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Report on the 2016 Proficiency Test of the European Union Reference Laboratory for Mycotoxins for the network of National Reference Laboratories

Determination of aflatoxin B1 in defatted peanut powder

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Contents

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Table 1: Participating laboratories

Organisation	Country
AGES GmbH	Austria
LVA GmbH	Austria
CODA-CERVA	Belgium
Central Laboratory for Chemical Testing and Control, BFSA	Bulgaria
E.C. Inspekt d.o.o.	Croatia
Andrija Stampar Teaching Institute for Public Health	Croatia
State General Laboratory	Cyprus
UKZUZ - Central Institute for Supervising and Testing in Agriculture	Czech Republic
Czech Agriculture and Food Inspection Authority (CAFIA)	Czech Republic
DTU Food	Denmark
Danish Veterinary and Food Administration	Denmark
Agricultural Research Centre, laboratory for Residues and Contaminants	Estonia
Central Chemistry Laboratory of Health Board	Estonia
Finnish Customs Laboratory	Finland
Laboratoire SCL de Rennes	France
LUFA-ITL GmbH	Germany
CVUA Westfalen - Standort Hamm -	Germany
Federal Institute for Risk Assessment (BfR)	Germany
Feedstuffs control laboratory of Athens – Ministry of rural development and food	Greece
Chemical State Laboratory, Division of Piraeus and Aegean	Greece
National Food Chain Safety Office, Food And Feed Safety Directorate, Food Toxicological NRL	Hungary
National Food Chain Safety Office, Food and Feed Safety Directorate, Feed Investigation NRL	Hungary
The State Laboratory	Ireland
Public Analyst's Laboratory	Ireland
Istituto Superiore di Sanita'	Italy
Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia
National Food And Veterinary Risk Assessment Institute	Lithuania
Laboratoire national de santé	Luxembourg
Public Health Laboratory	Malta
RIKILT - Wageningen UR	Netherlands
The Norwegian Veterinary Institute	Norway
National Institute of Public Health - National Institute of Hygiene	Poland
National Veterinary Research Institute	Poland
ASAE - DRAL - LFQ	Portugal
Sanitary Veterinary and Food Safety Directorate Bucharest	Romania
DSVSA GALATI - LSVSA	Romania
Hygiene and Veterinary Public Health Institute	Romania
SP Laboratorija A.D.	Serbia
Regional Public Health Authority - RUVZ so sídlom v Poprade	Slovakia
State veterinary and food institute Dolný Kubín, Veterinary and food institute in Košice	Slovakia
University of Ljubljana, Veterinary Faculty, National Veterinary Institute	Slovenia
National laboratory for health, environment and food	Slovenia
Laboratorio de Salud Pública de Albacete (Junta de Comunidades de Castilla-La Mancha)	Spain
National Centre for food – Spanish consuming, food safety and nutrition agency	Spain
National Food Agency	Sweden
National Veterinary Institute, SVA	Sweden

Organisation	Country
Worcestershire Scientific Services	UK
Edinburgh Scientific Services	UK
Kent Scientific Services	UK
Tayside Scientific Services	UK
West Yorkshire Joint Services	UK
Public Analyst Scientific Services Ltd (Norwich)	UK
Hampshire Scientific Services	UK
Fera Science Ltd.	UK

Abstract

The Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the European Union Reference Laboratory (EURL) for Mycotoxins. One of its core tasks is to organise proficiency tests (PTs) among appointed National Reference Laboratories (NRLs).

This report presents the results of the PT on the determination of aflatoxins in defatted peanut powder.

The test items for this PT were two contaminated defatted peanut powder samples. The materials were produced by the JRC and dispatched to the participants mid October 2016. Each participant received one bottle per test material containing approximately 55 g each.

Fifty-six participants from thirty countries (among them 40 NRLs and 16 official food control laboratories) registered for the exercise and 54 sets (Sample A and B) of results were reported.

The assigned values, established by an exact-matching double isotope dilution mass spectrometric technique, were 2.80 µg/kg (± 0.19 µg/kg) aflatoxin B1 in sample A and 3.20 µg/kg (± 0.20 µg/kg) in sample B.

Participants' results were rated with z-scores and zeta-scores for aflatoxin B1 in accordance with ISO 13528:2015. The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the PT, whereas the zeta-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value.

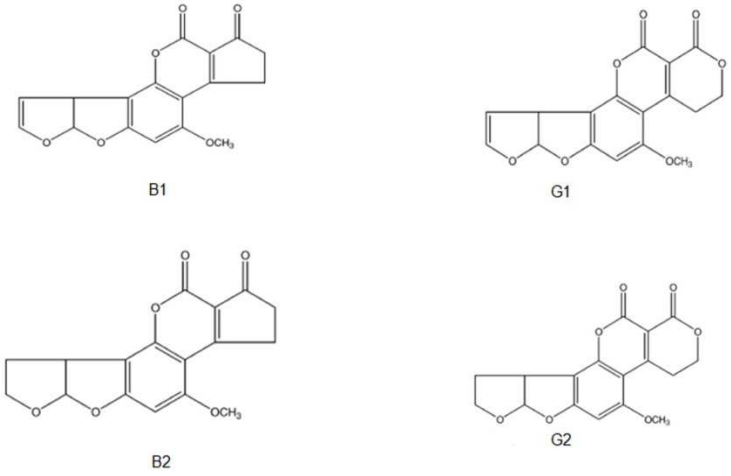
Only z-scores were used for the evaluation whether an individual laboratory underperformed. In total, 96 % of the attributed z-scores were below an absolute value of two for sample A and and 92 % for sample B. This indicates that most of the participants performed satisfactorily. The few participants that had z-scores above an absolute value of 2 will have to investigate the reasons for the deviation (root-cause analysis) and report the planned corrective actions to the EURL.

1 Introduction

Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. *A. flavus* produces aflatoxins B only, while the other species grow both aflatoxin B and aflatoxin G. They contaminate mainly maize and peanuts, but also other food and feed materials like nuts and spices.

Due to their carcinogenic, hepatotoxic, teratogenic and mutagenic effect in humans and farm animals (Wild and Turner, 2002) aflatoxins are a threat to public health. Aflatoxin B1 (Figure 1) is the most potent hepatocarcinogen known in mammals and it is classified by the International Agency of Research on Cancer (IARC, 1993) as a Group 1 carcinogen.

Figure1: Formula of Aflatoxin B1, Aflatoxin G1, Aflatoxin B2 and Aflatoxin G2



The most frequently used method for aflatoxin determination is high performance liquid chromatography (HPLC) with Kobra Cell derivatisation and fluorescence detection (Yao *et al.,* 2015; EN 14123:2003). Other successfully employed methods for the determination of aflatoxins are liquid chromatography coupled to mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS). Screening methods, like enzyme-linked immuno sorbent assay (ELISA) (Ardic *et al*., 2008) and immunochromatography (Shim *et al*., 2007) can be used for the determination of total aflatoxins.

Peanuts are a valuable source of vegetable proteins and often the oil is used for human consumption while the protein remainder is fed to animals. The maximum levels of aflatoxin B1 in animal feed are laid down in Directive 2002/32/EC.

2 Scope

As stated in Article 32 of Regulation (EC) No 882/2004, one of the core duties of the EURL is to organise proficiency tests (PTs) for the benefit of staff of NRLs. The scope of this PT was to test the competence of the appointed NRLs to determine the amount of aflatoxin B1 in defatted peanut powder. All invited laboratories were allowed to use their method of choice.

The EURL Mycotoxins performed the assessment of the measurement results on the basis of requirements laid down in legislation and followed the administrative and logistic procedures of ISO/IEC 17043:2010. The PT activities of the EURL are performed under ISO/IEC 17043:2010 accreditation.

2.1 Confidentiality

Confidentiality of the participant's results towards third parties is guaranteed by transmission of data through a dedicated web-based interface and a secure databank hosted by the JRC. European Commission rules on data protection were strictly followed as well.

3 Time Frame

The PT was opened for registration on 1th of July 2016 and the deadline for registration was 19th of July 2016. (Annex 1) The samples were dispatched to the participants on 18th and 19th of October 2016. The deadline for reporting the results was the 9th of December 2016.

4 Material

4.1 Preparation

The test materials used in this study were one naturally contaminated peanut powder (sample A) and one spiked peanut powder (sample B). The material for sample A was available at the JRC as fine powder. It was homogenised in a tumble mixer prior designation as test material.

The material for sample B was prepared by fortifying a small portion of peanut powder with an aliquot of a solution of all 4 aflatoxins. This fortified portion was blended with blank material. This blend was subsequently ground with a Retsch ZM 200 ultra-centrifugal mill, using sieves size of 1.0 mm and 0.5 mm.

Both materials were packed in amber plastic containers, taking portions from different places of the lot at random. The total sample size was ca. 55 g.

4.2 Homogeneity

To verify the homogeneity of the test materials, 10 units per material (Sample A and Sample B) were randomly selected. Two independent determinations per bottle were performed using a liquid chromatography isotope dilution tandem mass spectrometry detection (LC-ID-MS/MS) based method. The order of measurements of the batch was randomised. Homogeneity was evaluated according to the International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (Thompson *et al.*, 2006). The materials proved to be adequately homogeneous (Annex 2).

4.3 Stability

The stability study was conducted following an isochronous experimental design (Lamberty *et al.*, 1995); -18 °C was chosen as reference temperature for sample storage. Stability was evaluated according to the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (Thompson *et al.*, 2006). The materials proved to be adequately stable at 25 °C, 4 °C and -18 °C for 6 weeks. The results of the study are listed in Annex 3.

