were rated with satisfactory z-scores ($|z| \leq 2$), 11% of the results fell into the questionable range with $2<|z|<3$ and 8% of the results fell into the unsatisfactory range with $|z| \geq 3$. Four participants achieved optimal performance for both materials by reporting the individual quantitative results for mycotoxins that were satisfactory, with absence of false negative results and reporting within the indicated deadline. With respect to the sum of the aflatoxins, for results of both materials combined, 79% of submitted results were satisfactory and 23 participants showed satisfactory performance for both materials. Three participants achieved also satisfactory results for the sum of aflatoxins in both materials combined but submitted the results after the deadline. In this PT, 18 false negative (FN) result were reported, three FN results for material A and 15 FN results for material B.

Table 1a *Summary of proficiency materials parameters and participants' performance – number of laboratories reporting quantitative values, <LOQ and false negative (FN) results.*

		Assigned value	Uncertainty	Robust RSD_R[1)]	No of labs out of 33 reporting		
mycotoxins	**Matrix**	**(µg/kg)**	**(µg/kg)**	**(%)**	**Quant. value**	**<LOQ**	**FN**
AFB1	A	21.2	0.670	18	51		
	B	1.54	0.146	51	45	2	1
AFB2	A	0.976	0.043	24	45	4	2
	B	0.459	0.051	47	28	18	2
AFG1	A	2.33	0.096	23	48	1	1
	B	1.56	0.158	51	40	7	4
AFG2	A	0.203	0.027	50	21	28	
	B	1.29	0.148	56	37	9	6
Sum Aflatoxins	A	24.5	0.797	19	51		
	B	4.43	0.468	57	45	2	2
OTA	A	9.32	0.355	22	50		
	B	5.85	0.276	26	48	0	

Matrix: A= maize flour, B= cocoa powder.

[1)] robust relative standard deviation (interlaboratory RSD based on participants' results).

Table 1b *Summary of proficiency materials parameters and participants' performance – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

		Assigned value	z-scores[1)]			Labs out of 53 with Accept. z-score	
			Satisfact.	Quest.	Unsatisf.		
mycotoxins	**Matrix**	**(µg/kg)**	**(%)**	**(%)**	**(%)**	**No[2)]**	**%[2)]**
AFB1	A	21.2	96	2	2	49	92
	B	1.54	67	26	7	31	58
AFB2	A	0.976	89	6	4	42	79
	B	0.459	63	20	17	19	36
AFG1	A	2.33	96	2	2	47	89
	B	1.56	57	30	14	25	47
AFG2	A	0.203	67	-	33	14	26
	B	1.29	60	19	21	26	49
Sum Aflatoxins	A	24.5	96	2	2	49	91
	B	4.43	60	28	13	28	53
OTA	A	9.32	96	4	-	48	92
	B	5.85	96	4	-	46	87

Matrix: A= maize flour, B= cocoa powder.

[1)] calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted here as unsatisfactory z-score. The sum of % may deviating from 100% due to rounding.

[2)] the number and percentage here means: analyte determined, method with a sufficiently low LOQ to allow quantification, and obtaining a satisfactory z-score.

From the results obtained in this PT it can be concluded that the majority of the participants have an analytical method available for quantifying aflatoxins B1, B2, G1, G2 and OTA with sufficiently low LOQs in maize flour, and that the interlaboratory variability is within the target RSD_R. For cocoa powder, on the other hand, there was a considerable variation in the reported results, with a relatively high number of questionable and unsatisfactory results, and high robust RSD_Rs were obtained. In this respect, it should be mentioned that for many NRLs cocoa powder is a new matrix for the determination of individual aflatoxins and OTA. Further efforts should focus on improvement of the methods, in order to produce reliable data.

1 Introduction

Mycotoxins chosen for quantification in this PT were aflatoxins B1, B2, G1, G2 (AFB1, AFB2, AFG1, AFG2) and ochratoxin A (OTA).

For feed, a maximum level (ML) for AFB1 is defined in Directive 2002/32/EC [3], and for OTA a guidance value has been set in Recommendation 2006/576/EC [6]. The EU regulation for aflatoxins and OTA in selected foodstuffs is listed in Regulation (EC) No 2023/915 [9]. According to this regulation, the ML for OTA content in cocoa is set at 3 µg/kg while for different type of cereals and cereals derived products the levels range from 2-8 µg/kg.

The limits of quantification (LOQs) requirements for selected mycotoxins will become regulated for food in the new edition of Regulation (EC) No 401/2006 [2]. The Limit of Quantification (LOQ) for aflatoxin B1 in processed cereal-based foods for infants and young children, baby foods, and dietary foods designed for infants is to be set at ≤ 0.1 µg/kg. For all other foods, the LOQ for aflatoxins B1, B2, G1, and G2 should be established at ≤ 1 µg/kg. For OTA the LOQ requirement is set only for cocoa powder ≤ 3 µg/kg. In all other cases the LOQ should be ≤ 0.5x the ML and should preferably be lower ≤ 0.2x the ML.

Proficiency testing is conducted to provide participants with a tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and is demanded by ISO/IEC 17025:2017 [5]. Organisation of proficiency tests (PT) is one of the tasks of the European Union Reference Laboratories (EURLs) [1]. Here, the primary goal is to assess the proficiency of NRLs. To facilitate NRLs in their task, official laboratories (OLs) can also participate, in consultation with their NRL.

2 PT material

2.1 Scope of the PT

This proficiency test (PT) focused on the mycotoxins AFB1, AFB2, AFG1, AFG2 and OTA in maize flour and cocoa powder as representative matrices. The material (A) was a naturally contaminated maize. The cocoa (material B) was spiked to reach target concentrations of 2 µg/kg for individual aflatoxins and OTA at 5 µg/kg.

2.2 Material preparation

To obtain maize flour the maize was milled using a centrifugal mill (ZM 200, Retsch) to obtain a particle size of 500 µm. The total weight of this material was 4.5 kg. The maize flour was naturally contaminated with the mycotoxins of interest in this PT. Cocoa (material B) was spiked with AFB1, AFB2, AFG1, AFG2 and OTA. The total weight of the cocoa material was 4.5 kg, as well.

Cocoa powder was fortified by adding a solution of the mycotoxins in acetonitrile/water (44:56; v/v), targeting the levels for individual aflatoxins at 2 µg/kg and OTA at 5 µg/kg. The maize flour and cocoa powder were mixed with 5.9 L and 9 L of water, respectively, and further homogenised using an industrial mixer (brand Topcraft) according to in-house standard operating procedure [8]. The obtained slurries were air dried in a fume hood and subsequently homogenised in a Stephan cutter UMC12 and stored in a freezer until use.

2.3 Sample identification

After homogenisation, both materials were divided into sub-portions of approximately 50 g and stored in polypropylene, airtight closed containers in the freezer until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was "2023/EURL PT MP/mycotoxins/xxx", in which the three-digit number of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each participant. Each sample set consisted of one randomly selected sample of each of the materials. The codes of the samples are shown in Annex 2. The samples for homogeneity and stability testing were randomly selected out of materials A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, 10 containers of materials A and B were analysed in duplicate.

Method in brief (QuEChERS): Mycotoxins were extracted from the prewetted sample using acidified acetonitrile. After shaking, magnesium sulphate was added and shaken again, resulting in a phase separation and the solution was centrifuged. The aliquot of the organic phase was diluted (1+1, v/v) with methanol. Analysis was done by high performance chromatography coupled with tandem mass spectrometry (LC-MS/MS).

The homogeneity of both materials was evaluated according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [10] and ISO 13528:2015 [11]. The between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) were compared with the standard deviation for

proficiency assessment (σ_P). The method applied for homogeneity testing is considered suitable if $s_w < 0.5 \times \sigma_P$, and a material is considered adequately homogeneous if $s_s < 0.3 \times \sigma_P$.

The mycotoxins in material A for the homogeneity study fulfilled the criteria. Aflatoxin G2 in material B was the only analyte for which one of the criteria did not comply due to the high variation (RSD_r 13.2%). Despite this deviation, it was decided not to repeat the measurements since all other components showed sufficient homogeneity and that cocoa was spiked with a single solution containing all analytes of interest. Thus, the material B was considered homogeneous.

The results of the homogeneity study (grand means with the corresponding RSD_r) are presented in Table 2. The statistical evaluation is presented in Annex 3.

The mycotoxins in both materials demonstrated to be sufficiently homogeneous for use in this PT.

Table 2 *Concentrations of mycotoxins in materials A and B obtained during homogeneity testing.*

Compound	Material A: maize flour		Material B: cocoa powder	
	Conc. (µg/kg)	RSD_r (%)	Conc. (µg/kg)	RSD_r (%)
AFB1	30.1	4	2.30	10
AFB2	1.13	5	<1*	-
AFG1	3.36	5	2.48	9
AFG2	<1*	-	1.35	13
OTA	11.8	4	6.59	6

* below the method`s LOQ of 1 µg/kg.

2.5 Stability of the materials

The stability of the mycotoxins in the PT materials was assessed [10, 11]. On May 5th, 2023, the day of distribution of the PT samples, six randomly selected containers of material A and B were stored in an ultra-freezer(-80°C). Under these conditions it is assumed that the mycotoxins are stable in the materials. Additionally, six samples of each material were stored in a freezer (≤-18°C).

On the 28th of June 2023, 51 days after distribution of the samples, six samples of materials A and B, stored in the ultra-freezer and freezer, were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated. The stability of the maize flour samples was assessed using the QuEChERS method described under 2.4. Cocoa powder was analysed using an alternative method that included sample purification with immunoaffinity columns (IAC).

Method in brief for mycotoxins in cocoa powder: mycotoxins were extracted from the homogenized sample by addition of methanol/water/hexane using an Ultra Turrax. After filtration of the sample extract, an aliquot was ten times diluted with phosphate buffered saline/polysorbate 20 and extracted/purified using Multimycotoxin IAC columns. The mycotoxins were eluted from the IAC columns with methanol. Analysis was done by high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

It was determined whether a consequential instability of the analytes had occurred [10,11] in the materials stored in the freezer. A consequential instability is observed when the average value of an analyte concentration in the samples stored in the freezer is more than $0.3\sigma_P$ below the average value of the analyte concentration in the samples stored in the ultra-freezer. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 4. For the analytes in both materials none of the tested storage conditions caused a consequential instability. The mycotoxins in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

Invitations to the NRL network were sent out on April 3rd, 2023 (Annex 5). Fifty-four laboratories registered for the PT and 53 participants (Annex 1) reported their results of which three reported their results after the deadline. One participant was unable to report results due to instrument issues.

Out of 53 participating laboratories, 37 were NRLs from 23 EU Member States, Iceland, Norway, Switzerland, and Serbia and 16 were Official Laboratories (from 4 EU Member States and Switzerland). Each participant was free to use their method of choice reflecting their routine procedures.

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application designed for proficiency tests. The sample sets with the corresponding numbers, consisting of two coded samples (Annex 2) were sent on dry ice to the participants on May 8th, 2023. The participants were asked to store the samples in the freezer and to analyse the samples according to their routine method. As reported by participants, all samples were received in good order.

The samples were accompanied by a letter describing the requested analysis (Annex 6) and an acknowledgement of receipt form. In addition, each participant received instructions by e-mail on how to use the web application to report the results. Comprehensive details regarding the analytical methodology employed for the identification and measurement of mycotoxins have been requested via questionnaire. This included information on the sample processing, chromatography, detection technique, calibration strategies as well as the specified limits of quantification (LOQs) and recovery data.

For each material a total of six results, comprised of the individual mycotoxins AFB1, AFB2, AFG1,AFG2, OTA and the sum of the aflatoxins was requested. The deadline for submitting the quantitative results was June 19th, 2023, allowing the participants six weeks for analysis of the test samples. With the exception of three participants, all results were submitted before the deadline.

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [10], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [11] in combination with the insights published by the Analytical Methods Committee [12, 13] regarding robust statistics.

The evaluation of results was based on assigned values and the standard deviation for proficiency assessment (σ_P). From this, z-scores were calculated to classify the participants' performance. Detailed information on the methods used for the statistical evaluation can be found in the background document 'EURL-MP-background doc_001 (v1.1) Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed' which is available from the EURL mycotoxins & plant toxins website [4].

4.1 Calculation of the assigned value

The robust mean was used as consensus value in this PT. The consensus value was calculated based on the results provided by participating NRLs and OLs and was used as the assigned value. The values and their uncertainties are summarised in Table 1 in the Summary section.

4.2 Standard deviation for proficiency assessment (σ_P)

A fixed relative target standard deviation for proficiency assessment of 25% was used, irrespective of the mycotoxin, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and best practises for mycotoxin and plant toxin determination in food and feed. The rationale behind this is provided in the background document 'EURL-MP PT performance assessment' on the EURL-MP website [4].

4.3 Quantitative performance (z-scores)

For evaluation of the results submitted by the participant, z-scores are calculated based on the assigned value, its uncertainty, and the standard deviation for proficiency assessment. When the uncertainty of the assigned value is negligible and no instability of the analytes in the material is observed, z-scores are calculated by:

$$z = \frac{x - C}{\sigma_p} \qquad \text{Equation 1}$$

where:

z	=	z-score;
x	=	the result of the laboratory;
C	=	assigned value, here the consensus value;
σ_P	=	standard deviation for proficiency assessment.

The z-score compares the participants' deviation from the assigned value, taking the target standard deviation (as accepted for the proficiency test) into account. Based on z-score the performance of the laboratory is interpreted as indicated in Table 3.

Table 3 *Classification of z-scores.*

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

If the uncertainty of the assigned value and, if applicable, instability of the analyte in the PT material is not negligible, this is taken into account in the determination of the z-score. If applicable, this is indicated by assigning a z'-, z_i-, or z_i'-score. For details see the background document 'EURL-MP PT performance assessment' on the EURL-MP website [4].

In this PT, the uncertainty of the assigned values for a number of results in both materials were not negligible and this was taken into account in the assignment of the z-scores (z') for following:
- AFG2 for material A; and
- all individual aflatoxins and the sum of the aflatoxins for material B.

4.4 Evaluation of non-quantified results

Reported results, e.g. 'detected' or 'not detected', without specification of LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the specific analyte or analyte group/matrix. Non reported results for analytes or analyte groups are to be interpreted as unsatisfactory performance.

4.5 False positive and false negative results

A false positive is a quantitative result reported by the participant while the analyte is not detected in the PT material by the organiser, and/or not detected by most of the other participants. A threshold is then applied, above which results are considered false positives, indicated as FP. False positives are to be interpreted as unsatisfactory performance.

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', an assessment is made to judge whether such results should be classified as a false negative. This is the case when the 'proxy-z-score', calculated by using the reported LOQ-value as result, is <-2. False negatives are indicated as 'FN'. False negatives are to be interpreted as unsatisfactory performance.

5 Performance assessment

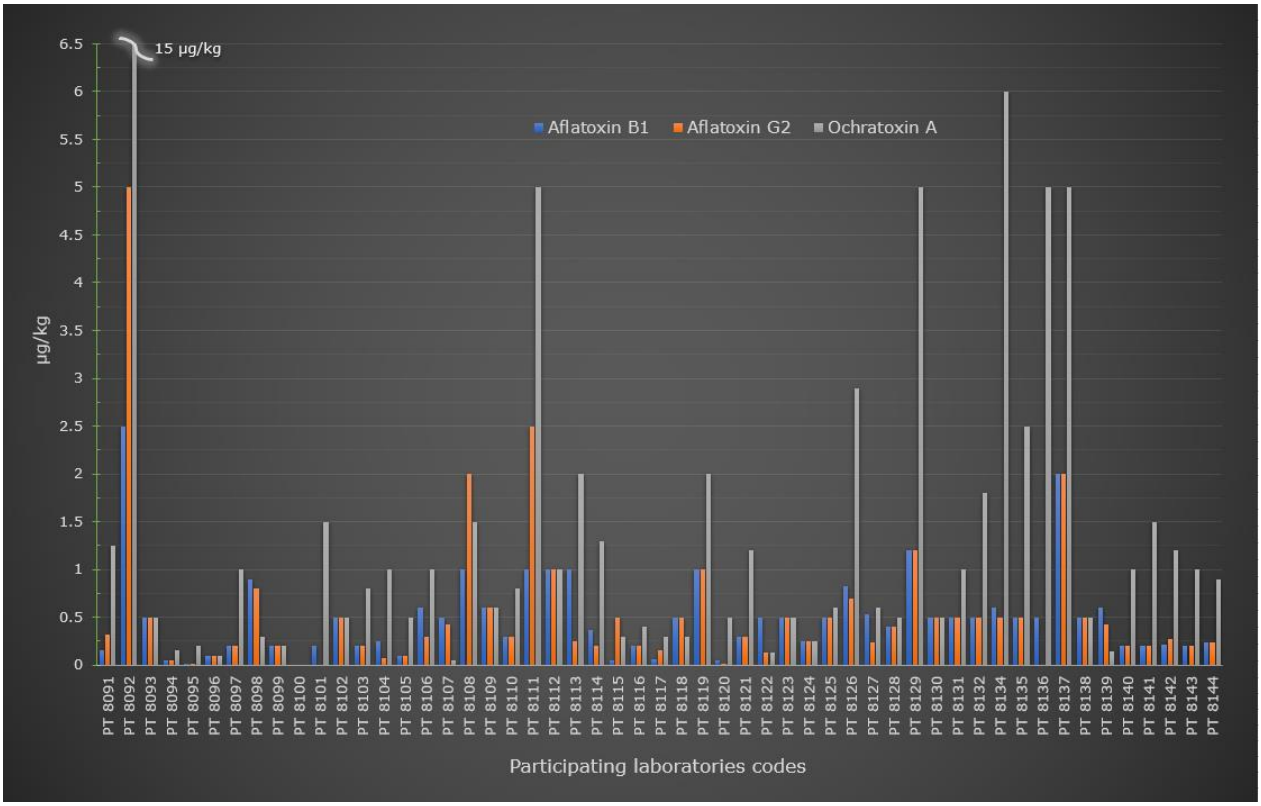
5.1 Scope and LOQ

Forty-six participants analysed both of the samples. Out of these 46 participants, 44 reported for both material A and B a total of 12 results, comprised of the mycotoxins aflatoxins B1, B2, G1, G2 and OTA and the sum of aflatoxins, as requested. One participant submitted a total of ten results due to the absence of aflatoxins AFB2 and AFG2 in their assessment of cocoa powder. The other participant reported six results, excluding OTA in maize flour and aflatoxins in cocoa powder from their scope.