4.4 Distribution

The test materials were dispatched at ambient temperature on 18th and 19th of October 2016. The samples were mostly received within 24 hours after dispatch.

Each participant received:

a) two units containing approximately 55 g of each test material

b) an accompanying letter with instructions on sample handling and reporting (Annex 4)

c) a material receipt form (Annex 5)

d) laboratory specific reporting files with a lab code by email

5 Instruction to participants

The laboratories were asked to report the recovery corrected levels as well as their expanded measurement uncertainty in µg/kg (coverage factor k=2) for the aflatoxins they are analysing in their laboratory on a routine basis.

Results were reported by the participants using the RingDat software, which is part of the ProLab software. Laboratory specific files generated by the ProLab software were sent to each laboratory individually (personal files) by email. A specific questionnaire was also included. The questionnaire was intended to provide further information on method and laboratory details to allow conclusions on possible individual and common effects observed for possible follow-up procedures. A copy of the questionnaire is shown in Annex 6.

Participants received the information that the materials were shipped at ambient temperature and that upon arrival the materials needed to be stored immediately at -18 °C until the analysis is performed.

6 Reference values and their uncertainties

The assigned values of aflatoxin B1 and their uncertainties for the test samples were established by "Exact-Matching Double Isotope Dilution Mass Spectrometry" at the JRC. This methodology is considered to provide the highest degree of accuracy of the assigned value and traceability to SI units.(Mackay *et al*. 2003). The assigned values were 2.80 µg/kg (Sample A) and 3.20 µg/kg (Sample B) for aflatoxin B1. The expanded uncertainties (k=2) of the respective assigned values were 0.19 µg/kg and 0.20 µg/kg. Other aflatoxins are not regulated for animal feed. As a result the reported values for other aflatoxins than aflatoxin B1 are reported in Tables 4 and 5 for information only.

7 Evaluation of the results

7.1 General observations

Fifty-six participants from 27 countries (among them 40 NRLs and 16 official food control laboratories) registered to the exercise [Table 3] and 54 sets of results were reported.

The laboratories were free to use their method of choice. Forty-one laboratories used HPLC-FLD, eleven analysed the samples with LCMS and two used ELISA.

7.2 Scores and evaluation criteria

Individual laboratory performance was assessed in terms of z and zeta (ζ) scores in accordance with ISO 13528:2015 and the IUPAC International Harmonised Protocol.

$$z = \frac{x_{lab} - x_{ref}}{\sigma_p} \qquad \textbf{Equation 1}$$

$$\zeta = \frac{x_{Lab} - x_{ref}}{\sqrt{u_{lab}^2 + u_{ref}^2}} \qquad \textbf{Equation 2}$$

where:

x_{lab} is the measurement result reported by a participant

x_{ref} is the reference value (assigned value)

u_{lab} is the standard uncertainty reported by a participant

u_{ref} is the standard uncertainty of the reference value

σ_p is the standard deviation for proficiency assessment (target standard deviation)

σ_p was calculated using the Horwitz equation, modified by Thompson [10] for analyte concentrations < 120 µg/kg:

- for analyte concentration < 120 µg/kg

$$\sigma_p = 0.22\ C \qquad \textbf{Equation 3}$$

where:

C is the concentration of the measurand (assigned value, x_{ref},) expressed as a dimensionless mass ratio, e.g. 1 µg/kg = 10^{-9}, 1 mg/kg = 10^{-6}

The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, σ_p. The z-score is interpreted as:

$\|z\| \leq 2$	indicates satisfactory performance
$2 < \|z\| < 3$	indicates questionable performance
$\|z\| \geq 3$	indicates unsatisfactory performance

The zeta (ζ)-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value. The ζ-score is the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value, its uncertainty as well as the uncertainty of the assigned values.

The interpretation of the ζ-score is similar to the interpretation of the z-score:

$\|\zeta\| \leq 2$	indicates satisfactory performance
$2 < \|\zeta\| < 3$	indicates questionable performance
$\|\zeta\| \geq 3$	indicates unsatisfactory performance

An unsatisfactory performance based on a |ζ|-score ≥3 might be due to an underestimation of the uncertainty, or to a large deviation from the reference value, or to a combination of the two factors.

7.3 Laboratory results and scoring

Statistical evaluation of the results was performed using MS Excel and ProLab software 8. The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528:2015

Summaries of the statistical evaluation for Aflatoxin B1 are presented in Table 2.
The calculated z-and ζ-scores for aflatoxin B1 in sample A and sample B are listed in Table 3.
Graphs with the z-and ζ-scores are in Annex 7.

Table 2. Summary statistics of the results for aflatoxin B1 in sample A & sample B.

Aflatoxin B1		**Sample A**	**Sample B**
Number of results		54	54
Range of results	µg/kg	0.19 - 3.9	0.22 - 4.36
Robust mean of results of participants	µg/kg	2.37	2.85
Assigned value	µg/kg	2.80	3.20
Expanded uncertainty (k=2) of the assigned value	µg/kg	0.19	0.20
standard deviation for proficiency assessment σ_p	µg/kg	0.62	0.70
Number (percentage) of results |z| > 2.0		4 (7 %)	2 (4 %)

Table 3. Analytical results, z-and ζ-scores for aflatoxin B1 in sample A and sample B. Colour code: green- satisfactory performance, yellow – questionable performance, red – unsatisfactory performance. Laboratories that didn't report any results are marked as "No result"

	Sample A Aflatoxin B1					Sample B Aflatoxin B1				
Lab code	**Sample A µg/ kg**	**U lab (k=2)**	**u lab**	**Z-Score**	**ζ-score**	**Sample B µg/ kg**	**U lab**	**u lab**	**Z-Score**	**ζ-score**
LC0002	2.11	0.3	0.15	-1.1	-3.9	2.51	0.30	0.15	-1.0	-3.8
LC0003	2.47	0.49	0.24	-0.5	-1.3	2.82	0.56	0.28	-0.5	-1.3
LC0004	2.93	0.88	0.44	0.2	0.3	3.30	0.99	0.49	0.1	0.2
LC0005	2.90	0.87	0.43	0.2	0.2	3.5	1.10	0.55	0.4	0.5
LC0006	2.36	0.6	0.3	-0.7	-1.4	2.88	0.73	0.36	-0.5	-0.8
LC0007	1.68	0.3	0.15	-1.8	-6.3	2.68	0.48	0.24	-0.7	-2.0
LC0008	3.07	0.98	0.49	0.4	0.5	3.66	1.20	0.60	0.7	0.8
LC0009	3.90	0.78	0.39	1.8	2.7	3.3	0.67	0.33	0.1	0.3
LC0010	1.21	0.5	0.25	-2.6	-5.9	1.94	0.80	0.40	-1.8	-3.1
LC0011	2.38	0.38	0.19	-0.7	-2	2.89	0.46	0.23	-0.4	-1.2
LC0012	3.00	1.2	0.6	0.3	0.3	3.4	1.40	0.70	0.3	0.3
LC0013	No result	No result	No result	No result	No result	No result	No result	No result	No result	No result
LC0014	2.57	0.07	0.04	-0.4	-2.3	3.06	0.12	0.06	-0.2	-1.2
LC0015	3.17	1.27	0.64	0.6	0.6	3.49	1.39	0.70	0.4	0.4
LC0016	2.23	0.59	0.29	-0.9	-1.8	2.83	0.72	0.36	-0.5	-1.0
LC0017	1.84	0.5	0.25	-1.6	-3.6	2.68	0.72	0.36	-0.7	-1.4
LC0018	3.27	1.9	0.95	0.8	0.5	3.97	2.30	1.15	1.1	0.7
LC0019	2.63	0.7	0.35	-0.3	-0.5	2.9	0.77	0.39	-0.4	-0.8
LC0020	No result	No result	No result	No result	No result	No result	No result	No result	No result	No result
LC0022	2.23	0.85	0.42	-0.9	-1.3	2.72	1.04	0.52	-0.7	-0.9
LC0023	1.83	0.32	0.16	-1.6	-5.2	2.25	0.40	0.20	-1.3	-4.2
LC0024	1.67	0.42	0.21	-1.8	-4.9	1.83	0.46	0.23	-1.9	-5.5
LC0025	3.41	0.51	0.25	1	2.2	2.82	0.42	0.21	-0.5	-1.6
LC0026	2.01	0.4	0.2	-1.3	-3.6	2.64	0.53	0.27	-0.8	-2.0
LC0027	1.68	0.67	0.34	-1.8	-3.2	2.05	0.82	0.41	-1.6	-2.7
LC0028	3.84	0.36	0.18	1.7	5.1	4.36	0.36	0.18	1.6	5.6
LC0029	1.97	0.77	0.39	-1.3	-2.1	2.68	1.07	0.54	-0.7	-1.0
LC0030	1.80	0.36	0.18	-1.6	-4.9	2.15	0.43	0.21	-1.5	-4.4
LC0031	0.19	0.06	0.03	-4.2	-26.3	0.22	0.07	0.03	-4.2	-28.3
LC0032	1.80	0.58	0.29	-1.6	-3.3	3.8	1.21	0.60	0.9	1.0
LC0033	2.65	0.7	0.35	-0.3	-0.4	3.09	0.82	0.41	-0.2	-0.3
LC0035	2.29	0.71	0.35	-0.8	-1.4	3.06	0.95	0.47	-0.2	-0.3
LC0036	2.90	0.5	0.25	0.2	0.4	3.48	0.63	0.31	0.4	0.8
LC0037	2.62	1.05	0.52	-0.3	-0.3	3.14	1.26	0.63	-0.1	-0.1
LC0038	2.65	0.8	0.4	-0.2	-0.4	3.22	1.00	0.50	0.0	0.0
LC0039	1.12	0.49	0.24	-2.7	-6.4	1.25	0.55	0.28	-2.8	-6.7
LC0040	2.56	0.38	0.19	-0.4	-1.1	2.91	0.44	0.22	-0.4	-1.2
LC0041	2.04	0.14	0.07	-1.2	-6.4	2.42	0.11	0.06	-1.1	-6.8
LC0042	2.79	0.61	0.3	0	0	3.43	0.75	0.38	0.3	0.6
LC0043	2.09	0.63	0.32	-1.2	-2.2	1.92	0.58	0.29	-1.8	-4.2
LC0044	2.78	0.67	0.34	0	-0.1	3.63	0.87	0.43	0.6	1.0
LC0045	2.10	0.8	0.4	-1.1	-1.7	2.5	1.00	0.50	-1.0	-1.4
LC0046	3.20	0.3	0.15	0.6	2.3	4.2	0.40	0.20	1.4	4.5
LC0047	2.11	0.63	0.32	-1.1	-2.1	2.70	0.81	0.41	-0.7	-1.2
LC0048	1.87	0.09	0.05	-1.5	-8.8	2.30	0.12	0.06	-1.3	-7.8
LC0049	2.30	0.23	0.12	-0.8	-3.4	4.3	0.65	0.32	1.6	3.3
LC0050	2.50	0.4	0.2	-0.5	-0.6	2.4	0.40	0.20	-1.1	-1.6
LC0051	1.50	0.75	0.37	-2.1	-3.4	1.77	0.89	0.44	-2.0	-3.1

	Sample A Aflatoxin B1					Sample B Aflatoxin B1				
Lab code	Sample A µg/ kg	U lab (k=2)	u lab	Z-Score	ζ-score	Sample B µg/ kg	U lab	u lab	Z-Score	ζ-score
LC0052	2.35	0.73	0.36	-0.7	-1.2	2.95	0.92	0.46	-0.4	-0.5
LC0053	3.00	1.2	0.6	0.3	0.3	3.2	1.30	0.65	0.0	0.0
LC0054	2.00	0.27	0.14	-1.3	-4.8	2.2	0.30	0.15	-1.4	-5.5
LC0055	1.80	0.13	0.07	-1.6	-8.7	2.19	0.16	0.08	-1.4	-7.9
LC0056	2.31	0.35	0.17	-0.8	-2.5	2.37	0.36	0.18	-1.2	-4.0
LC0057	2.81	1.52	0.76	0	0	3.36	1.77	0.89	0.2	0.2

Table 4. Summary of the results for aflatoxin B2 in sample A & aflatoxin B2, aflatoxin G1 and aflatoxin G2 in sample B.

		Sample A Aflatoxin B2	Sample B Aflatoxin B2	Sample B Aflatoxin G1	Sample B Aflatoxin G2
Number of results		54	54	54	54
Range of results	µg/kg	0.09 – 0.63	0 – 1.9	0 – 3	0 – 2.1
Robust mean of results of participants	µg/kg	0.18	0.67	1.56	0.63

Table 5. Analytical results for aflatoxins B2 in sample A and aflatoxin B2, aflatoxin G1 and aflatoxin G2 in sample B. Laboratories that didn't report any results are marked as "No result", all other results are the figures that the laboratories reported.

	Sample A Aflatoxin B2	Sample B Aflatoxin B2	Sample B Aflatoxin G1	Sample B Aflatoxin G2
Lab code	µg/kg	µg/kg	µg/kg	µg/kg
LC0002	0.15	0.61	1.31	0.57
LC0003	0.22	0.66	1.62	0.91
LC0004	0	0	0	0
LC0005	< 0.50	0.9	1.8	0.7
LC0006	< 0.50	0.72	1.37	0.72
LC0007	No result	No result	No result	No result
LC0008	< 0.20	0.78	2.04	0.73
LC0009	< 2.50	< 2.50	< 2.50	< 2.50
LC0010	< 0.10	0.48	1.44	0.56
LC0011	< 0.20	0.66	1.59	0.53
LC0012	< 0.50	0.8	1.7	0.8
LC0013	No result	No result	No result	No result
LC0014	0.17	0.79	1.62	0.7
LC0015	< 5.00	< 5.00	1.57	< 5.00
LC0016	0.16	0.7	1.33	0.65
LC0017	< 0.25	0.6	1.44	0.58
LC0018	0.63	1.28	2.17	0.7
LC0019	0.17	0.75	1.67	0.7
LC0020	No result	No result	No result	No result
LC0022	0.17	0.64	1.56	0.6
LC0023	0.09	0.48	1.32	0.39
LC0024	0.16	0.66	1.39	0.61
LC0025	0.19	0.65	1.46	0.58
LC0026	0.12	0.56	1.69	0.51
LC0027	0.11	0.47	1.1	0.3

	Sample A Aflatoxin B2	Sample B Aflatoxin B2	Sample B Aflatoxin G1	Sample B Aflatoxin G2
Lab code	µg/kg	µg/kg	µg/kg	µg/kg
LC0028	0.51	1.88	2.22	0.55
LC0029	0.19	0.67	1.19	0.37
LC0030	0.11	0.44	1.23	0.38
LC0031	0.13	0.06	0.11	0.02
LC0032	0.2	0.9	1.9	0.7
LC0033	< 0.20	0.49	1.28	0.48
LC0035	0.19	0.74	1.49	0.61
LC0036	0.27	0.81	1.7	0.65
LC0037	0.19	0.63	1.49	0.42
LC0038	0.28	0.88	1.88	0.67
LC0039	0.1	0.36	0.85	0.38
LC0040	0.35	0.99	2.56	1.77
LC0041	0.14	0.54	1.23	0.44
LC0042	0.16	0.82	1.88	0.77
LC0043	< 0.30	1.92	2.23	2.06
LC0044	0.19	0.82	2.08	0.77
LC0045	< 0.20	0.6	1.3	0.5
LC0046	0.29	1	2	1.1
LC0047	< 0.30	0.66	1.47	0.58
LC0048	0.15	0.54	1.35	0.57
LC0049	0.12	0.83	3	1.2
LC0050	0.22	0.62	1.73	0.52
LC0051	< 0.20	0.43	0.87	0.33
LC0052	0.16	0.69	1.75	0.6
LC0053	< 1.00	< 1.00	1.8	< 2.50
LC0054	0.14	0.49	1.25	0.47
LC0055	0.11	0.52	1.06	0.39
LC0056	0.2	0.74	1.24	0.64
LC0057	0.27	0.77	1.64	< 1.00

For the 2 laboratories using ELISA the z-scores for total aflatoxins were calculated with the assigned value for aflatoxin B1 and the robust mean from all the other participants for aflatoxin B2, aflatoxin G1 and aflatoxin G2 for indicative purposes.

Table 6. Analytical results and z-scores for aflatoxins in sample A and in sample B.

	Sample A AFLA total		Sample B AFLA total	
Lab code	µg/kg	Z-Score	µg/kg	Z-Score
LC0021	4.14	1.8	5.10	-0.7
LC0034	3.15	0.3	4.52	1.1

Figure 1. Sigmoidal plot of individual results reported for aflatoxin B1 in sample A