Participants PT8091, PT8092, PT8101 (reported only AFB1, OTA and the sum of the aflatoxins), PT8128 and PT8136 analysed only material A (reported only AFB1, OTA and the sum of the aflatoxins) and participant PT8107 and PT8127 analysed only material B.

The LOQs provided by the participants are presented in Annex 7. For the aflatoxins, LOQs reported by the participants varied from 0.006 to 5 µg/kg, with the majority (43 participants) in the range 0.1-1 µg/kg. For OTA, LOQs ranged from 0.05-15 µg/kg with the majority (38 participants) reported LOQs below 1.5 µg/kg and 14 participants reported LOQs above 1.5 µg/kg with two laboratories reporting LOQ values of 6 and 15 µg/kg, each.

The reported LOQ values for AFB1, AFG2 and OTA in maize are plotted below in Graph 1.



Graph 1 *Plotted LOQs for AFB1, AFG2 and OTA in maize as reported by participating laboratories. Laboratory PT8100 did not report their LOQs. For more details, see Annex 7.*

5.2 Analytical methods

All participating laboratories were asked to fill in a questionnaire addressing their accreditation, the conditions used for sample preparation, chromatographic separation, detection, quantification and calibration (Annex 8). Eight participants did not complete the questionnaire. Three of these participants provided limited information about the analysis and analytical method via the web application.

Most of the methods used were internally developed and accredited according to ISO 17025. Among seventeen participants using a multi-method for mycotoxins, ten utilized MS/MS, one used HRMS, five used fluorescence detection, and one used fluorescence for aflatoxin determination and MS/MS for OTA. Thirteen participants used separate methods for the analysis of aflatoxins and OTA. Those participants employed different extraction solvents for aflatoxins and OTA, along with immunoaffinity clean-up. The identification and quantification utilized the same detection technique (LC-MS/MS or FLD), with exception of two participants. Nineteen participants used more than one and up to four different methods in evaluating the PT samples. Dedicated IAC columns were used for the aflatoxins or the individual mycotoxin OTA, along with dedicated detection technique (MS/MS or fluorescence). Instrumental measurement were consistently based on LC.

The sample size used by the participants varied from 2–50 g, with 5 g being the most common. For most of the participants, the choice for extraction solvent was acetonitrile (ACN), with methanol being slightly less commonly chosen. The composition of extraction solvents varied considerably among laboratories, ranging from neutral to basic or acidified, often combining organic solvents with aqueous component. In case a separate extraction solvent for OTA was used, 43% of the participants used mixtures of ACN/water, 37% used mixtures of methanol/water, 17% used basic aqueous and one participant used methyl-tert-butyl ether (3%).

There were also participants who used the same extraction solvent for aflatoxins and OTA in maize flour or cocoa powder. For maize flour, the most often used extraction solvents were acetonitrile or acidified acetonitrile with formic or acetic acid. For cocoa powder, methanol was used as the primary organic phase in the extraction solvent. The combination of the extraction solvents included methanol in combination with water or methanol in combination with water and acetonitrile. Extraction methods involved mechanical shaking, blenders or ultraturrax, with extractions times ranging from 1 to 75 min.

Roughly half of the labs used LC-MS/MS for the analysis of the extracts and employed SPE or IAC clean-up. Some participants opted for the sample extracts dilution or used the QuEChers method. The other half, that used LC-fluorescence for detection commonly applied IAC clean-up. Additionally, one participant applied LC-HRMS for detection.

Among participants employing LC-MS/MS-based methods, a majority (63%) used isotope-labelled standards for quantification, either added to the final extract (50%) or before extraction (50%). The quantification involved standards prepared in solvents or matrix. For LC-MS/MS-based methods, the prevalent approach was quantification based on calibration standards prepared in solvents, with a few participants opting for matrix-matched standard calibration. For LC-fluorescence-based methods, quantification was typically carried out using calibration standards prepared in solvents.

5.3 Performance

A summary of the statistical evaluation of the PT results is presented in Tables 6 and 7. These tables include all relevant parameters: the assigned value (A), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results. In case the uncertainty of the assigned value did not comply with the criterion $u \leq 0.3\sigma_p$, the uncertainty of the assigned value was taken into account in the evaluation of the z-scores (calculating the z' -score).

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for the mycotoxins in materials A (maize flour) and B (cocoa powder) are summarised in Annex 9 and 10, respectively. A summary of the performance of the participants in this PT is provided in Annex 11.

Table 4 *Summary of statistical evaluation of the PT results on mycotoxins in material A (maize flour).*

	AFB1	AFB2	AFG1	AFG2	Sum aflatoxins	OTA
A (µg/kg)	21.2	0.976	2.33	0.203	24.5	9.32
u (µg/kg)	0.670	0.043	0.096	0.027	0.797	0.355
σ_p (µg/kg) (25%)	5.31	0.244	0.583	0.051	6.13	2.33
u>0.3σ_p	No	No	No	Yes	No	No
robust σ (µg/kg)	3.83	0.229	0.531	0.100	4.55	2.01
robust σ (%)	18.0	23.5	22.8	**49.5**	18.6	21.5
# reported	51	49	49	49	51	50
"<", nd, detected		4	1	28		
# quantitative results	51	45	48	21	51	50
|z|≤ 2	49	42	47	14	49	48
2<|z|<3	1	3	1	0	1	2
|z|≥ 3	1	0	0	7	1	0
FN		2	1	0	0	0
S z-scores (%)	96	89	96	67	96	96

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

Table 5 *Summary of statistical evaluation of the PT results on mycotoxins in material B (cocoa powder).*

	AFB1	AFB2	AFG1	AFG2	Sum aflatoxins	OTA
A (µg/kg)	1.54	0.459	1.56	1.29	4.43	5.85
u (µg/kg)	0.146	0.051	0.158	0.148	0.468	0.276
σ_p (µg/kg) (25%)	0.385	0.115	0.389	0.323	1.11	1.46
u>0.3σ_p	Yes	Yes	Yes	Yes	Yes	No
robust σ (µg/kg)	0.784	0.216	0.797	0.719	2.51	1.53
robust σ (%)	**50.9**	**47.1**	**51.2**	**55.7**	**56.8**	**26.2**
# reported	47	46	47	46	47	48
"<", nd, detected	2	18	7	9	2	0
# quantitative results	45	28	40	37	45	48
|z|≤ 2	31	19	25	26	28	46
2<|z|<3	12	6	13	8	13	2
|z|≥ 3	2	3	2	3	4	0
FN	1	2	4	6	2	0
S z-scores (%)	67	63	57	60	60	96

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

For the individual mycotoxins in material A, 92% of the results were rated with satisfactory z-scores (|z|≤ 2), 3% of the results fell into the questionable range with 2<|z|<3 and 5% of the results fell into the unsatisfactory range with |z|≥ 3 (Table 4). For material B this was 70%, 19% and 11%, respectively (Table 5). Overall, 81% percent of the results obtained for both materials (A and B) were rated with satisfactory z-scores (|z|≤ 2), 11% of the individual mycotoxins results fell into the questionable range with 2<|z|<3 and 8% of the results fell into the unsatisfactory range with |z|≥ 3.

In case of the sum of the aflatoxins B1, B2, G1 and G2, for material A, 96% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 2% of the results fell into the questionable range with $2<|z|<3$ and 2% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 4). For the sum of the aflatoxins in material B this was 60%, 28% and 13%, respectively (Table 5). Regarding the sum of aflatoxins, for both materials, 79% of submitted results were satisfactory.

In Annex 11 an overview of the overall performance for each participant in this PT is provided. Given the significant performance variation for maize and cocoa, the performance overview of the laboratories was further presented for each of the materials in separate tables.

For the two materials combined, a maximum of 10 satisfactory z-scores, based on quantitative results for the individual mycotoxins could be obtained, and '10 out of 10' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. Out of 53 participants, four participants achieved optimal performance for both materials by detecting the individual mycotoxins with correct quantification, the absence of false negative results and reporting within the deadline. For the other 49 participants, false negative (FN) results were reported or one or more non-satisfactory z-scores were obtained. With respect to the sum of aflatoxins, 23 participants showed satisfactory performance and three participants also achieved satisfactory results for the sum of aflatoxins though reported after the deadline.

Among all enrolled participants for cocoa, 96% achieved satisfactory results for OTA, whereas only 60% did so for the total aflatoxin, highlighting a clear need for improvement in this area.

Regarding maize results, 96% of participants demonstrated good performance concerning the total aflatoxin levels in maize and 96% performed well regarding evaluation of AFB1, both values relevant in the EC 915/2032. For OTA in maize the percentage of successful reporting was 96%.

In total, 18 FN results were reported. For material A three FN results were reported: two for AFB2 and one for AFG1. For material B 15 FN results were reported: one FN for AFB1, two FNs for AFB2, four FNs for AFG1, six FNs for AFG2 and two FNs for the sum of aflatoxins.

5.4 Robust relative standard deviation

The robust relative standard deviation (RSD_R) was calculated according to ISO13528:2015 [12] for informative purpose. In this study it was used as an estimation of the interlaboratory variability. The RSD_R values are given in Table 1 (the Summary section), in Tables 6 and 7 (Section 5.3) and in Annex 9 and 10.

For maize, all the RSD_R of the reported results (ranging between 18 – 24%) were below the target standard deviation (25%), except for aflatoxin G2 (50%). The assigned concentration for AFG2 was 0.203 µg/kg, for most of the participants being close or below their method`s LOQ, and thus being likely the cause of the higher variation. For cocoa, all the RSD_R values of the reported results (ranging between 26 – 56%) were above the target standard deviation (25%) indicating higher variability when compared to maize results. This suggests that there is a room for improving the methods used to evaluate cacao potentially leading to more consistent and accurate results among the laboratories.

The RSD_R values for the total sum of the aflatoxins was 19% for material A, falling below the intended standard deviation of 25%. However, for material B, for the same set of results the RSD_R was 57%, significantly surpassing the target standard deviation of 25%.

6 Conclusions

Fifty-three laboratories, of which 37 were NRLs for mycotoxins and/or plant toxins in food and feed (from 23 EU Member States plus Iceland, Norway, Switzerland, Serbia) and 16 OLs (from four EU Member States plus Switzerland) participated in the PT on quantitative determination of aflatoxins B1, B2, G1, G2 and OTA in maize flour and cocoa powder.

Out of 53 participants, 44 reported for both material A and B a total of 12 results, comprised of the results of individual mycotoxins and the sum of aflatoxins.

The methods utilized by the participants in this PT relied on MS/MS or fluorescence detection, preceded by LC separation. The LOQs provided by the participants are given in Annex 7.

For both materials, most of the participants reported LOQs for aflatoxins which meet the criteria (the upcoming edition of Regulation (EC) No 401/2006) for (non-baby) food or feed matrices. For OTA we see higher incidence of LOQ values that would not meet the required criteria, considering the maximum limit (of 3 µg/kg for cereals and cocoa) defined in EC 915/2023.

For individual mycotoxins in material A, satisfactory results varied from 89 to 96%, with exception for AFG2. Ninety-two percent of the results were satisfactory. The RSD_R of the reported results (18–24%) for individual mycotoxins were below the target standard deviation (25%), except for AFG2 (50%). For material B, satisfactory results varied from 57 to 67%, except for OTA (96%). Seventy percent of the results were satisfactory. For aflatoxins the RSD_R varied from 47 to 56% and for OTA the RSD_R was 26%, all above the target value of 25%. With respect to the sum of aflatoxins, for material A and B, respectively, 96% and 60% of the results were satisfactory. The RSD_R for material A and B was 19% and 57%, respectively.

Overall, for individual mycotoxins in both materials combined (10 results), 81% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 11% of the results fell into the questionable range with $2<|z|<3$ and 8% of the results fell into the unsatisfactory range with $|z| \geq 3$. Four participants demonstrated a satisfactory performance. For the sum of aflatoxins in both materials combined (two results), 79% of the results were satisfactory with 26 participants having a satisfactory performance. In total 18 FN results were reported.

High variation in the reported results of the aflatoxins for cocoa powder resulted in relatively high robust RSD_R values (47 to 56%). This implies that the methods used were not suitable enough for evaluation of the aflatoxins at the levels present in this matrix. The proficiency test results suggest that improved methods are required for quantifying both OTA and, to a greater extent, aflatoxins in cocoa powder.

From the results obtained for maize flour it can be concluded that the majority of participants have an analytical method available for quantification of the aflatoxins B1, B2, G1, G2 and OTA with sufficiently low LOQs and satisfactory performance.

References

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[2] Working document 10672R6/2021, Commission Implementing Regulation (EU) …/... of XXX laying down the methods of sampling and analysis for the control of mycotoxins toxins in food and repealing Regulation (EC) No 401/2006.

[3] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Off. J. European Union, L 140, 30.5.2002, 10-21.

[4] EURL mycotoxins & plant toxins, 2019. Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed. EURL-MP-background doc_001 (version 1). https://www.wur.nl/en/research-results/research-institutes/food-safety-research/reference-laboratory/european-union-reference-laboratory/eurl-mycotoxins-plant-toxins/library-eurl-mp.htm#eurlmp_background_documents.

[5] ISO/IEC 17025:2017(E). 2017. General requirements for the competence of testing and calibration laboratories.

[6] Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding

[7] ISO/IEC 17043:2010. Conformity assessment - General requirements for proficiency testing.

[8] WFSR SOP-A-0989 – Preparation of PT materials and PT samples.

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[12] Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

[13] Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 2. Inter-laboratory trials. Analyst. 114:1699-1702.

[14] Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.

Annex 1 List of participants

Country	Organisation
AUSTRIA*	AGES GmbH
BELGIUM*	CER Groupe
BULGARIA*	Bulgarian Food Safety Agency
CROATIA*	A. Stampar Teaching Institute of Public Health
CYPRUS*	Feeding Stuffs Quality Control Laboratory - Analytical Laboratories Section
CYPRUS*	State General Laboratory
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
CZECH REPUBLIC*	Central Institute for Supervising and Testing in Agriculture
DENMARK*	Danish Veterinary and Food Administration
ESTONIA*	Agricultural Research Centre
FINLAND*	Finnish Customs Laboratory
FRANCE*	SCL
FRANCE***	Labocea
GERMANY**	Eurofins WEJ Contaminants
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GERMANY***	Chemisches und Veterinaruntersuchungsamt Rheinland
GERMANY***	Agrolab Lufa GmbH
GREECE*	General Chemical State Laboratory
GREECE*	Ministry of Rural Development & Food/Directorate of Animal Feed & Pasturelands
HUNGARY*	Food Chain Safety Laboratory Directorate
HUNGARY*	National Food Chain Safety Office
IRELAND*	The State Laboratory
IRELAND*	The Public Analyst's Laboratory
ITALY*	Istituto Superiore di Sanita
ITALY***	USL Toscana Centro
ITALY***	ATS Val padana
ITALY***	ATS Milano Citta metropolitana
ITALY***	APPA BZ
LATVIA*	Institute of Food Safety, Animal Health and Environment "BIOR"
LITHUANIA*	National Food and Veterinary Risk Assessment Institute
LUXEMBOURG*	Laboratoire National de Sante
NORWAY**	Norwegian Veterinary Institute
PORTUGAL*	Autoridade Seguranca Alimentar e Economica
PORTUGAL*	National Institute of Agrarian and Veterinary Research
ROMANIA*	Institute for Hygiene and Veterinary Public Health
ROMANIA*	Directia Sanitara Veterinara si pentru Siguranta Alimentelor (DSVSA) Bucuresti
SERBIA**	SP Laboratorija A.D.
SLOVAKIA*	Regional Public Health Authority in Poprad (RUVZ)
SLOVAKIA*	Veterinary and food institute Kosice
SPAIN*	Spanish Agency for consumer affairs, Food Safety and Nutrition
SPAIN***	Direccion general de salud publica
SPAIN***	Laboratorio de Sanidad Animal de Gijon
SPAIN***	Eurofins Ecosur, S.A.
SPAIN***	Centro Nacional de Technologia Y Seguridad Alimentaria (CNTA)
SPAIN***	Madrid Salud, Ayuntamiento de Madrid
SPAIN*	Ministry of Agriculture, Fisheries and Food
SPAIN***	Laboratori Agroalimentari
SPAIN***	Ainia
SPAIN***	Laboratorio Agroambiental DGA
SWEDEN*	Swedish Food Agency
SWEDEN*	Statens Veterinarmedicinska Anstalt
SWITZERLAND**	Kantonales Laboratorium Bern
SWITZERLAND***	Amt fur Verbraucherschutz

* National Reference Laboratory (NRL) of EU Member State.

** National Reference Laboratory (NRL) of the European Free Trade Association (Eurofins WEJ Contaminants = Iceland) and Serbia.