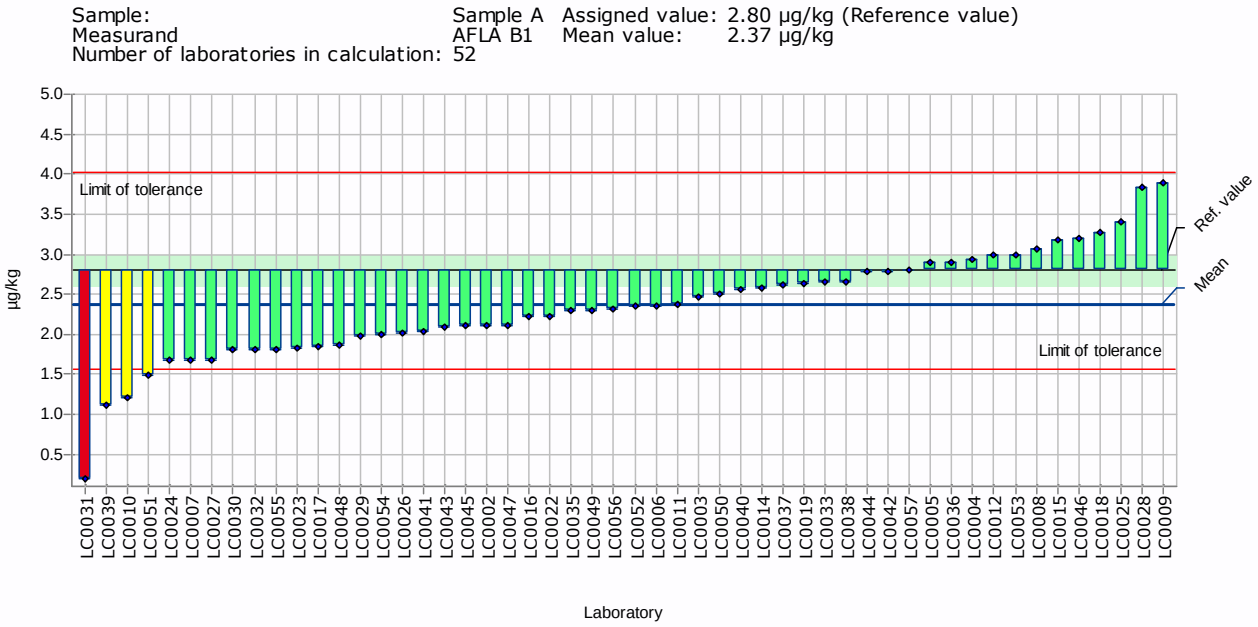
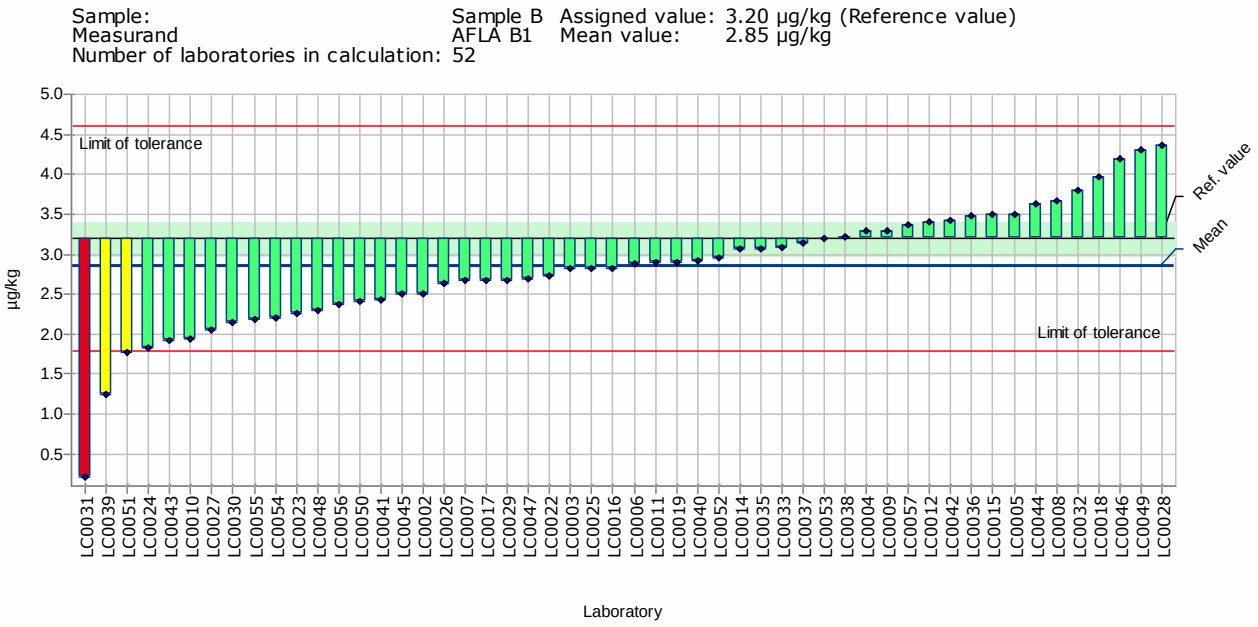


Figure 2. Sigmoidal plot of individual results reported for aflatoxin B1 in sample B



7.4 Evaluation of questionnaire

All laboratories that reported result filled in the questionnaire. The summary of the answers is presented in Annex 8.

The main technique used to determine aflatoxins in defatted peanut powder is HPLC-FLD, followed by LC-MS. Two laboratories used ELISA and reported only total aflatoxins.

8 Conclusions

Fifty-four laboratories participated in this study and, for the determination of aflatoxin B1, the performance of most of the participants based on their z-scores was satisfactory (> 92 %).

Most laboratories reported acceptable recoveries for all aflatoxins. (Annex 9)

In line with observations of previous PTs organised by the EURL for Mycotoxins, performance of the laboratory based on their ζ-scores were not as satisfactory as when based on the z-scores, which indicates that the respective participants should review their uncertainty estimation.

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List of abbreviations and definitions

AFLA	Aflatoxin
ELISA	Enzyme-Linked Immuno Sorbent Assay
EC	European Commission
EN	European Standard
EU	European Union
EURL	European Union Reference Laboratory
FLD	Fluorescence detector
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PT	Proficiency Test

List of figures

List of tables

Annexes

Annex 1. Announcement- opening of registration

From: JRC IRMM EURL MYCOTOX
Sent: Friday, July 01, 2016 2:22 PM
To: JRC IRMM EURL MYCOTOX
Cc: STROKA Joerg (JRC-GEEL); CUBERO LEON Elena (JRC-GEEL); BOUTEN Katrien (JRC-GEEL)
Subject: PT 2016 Aflatoxins in defatted peanut powder

Dear colleagues

On behalf of the European Union Reference Laboratory for Mycotoxins (EURL Mycotoxins), I have the pleasure to announce the opening for registration to the inter-laboratory comparison/proficiency test on the determination of aflatoxins in defatted peanut powder.

According to Regulation (EC) No 882/2004 it is obligatory for EU National Reference Laboratories to participate. The main PT samples will be dispatched in October 2016, the mixed aflatoxins solution will be dispatched directly after the registration period.

The deadline for **registration is 19th July 2016**.

Technical details on the PT design:

Prior to the main PT (aflatoxins in defatted peanut powder) you will receive a vial with a mixed aflatoxins solution.

For all laboratories we will ask to measure the exact content of aflatoxins in the solution against their standards.

For laboratories that use LC with fluorescence detection you might remember that we asked some time ago to report fluorescence response factors for aflatoxins. We have gathered sufficient data that supports the assumption that a single calibration with AfB1 is sufficient for compliance judgement (under given conditions). To further substantiate this finding we ask you to use the calibrants in the vial (a dilution scheme will be supplied) to determine response factors prior the main PT. This means injecting the solution at least in duplicate around 2 and 1 month before.

When reporting your results we will – in addition – recalculate individual results and compare the overall performance of results (not your individual) for both scenarios. We hope that these findings might trigger some discussion if a single point as well as a single aflatoxin calibration is “fit-for-purpose”.

For NRLs the participation is free of charge.

The participation fee for official food control laboratories is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. Enrolled control laboratories will be contacted for payment details upon registration.

Confidentiality of results is guaranteed.

Please register at the following link: https://ec.europa.eu/eusurvey/runner/MYCO-PT-2016-Aflatoxins

Thank you in advance for your consideration.

EURL Mycotoxins Operating Manager

EURL Mycotoxins

European Commission
Joint Research Centre
Retieseweg 111
B-2440 Geel, Belgium
Phone: +32 (0)14 571231
Fax: +32 (0)14 571343

Interlaboratory comparison for the determination of aflatoxins in defatted peanut powder.

Fields marked with * are mandatory.

1

On behalf of the European Union Reference Laboratory for Mycotoxins (EURL Mycotoxins), I have the pleasure to announce the opening for registration to the inter-laboratory comparison/proficiency test on the determination of aflatoxins in peanut powder.

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Confidentiality of results is guaranteed.

Thank you in advance for your consideration.

EURL Mycotoxins Operating Manager

2

*Contact person

*Second contact person

*Organisation

*Address

*Postcode

*City

*Country

*Telephone number

Fax

*Email address

3

*The instrument used to determine aflatoxins is:

- HPLC-FLD
- LCMS
- other

Additional comments

4

Annex 2. Homogeneity study

Sample A

AFB1:

Content: 2.11 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs

	Mean Sq	replicates	Variance	sd
UNIT	0.042502	4	0.001927	0.044
Repeatability	0.034793		0.034793	0.190
Reproducibility			0.036721	0.190

Test for sufficient homogeneity acc. to Harmonized Protocol PTs

	s_sam^2	s_an^2	s_all^2	F1	F2	critical	Homogenous	s_an/s_p<0.5
Results	0.001927	0.034793	0.01939335	1.88	1.01	0.0716	TRUE	TRUE

AFB2:

Content: 0.38 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs

	Mean Sq	replicates	Variance	sd
UNIT	0.024864	4	0.0024964	0.05
Repeatability	0.014878		0.0148780	0.12
Reproducibility			0.0173744	0.13

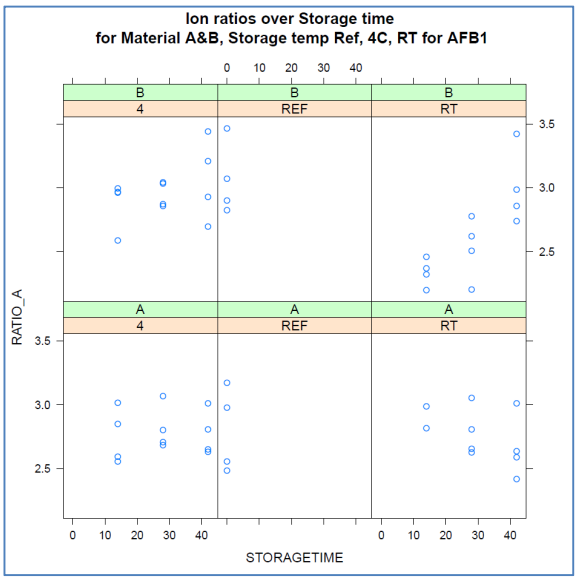
Test for sufficient homogeneity acc. to Harmonized Protocol PTs

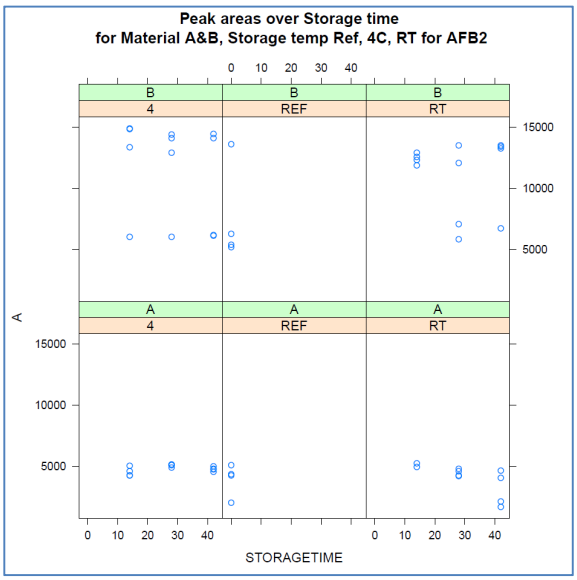
	s_sam^2	s_an^2	s_all^2	F1	F2	critical	Homogenous	s_an/s_p<0.5
Results	0.0024964	0.014878	0.0006290064	1.88	1.01	0.0162	TRUE	FALSE