*** Official Laboratory (OL).

Annex 2 Codification of the samples

Participant's code	Material A*	Material B*
PT8091	185	297
PT8092	537	682
PT8093	727	769
PT8094	928	711
PT8095	238	958
PT8096	345	632
PT8097	329	436
PT8098	543	936
PT8099	536	140
PT8100	507	680
PT8101	502	878
PT8102	265	659
PT8103	777	882
PT8104	474	748
PT8105	115	674
PT8106	908	849
PT8107	657	650
PT8108	484	601
PT8109	662	278
PT8110	295	788
PT8111	285	653
PT8112	203	373
PT8113	439	894
PT8114	197	339
PT8115	258	810
PT8116	142	863
PT8117	792	664
PT8118	706	985
PT8119	249	155
PT8120	149	923
PT8121	774	201
PT8122	462	758
PT8123	949	132
PT8124	207	821
PT8125	853	267
PT8126	813	510
PT8127	447	308
PT8128	429	272
PT8129	980	146
PT8130	153	597
PT8131	971	950
PT8132	374	218
PT8134	753	666
PT8135	422	125
PT8136	315	868
PT8137	990	723
PT8138	482	611
PT8139	547	648
PT8140	968	848
PT8141	667	862
PT8142	291	852
PT8143	965	684
PT8144	660	424

* All sample codes start with 2023/EURL PT MP/mycotoxins/.

Annex 3 Statistical evaluation of homogeneity data

	Aflatoxin B1 (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	31.5	30.0
Hom/A002	29.7	30.0
Hom/A003	30.1	31.6
Hom/A004	29.5	29.6
Hom/A005	27.8	29.6
Hom/A006	28.6	28.4
Hom/A007	29.3	29.9
Hom/A008	30.0	30.8
Hom/A009	31.3	33.1
Hom/A010	30.9	31.1
Grand mean	30.1	
Cochran's test		
C	0.266	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.54	
s_x	1.13	
s_w	0.780	
s_s	0.982	
Critical= 0.3 σ_P	2.26	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	Aflatoxin B2 in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	1.28	1.17
Hom/A002	1.13	1.13
Hom/A003	1.18	1.15
Hom/A004	1.08	1.04
Hom/A005	1.10	1.14
Hom/A006	1.07	1.11
Hom/A007	1.07	1.14
Hom/A008	1.07	1.17
Hom/A009	1.19	1.09
Hom/A010	1.04	1.18
Grand mean	1.13	
Cochran's test		
C	0.315	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.282	
s_x	0.045	
s_w	0.056	
s_s	0.021	
Critical= 0.3 σ_P	0.084	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Aflatoxin G1 in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	3.20	3.30
Hom/A002	3.36	3.30
Hom/A003	3.24	3.43
Hom/A004	3.43	3.17
Hom/A005	3.47	3.37
Hom/A006	3.16	3.08
Hom/A007	3.28	3.73
Hom/A008	3.54	3.05
Hom/A009	3.57	3.39
Hom/A010	3.39	3.67
Grand mean	3.36	
Cochran's test		
C	0.349	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.839	
s_x	0.128	
s_w	0.185	
s_s	0.000	
Critical= 0.3 σ_P	0.252	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ochratoxin A in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	11.7	11.8
Hom/A002	11.8	11.7
Hom/A003	11.4	11.5
Hom/A004	12.5	11.7
Hom/A005	11.6	12.0
Hom/A006	12.2	11.8
Hom/A007	12.7	12.5
Hom/A008	12.0	10.8
Hom/A009	12.2	11.0
Hom/A010	11.8	11.8
Grand mean	11.8	
Cochran's test		
C	0.368	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.96	
s_x	0.348	
s_w	0.442	
s_s	0.153	
Critical= 0.3 σ_P	0.887	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Aflatoxin B1 in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	2.49	2.23
Hom/B002	2.30	2.72
Hom/B003	2.36	2.60
Hom/B004	2.36	2.38
Hom/B005	2.17	2.36
Hom/B006	2.33	2.33
Hom/B007	2.49	2.39
Hom/B008	2.38	1.96
Hom/B009	2.20	2.25
Hom/B010	1.91	1.81
Grand mean	2.30	
Cochran's test		
C	0.328	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.575	
s_x	0.189	
s_w	0.164	
s_s	0.150	
Critical= 0.3 σ_P	0.173	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Aflatoxin G1 in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	2.95	2.68
Hom/B002	2.71	2.32
Hom/B003	2.64	2.39
Hom/B004	2.51	2.66
Hom/B005	2.46	2.48
Hom/B006	2.48	2.75
Hom/B007	2.41	2.55
Hom/B008	2.21	2.29
Hom/B009	2.22	2.20
Hom/B010	2.66	2.12
Grand mean	2.48	
Cochran's test		
C	0.416	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.621	
s_x	0.176	
s_w	0.187	
s_s	0.115	
Critical= 0.3 σ_P	0.186	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	Aflatoxin G2 in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	1.50	1.47
Hom/B002	1.71	1.37
Hom/B003	1.29	1.19
Hom/B004	1.64	1.31
Hom/B005	1.09	1.34
Hom/B006	1.38	1.43
Hom/B007	1.68	1.19
Hom/B008	1.17	1.23
Hom/B009	1.30	1.24
Hom/B010	1.13	1.36
Grand mean	1.35	
Cochran's test		
C	0.400	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.338	
s_x	0.129	
s_w	0.173	
s_s	0.041	
Critical= 0.3 σ_P	0.101	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	NOT ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between sample standard deviation.

	Ochratoxin A in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	6.28	6.38
Hom/B002	5.99	6.30
Hom/B003	6.48	6.55
Hom/B004	6.36	6.64
Hom/B005	6.19	6.59
Hom/B006	6.89	6.58
Hom/B007	6.56	6.74
Hom/B008	7.24	6.77
Hom/B009	7.07	7.27
Hom/B010	6.03	6.93
Grand mean	6.59	
Cochran's test		
C	0.523	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.65	
s_x	0.310	
s_w	0.278	
s_s	0.239	
Critical= 0.3 σ_P	0.494	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Annex 4 Statistical evaluation of stability data

Stability evaluation for aflatoxin B1 in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	29.5	29.9
	29.6	29.9
	30.2	30.6
	28.3	29.3
	30.7	31.9
	29.5	31.9
Average amount (µg/kg)	29.6	30.6
n	6	6
st. dev (µg/kg)	0.809	1.10
Difference		-0.950
$0.3*\sigma_P$		2.22
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for aflatoxin B2 in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	1.06	1.01
	1.12	1.15
	1.04	1.08
	1.08	1.00
	1.25	1.11
	1.09	1.11
Average amount (µg/kg)	1.11	1.08
n	6	6
st. dev (µg/kg)	0.075	0.060
Difference		0.030
$0.3*\sigma_P$		0.083
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for aflatoxin G1 in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	3.87	3.80
	3.49	3.44
	3.66	3.46
	3.80	3.90
	3.67	3.50
	3.49	3.43
Average amount (µg/kg)	3.663	3.588
n	6	6
st. dev (µg/kg)	0.156	0.207
Difference		0.075
$0.3*\sigma_P$		0.275
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ochratoxin A in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	12.9	13.3
	13.0	12.2
	11.2	12.8
	14.5	12.3
	13.3	13.4
	12.3	12.1
Average amount (µg/kg)	12.9	12.7
n	6	6
st. dev (µg/kg)	1.09	0.571
Difference		0.183
$0.3*\sigma_P$		0.965
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for aflatoxin B1 in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	1.77	1.62
	1.94	1.68
	1.73	1.94
	2.04	1.84
	1.95	1.85
	1.95	1.76
Average amount (µg/kg)	1.90	1.78
n	6	6
st. dev (µg/kg)	0.120	0.118
Difference		0.115
$0.3*\sigma_P$		0.142
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for aflatoxin G1 in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	2.09	2.17
	2.06	1.94
	1.95	2.10
	1.89	1.93
	1.73	2.35
	1.94	1.80
Average amount (µg/kg)	1.94	2.05
n	6	6
st. dev (µg/kg)	0.129	0.198
Difference		-0.105
$0.3*\sigma_P$		0.146
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for aflatoxin G2 in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	1.74	1.71
	1.95	1.99
	2.10	1.82
	2.08	1.98
	1.93	2.14
	2.08	2.00
Average amount (µg/kg)	1.98	1.94
n	6	6
st. dev (µg/kg)	0.138	0.152
Difference		0.040
$0.3*\sigma_P$		0.149
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ochratoxin A in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	51
Calculated amounts (µg/kg)	6.99	8.60
	7.64	8.54
	8.13	8.17
	7.70	7.37
	7.45	7.56
	8.35	7.91
Average amount (µg/kg)	7.71	8.03
n	6	6
st. dev (µg/kg)	0.485	0.505
Difference		-0.315
$0.3*\sigma_P$		0.578
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

NRLs mycotoxins & plant toxins

Dear Colleague,

The EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), will organize a proficiency test regarding the mycotoxins aflatoxin B1, B2, G1, G2 and ochratoxin A in food and feed matrices (EURLPT-MP10). This proficiency test will focus on the quantification of these mycotoxins and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013).

I would like to invite you to participate in this proficiency test.

A maximum level for aflatoxin B1 in feed is laid down in Directive 2002/32/EC, and for ochratoxin A a guideline level has been set in Recommendation 2006/576/EC and its amendments. Harmonised EU regulation for aflatoxins and ochratoxin A in foodstuffs is laid down in Regulation (EC) No 1881/2006 and its amendments. Currently there are no harmonised limits set for aflatoxins in cocoa. However, screening of this matrix/toxins combination is of relevance, based on reports of co-occurrence.

The primary goal of this proficiency test is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of multiple mycotoxins in food and feed matrices.

According to Regulation (EU) 2017/625, all EU National Reference Laboratories (NRLs) mycotoxins & plant toxins in food and/or feed are mandatory to participate.

The following matters are important for participation in this proficiency test:

1. Test materials
   One test sample of maize flour and one test sample of cocoa powder will be provided.
   The test amount sent will be approximately 50 g.

2. Shipment of the test materials
   Test materials will be sent in May 2023. The distribution of the test materials will be announced by e-mail. The deadline for reporting is strict and will be six weeks after the shipment of the samples.

3. Scope of the analysis

Wageningen Food Safety Research

Natural toxins

DATE
April 3, 2023

SUBJECT
Invitation EURL mycotoxins & plant toxins proficiency test aflatoxins and ochratoxin A in maize and cocoa 2023 (EURLPT-MP10)

POSTAL ADDRESS
P.O. Box 230
6700 AE Wageningen
The Netherlands

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Wageningen Campus
Building 123
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INTERNET
www.wur.nl

CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31(0) 614 323 017

EMAIL
pt.wfsr@wur.nl

Both materials are to be analysed for the aflatoxins B1, B2, G1, G2, the sum of aflatoxins and ochratoxin A. The participants should provide their own analytical standards.

4. Questionnaire
A questionnaire will be send electronically. In this questionnaire the particants will be asked to provide information about the laboratory method(s) used. This information is necessary to conduct a more in depth analysis of the results obtained in this proficiency test.

5. Report
- Preliminary results of this proficiency test will be reported to the participants in September 2023.
- The report is expected to be published in December 2023.
- Results of the proficiency test will be presented anonymously.
- Disclosure of the results of the NRLs to the representative of the European Commission is foreseen after the report is published.
- The follow-up protocol on proficiency tests from DG Santé will be applied.

6. Additional information
- WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
- WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

7. Costs
- Participation is free of charge for NRLs.
- Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee for OLs is €270.- (ex. VAT) as a compensation for the preparation and transportation of the samples.
- If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back **before April 21th 2023**: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiency test,

D. Pereboom

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins in food and feed
Wageningen Food Safety Research

Annex 6 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of mycotoxins aflatoxin B1, B2, G1, G2 and ochratoxin A in maize and cocoa.

The parcel shipped to you should contain:

- One material consisting of a maize flour and one material consisting of cocoa. Each test material unit contains approximately 50 grams of the homogenised test material.

Instructions:

- After arrival the samples should be stored in the freezer.
- Please fill in the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples by e-mail (pt.wfsr@wur.nl).
- Before analysis, (re)homogenise the samples according to your laboratory's procedure.
- Treat the test material as a sample for routine analysis, according to your laboratory procedure.
- Report for each material 6 results:
  1. Aflatoxin B1
  2. Aflatoxin B2
  3. Aflatoxin G1
  4. Aflatoxin G2
  5. Sum of aflatoxin B1, B2, G1 and G2
  6. Ochratoxin A
- Reporting:
  - For mycotoxins found, report all analytical results in **µg/kg material as received** (so for feed laboratories, **do NOT report on 12% moisture basis**)
  - For mycotoxins included in your method but not found, so below your LOQ, report as '**<[value µg/kg]**', e.g. < 5 µg/kg.
  - In case a mycotoxin is not included in the scope of your method, so not measured, report as '**nt**' (not tested).
- Results reported in any other format (e.g. nd, detected, <LOQ, etc) will be regarded as not tested, 'nt'.

Wageningen Food Safety Research

DATE
May 8, 2023

SUBJECT
Instructions proficiency test regarding the mycotoxins aflatoxin B1, B2, G1, G2 and ochratoxin A in maize and cocoa

YOUR REFERENCE
NR

OUR REFERENCE
WFSR/ 2310179

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CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

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- Please use the web application to submit the results for the test samples (https://crlwebshop.wur.nl/ordsp/f?p=107:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- Provide detailed information in the questionnaire on the analysis of the mycotoxins and the analytical method used and send it back to us by e-mail (pt.wfsr@wur.nl).
- The deadline for submitting test-results for this proficiency test is **19th of June 2023.**

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

Please contact me in case you have any questions or need any assistance.

With kind regards,

D. Pereboom

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research (WFSR)
The Netherlands

Annex 7 Scope and LOQ

	LOQ (µg/kg)				
Lab code	**Aflatoxin B1**	**Aflatoxin B2**	**Aflatoxin G1**	**Aflatoxin G2**	**Ochratoxin A**
PT 8091	0.156	0.313	0.313	0.313	1.25
PT 8092	2.5	5	2.5	5	15
PT 8093	0.5	0.5	0.5	0.5	0.5
PT 8094	0.05	0.05	0.05	0.05	0.15
PT 8095	0.006	0.006	0.006	0.006	0.2
PT 8096	0.1	0.1	0.1	0.1	0.1
PT 8097	0.2	0.2	0.2	0.2	1
PT 8098	0.9	0.9	0.8	0.8	0.3
PT 8099_A_B	0.2/0.5	0.2/0.5	0.2/0.5	0.2/0.5	0.2/0.5
PT 8100					
PT 8101	0.2				1.5
PT 8102	0.5	0.5	0.5	0.5	0.5
PT 8103	0.2	0.2	0.2	0.2	0.8
PT 8104	0.25	0.07	0.25	0.07	1
PT 8105	0.1	0.1	0.1	0.1	0.5
PT 8106	0.6	0.3	0.6	0.3	1
PT 8107	0.50	0.42	0.63	0.42	0.05
PT 8108	1	1	1	2	1.5
PT 8109_A/B	0.6/06	0.6/0.6	0.6/0.6	0.6/0.6	0.6/3
PT 8110	0.3	0.3	0.3	0.3	0.8
PT 8111	1	1	2.5	2.5	5
PT 8112	1	1	1	1	1
PT 8113	1	0.25	1	0.25	2
PT 8114_A/B	0.37/0.91	0.20/0.45	0.41/1.0	0.20/0.5	1.3/3.3
PT 8115_A/B	0.05/0.5	0.5/0.5	0.5/0.5	0.5/2	0.3/2
PT 8116	0.2	0.2	0.2	0.2	0.4
PT 8117	0.06	0.15	0.2	0.15	0.3
PT 8118_A/B	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.3/1
PT 8119	1	1	1	1	2
PT 8120	0.05	0.012	0.05	0.012	0.50
PT 8121	0.3	0.3	0.3	0.3	1.2
PT 8122	0.5	0.13	0.5	0.13	0.13
PT 8123	0.5	0.5	0.5	0.5	0.5
PT 8124	0.25	0.25	0.25	0.25	0.25
PT 8125	0.5	0.5	0.5	0.5	0.6
PT 8126	0.83	1.26	0.74	0.69	2.9
PT 8127	0.53	0.15	0.32	0.24	0.6
PT 8128	0.4	0.4	0.4	0.4	0.5
PT 8129	1.2	1.2	1.2	1.2	5
PT 8130	0.5	0.5	0.5	0.5	0.5
PT 8131	0.5	0.5	0.5	0.5	1.0
PT 8132	0.5	0.5	0.5	0.5	1.8
PT 8134	0.6	0.5	0.6	0.5	6
PT 8135	0.5	0.5	0.5	0.5	2.5
PT 8136	0.5				5
PT 8137	2	2	2	2	5
PT 8138	0.5	0.5	0.5	0.5	0.5
PT 8139	0.6	0.33	0.39	0.42	0.14
PT 8140	0.2	0.2	0.2	0.2	1
PT 8141	0.2	0.2	0.2	0.2	1.5
PT 8142_A/B	0.21/0.32	0.19/0.33	0.22/0.44	0.27/0.30	1.2/1.4
PT 8143	0.2	0.2	0.2	0.2	1
PT 8144	0.24	0.24	0.24	0.24	0.9

* (A)= material A (maize flour) and (B)= material B (cocoa powder).

Annex 8 Analytical method details

Lab	Column	Column length	Total run time	Mobile phase	Detection technique	Retention time (min)				
code		(mm)	(min)			AFB1	AFB2	AFG1	AFG2	OTA
PT8091	EVO, C18, 100 × 1.0 mm (100 Å), 1.7 µm	100	15	A: 5 mM ammonium formate + 0.1% formic acid in H_2O; B: 98% MeOH	MS/MS	7.55	7.34	7.17	6.97	9.53
PT8092	Waters, CORTECS C18, 100 x 2.1 mm, 2.7um	100	14	A: 2 mM ammonium acetate with 0.1% formic acid in H_2O B: 2 mM ammonium acetate with 0.1% formic acid in MeOH	MS/MS	7.45	7.12	6.78	6.42	9.51
PT8093	Waters, Acquity UPLC HSS T3, 150 x 2.1 mm, 1.8 µm, with a precolumn	150	9	A: 10 mM ammonium formate in water B: 10 mM ammonium formate in methanol	MS/MS	4.1	3.9	3.5	3.3	4.7
PT8094	Zorbax Eclipse XDB-C18		18	Aflas: acetonitrile/methanol/acetic acid 2% 18/27/55 OTA: Acetonitrile/acetic acid 2% 45/55	other	8.1	7	6.2	5.5	9.1
PT8095	Waters, Aquity C18		45	water-methanol-acetonitrile 620-190-190 + 119 mg KBr + 100 uL HNO_3 65%	Aflas FLD; OTA MS/MS	14.4	11.8	10.2	8.5	13.4
P8096	Phenomenex, Kinetex XB-C18, 50 x 2.1 mm, 2.6 µm	50	14	Aflas: A: ammonium-formate 10 mmol B: Methanol OTA: A: formic acid 0.1% B: methanol	MS/MS	4.8	4.6	4.4	4.1	4.2
PT8097										
PT8098	Aflas: Purospher Star, RP18e, 250 x 4.6 mm, 5 µm OTA: Phenomenex, Kinetex XB-C18, 150 x 4.6 mm, 2.6 µm	Aflas: 250 OTA: 150	Aflas:25 OTA:15	Aflas: H_2O:ACN:MeOH (3:1:1) (v/v/v) OTA: ACN:H_2O:acetic acid (49.5:49:5:1)	other	15.1	12.3	11.2	9.4	6.8
PT8099	Waters, Aqcuity BEH C18, 100 x 2.1 mm, 1.7 µm	100	12	A: water:formic acid (0.1%)) B: acetonitrile:formic acid (0.1%)	MS/MS	6.7	6.5	6.5	6.3	8.0
PT8100										
PT8101	Symmetry C18, 250 x 4.6, 5 µm	250	15	water/methanol/acetonitrile (50:40:10)	FLD					
PT8102	Aflas: Zorbax Eclipse Plus C18, 100 x 2.1mm, 1.8 µm OTA: Supelcosil LC-18, 250 x 4.6 mm, 5 µm	Aflas: 100 OTA: 250	Aflas:20 OTA:30	Aflas: A: 0,2 mM NH4F B: methanol OTA: acetonitrile/water/acetic acid (49:49:2)	Aflas: MS/MS OTA: FLD	4.97	4.69	4.28	3.88	9.61
PT8103	Aflas: Inertsil ODS-2, 150 mm x 4.6 mm, 5 µm OTA: Lichrosorb RP-18, 200 mm x 4.6 mm, 5 µm	Aflas: 150 OTA:200	Aflas:15 OTA:10	Aflas:H_2O+acetonitrile+ethanol (60:10:30;v/v); 119 mg KBr + 100 µl HNO_3 for 1 l OTA: acetonitrile + H_2O+acetic acid (99:99:2; v/v)	FLD	10.5	8.7	7.2	6.1	7.3

Lab code	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Retention time (min)				
						AFB1	AFB2	AFG1	AFG2	OTA
PT8104	Aflas: Phenomenex, C18, 150 x 4.6 mm 5µm 100A OTA: Kinetex C18, 50 x 3 mm, 1.7 µm	Aflas: 150 OTA: 50	Aflas:18 OTA:10	Aflas: methanol/water/KBr/HNO_3 OTA: A: water + 0.1% formic acid + 0.5 mM ammonia acetate B: acetonitrile + 0.1% formic acid + 0.5 mM ammonia acetate	Aflas: FLD; OTA: MS/MS	11.086	8.916	7.337	6.022	6.2
PT8105	Macherey Nagel, GmbH & Co KG C18, 250 x 3 mm	250	12	ACN/MeOH/H_2O/HNO_3/KBr	FLD	8.9	7.6	6.6	5.8	8.7
PT8106 (A)	Aflas: Supelco, Ascentis, Phenyl-Hexyl, 100 x 4.6 mm, 2.7 µm OTA: Zorbax Eclipse Plus C18, 4.6 x 150 mm, 5 µm	Aflas: 100 OTA: 150	Aflas:15 OTA:10	Aflas: water/methanol/acetonitrile/acetic acid (41:29:29:1) OTA: water/acetonitrile/acetic acid (50:49:1)	other	16.5	13.4	11.6	9.7	7.3
PT8106 (B)	Aflas: Zorbax Eclipse XDB-C18, 4.6 x 250 mm, 5 µm OTA: Zorbax Eclipse XDB-C18, 4.6 x 250 mm, 5 µm	250	Aflas:20 OTA:20	water/methanol/acetonitrile (56:28:16)	other	16.5	13.5	11.7	9.7	3.9
PT8107	Aflas: Spherisorb C18 ODS2, 250 x 4.6 mm, 5 µm OTA: Spherisorb C18 ODS1, 250 x 4.6 mm, 5 µm	250	20	Aflas: water/methanol/acetonitrile (54:28:18) OTA: acetic acid/ methanol (40:60)	other	11.5	10.1	8.6	7.7	10.5
PT8108	C18		15	A: water with 0,1% formic acid B: acetonitrile	HRMS					
PT8109 (A)	Aflas: Lichrospher 100 RP-18 (endcapped), 125 x 4.0 mm, 5 µm OTA: Zorbax SB C18 (encapped), 250 x 4.6 mm, 5 µm	Aflas: 125 OTA: 250	Aflas:20 OTA:30	Aflas: methanol/acetonitrile/water/nitric acid 4 M potassium bromide (410 g:265 g:1200 g:700 µL:240 mg) OTA: methanol/acetonitrile/water (35:35:29:1; v/v)	FLD	12.35	10.23	9.02	nd -	7.987
PT8109 (B)	Aflas: Lichrospher 100 RP-18 (endcapped), 125 x 4.0 mm, 5 µm OTA: Zorbax SB C18 (encapped), 250 x 4.6 mm, 5 µm	Aflas: 125 OTA: 250	Aflas:20 OTA:30	Aflas: methanol/acetonitrile/water/nitric acid 4M/KBr (410 g/265 g/1200 g/700 µL/240 mg) OTA: Methanol/Acetonitrile/Water (28/28/39/1) (v/v)	FLD	12.34	10.24	9.02	7.61	18.32
PT8110 (A)	Hypersil Gold, 2.1 x 50 mm, 1.9 µm	50	13	A: 5 mM ammonium formate+0,1% formic acid in H_2O, B: 5 mM ammonium formate + 0,1% formic acid in MeOH	MS/MS	7.34	7.1	6.83	6.62	10.08
PT8110 (B)	Zorbax Eclipse C18, 250 x 4.6 mm, 5 µm	250	Aflas:25 OTA:13	Aflas: H_2O: ACN: MeOH = 3: 1: 1 OTA: ACN: H_2O: acetic acid = 480: 510: 10	other	12.41	10 14	9	7.47	10.65
PT8111	Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	11	A: H_2O + 0.1% formic acid B: MeOH + 0.1% formic acid + 1mM ammonium formate	MS/MS	3.93	3.78	3.6	3.43	5.21
PT8112										
PT8113	Acquity BEH C18, 2,1 x 50 mm, 1.7 µm	50	5	A: methanol/water 0,1% acetic acid/acetonitrile 20/75/5 B: methanol/water 0,1% aacetic acid/acetonitrile 65/15/20	FLD	3.1	2.9	2.8	2.5	4.5
PT8114	Waters, Xbridge Premier BEH C 18, 150 x 2.1mm, 2.5 µm, pore size 130A	150	22	A: $H_2$0 + 5mM ammonium formate + 0.1% formic acid B: MeOH + 5mM ammonium formate + 0.1% formic acid	MS/MS	13.78	13.27	12.78	12.15	17.37
PT8115	Water, C18		15	water, methanol	MS/MS	7.3	7.1	6.8	6.6	9.7
PT8116					FLD					
PT8117	Waters, Symmetry C18, 250 x 4.6 mm, 5 µm	250	Aflas:25 OTA:12	Aflas: methanol: water (20: 80) (for kobra cell) OTA: acetonitrile:methanol: acetic acid 1% (33:33:36)	other	21	16	13	10	6.5

Lab code	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Retention time (min)				
						AFB1	AFB2	AFG1	AFG2	OTA
PT8118	Zorbax Eclipse Plus C18 RRHD, 2.1 x 50 mm, 1.8 µm	50	27	A: 10 mM ammonium formate, pH 3.0 B: 0,2% formic acid in methanol	MS/MS	6.38	6.05	5.69	5.34	9.18
PT8119	ASCENTIS EXPRESS		17	A: water/ammonium formate 5 mM/formic acid 0.1% B: methanol/ammonium formate 5 mM/formic acid 0.1%	MS/MS	4.2	4	3.8	3.6	7.2
PT8120	Kinetex, C18, 100 x 4.6 mm, 5 µm	100	15	Aflas: water:acetonitrile:methanol (60:20:20) OTA: 1% acetic acid: acetonitrile (49:51)	FLD					
PT8121										
PT8122	Waters, Spherisorb ODS 2, 250 mm	250	10	ACN/H_2O/AcCOOH (51:47:2)	FLD					
PT8123	Sunfire, C18, 150 x 4.6 mm, 5 µm	150	20	Aflas: water/methanol (60:40) + KBr + HNO3 OTA: ACN/water/acetic acid (51:47:2)	other	12.8	10.1	8.2	6.6	6.3
PT8124	Aquity UPLC HSS T3, 100 x 2.1 mm x 1.8 µm	100	18	A: Ammonium formate 0.2 mM in water + 0.1% formic acid B: Ammonium formate 0.2 mM in methanol + 0.1% formic acid	MS/MS	9.16	8.95	8.65	8.4	10.79
PT8125	Agilent, ZORBAX Bonus-RP, 150 x 2.1 mm, 3.5 µm	150	19	A: formic acid 0.15% in water (10 mmol ammonium formate);formic acid (0.05%) in MeOH	MS/MS	7.41	7.2	7.07	6.83	9.36
PT8126	Thermoscientific, ODS Hypersil, 250 x 4.6 mm	250	25	ACN:MeOH:H_2O (20:20:60; v/v), acetic acid, KBr	other	15.5	12.6	10.6	9.09	10.4
PT8127	Aflas: supel coil LC-18, 250 x 4.6 mm, 5 µm OTA: Sphereclone 5u ODS (2), 150 x 4.6 mm, 5 µm	Aflas: 250 OTA: 150	Aflas:30 OTA:20	Aflas: MeOH-ACN-H_2O (20:20:60) OTA: MeOH-ACN-H_2O-acetic acid (28:28:39:1)	FLD	17.506	14.878	13.59	11.757	8.549
PT8128	Inertsil ODS-3, 250 x 4.6 mm, 5 µm	250	30	Aflas: H_2O/ACN/MeOH (60:20:20) + 119 mg/l KBr + 350 µl de HNO_3 4 M OTA: phosphate buffer PH=2.5 /MeOH (40:60)	other	16.55	13.7	12.44	10.45	10.92
PT8129	Cortecs UPLC T3, 100 x 2.1 mm, 1.6 µm	100	15	A: 0,5% formic acid, 5 nM ammonium formate in water; B: 0,5% formic acid, 5 nM ammonium formate in 50:50 MeOH/ACN	MS/MS	7.07	6.57	6.24	7.84	9.56
PT8130	Zorbax C18, 75 x 3 mm	75	20	A: water B: MeOH	MS/MS	9.6	9.4	9.2	9.05	10.95
PT8131_FLD	Waters, Symmetry C18, 250 x 5 mm, 4.6 µm,	250	27	tetrahydrofuran:H_2O (79:21)	FLD	18.67	13.34	15.03	11.37	13.04
PT8131_LC-MS	Phenomenex Gemini-NX C18, 150 x 2 mm, 5.0 µm	150	18	A: H_2O ammonium acetate 1 mM, 0,1% acetic acid B: MeOH 0,1% acetic acid	MS/MS					
PT8132 (A)					MS/MS					
PT8132 (B)					FLD					
PT8133										
PT8134 (A)	Machery-Nagel, EC 250/4.6 Nucleosil, 100-5 C18, 250 x 4.6 mm, 5 µm	250	Aflas:15 OTA:12	Aflas: water: acetonitrile:methanol, KBr, HNO_3 4 mol/l OTA maize water:acetonitrile:acetic acid	FLD					
PT8134 (B)	Machery-Nagel EC 250/4.6 Nucleosil 100-5 C18, 250 x 4.6 mm, 5 µm	250		Aflas: water:acetonitrile:methanol, KBr, HNO_3 4 mol/l OTA: water:methanol:acetonitrile:acetic acid	FLD					

Lab code	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Retention time (min)				
						AFB1	AFB2	AFG1	AFG2	OTA
PT8135	ACQUITY Premier BEH C18, 100 2.1 mm, 1.70 µm	100	14	A: 1 mM ammonium acetate in water with 0.5% acetic acid and 0.1% formic acid; B: methanol with 0.5% acetic acid and 0.1% formic acid	MS/MS	7.49	7.17	6.86	6.52	9.34
PT8136	Agilent Technologies, LC-18, 150 x 4.6 mm, 5µm	150		AFB1: H_2O:methanol:acetonitrile (55:27:18) OTA: acetonitrile:methanol:3% acetic acid (35:35:30)	other	12.4				4.6
PT8137					FLD					
PT8138	Aflas: C18, Zorbax, 150 x 2.1 mm OTA: Luna C18, 150 x 4.6 mm, 5 µm	150	Aflas:15 OTA:20	A: 0.1% formic acid in H20; B: 0.1% formic acid in ACN ACN:H_2O:HAc	Aflas: MS/MS OTA: other	5.4	5.1	5	4.8	8.5
PT8139	Waters, HSS T3, 150 × 2.1mm, 1.8 µm		14	A:2 mM ammonium formate 0,1% formic acid in H_2O B: 2 mM ammonium formate 0,1% formic acid in MeOH	MS/MS	5.3	5.6	5	4.85	7.3
PT8140	Aflas: ACE 5 C18, 150 x 4.6mm, 5 µm OTA: ACE 3 C18, 150 x 4.6 mm, 3 µm	150	Aflas:20 OTA:8	Aflas: 120 mg KBr+ 350 µl 4 M HNO_3 in water:ACN:MeOH (650:160:190) OTA: 2% acetic acid in water:ACN, (47:53)	FLD	13.3	10.5	9.1	7.3	6.1
PT8141	Acquity UPLC HSS T3, 100 x 2.1 mm, 1.8 µm	100		A: water:methanol:ammonium formate/formic acid (90:10:0.1:0.05) B: methanol/water/ammonium formate/formic acid (95:5:0.1:0.05)	MS/MS	3.31	3.17	2.96	2.80	3.40
PT8142	Aflas: Agilent Eclipse XDB-C18, 150 x 4.6 mm, 5 µm OTA: LiChrospher RP-18, 125 x 4 mm, 5 µm	Aflas: 150 OTA:125	20	Aflas: ACN:MeOH:H_2O OTA: ACN:H_2O:acetic acid	FLD	8.28	7.07	6.30	5.21	9.84
PT8143					Aflas: FLD OTA: MS/MS					
PT8144	C18		35	acetonitrile/water/acetic acid (49:49:1)	other	13	10	8	6	9

A = material A (maize flour); B= material B (cocoa powder); Aflas=aflatoxins B1, B2, G1, G2; OTA = ochratoxin A; ACN = acetonitrile; MeOH = methanol; HNO_3 = nitric acid.