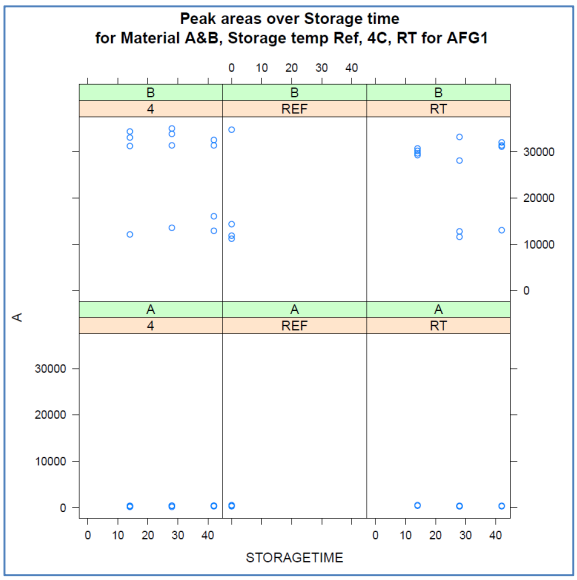
Sample B

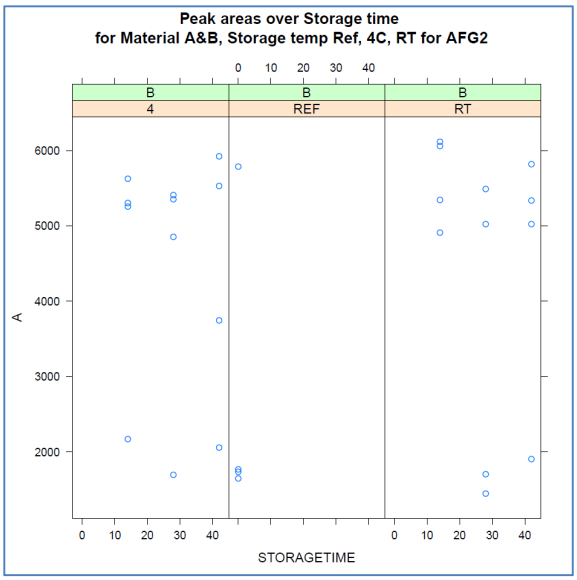
```
AFB1:

Content: 3.10 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs
                Mean Sq replicates Variance   sd
UNIT            0.267033          4 0.057147 0.24
Repeatability   0.038444            0.038444 0.20
Reproducibility                     0.095591 0.31


Test for sufficient homogeneity acc. to Harmonized Protocol PTs

         s_sam^2   s_an^2    s_all^2   F1   F2 critical Homogenous s_an/s_p<0.5
Results 0.057147 0.038444 0.04186116 1.88 1.01    0.118       TRUE          TRUE

AFB2:

Content: 0.81 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs
                Mean Sq replicates  Variance    sd
UNIT            0.0211136          4 0.0033609 0.058
Repeatability   0.0076702            0.0076702 0.088
Reproducibility                      0.0110310 0.110

Test for sufficient homogeneity acc. to Harmonized Protocol PTs

          s_sam^2    s_an^2     s_all^2   F1   F2 critical Homogenous s_an/s_p<0.5
Results 0.0033609 0.0076702 0.002857972 1.88 1.01   0.0131       TRUE          TRUE

AFG1:

Content: 1.11 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs
                Mean Sq replicates  Variance    sd
UNIT            0.039226           4 0.0075762 0.087
Repeatability   0.008922             0.0089218 0.094
Reproducibility                      0.0164980 0.130

Test for sufficient homogeneity acc. to Harmonized Protocol PTs

          s_sam^2    s_an^2     s_all^2   F1   F2 critical Homogenous s_an/s_p<0.5
Results 0.0075762 0.0089218 0.005367028 1.88 1.01   0.0191       TRUE          TRUE


AFG2:

Content: 0.33 ug/kg
Analysis of Variance Table
Variance estimates for repeatability and reproducibility

Response: AM_obs
                Mean Sq replicates  Variance    sd
UNIT            0.0111001          4 0.0004526 0.021
Repeatability   0.0092896            0.0092896 0.096
Reproducibility                      0.0097422 0.099


Test for sufficient homogeneity acc. to Harmonized Protocol PTs

          s_sam^2    s_an^2      s_all^2   F1   F2 critical Homogenous s_an/s_p<0.5
Results 0.0004526 0.0092896 0.0004743684 1.88 1.01   0.0103       TRUE         FALSE
```

Annex 3. Stability study









Annex 4. Sample accompanying letter

Ref. Ares(2016)5983147 - 18/10/2016

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

Geel, 18 October 2016

2016 Proficiency testing of National Reference Laboratories (NRLs) and Official Control Laboratories (OCLs) on the determination of aflatoxins in defatted peanut powder

Dear Participant,

Please read the following information carefully before starting any analysis. The 2016 PT on aflatoxins in peanut powder aims to assess the content in two naturally contaminated test samples ("Sample A" and "Sample B").

The materials are shipped at ambient temperature. After receipt freeze the samples immediately at -18 °C until the analysis is performed.

Please confirm the parcel's receipt by e-mail upon arrival, by using the "Materials Receipt Form". If any material is damaged, request new material immediately.

Report the recovery corrected value (µg/kg), including your recovery (%) and measurement uncertainty (µg/kg) for a coverage factor of 2 (k=2).

Participants that indicated they will use HPLC-FLD detection and have received the standard solution at the end of July should inject the diluted standard solution 3 times when analysing the samples (Set 3). Report the area of all injections of the standards in the results table (3 sets of 3 injections).

For participants only using LC-MS there is a 1 mL standard solution included in the package. The concentrations of the aflatoxins are: Aflatoxin B1: 80.1 ng/mL; Aflatoxin B2: 27.2 ng/mL; Aflatoxin G1: 84.3 ng/mL and Aflatoxin G2: 28.1 ng/mL.

Data generated by the participants will be collected by using the software RingDat, supplementary to ProLab software, that has been used for professional data handling and statistical analyses of interlaboratory tests results. You should have received two files attached to this email for reporting the results. The instructions on how to use the software RingDat can be found in the Annex 1 at the end of this document.

The deadline for reporting the PT results is the 9th December 2016.

Please keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to costumers, accreditation bodies and analysts alike.

Should you need any further assistance, please do not hesitate to contact us.

Katrien Bouten
(on behalf of the Operating Manager of the EURL Mycotoxins)

E-mail: JRC-EURL-MYCOTOX@ec.europa.eu
Tel: +32-14-571231

Cc: Frans Verstraete, Hendrik Emons, Joerg Stroka

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 229. https://ec.europa.eu/jrc/
E-mail: JRC-EURL-MYCOTOX@ec.europa.eu

Annex 1: Instructions for reporting the results using RingDat.

1. Download the updated version of the data entry program (called RingDat) free from the QuoData web page using following link: http://quodata.de/ringdat_en.php

User: *ringdat*

Password: *prolabdata*

Alternatively, in case you already have Ringdat, you can update it via the "Programm-update" button.

2. Save the two lab specific files with the extension "***.Lab**" and "***.LA2**" attached to this email in the same folder as RingData.exe.

The name of each laboratory and the samples are codified by the software, so that each participant will receive samples with unique codified numbers (i.e., LC0001).

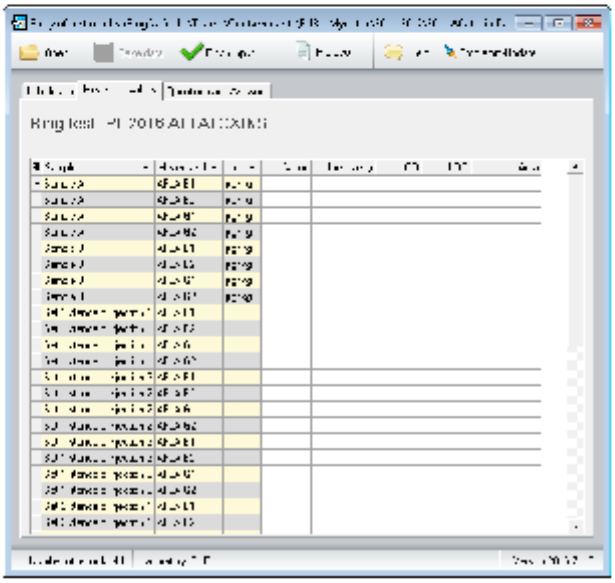
- The "***.LA2**" file contains information about the participant – laboratory name and laboratory code.
- The "***.LAB**" file is unique to each laboratory and contains information about the samples and measurands that have to be analysed and reported.

3. Start the RingDat.exe program and open "***.LAB**" file to access your workspace.

- The first tab contains detailed information about the laboratory (Lab details).
- The second tab contains a table for entering the results for every measurand/ sample combination (Measured values).
- The third tab contains a general questionnaire (Questions and Answers).

4. Fill in the results table ("Measured values") with your data. Please find below some captures of the RingDat pages that have been configured for this PT.