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract Loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standard
PT8091	2	10 ml of H_2O and 10 ml of HCN/acetic acid (995/5)	10	mechanical shaking	30	SPE	Isolute	2	0.8	U-OTA; U-AFB1; U-AFB2; U-AFG1; U-AFG2
PT8092	2	acetonitrile:water	16	mechanical shaking	20	dilution			0.05	C13
PT8093	5	H_2O-ACN (80-20) with 2% formic acid	15	mechanical shaking	75	LLE				AFB1 C13, OTA C13
PT8094	12.5	Aflas: methanol/water 80/20 OTA: acetonitrile/water 60/40	100	mechanical shaking	Aflas: 30 OTA: 60	SPE	Rbiopharm Aflaprep Ochraprep	Aflas: 1 OTA: 4	Aflatoxins: 0.083 Ochratoxins: 0.25	
PT8095	10	MeOH:H_2O (80-20) + 25 g NaCl	120	shaking (hand/vortex)	60	SPE	IAC	50	1	
PT8096	10	acetonitrile:water (9+1 v/v)	40	mechanical shaking	60	SPE	Aflas: Mycosep 226 OTA: Mycosep 229	Aflas: 3 OTA: 5	0.25	13C-marked Toxins
PT8097	Not reported									
PT8098	12.5	MeOH/H_2O	50	blender	3	SPE	Aflas: IAC, R-Biopharm OTA: IAC, Vicam			
PT8099 (A)	Aflas: 25 OTA: 25	Aflas: methanol:water + cyclohexane OTA: ACN:water	100 -300 ml depending on the protocol	blender	3	SPE	Aflas: IAC: Aflaprep, OTA: IAC, Ochraprep, Rhone diagnostics	4,5 - 10 ml depending on the protocol	0,06 - 2,27 g depending on the extraction protocol	
PT8099 (B)	Aflas: 25 OTA: 5	Aflas: methanol:water OTA: 2% sodium bicarbonate in water		blender	3	SPE	Aflas: IAC: Aflaprep, OTA: IAC, Ochraprep, Rhone diagnostics	4,5 - 10 ml depending on the protocol	0,06 - 2,27 g depending on the extraction protocol	
PT8100	Not reported									
PT8101	50	acetone:water 85/15	250	mechanical shaking	30	SPE	AflaStar/ROMER	2.5	0.1	
PT8102	Aflas: 2 OTA: 5	Aflas: 1% formic acid in acetonitrile OTA: acetonitril/water (75:25)	Aflas: 10 OTA: 100	Aflas: mechanical shaking OTA: ultraturrax	Aflas: 30 OTA: 2	Aflas: none OTA: SPE	Aflas: - OTA: IAC column R-BIOPHARM	4	Aflas: 0.2 OTA: 0.2	Aflas: aflatoxin B1 13C17, aflatoxin B2 13C17, aflatoxin G1 13C17, aflatoxin G2 13C17 OTA: no
PT8103	25	Aflas: methanol+H_2O+NaCl OTA: acetonitril + H_2O	100	blender	3	IAC	R-Biopharm	10	Aflas: 0.25 OTA: 0.167	

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract Loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standard
PT8104 (A)	Aflas: 6.25 OTA: 2.5	Aflas: methanol:water (80:20) OTA: acetonitrile, water and 0.1% formic acid	Aflas: 25 OTA: 10	mechanical shaking	Aflas: 10 OTA: 20	Aflas: SPE OTA: QuEChERS	Aflas: AflaStar R, IAC Column, Romer labs	Aflas: 4	Aflas: - OTA: 0.125	OTA: U-[$^{13}C_{20}$]-Ochratoxin A
PT8104 (B)	Aflas: 6.25 OTA: 5	Aflas: methanol:water (80:20) OTA: 80% methanol in water	Aflas: 25 OTA: 25	mechanical shaking	Aflas: 10 OTA: 10	SPE	Aflas: AflaStar R, IAC Column, Romer labs OTA: OchraStar R (RomerLabs)	4	Aflas: - OTA: 1.6	OTA: U-[$^{13}C_{20}$]-Ochratoxin A
PT8105 (A)	6	MeOH/ H_2O	50	mechanical shaking	2	SPE	easy Extrakt, R-Biopharm	3	0.36	
PT8105 (B)	2.5	ACN/MeOH/H_2O	25	mechanical shaking	2	SPE	easy Extrakt, R-Biopharm	3	0.3	
PT8106 (A)	12.5	Aflas: methanol:water (80:20) OTA: acetonitrile:water (60:40)	Aflas: 100 OTA: 50	mechanical shaking	Aflas: 40 OTA: 30	SPE	Aflas: IAC Rbiopharm OTA: IAC Romer	Aflas: 55 OTA: 48	Aflas: 0.25 OTA: 1	
PT8106 (B)	Aflas: 5 OTA: 10	Aflas: methanol OTA: methanol:water (60:40)	Aflas: 50 OTA: 100	blender	Aflas: 3 OTA: 3	SPE	IAC Romer	Aflas: 25 OTA: 80	Aflas: 1 OTA: 0.5	
PT8107	5	Aflas: methanol/water OTA: methyl-tert-butylether	Aflas: 75 OTA: 100	ultraturrax	3	SPE	VICAM (IAC)	Aflas: 10 OTA: 30	Aflas: 3 OTA: 46.9	
PT8108	2	Acetonitrile 80%	2*10	mechanical shaking	60	none				Zearalanone
PT8109 (A)	25	Aflas: methanol:water (80:20) OTA: acetonitrile:water (80:20)	100	blender	Aflas: 3 OTA: 2	SPE	Aflas: IAC R-BIOPHARM AFLARHONE WIDE ref P116/500 OTA: IAC R-BIOPHARM OCHRARHONEWIDE ref 119/100	Aflas: 10 OTA: 10	Aflas: 0.625 OTA: 1	
PT8109 (B)	Aflas: 25 OTA: 12.5	Aflas: methanol/water (80:20) OTA: methanol/water (80:20)	100	Aflas: blender OTA: mechanical shaking	Aflas: 3 OTA: 40	SPE	Aflas: IAC R-BIOPHARM AFLARHONE WIDE ref P116/500 OTA: IAC R-BIOPHARM OCHRARHONEWIDE ref 119/100	Aflas: 10 OTA: 4	Aflas: 0.625 OTA: 0.333	
PT8110 (A)	2.5	acetonitrile:water:acetic acid (79:20:1)	Aflas: 20 OTA: 10	mechanical shaking	60	none				13C17-AFB1, 13C17-AFB2, 13C17-AFG1, 13C17-AFG2, 13C20-OTA

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract Loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standard
PT8110 (B)	Aflas: 5 OTA: 2.5	60% MeOH	Aflas: 20 OTA: 10	mechanical shaking	Aflas: 60 OTA: 30	SPE	Aflas: IAC (3ml), Protealmmun OTA: IAC (3ml), Protealmmun	Aflas: 10 OTA: 5	Aflas: 0.6 OTA: 2	
PT8111	2	ACN + 0.1% formic acid in water (1:1)	20	mechanical shaking	20	dilution				
PT8112	Not reported									
PT8113	25	methanol:water (80:20)	100	mechanical shaking	15	SPE	AflaOchra LC (VICAM)	5	1.25	
PT8114 (A)	5	ACN containing 1% acetic acid	10	mechanical shaking	10	SPE	IAC Aflaochra Prep, r-biopharm	Aflas: 0.2 OTA: 2	Aflas: 0.5 OTA: 5	Aflatoxin G2: 13C17, Aflatoxin B1, B2 und G1: D3 Ochratoxin A: D5
PT8114 (B)	2	ACN containing 1% acetic acid	10	mechanical shaking	10	SPE	IAC Aflaochra Prep, r-biopharm	2	2	Aflatoxin G2: 13C17, Aflatoxin B1, B2 und G1: D3 Ochratoxin A: D5
PT8115 (A)	5	ACN	10	shaking (hand/vortex)	30	other				
PT8115 (B)		methanol:water:ACN	150	shaking (hand/vortex)	60	SPE	IAC, BIOPHARM	20	2.5	
PT8116		Aflas: MeOH H_2O (80:20) OTA: 60% ACN				SPE	IAC			
PT8117 (A)	Aflas: 25 OTA: 12.5	methanol:water (80:20)	Aflas: 100 OTA: 100	mechanical shaking	Aflas: 40 OTA: 40	SPE	IAC Ochraprep R-Biopharm	Aflas: 70 OTA: 4	Aflas: 1 OTA: 0.5	
PT8117 (B)	Aflas: 5 OTA: 12.5	Aflas: acetonitrile:water (80:20) OTA: methanol:water (80:20)	Aflas: 10 OTA: 100	Aflas: shaking (hand/vortex) OTA: mechanical shaking	Aflas: 10 OTA: 40	SPE	IAC Ochraprep R-Biopharm	Aflas: 25 OTA: 4	Aflas: 1 OTA: 0.5	
PT8118	25	acetonitril:water:acetic acid (79:20:1)	100	mechanical shaking	30	other			0.25	C13 (Romer lab)
PT8119 (A)	5	ACN	10	shaking (hand/vortex)	5	QUECHERS			0.25	13C
PT8119 (B)	2	methanol:water (80:20)	15	shaking (hand/vortex)	5	SPE	IAC, R-Biopharm	10		13C
PT8120 (A)	2	Aflas: 75% methanol OTA: 60% acetonitrile	20	shaking (hand/vortex)	5	SPE	Aflaprep, Ochraprep	5	1	
PT8120 (B)		Aflas: 75% methanol OTA:1% $NaHCO_3$	20	shaking (hand/vortex)	5	SPE	Aflaprep, Ochraprep	5	1	
PT8121	Not reported									
PT8122	40	$NaHCO_3$ 1% water:MeOH (60:40; v/v)	200	ultraturrax	2	SPE	R-Biopharm	20	0.2	

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract Loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standard
PT8123 (A)	10	Aflas: water/methanol (40:60) OTA: 1% sodium bicarbonate	100	ultraturrax	4	other				
PT8123 (B)	10	Aflas: ACN/water (75:25) OTA: 1% sodium bicarbonate	100	ultraturrax	4	other				
PT8124	12.5	methanol/water (70:30)	50	ultraturrax	3	SPE	IAC, R-Biopharm	20	0.21	
PT8125 (A)	5	acetonitrile:water (1:1)	20	mechanical shaking	15	none				13C-labeled ISTDs
PT8125 (B)	2	acetonitrile:water (1:1)	20	mechanical shaking	15	SPE	IAC, AflaOTA 25 (RomerLabs)	25	1	13C-labeled ISTDs
PT8126 (A)	5	Aflas:MeOH:H_2O (70:30; v/v) OTA: ACN:H_2O (60:40; v/v)	20	mechanical shaking	30	SPE	IAC, Romer Labs	4	1	
PT8126 (B)	5	Aflas: MeOH:H_2O 70:30 v/v OTA: 3% $NaHCO_3$:MeOH (50:50; v/v)	20	mechanical shaking	30	SPE	IAC, Romer Labs	4	1	
PT8127	Aflas: 5 OTA: 3.5	Aflas: MeOH:ACN:H_2O (35:40:25) OTA: MeOH:H_2O (80:20)	Aflas: 40 OTA: 28	shaking (hand/vortex)	Aflas: 3 OTA: 40	SPE	IAC	Aflas: 30 OTA: 55	Aflas: 0.0625 OTA: 0.5	
PT8128	10	Aflas: MeOH:H_2O (60/40) OTA: ACN:H_2O (60/40)	Aflas: 250 OTA: 200	mechanical shaking	30	SPE	Aflas: IAC Aflaprep, R-Biopharm OTA: IAC, Ochraprep. R-Biopharm	Aflas: 20 OTA: 4	Aflas: 0.04 OTA: 0.05	
PT8129	5	acetonitrile/water/formic acid	25	mechanical shaking	60	SPE	Oasis Prime HLB (Waters)	3	0.8	Internal standard of 13C, one for each analyte
PT8130	(A): 8 (B): 5	acetonitrile/water	40	mechanical shaking	90	none				C13 marked internal Standard for each analyte
PT8131 (A)	10	Aflas: MeOH:H_2O OTA: Acetonitrile:H_2O	Aflas: 40 OTA: 40	mechanical shaking	30	SPE	IAC		0.033	Aflas: MS/MS: [13C17] Aflatoxins B1, B2, G1, G2
PT8131 (B)	10	Aflas:MeOH:H_2O OTA: 1% $NaHCO_3$ in H_2O	Aflas: 40 OTA: 200	mechanical shaking	30	SPE	IAC		Aflas: 0.167 OTA: 0.25	Aflas: MS/MS: [13C17] Aflatoxins B1, B2, G1, G2
PT8132 (A)		ACN + formic acid		mechanical shaking		SPE	d-SPE			MS/MS: Aflatoxin B1 C-13
PT8132 (B)		methanol;water		ultrasonic extraction		SPE	IAC			
PT8134 (A)	5	Aflas: methanol 70%+NaCl OTA: methanol:$NaHCO_3$ 3%	Aflas: 25 OTA: 40	shaking (hand/vortex)	30	Aflas: Easi extract OTA: SPE	Easi extract aflatoxin, Ochraprep	Aflas: 15 OTA: 50	Aflas: 0.33 OTA: 0.05	

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract Loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Internal standard
PT8134 (B)	5	Aflas: acetonitrile 70% OTA: methanol 80%	Aflas: 20 OTA: 40		30	Aflas: Easi extract OTA: SPE	Easi extract aflatoxin, Ochraprep	Aflas: 30 OTA: 55	Aflas: 0.08 OTA: 0.25	
PT8135	5	acetonitrile 85/water (85:15)	25	mechanical shaking	120	dilution			0.02	
PT8136	Aflas: 50 OTA: 25	Aflas: acetone:H_2O OTA: methanol:3% $NaHCO_3$	Aflas: 250 OTA: 200	mechanical shaking	Aflas: 30 OTA: 40	SPE	IAC			
PT8137						SPE	IAC			
PT8138	Aflas: 20 OTA: 2	ACN:H_2O	Aflas: 100 OTA: 25	mechanical shaking	Aflas: 120 OTA: 30	SPE	Aflas: Vicam IAC OTA: Romerlab	Aflas: 4 OTA: 5	Aflas: 2 OTA: 0.08	
PT8139 (A)	5	H_2O:acetonitrile:acetic acid	20	mechanical shaking	20	other			0.5	C13 Aflatoxins and C13 OTA
PT8139 (B)	2	H_2O/acetonitrile/acetic acid	20	mechanical shaking	20	SPE	Ochraprep, r-biopharm	2	1	C13 Aflatoxins and C13 OTA
PT8140	5	Aflas: acetone/water (85:15) OTA: 3% sodium bicarbonate in water:methanol (50:50)	Aflas: 25 OTA: 50	mechanical shaking	60	SPE	Aflas: IAC, Aflastar OTA: Ochrastar	Aflas: 2.5 OTA: 10	Aflas: 0.17 OTA: 1.67	
P8141	Aflas: 20 OTA: 10	Aflas: acetonitrile:water (80:20) OTA: acetonitrile:water:formic acid (74:25:1)	Aflas: 100 OTA: 50	mechanical shaking	Aflas: 45 OTA: 60	Aflas: SPE OTA: dilution	Aflas: Waters Vicam AflaTest	Aflas: 1	Aflas: 0.05 OTA: 0.1	
PT8142	Aflas: 5 OTA: 10	Aflas: MeOH/H_2O, OTA: $NaHCO_3$	Aflas: 50 OTA: 200	shaking (hand/vortex)	30	SPE	Aflas: Aflaprep, R-Biopharm OTA: Ochraprep, R-Biopharm	Aflas: 20 OTA: 20	Aflas: 2 OTA: 3	
PT8143		chloroforme acetonitrile/water/acetic acid				SPE	IAC			
PT8144	10	methanol/water	40	shaking (hand/vortex)	20	SPE	R biopharm	50		

(A)= material A (maize flour); B= material B (cocoa powder); Aflas=aflatoxins B1, B2, G1, G2; OTA = ochratoxin A; ACN = acetonitrile; MeOH = methanol; $NaHCO_3$ = sodium hydrogencarbonate; NaCl = sodium chloride.

Annex 9 Results material A (maize flour)

	Material A					
	Aflatoxin B1 **A: 21.2 µg/kg** **u: 0.670 µg/kg** **σ_p: 5.31 µg/kg (25%)** **robust σ: 3.83 µg/kg (18.0%)**		**Aflatoxin B2** **A: 0.976 µg/kg** **u: 0.043 µg/kg** **σ_p: 0.244 µg/kg (25%)** **robust σ: 0.229 µg/kg (23.5%)**		**Aflatoxin G1** **A: 2.33 µg/kg** **u: 0.096 µg/kg** **σ_p: 0.583 µg/kg (25%)** **robust σ: 0.531 µg/kg (22.8%)**	
Lab code	**Result µg/kg**	**z-score**	**Result µg/kg**	**z-score**	**Result µg/kg**	**z-score**
PT8091	17.8	-0.65	0.9	-0.31	2.3	-0.05
PT8092	24.75	0.66	1.05	0.30	2.6	0.46
PT8093	16.2	-0.95	0.83	-0.60	1.5	-1.42
PT8094	14.36	-1.30	0.66	-1.29	1.48	-1.46
PT8095	27.71	1.22	1.17	0.80	2.98	1.12
PT8096	22.0	0.14	0.6	-1.54	2.2	-0.22
PT8097	24.50	0.61	1.05	0.30	2.29	-0.07
PT8098	18.28	-0.56	0.75	-0.93	1.29	-1.79
PT8099	17.0	-0.80	0.80	-0.72	1.9	-0.74
PT8100	25.7	0.84	1.39	1.70	3.35	1.75
PT8101	21.1	-0.03	nt		nt	
PT8102	25.91	0.88	1.45	1.94	3.19	1.48
PT8103	19.7	-0.29	0.94	-0.15	1.84	-0.84
PT8104	24.7	0.65	1.17	0.80	2.46	0.22
PT8105	16.08	-0.97	0.68	-1.21	1.78	-0.94
PT8106	22.193	0.18	0.970	-0.02	2.270	-0.10
PT8108	32.3	**2.08**	1.66	**2.80**	3.2	1.49
PT8109	19	-0.42	0.89	-0.35	1.9	-0.74
PT8110	24.1	0.54	0.5	-1.95	2.5	0.29
PT8111	23.0	0.33	<1	[0.10]	2.57	0.41
PT8112	17.03	-0.79	<1	[0.10]	2.21	-0.21
PT8113	17.99	-0.61	0.86	-0.47	1.85	-0.82
PT8114	21.10	-0.03	0.98	0.02	2.26	-0.12
PT8115	19.46	-0.34	0.59	-1.58	1.65	-1.17
PT8116	18.6	-0.50	0.9	-0.31	2.0	-0.57
PT8117	19.6	-0.31	1.0	0.10	2.6	0.46
PT8118	25.3	0.76	1.5	**2.15**	3.4	1.84
PT8119	20.7	-0.10	1.10	0.51	2.2	-0.22
PT8120	18.76	-0.47	0.843	-0.54	2.017	-0.54
PT8121	29.2	1.50	1.19	0.88	2.97	1.10
PT8122	18.84	-0.45	0.80	-0.72	1.94	-0.67
PT8123	21.16	-0.02	0.88	-0.39	1.89	-0.76
PT8124	16.123	-0.96	0.767	-0.86	1.659	-1.15
PT8125	23.29	0.38	1.25	1.12	2.63	0.51
PT8126	24.299	0.57	0.881	-0.39	2.638	0.53
PT8128	23.35	0.40	1.15	0.71	2.68	0.60
PT8129	25.4	0.78	1.3	1.33	2.8	0.81
PT8130	20.6	-0.12	0.9	-0.31	2.2	-0.22
PT8131	20.7	-0.10	0.9	-0.31	2.3	-0.05
PT8132	18.2	-0.57	1.2	0.92	3.4	1.84
PT8134	19.4	-0.35	0.94	-0.15	2.26	-0.12
PT8135	19.0	-0.42	1.1	0.51	2.4	0.12
PT8136	22.267	0.19	nt		nt	
PT8137	23.2	0.37	1.1	0.51	2.1	-0.39
PT8138	21.6	0.07	0.96	-0.06	2.26	-0.12
PT8139	23.2	0.37	<0.33	**[-2.65] FN**	2.4	0.12
PT8140	29.433	1.54	1.024	0.20	4.062	**2.97**
PT8141	22.26	0.19	1.57	**2.44**	3.01	1.17
PT8142	1.70	**-3.68**	<0.19	**[-3.22] FN**	<0.22	**[-3.62] FN**
PT8143	24.01	0.52	0.92	-0.23	2.55	0.38
PT8144	17.25	-0.75	0.75	-0.93	1.8	-0.91

A = consensus value (robust mean).
u = uncertainty of consensus value.
σ_p = target standard deviation for proficiency test.
robust σ = robust (relative) standard deviation based on participants' results.
nt = not tested.
Participant PT 8107 and PT8127 didn't analyse material A.

	Material A					
	Aflatoxin G2 **A: 0.203 µg/kg** **u: 0.027 µg/kg** **σ_p: 0.051 µg/kg (25%)** **robust σ: 0.100 µg/kg (49.5%)**		**Sum aflatoxins** **A: 24.5 µg/kg** **u: 0.797 µg/kg** **σ_p: 6.13 µg/kg (25%)** **robust σ: 4.55 µg/kg (18.6%)**		**Ochratoxin A** **A: 9.32 µg/kg** **u: 0.355 µg/kg** **σ_p: 2.33 µg/kg (25%)** **robust σ: 2.01 µg/kg (21.5%)**	
Lab code	**Result µg/kg**	**z'-score**	**Result µg/kg**	**z-score**	**Result µg/kg**	**z-score**
PT8091	0.0	**-3.52**	21	-0.57	8.4	-0.39
PT8092	<5		28.4	0.63	10.65	0.57
PT8093	<0.5		18.6	-0.97	8.3	-0.44
PT8094	0.09	-1.96	16.59	-1.29	6.82	-1.07
PT8095	0.19	-0.22	32.05	1.23	6.82	-1.07
PT8096	<0.1	[-1.78]	24.8	0.05	8.0	-0.57
PT8097	<0.2	[-0.05]	27.84	0.54	8.1	-0.52
PT8098	<0.3		20.32	-0.69	11.17	0.80
PT8099	<0.2	[-0.05]	19.8	-0.77	10.3	0.42
PT8100	0.41	**3.60**	30.9	1.04	9.85	0.23
PT8101	nt		21.1	-0.56	8.6	-0.31
PT8102	<0.5		30.55	0.98	9.11	-0.09
PT8103	<0.2	[-0.05]	22.7	-0.30	7.68	-0.70
PT8104	0.21	0.13	28.5	0.65	14.6	**2.27**
PT8105	0.14	-1.09	18.68	-0.95	9.37	0.02
PT8106	<0.3		25.433	0.15	9.068	-0.11
PT8108	<2		37.2	**2.07**	6.9	-1.04
PT8109	<0.6		22	-0.41	11.3	0.85
PT8110	<0.3		27.1	0.42	10.1	0.34
PT8111	<2.5		27.3	0.45	9.46	0.06
PT8112	<1		19.24	-0.86	11.16	0.79
PT8113	0.23	0.47	20.93	-0.59	7.77	-0.66
PT8114	0.16	-0.74	24.5	0.00	10.44	0.48
PT8115	<0.5		21.7	-0.46	5.72	-1.54
PT8116	0.5	**5.16**	22.0	-0.41	13.15	1.65
PT8117	0.2	-0.05	23.4	-0.18	2.96	**-2.73**
PT8118	0.48	**4.81**	30.8	1.02	7.8	-0.65
PT8119	<1		24.0	-0.09	6.0	-1.42
PT8120	0.158	-0.78	21.68	-0.46	13.88	1.96
PT8121	0.15	-0.92	33.51	1.47	6.8	-1.08
PT8122	0.15	-0.92	21.73	-0.46	nt	
PT8123	<0.5		23.94	-0.09	7.5	-0.78
PT8124	0.126	-1.33	18.675	-0.95	10.335	0.44
PT8125	<0.50		27.17	0.43	10.45	0.49
PT8126	0.13	-1.26	27.948	0.56	7.89	-0.61
PT8128	<0.40		27.44	0.48	12.27	1.27
PT8129	<1.2		29.7	0.84	13	1.58
PT8130	<0.5		23.7	-0.13	9.8	0.21
PT8131	<0.5		23.9	-0.10	11.1	0.77
PT8132	<1		22.8	-0.28	9.7	0.16
PT8134	<0.5		22.6	-0.31	8.2	-0.48
PT8135	0.1	-1.78	22.6	-0.31	9.4	0.04
PT8136	nt		22.267	-0.37	8.963	-0.15
PT8137	<2.0		26.4	0.31	9.3	-0.01
PT8138	<0.5		24.82	0.05	9.88	0.24
PT8139	0.8	**10.37**	26.4	0.31	11.1	0.77
PT8140	0.243	0.70	34.762	1.67	11.774	1.05
PT8141	0.88	**11.76**	27.72	0.52	10.44	0.48
PT8142	<0.27		1.70	**-3.72**	9.47	0.07
PT8143	<0.20	[-0.05]	27.48	0.48	9.00	-0.14
PT8144	0.55	**6.03**	20.35	-0.68	6.8	-1.08

A = consensus value (robust mean).
u = uncertainty of consensus value.
σ_p = target standard deviation for proficiency test.
robust σ = robust (relative) standard deviation based on participants' results.
nt = not tested.
Participant PT 8107 and PT8127 didn't analysed material A.

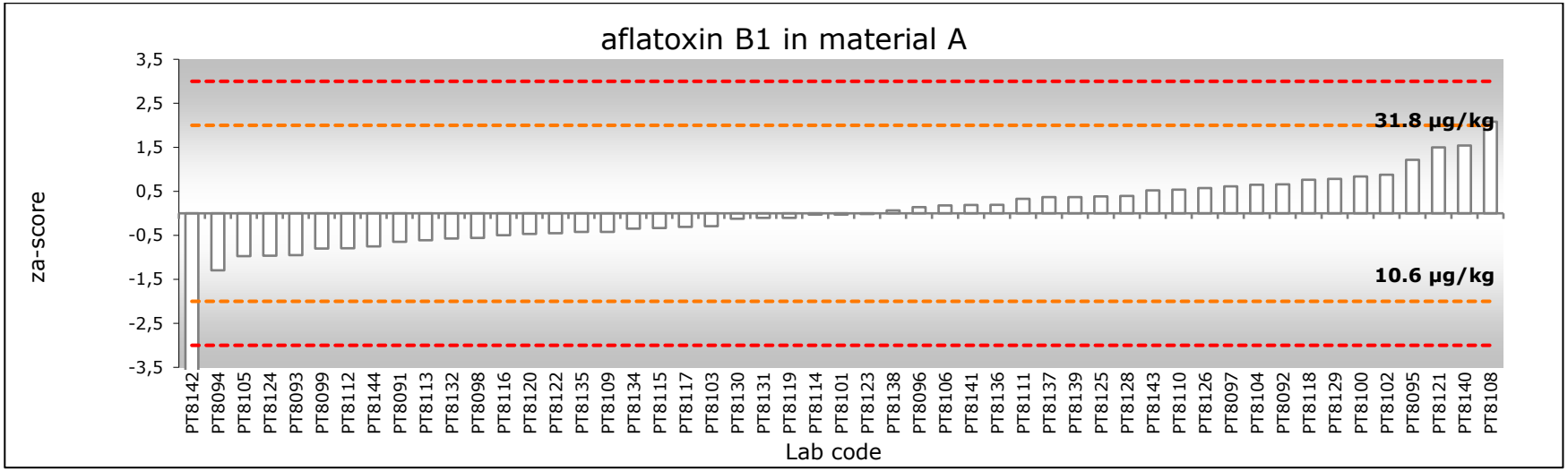

Figure 1 *Graphical representation of the z-scores for aflatoxin A in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

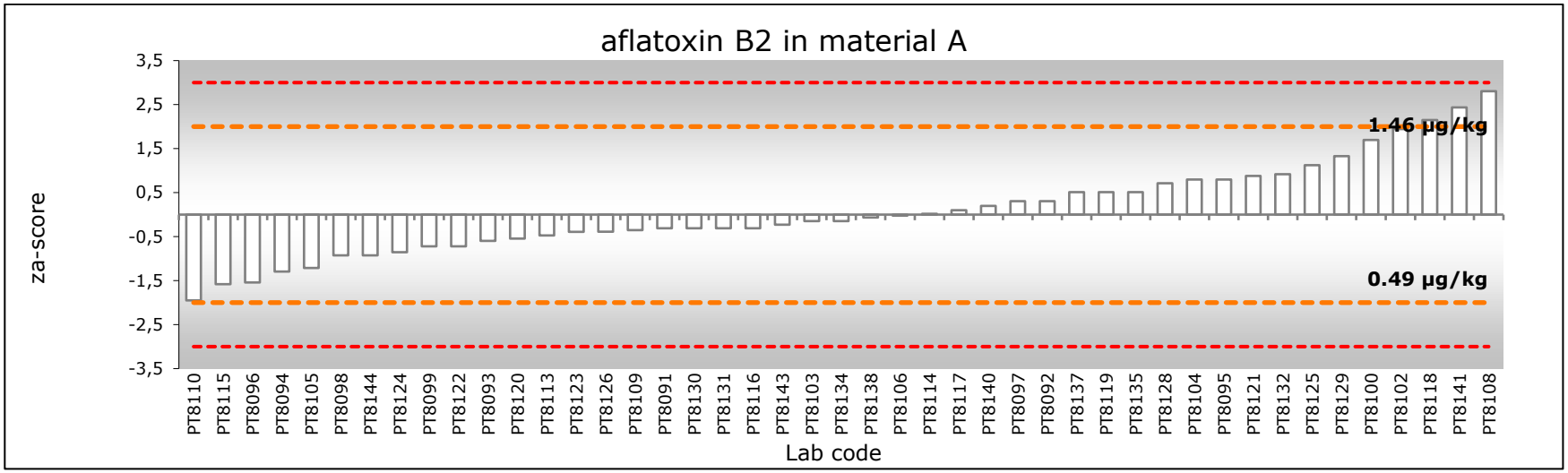

Figure 2 *Graphical representation of the z-scores for aflatoxin B2 in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

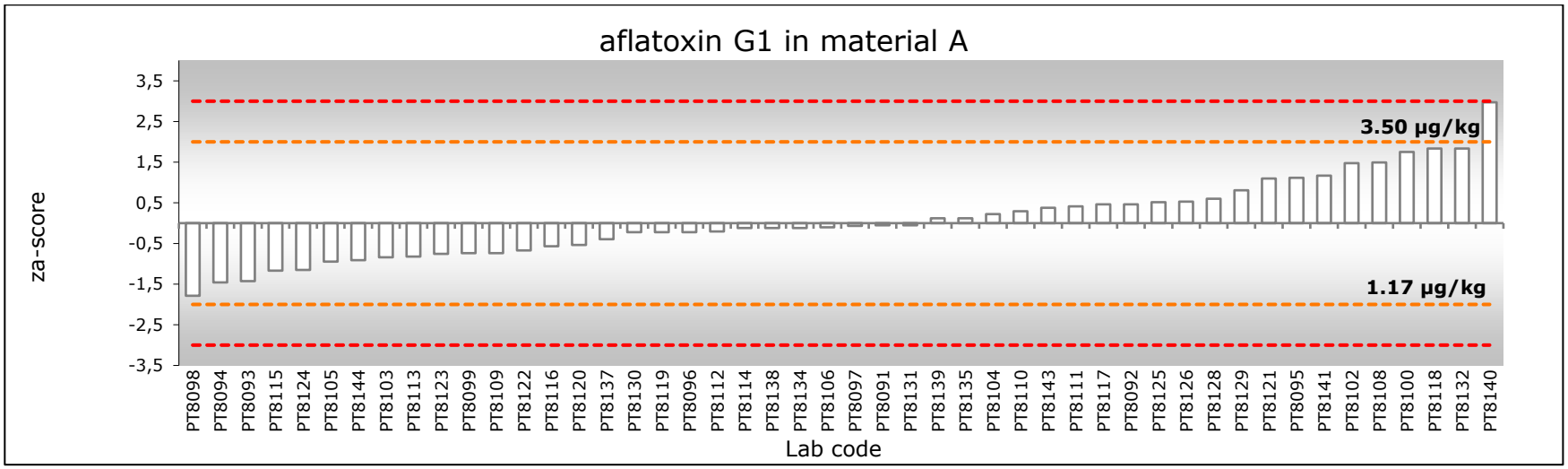

Figure 3 *Graphical representation of the z-scores for aflatoxin G1 in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

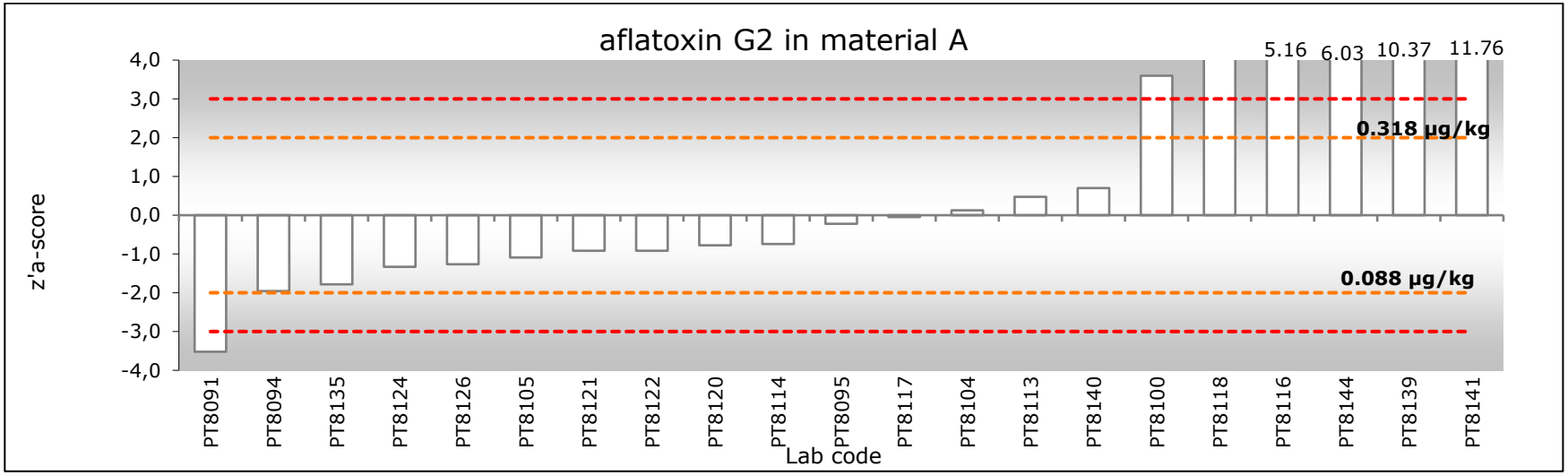

Figure 4 *Graphical representation of the z-scores for aflatoxin G2 in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

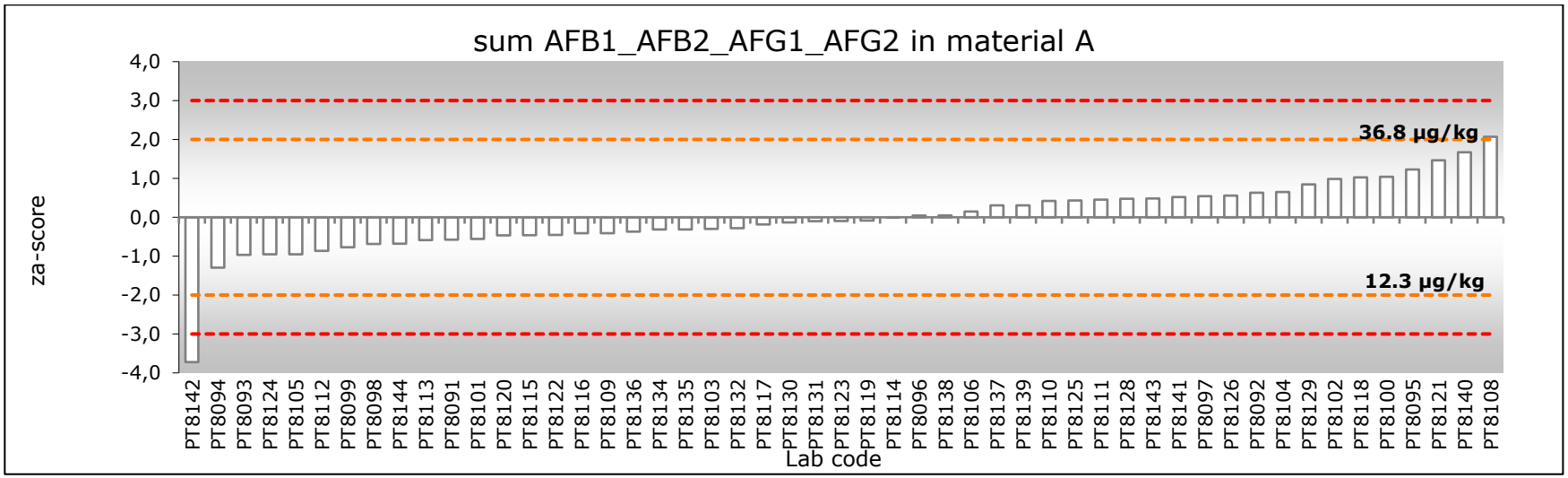

Figure 5 *Graphical representation of the z-scores for the sum aflatoxins in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