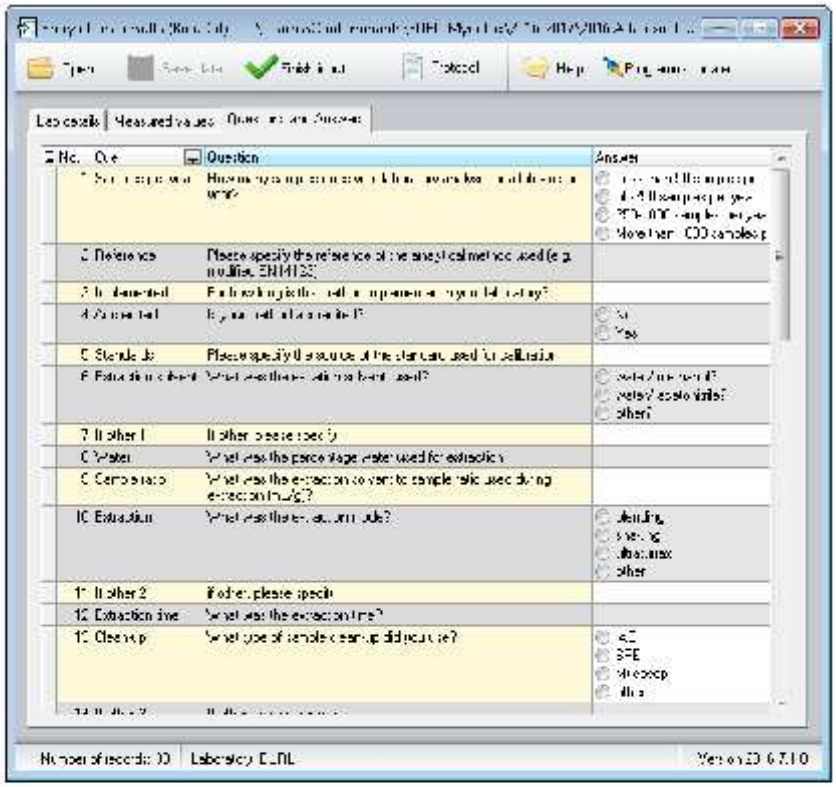
Figure 1 – Capture of the "Measured Values" page



2

5. Afterwards, please fill in the questionnaire on the next tab.

Figure 2 – Capture of the "Questions and Answers" page



6. After finishing the input, save the file using the button on the top of the window menu. You can change the inputs after saving the file as long as you haven't pushed "Finish input" button. At the end finalise the data entry by pressing the "Finish input" button.

7. Send both the "*.LAB" and "*.LA2" files back to us by e-mail to our functional mail box: JRC-EURL-MYCOTOX@ec.europa.eu

8. Should you want to correct some of your entries after finishing the input, you must use the original *.LAB file downloaded from the email and introduce all the information again (results and answers to the questionnaire).

Annex 5. Material receipt form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

Geel, 18 October 2016

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Member State:

NOTE: STORE MATERIAL IN A FREEZER AT -18 °C!

Please ensure that the items listed below have been received undamaged, and then check the relevant statement:

Date of receipt	
All items have been received undamaged	YES† / NO†
If NO, please list damaged items:	

Contents of the parcel:

a) 2 test materials (defatted peanut powder) for analysis:
- Sample A
- Sample B

b) Material receipt form

Your Signature / Stamp here:

Please e-mail the completed form to:

Katrien Bouten

JRC-EURL-MYCOTOX@ec.europa.eu

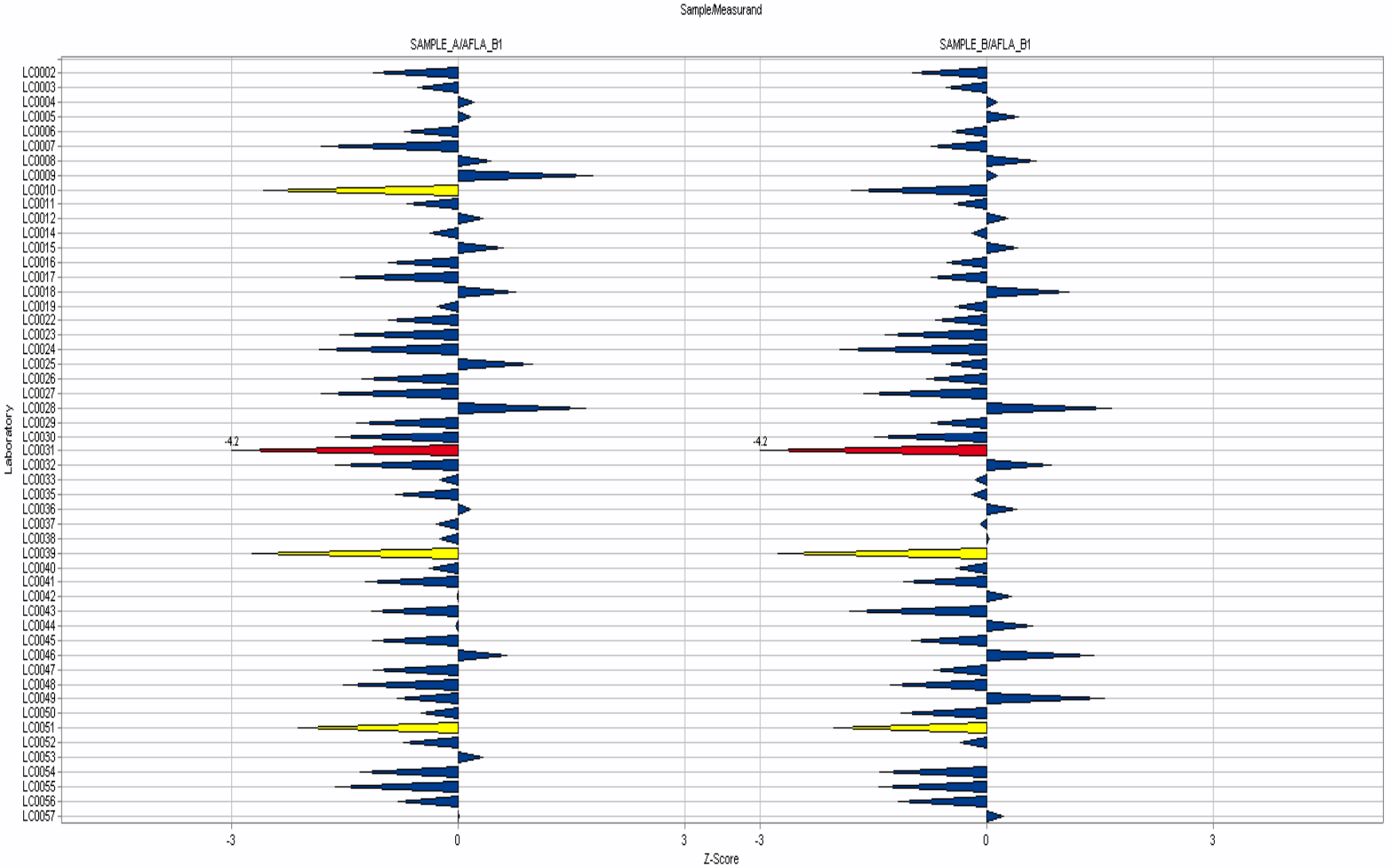
Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 231. https://ec.europa.eu/jrc/
E-mail: JRC-EURL-MYCOTOX@ec.europa.eu