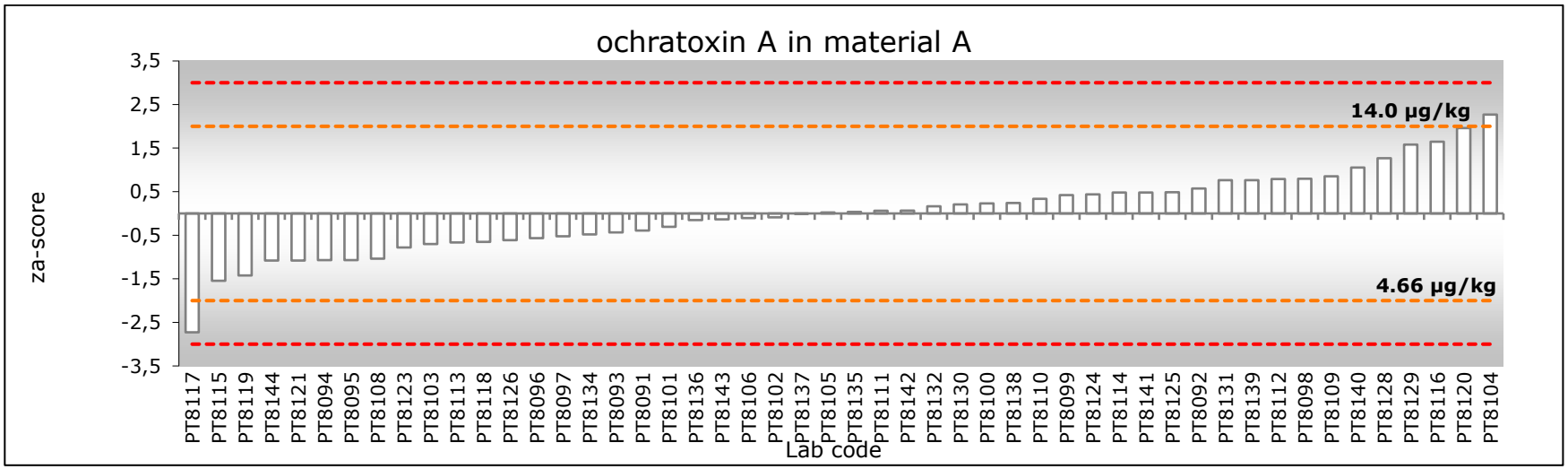

Figure 6 *Graphical representation of the z-scores for ochratoxin A in material A Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 10 Results material B (cocoa powder)

	Material B					
	Aflatoxin B1 **A: 1.54 µg/kg** **u: 0.146 µg/kg** **σ_p: 0.385 µg/kg (25%)** **robust σ: 0.784 µg/kg (50.9%)**		**Aflatoxin B2** **A: 0.459 µg/kg** **u: 0.051 µg/kg** **σ_p: 0.115 µg/kg (25%)** **robust σ: 0.216 µg/kg (47.1%)**		**Aflatoxin G1** **A: 1.56 µg/kg** **u: 0.158 µg/kg** **σ_p: 0.389 µg/kg (25%)** **robust σ: 0.797 µg/kg (51.2%)**	
Lab code	**Result µg/kg**	**z'-score**	**Result µg/kg**	**z'-score**	**Result µg/kg**	**z'-score**
PT8093	1.4	-0.34	<0.5		1.5	-0.14
PT8094	0.43	**-2.69**	0.12	**-2.70**	0.54	**-2.42**
PT8095	<0.1	**[-3.50]FN**	<0.1	**[-2.86]FN**	<0.1	**[-3.47]FN**
PT8096	1.6	0.15	0.2	**-2.06**	1.8	0.58
PT8097	0.56	**-2.38**	0.14	**-2.54**	0.53	**-2.45**
PT8098	< 0.9	[-1.55]	< 0.3	[-1.27]	<0.8	[-1.80]
PT8099	0.75	-1.92	<0.5	[0.33]	0.96	-1.42
PT8100	2.74	**2.92**	1.17	**5.66**	2.27	1.70
PT8102	1.60	0.15	0.63	1.36	1.83	0.65
PT8103	0.3	**-3.01**	<0.2	**[-2.06]FN**	0.37	**-2.83**
PT8104	1.82	0.68	0.51	0.41	2.12	1.34
PT8105	1.64	0.25	0.46	0.01	1.78	0.53
PT8106	0.632	**-2.20**	<0.3	[-1.27]	0.715	**-2.01**
PT8107	1.49	-0.12	<0.42	[-0.31]	1.61	0.13
PT8108	2.7	**2.82**	nt		3.8	**5.34**
PT8109	0.98	-1.36	<0.6		0.93	-1.49
PT8110	1.1	-1.07	<0.3	[-1.27]	0.7	**-2.04**
PT8111	1.88	0.83	<1		<2.5	
PT8112	1.53	-0.02	<1		1.94	0.91
PT8113	1.06	-1.16	0.16	**-2.38**	1.55	-0.02
PT8114	2.03	1.19	0.58	0.96	1.95	0.94
PT8115	1.58	0.10	<0.5		1.44	-0.28
PT8116	0.26	**-3.11**	0.29	-1.34	0.49	**-2.54**
PT8117	2.1	1.36	0.5	0.33	1.6	0.10
PT8118	2.0	1.12	0.55	0.73	1.8	0.58
PT8119	1.6	0.15	0.47	0.09	1.7	0.34
PT8120	0.896	-1.56	0.23	-1.82	1.183	-0.89
PT8121	0.85	-1.67	0.22	-1.90	0.88	-1.61
PT8122	nt		nt		nt	
PT8123	1.72	0.44	0.37	-0.71	1.32	-0.56
PT8124	0.406	**-2.75**	0.125	**-2.66**	0.515	**-2.48**
PT8125	2.28	1.80	0.69	1.84	1.94	0.91
PT8126	0.56	**-2.38**	0.132	**-2.60**	0.493	**-2.53**
PT8127	2.67	**2.75**	0.59	1.04	2.43	**2.08**
PT8129	2.2	1.61	<1.2		2.5	**2.25**
PT8130	1.5	-0.09	0.6	1.12	1.5	-0.14
PT8131	2.67	**2.75**	0.52	0.49	2.55	**2.36**
PT8132	1.4	-0.34	1.4	**7.49**	<0.5	**[-2.52]FN**
PT8134	1.75	0.51	<0.5	[0.33]	1.92	0.86
PT8135	1.6	0.15	0.5	0.33	1.8	0.58
PT8137	1.8	0.64	<2.0		<5.0	**[8.20]**
PT8138	0.5	**-2.52**	<0.5	[0.33]	<0.5	**[-2.52]FN**
PT8139	2.1	1.36	<0.33	[-1.03]	2.2	1.53
PT8140	2.309	1.87	0.449	-0.08	3.189	**3.89**
PT8141	2.38	**2.05**	1.27	**6.46**	2.53	**2.32**
PT8142	1.09	-1.09	<0.33	[-1.03]	<0.44	**[-2.66]FN**
PT8143	2.53	**2.41**	0.56	0.81	2.14	1.39
PT8144	1.76	0.54	0.50	0.33	0.55	**-2.40**

A = consensus value (robust mean).
u = uncertainty of consensus value.
σ_p = target standard deviation for proficiency test.
robust σ = robust (relative) standard deviation based on participants' results.
Participant PT8091, PT8092, PT8101, PT8128 and PT8136 didn' t analysed material B.

	Material B					
	Aflatoxin G2 **A: 1.29 µg/kg** **u: 0.148 µg/kg** **σ_p: 0.323 µg/kg (25%)** **robust σ: 0.719 µg/kg (55.7%)**		**Sum aflatoxins** **A: 4.43 µg/kg** **u: 0.468 µg/kg** **σ_p: 1.11 µg/kg (25%)** **robust σ: 2.51 µg/kg (56.8%)**		**Ochratoxin A** **A: 5.85 µg/kg** **u: 0.276 µg/kg** **σ_p: 1.46 µg/kg (25%)** **robust σ: 1.53 µg/kg (26.2%)**	
Lab code	**Result µg/kg**	**z'-score**	**Result µg/kg**	**z'-score**	**Result µg/kg**	**z-score**
PT8093	<0.5	**[-2.23]FN**	2.9	-1.27	6.7	0.58
PT8094	0.19	**-3.10**	1.28	**-2.62**	4.90	-0.65
PT8095	<0.1	**[-3.36]FN**	<0.1	**[-3.60]FN**	6.24	0.27
PT8096	1.0	-0.82	4.6	0.14	3.3	-1.74
PT8097	0.61	-1.92	1.84	**-2.15**	5.4	-0.31
PT8098	< 0.8	[-1.38]	< 0.9	**[-2.94]FN**	3.39	-1.68
PT8099	<0.5	**[-2.23]FN**	1.7	**-2.27**	5.9	0.04
PT8100	2.03	**2.08**	8.21	**3.15**	7.32	1.01
PT8102	1.36	0.19	5.42	0.83	6.66	0.56
PT8103	0.4	**-2.51**	1.24	**-2.65**	6.10	0.17
PT8104	1.64	0.98	6.09	1.38	7.62	1.21
PT8105	1.33	0.11	5.1	0.56	6.52	0.46
PT8106	0.716	-1.62	2.063	-1.97	5.506	-0.23
PT8107	1.77	1.35	4.87	0.37	6.3	0.31
PT8108	nt		6.5	1.72	3.6	-1.54
PT8109	1.19	-0.29	3.1	-1.10	7.2	0.93
PT8110	1	-0.82	2.8	-1.35	4.4	-0.99
PT8111	<2.5		4.88	0.38	6.35	0.34
PT8112	1.34	0.14	4.81	0.32	6.81	0.66
PT8113	0.45	**-2.37**	3.22	-1.00	4.91	-0.64
PT8114	1.82	1.49	6.38	1.62	6.27	0.29
PT8115	1.11	-0.51	5.62	0.99	4.13	-1.17
PT8116	0.43	**-2.43**	1.47	**-2.46**	4.44	-0.96
PT8117	1.2	-0.26	5.4	0.81	4.0	-1.26
PT8118	2.0	2.00	6.2	1.47	8.5	1.81
PT8119	2.0	2.00	5.7	1.06	3.6	-1.54
PT8120	1.12	-0.48	3.42	-0.84	5.602	-0.17
PT8121	0.43	**-2.43**	2.38	-1.70	2.5	**-2.29**
PT8122	nt		nt		3.73	-1.45
PT8123	0.64	-1.83	4.05	-0.31	3.8	-1.40
PT8124	0.480	**-2.29**	1.526	**-2.41**	6.000	0.10
PT8125	1.77	1.35	6.68	1.87	7.33	1.01
PT8126	0.719	-1.61	1.904	**-2.10**	5.502	-0.24
PT8127	1.88	1.66	7.5	**2.56**	7.0	0.79
PT8129	2.0	2.00	7.3	**2.39**	8.9	**2.09**
PT8130	1.5	0.59	5.1	0.56	6.5	0.45
PT8131	1.75	1.29	7.49	**2.55**	7.18	0.91
PT8132	1.3	0.02	4.1	-0.27	4.0	-1.26
PT8134	1.40	0.31	5.50	0.89	6.4	0.38
PT8135	1.8	1.43	5.7	1.06	5.9	0.04
PT8137	<5.0		1.8	**-2.19**	4.6	-0.85
PT8138	<0.5	**[-2.23]FN**	0.5	**-3.27**	7.08	0.84
PT8139	<0.42	**[-2.45]FN**	4.3	-0.11	7.0	0.79
PT8140	2.897	**4.52**	8.844	**3.67**	6.919	0.73
PT8141	2.62	**3.74**	8.80	**3.64**	8.09	1.53
PT8142	<0.30	**[-2.79]FN**	1.09	**-2.78**	4.27	-1.08
PT8143	2.24	**2.67**	7.47	**2.53**	6.50	0.45
PT8144	0.41	**-2.48**	3.22	-1.00	7.4	1.06

A = consensus value (robust mean).
u = uncertainty of consensus value.
σ_p = target standard deviation for proficiency test.
robust σ = robust (relative) standard deviation based on participants' results.
Participant PT8091, PT8092, PT8101, PT8128 and PT8136 didn' t analysed material B.

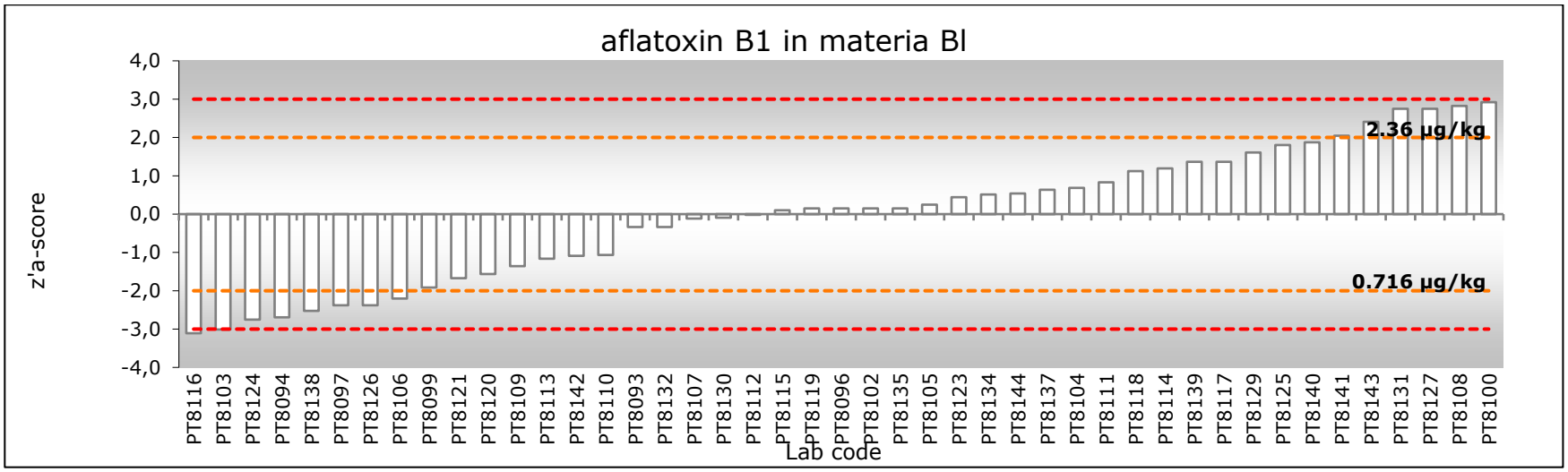

Figure 7 *Graphical representation of the z-scores for aflatoxin B1 in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

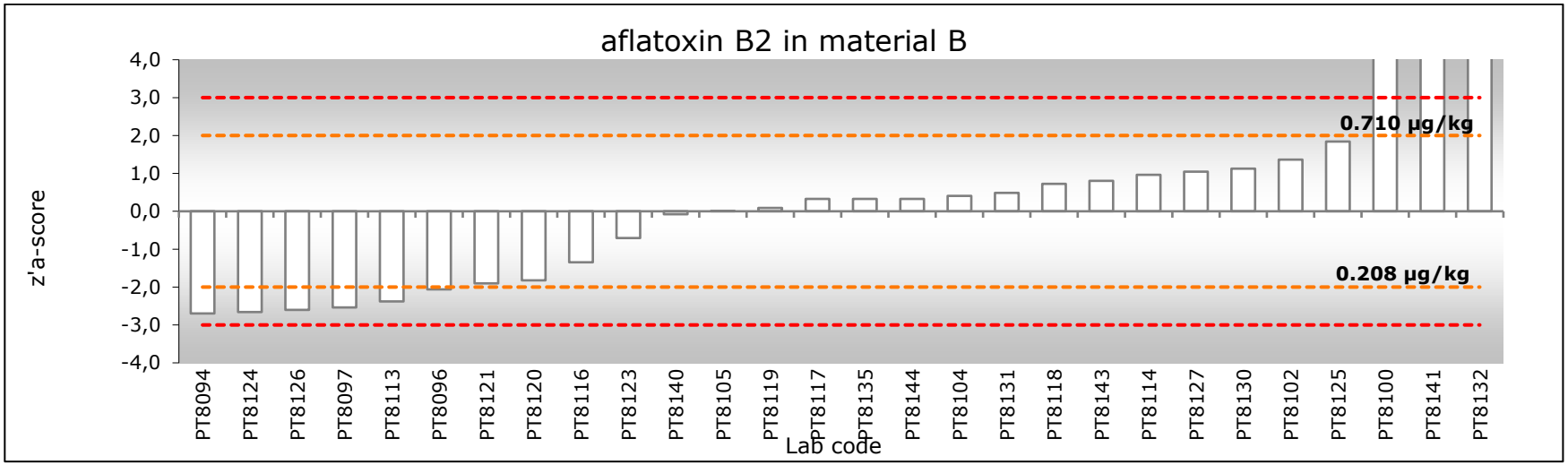

Figure 8 *Graphical representation of the z-scores for aflatoxin B2 in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