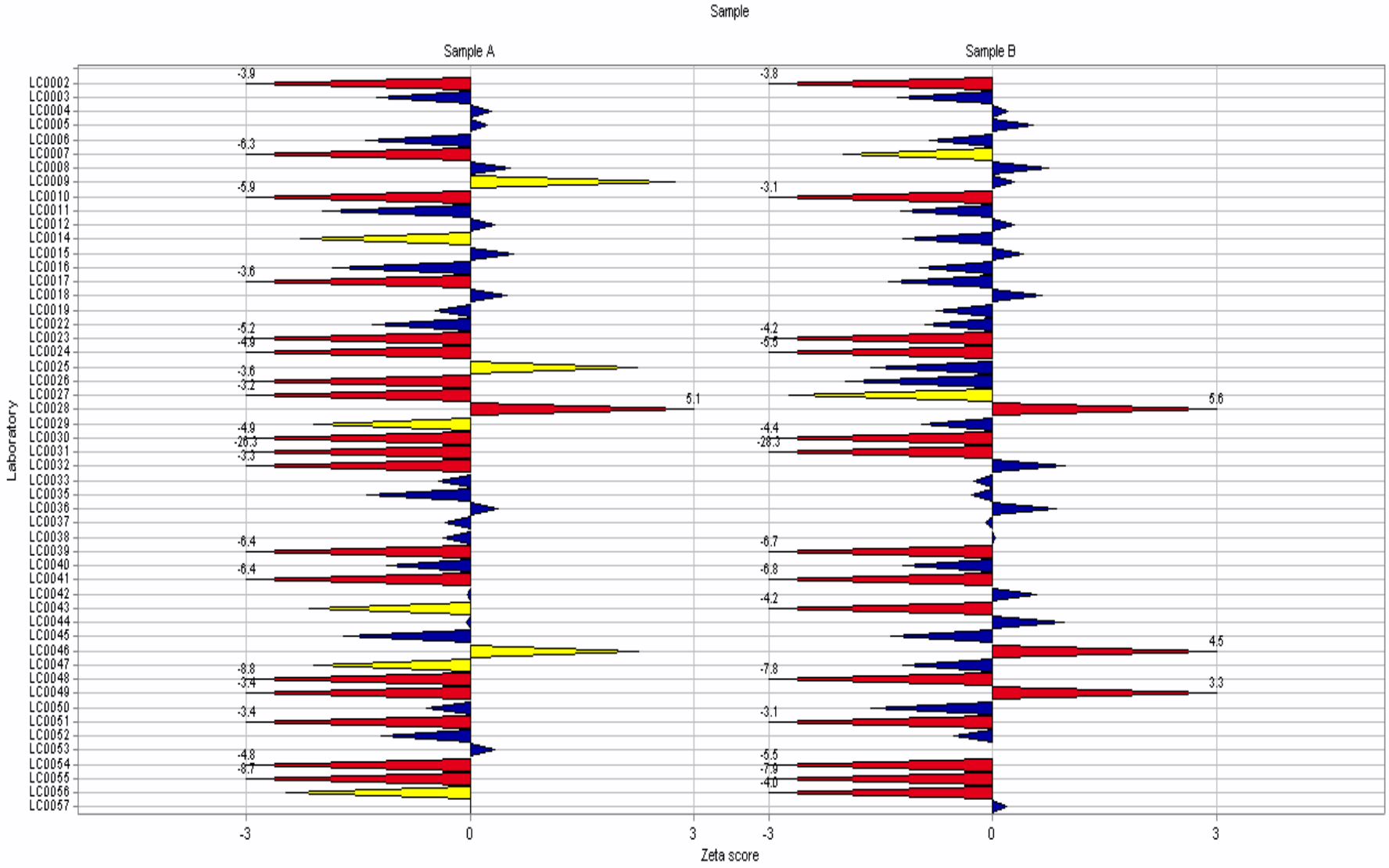
Annex 6. Questionnaire

No	Cue	Question	Answer
1	Samples per year	How many samples does your laboratory analyse for aflatoxins per year?	Less than 50 samples per year 50-250 samples per year 250-1000 samples per year More than 1000 samples per year
17	Reference	Please specify the reference of the analytical method used (e.g. modified EN14123)	
31	Implemented	For how long is this method implemented in your laboratory?	
2	Accredited	Is your method accredited?	No Yes
3	Standards	Please specify the source of the standard used for calibration:	
4	Extraction solvent	What was the extration solvent used?	water/ methanol? water/ acetonitrile? other?
32	If other 1	If other, please specify	
41	Water	What was the percentage water used for extraction	
5	Sample ratio	What was the extraction solvent to sample ratio used during extraction (mL/g)?	
6	Extraction	What was the extraction mode?	blending shaking ultraturrax
33	If other 2	if other, please specify	
7	Extraction time	What was the extraction time?	
8	Clean-up	What type of sample clean-up did you use?	IAC SPE Mycosep other
34	If other 3	If other, please specify	
9	IAC	If you used immuno affinity columns , please specify the brand:	R-Biopharm Vicam Neogen other
35	If other 4	If other, please specify	
10	Overnight stop 1	During the analysis did you need to include an overnight stop?	No Yes
11	Overnight stop 2	If yes, please state for which samples and at what state of the analysis:	
37	MP	What mobile phase did you use?	

No	Cue	Question	Answer
12	Detection	What type of detection method did you use?	HPLC_FLD LC-MS ELISA other
36	If other 5	If other, please specify	
21	Ex	If you used HPLC-FLD, please specify the detector settings - Extinction	
38	Em	If you used HPLC-FLD, please specify the detector settings - Emission	
39	Bandwidth	If you used HPLC-FLD, please specify the detector settings - Bandwidth	
16	LCMS	If you used LCMS, please state the ionisation mode and transitions used for the analysis	
14	ISTD (1)	If you used LCMS, did you used a stable istope labelled internal standard?	for aflatoxin B1 for aflatoxin B2 for aflatoxin G1 for aflatoxin G1 No
15	ISTD (2)	At what stage was the internal standard added? 	before extraction after extraction
23	ELISA	If you used ELISA, please specify the brand 	
13	Derivatisation	Which derivatisation method was applied?	KOBRA PBPB UV other
40	If other 6	If other, please specify	
18	Glassware	Did you used acid glassware?	No Yes
19	Daylight	Was protection against daylight applied?	No Yes
20	Problems	Did you encounter any problems during the analysis?	No Yes
25	If yes, what were the specified	If yes, what were the specified problems and to which samples they apply?	
26	Observations	Did you notice any unsual observations which, however, did not seem to have any effect on the results?	No Yes
27	If yes, describe	If yes, what were the observations and to which samples do they apply?	
28	Instructions	Did you find the instructions distributed for this PT adequate?	No Yes
29	If no, describe	If no, which parts do you think can be improved?	
30	Comments	Any other comments you wish to address?	

Annex 7. Aflatoxin B1 Z- & ζ-score graphs




Sample
Sample A
Sample B
Laboratory
LC0002
LC0003
LC0004
LC0005
LC0006
LC0007
LC0008
LC0009
LC0010
LC0011
LC0012
LC0014
LC0015
LC0016
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LC0046
LC0047
LC0048
LC0049
LC0050
LC0051
LC0052
LC0053
LC0054
LC0055
LC0056
LC0057
-3.9
-6.3
-5.9
-3.6
-5.2
-4.9
-3.6
-3.2
5.1
-4.9
-26.3
-3.3
-6.4
-6.4
-8.8
-3.4
-3.4
-4.8
-8.7
-3.8
-3.1
-4.2
-5.5
5.6
-4.4
-28.3
-6.7
-6.8
-4.2
4.5
-7.8
3.3
-3.1
-5.5
-7.9
-4.0
-3
0
3
Zeta score

Annex 8. Experimental details

Lab Code	How many samples does your laboratory analyses for aflatoxins per year?	Reference of the analytical method used	For how long is this method implemented in your laboratory?	Is your method accredited?	Source of the standard used for calibration
LC0002	250-1000 samples per year	F/0329 (VEMS)	21 years	yes	Sigma
LC0003	250-1000 samples per year	modified MSZ EN ISO 17375:2006	6 years	yes	Biopur MIX1 aflatoxin ROMERLABS
LC0004	50-250 samples per year	internal document SOP 10575.1	since 2012	yes	Sigma Aldrich
LC0005	More than 1000 samples per year	in house LC method		yes	Coring
LC0006	250-1000 samples per year	Internal method		yes	Sigma-Aldrich
LC0007	50-250 samples per year	inhouse method based on ISO17375:2006	since 2012	yes	SIGMA
LC0008	250-1000 samples per year	In-house method	more than 10 years	yes	SigmaAldrich
LC0009	250-1000 samples per year	In house method		yes	Biopure
LC0010	50-250 samples per year	modified EN14123	12 years	no	Sigma
LC0011	50-250 samples per year	ISEN14123-2007	9	yes	IRMM
LC0012	250-1000 samples per year	in house method	one year	no	
LC0014	Less than 50 samples per year	according EN 14123	8 years	yes	Romer Labs "Biopure"
LC0015	50-250 samples per year	modified AB Sciex Technical Note	3 years	yes	Romer
LC0016	50-250 samples per year	in-house method	since 2003	yes	
LC0017	250-1000 samples per year	Modified EN 14123	10 years +	no	Sigma-Aldrich
LC0018	Less than 50 samples per year	In-house validated metod	2007	yes	Biopure
LC0019	250-1000 samples per year	AOAC 991.31 (2010) Final Action)	Since 1995	yes	Sigma Aldrich
LC0021	Less than 50 samples per year	manufacturer`s (Neogen) instruction for use of ELISA kit	5 years	yes	
LC0022	50-250 samples per year	N/A	10 Years	yes	R-Biopharm Rhone
LC0023	250-1000 samples per year	In house method	8 years	yes	Trilogy
LC0024	50-250 samples per year	R-Biopharm Rhône, Immunoaffinity columns, Instructions for use	14 years (since 2002)	yes	Romer Labs (Biopure)
LC0025	50-250 samples per year	EN 14123:2007	5 years	yes	Biopure
LC0026	50-250 samples per year	modified EN14123	2 year	no	R-Biopharm
LC0027	250-1000 samples per year	modified EN14123	20 years	yes	Romer Labs
LC0028	50-250 samples per year	In house method	20 years	yes	Sigma
LC0029	50-250 samples per year			yes	
LC0030	50-250 samples per year	AOAC Official method 991.31	over 10 years	yes	Biopure, Romer labs
LC0031	50-250 samples per year	R-Biopharm Easi-extract aflatoxin	10 years	yes	R-Biopharm