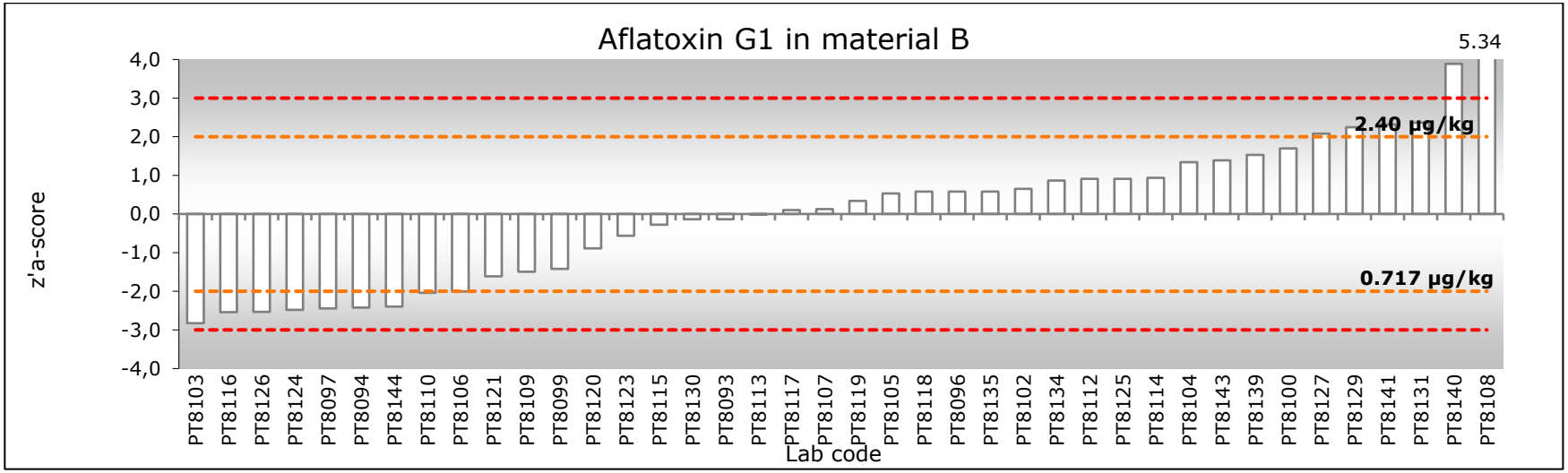

Figure 9 *Graphical representation of the z-scores for aflatoxin G1 in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

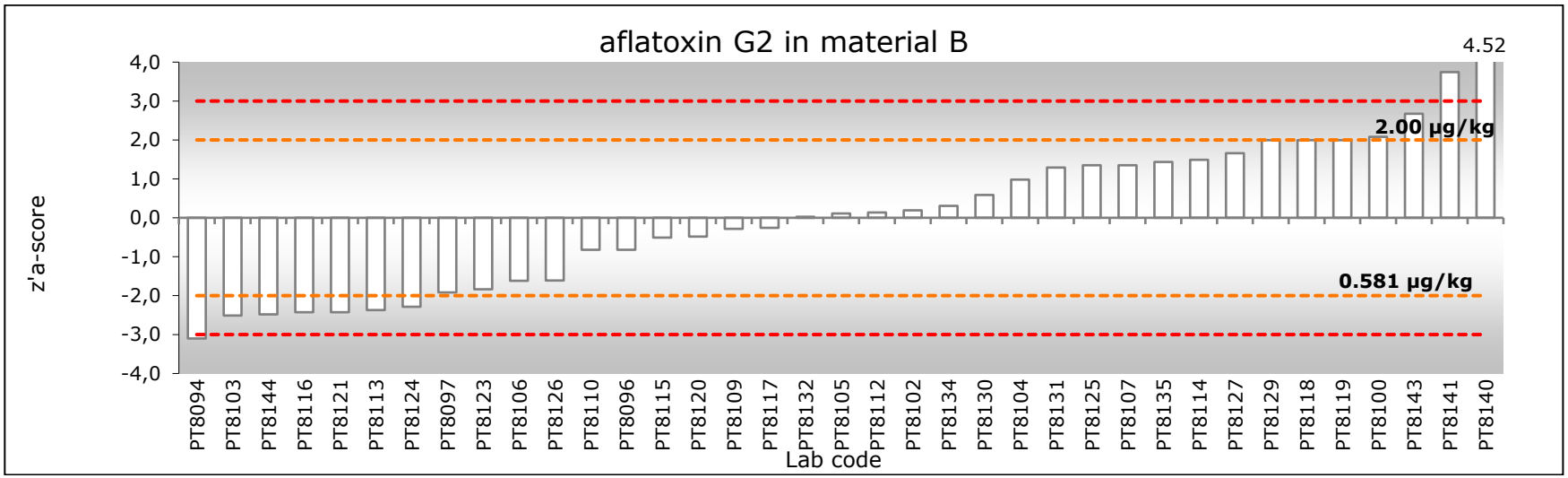

Figure 10 *Graphical representation of the z-scores for aflatoxin G2 in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

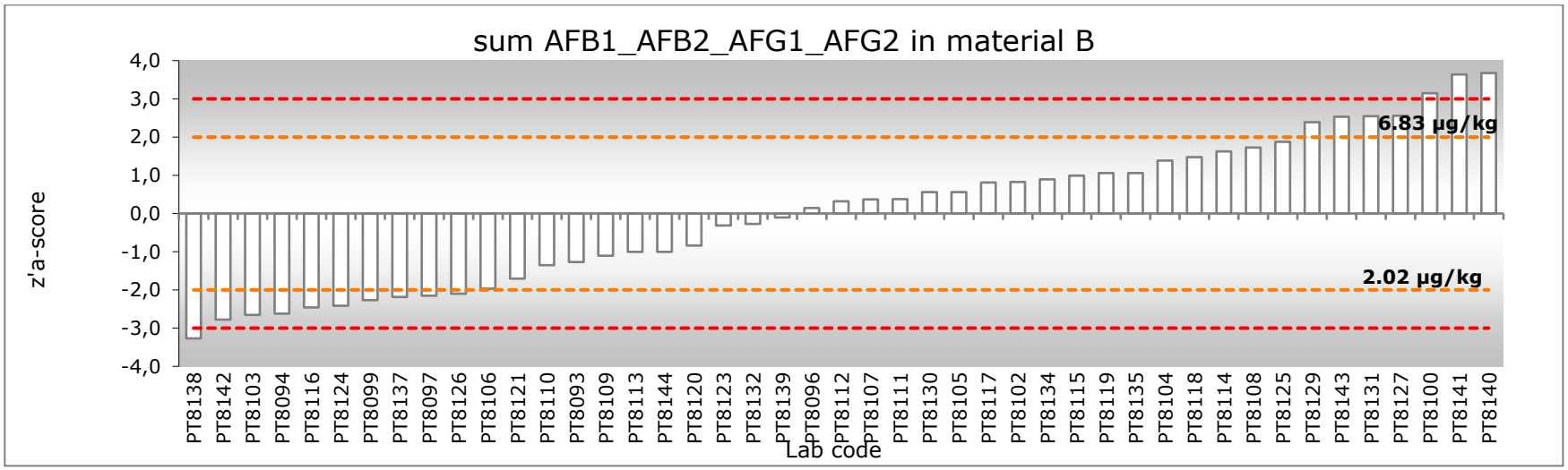

Figure 11 *Graphical representation of the z-scores for the sum aflatoxins in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

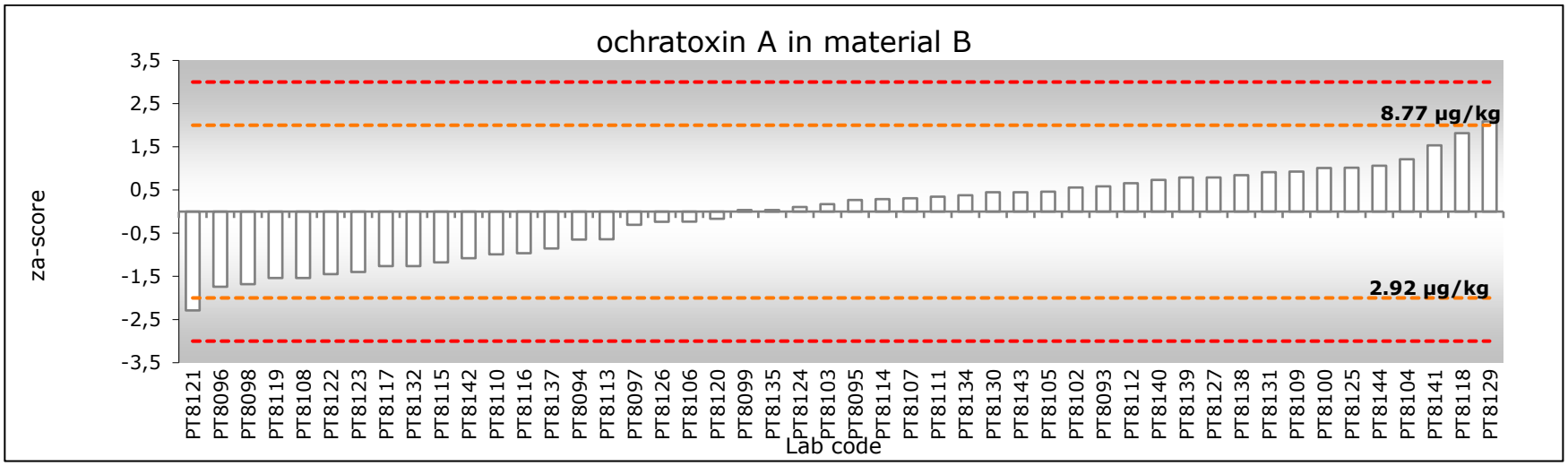

Figure 12 *Graphical representation of the z-scores for ochratoxin A in material B Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3*

Annex 11 Overview performance per laboratory

Lab code	Individual mycotoxins in maize and cocoa Satisfactory performance *	Sum aflatoxins in maize and cocoa Satisfactory performance *	FN
PT8091	4 out of 10	1 out of 2	
PT8092	4 out of 10	1 out of 2	
PT8093	7 out of 10	**2 out of 2**	1
PT8094	6 out of 10	1 out of 2	
PT8095	6 out of 10	1 out of 2	5
PT8096	8 out of 10	**2 out of 2**	
PT8097	6 out of 10	1 out of 2	
PT8098	5 out of 10	1 out of 2	1
PT8099	7 out of 10	1 out of 2	1
PT8100	6 out of 10	1 out of 2	
PT8101	2 out of 10	1 out of 2	
PT8102	9 out of 10	**2 out of 2**	
PT8103	5 out of 10	1 out of 2	1
PT8104	9 out of 10	**2 out of 2**	
PT8105	**10 out of 10**	**2 out of 2**	
PT8106	6 out of 10	**2 out of 2**	
PT8107	4 out of 10	1 out of 2	
PT8108	3 out of 10	1 out of 2	
PT8109	8 out of 10	**2 out of 2**	
PT8110	7 out of 10	**2 out of 2**	
PT8111	5 out of 10	**2 out of 2**	
PT8112	7 out of 10	**2 out of 2**	
PT8113	8 out of 10	**2 out of 2**	
PT8114	**10 out of 10**	**2 out of 2**	
PT8115	8 out of 10**	***2 out of 2***	
PT8116	6 out of 10	1 out of 2	
PT8117	9 out of 10**	***2 out of 2***	
PT8118	8 out of 10	**2 out of 2**	
PT8119	9 out of 10	**2 out of 2**	
PT8120	**10 out of 10**	**2 out of 2**	
PT8121	8 out of 10	**2 out of 2**	
PT8122	5 out of 10	1 out of 2	
PT8123	9 out of 10	**2 out of 2**	
PT8124	6 out of 10	1 out of 2	
PT8125	9 out of 10	**2 out of 2**	
PT8126	7 out of 10	1 out of 2	
PT8127	3 out of 10	0 out of 2	
PT8128	4 out of 10	1 out of 2	
PT8129	6 out of 10	1 out of 2	
PT8130	9 out of 10	**2 out of 2**	
PT8131	7 out of 10	1 out of 2	
PT8132	7 out of 10	**2 out of 2**	1
PT8134	8 out of 10	**2 out of 2**	
PT8135	**10 out of 10**	**2 out of 2**	
PT8136	2 out of 10	1 out of 2	
PT8137	6 out of 10	1 out of 2	
PT8138	5 out of 10	1 out of 2	2
PT8139	6 out of 10	**2 out of 2**	2
PT8140	7 out of 10	1 out of 2	
PT8141	4 out of 10	1 out of 2	
PT8142	3 out of 10	0 out of 2	4
PT8143	7 out of 10	1 out of 2	
PT8144	7 out of 10**	***2 out of 2***	

* Satisfactory performance here means a quantitative result with a satisfactory z-score was obtained for the individual mycotoxins or the total sum of aflatoxins present in material A and B. Results reported as <LOQ are not considered a satisfactory z-score.

** reported results after the deadline.

Lab code	Material A			Material B		
	Individual mycotoxins Satisfactory performance	Sum aflatoxins Satisfactory performance	FN	Individual mycotoxins Satisfactory performance	Sum aflatoxins Satisfactory performance	FN
PT8091	4 out of 5	**1 out of 1**		0 out of 5	0 out of 1	
PT8092	4 out of 5	**1 out of 1**		0 out of 5	0 out of 1	
PT8093	4 out of 5	**1 out of 1**		3 out of 5	**1 out of 1**	1
PT8094	**5 out of 5**	**1 out of 1**		1 out of 5	0 out of 1	
PT8095	**5 out of 5**	**1 out of 1**		1 out of 5	0 out of 1	5
PT8096	4 out of 5	**1 out of 1**		4 out of 5	**1 out of 1**	
PT8097	4 out of 5	**1 out of 1**		2 out of 5	0 out of 1	
PT8098	4 out of 5	**1 out of 1**		1 out of 5	0 out of 1	1
PT8099	4 out of 5	**1 out of 1**		3 out of 5	0 out of 1	1
PT8100	4 out of 5	**1 out of 1**		2 out of 5	0 out of 1	
PT8101	2 out of 5	**1 out of 1**		0 out of 5	0 out of 1	
PT8102	4 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8103	4.out of 5	**1 out of 1**		1 out of 5	0 out of 1	1
PT8104	4 out of 5	**1 out of 1**		**5.out of 5**	**1 out of 1**	
PT8105	**5 out of 5**	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8106	4 out of 5	**1 out of 1**		2 out of 5	**1 out of 1**	
PT8107	0 out of 5	0 out of 1		4 out of 5	**1 out of 1**	
PT8108	2 out of 5	0 out of 1		1 out of 5	**1 out of 1**	
PT8109	4 out of 5	**1 out of 1**		4 out of 5	**1 out of 1**	
PT8110	4 out of 5	**1 out of 1**		3 out of 5	**1 out of 1**	
PT8111	3 out of 5	**1 out of 1**		2 out of 5	**1 out of 1**	
PT8112	3 out of 5	**1 out of 1**		4 out of 5	**1 out of 1**	
PT8113	**5 out of 5**	**1 out of 1**		3 out of 5	**1 out of 1**	
PT8114	**5 out of 5**	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8115	4 out of 5**	**1 out of 1****		4 out of 5**	**1 out of 1***	
PT8116	4 out of 5	**1 out of 1**		2 out of 5	0 out of 1	
PT8117	4 out of 5**	**1 out of 1****		**5 out of 5****	**1 out of 1****	
PT8118	3 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8119	4 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8120	**5 out of 5**	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8121	**5 out of 5**	**1 out of 1**		3 out of 5	**1 out of 1**	
PT8122	4 out of 5	**1 out of 1**		1 out of 5	0 out of 1	
PT8123	4 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8124	**5 out of 5**	**1 out of 1**		1 out of 5	0 out of 1	
PT8125	4 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8126	**5 out of 5**	**1 out of 1**		2 out of 5	0 out of 1	
PT8127	0 out of 5	0 out of 1		3 out of 5	0 out of 1	
PT8128	4 out of 5	**1 out of 1**		0 out of 5	0 out of 1	
PT8129	4 out of 5	**1 out of 1**		2 out of 5	0 out of 1	
PT8130	4 out of 5	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8131	4 out of 5	**1 out of 1**		3 out of 5	0 out of 1	
PT8132	4 out of 5	**1 out of 1**		3 out of 5	**1 out of 1**	1
PT8134	4 out of 5	**1 out of 1**		4 out of 5	**1 out of 1**	
PT8135	**5 out of 5**	**1 out of 1**		**5 out of 5**	**1 out of 1**	
PT8136	2 out of 5	**1 out of 1**		0 out of 5	0 out of 1	
PT8137	4 out of 5	**1 out of 1**		2 out of 5	0 out of 1	
PT8138	4 out of 5	**1 out of 1**		1 out of 5	0 out of 1	2
PT8139	3 out of 5	**1 out of 1**	1	3 out of 5	**1 out of 1**	1
PT8140	4 out of 5	**1 out of 1**		3 out of 5	0 out of 1	
PT8141	3 out of 5	**1 out of 1**		1 out of 5	0 out of 1	
PT8142	1 out of 5	0 out of 1	2	2 out of 5	0 out of 1	2
PT8143	4 out of 5	**1 out of 1**		3 out of 5	0 out of 1	
PT8144	4 out of 5**	**1 out of 1****		3 out of 5**	**1 out of 1****	

* Satisfactory performance here means a quantitative result with a satisfactory z-score was obtained for the individual mycotoxins or the total sum of aflatoxins present in material A and B. Results reported as <LOQ are not considered a satisfactory z-score.

** reported results after the deadline.

Participants PT8091, PT8092, PT8101, PT8128 and PT8136 analysed only material A and participant PT8107 and PT8127 analysed only material B.

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