Lab Code	How many samples does your laboratory analyses for aflatoxins per year?	Reference of the analytical method used	For how long is this method implemented in your laboratory?	Is your method accredited?	Source of the standard used for calibration
LC0032	250-1000 samples per year	Inhouse method, modified EN14123	>25 years	yes	
LC0033	Less than 50 samples per year	inhouse method	>10 years	yes	Sigma
LC0034	250-1000 samples per year	Protocol Elisa of R-Biopharm	8 years	yes	Kit Elisa of R-Biopharm
LC0035	50-250 samples per year	EN 14123.	10 years	yes	Sigma-Aldrich
LC0036	50-250 samples per year		18 years	yes	SIGMA
LC0037	50-250 samples per year	Sulyok M et al. 2006. Rapid Communication in Mass Spectrometry 20, 2649-2659	2010	yes	biopure, Romer Labs Diagnostic GmbH, Tulln - Austria; office-europe@romerlabs.com
LC0038	50-250 samples per year	Internal method		yes	Sigma Aldrich
LC0039	50-250 samples per year	UNI EN 14123:2008	20	yes	BIOPURE
LC0040	50-250 samples per year			yes	
LC0041	250-1000 samples per year	own method	since 2013	yes	Supelco standard mix
LC0042	Less than 50 samples per year	modified EN14123 with LC-MS method	2	yes	romer
LC0043	250-1000 samples per year	EN 14123	15 years	yes	SIGMA
LC0044	50-250 samples per year	modified EN ISO 16050	6 years	yes	LGC Standards
LC0045	250-1000 samples per year	EN 14123	>10 years	yes	Biopure, aflatoxin mix
LC0046	Less than 50 samples per year		6 years	yes	
LC0047	50-250 samples per year	modified EN 14123	6 years	yes	Biopure Romer labs
LC0048	250-1000 samples per year	§ 64 LFGB L23.05-2 and § 64 LFGB L15.00-2	20 years	yes	Romer Labs Diagnostic GmbH
LC0049	Less than 50 samples per year			no	
LC0050	Less than 50 samples per year	Application notes for quantitative determination of aflatoxins in various foods by immunoaffinity chromatography, Rhone-diagnostic Technologies Ltd.	15 Years	no	R-biopharm
LC0051	50-250 samples per year		13 years	yes	Romer
LC0052	250-1000 samples per year	modified EN14123	10 years	yes	SUPELCO
LC0053	50-250 samples per year	/	5 years	yes	Biopure
LC0054	50-250 samples per year	EN 14123	2007	yes	LGC Standards
LC0055	50-250 samples per year	In house.	10 years	yes	R-Biopharm
LC0056	More than 1000 samples per year	Modified EN ISO 16050	12	yes	Sigma Aldrich (A6636; A9887; A0138; A0263)
LC0057	250-1000 samples per year	Zachariasova M, Analytica Chimica Acta, 2010, 662, 51-61	4 years	yes	Sigma Aldrich

Lab Code	Extraction solvent	% Water in extraction solvent	What was the solvent to sample ratio used during extraction (mL/g)	Extraction mode	Extraction time
LC0002	water/ methanol	70	250ml/50g (5ml/1g)	blending	1 minute
LC0003	water/acetone		5	shaking	60 min
LC0004	0,1 % HCOOH in Water+ACN	50	5	shaking	20 min
LC0005	water/ acetonitrile	20	5	shaking	90 min
LC0006	water/ methanol	20	1.6	blending	2 min
LC0007	acetone/water	15	250ml/50g	shaking	30min
LC0008	water/ acetonitrile	20	5 mL/g	shaking	45 minutes
LC0009	1 % acetic acid in acetonitril		10	shaking	30 min
LC0010	water/ methanol	20	4	ultraturrax	3 minutes
LC0011	water/ methanol	20	80	ultraturrax	2minutes
LC0012	water/ acetonitrile	30	5	shaking	2 h
LC0014	water/methanol/hexane containing 16.7 g/l NaCl	13	6	shaking	45 min
LC0015	water/ acetonitrile	20	8/2	shaking	20 mins
LC0016	water/ methanol	20		ultraturrax	3 minutes + 15' shaking
LC0017	water/methanol/acetonitrile	40	10mL/1g	ultraturrax	6 minutes
LC0018	water/ acetonitrile	16	100 ml/25 g	shaking	30 min
LC0019	water/ methanol	30	10	blending	2 min
LC0021	water/ methanol	30	5/1	shaking	approx. 50 minutes (3 minutes of shaking)
LC0022	water/ methanol	30	125/25	blending	1 minute
LC0023	water/ methanol	40	5/1	shaking	
LC0024	water/ methanol	40		shaking	1 hour
LC0025	water/ methanol	20	4	shaking	20
LC0026	water/ methanol	30	125/25	shaking	15
LC0027	water/ methanol	20	4	shaking	30 min
LC0028	water/ acetonitrile	40	0.4g of sample per 1ml of extraction solution	Blending and shaking	2min blending 30min shaking
LC0029	water/ acetonitrile	40	5	shaking	1 hour
LC0030	water/ methanol	37,5	4	shaking	20 minutes
LC0031	water/ methanol	20	2	ultraturrax	2 minutes
LC0032	water/ acetonitrile			ultraturrax	3 minutes
LC0033	water/acetone	25	4,2	blending	5 min
LC0034	water/ methanol	30	10 ml methanol/distilled water 70 % with 2 g sample	shaking	15 minut
LC0035	water/ methanol	20	4:1	blending	3 min

Lab Code	Extraction solvent	% Water in extraction solvent	What was the solvent to sample ratio used during extraction (mL/g)	Extraction mode	Extraction time
LC0036	water/ methanol	30	6.7	ultraturrax	3 min
LC0037	acetonitrile/water/formic acid (79/20/1) (v:v:v)	20	2 (20 ml extraction solvent, 10 g sample weight)	shaking	0.5 h
LC0038	water/ methanol	64	25:5	First Whirlemixer then shaking	5 sek + 15 min
LC0039	water/ methanol	20	4/1	blending	3 min
LC0040	water/ methanol	58		shaking	
LC0041	water/ methanol	30	4 ml/g	shaking	30 min
LC0042	water/ methanol	20	4	shaking	60 min
LC0043	water/methanol/hexane	20	4 ml/g	blending	3 minutes
LC0044	water/ methanol	30	5	shaking	5 minutes
LC0045	methanol/water/cyclohexane	16	5	blending	3 min
LC0046	water/ acetonitrile	40	4	shaking	60 min
LC0047	water/ methanol	20	10ml/1g	blending	3min
LC0048	water/ methanol	40	100 mL /25 g	shaking	45 min
LC0049	water/ methanol	20	12,5	blending	2 min.
LC0050	water/ methanol	40	250 ml/ 50 g	ultraturrax	3 minutes
LC0051	water/ methanol	20	8	shaking	45min
LC0052	water/ methanol	20	20 ml / 5 g	shaking	2 hours
LC0053	acetone/isopropanol/water/acetic acid	40	4.5	shaking	60 min
LC0054	water/ methanol	40	5	shaking	60 min
LC0055	water/ methanol	30	62.5/12.5	blending	2 minutes
LC0056	water/ methanol	20	4ml/g	shaking	60min
LC0057	acetonitrile/formic acid		5 mL/g	shaking	30 min.

Lab Code	What type of sample clean-up did you use?	Brand immuno affinity column	Did you need to include an overnight stop?	What mobile phase did you used?
LC0002	IAC	R-Biopharm	No	water:acetonitrile:methanol (60:10:30) containing 119mg of KBr and 350µl of 4M nitric acid
LC0003	IAC	ROMERLABS	No	Water/Acn/MeOH 68/16/16 +119mg KBr/l
LC0004	QuEChERS	-	No	A=0,1 % HCOOH in H2O; B=0,1 % HCOOH + 1mMHCOONH4 in MeOH
LC0005	none		No	
LC0006	IAC	R-Biopharm	No	
LC0007	IAC	LC-Tech	No	water/methanol/acetonitrile
LC0008	IAC	Vicam	No	KBr, HNO3, methanol, acetonitrile, water
LC0009			No	1 % formic acid/5 mM ammonium formate in water/ methanol
LC0010	IAC	R-Biopharm	No	Water:Methanol:Acetonitrile (6:3:2)
LC0011	IAC	Vicam	All samples. Extracts were stored in the freezer prior to dilution with water and IAC cleanup	45:55 Methanol : Water
LC0012	IAC	Vicam	No	0.1 % Formic acid in water and 0.1 % Formic acid in acetonitrile
LC0014	IAC	LC-Tech	All samples were stored at 4 °C after extraction for 18 hours.	mixture of water/methanol/acetonitrile 54/29/17 (v/v/v) containing 0.0119 % (w/v) KBr and 0.01 % (v/v) nitric acid (65 %)
LC0015	none		No	5mM Ammonium Acetate containing 0.5 % acetic acid in water/methanol
LC0016	IAC	R-Biopharm	after extraction for all samples	water/ACN/methanol (62/19/19) + HNO3 + KBr
LC0017	IAC	R-Biopharm	No	27 % Acetonitrile/58 % water/15 % methanol +120mg potassium bromide and 350µL 4N Nitric acid per litre
LC0018	MultiSep		For both samples: Sample extraction and clean-up day 1. Frozen extracts overnight. Evaporation, resolution, HPLC day 2.	Water:Acetonitrile:Methanol (900:180:240). 119 mg KBr+350 µl 4M HNO3 per liter.
LC0019	IAC	Vicam	No	Acetonitrile (20 %) :H2O (60 %) : MEOH (20 %)
LC0021			No	
LC0022	IAC	R-Biopharm	No	Water/Methanol/Acetonitrile 65/17.5/17.5 + 119mg KBr + 350µl 4M HNO3/litre
LC0023	IAC	Romer	No	Water 57:acetonitrile 5:methanol 38
LC0024	IAC	R-Biopharm	No	Methanol-acetonitrile-water with addition of HNO3 and KBr
LC0025	IAC	Romer Labs AlfaStar R	No	Water/MeOH/HNO3/KBr

Lab Code	What type of sample clean-up did you use?	Brand immuno affinity column	Did you need to include an overnight stop?	What mobile phase did you used?
LC0026	IAC	R-Biopharm	No	Water-Methanol-Acetonitrile (62:22:16)+KBr+HNO3
LC0027	IAC	R-Biopharm	No	acetonitril/methanol/aceticacid2 % (18/27/55; v/v/v), isocratic
LC0028	IAC	R-Biopharm	No	water acetonitrile methanol mix acidifed with acetic acid
LC0029	IAC	R-Biopharm	No	Acetonitrile + Methanol + water (18 + 27 + 55) 120 mg KBr, 350 µL 4 N salpetersyre / pr. litre mobilfase
LC0030	IAC	Vicam	No	Tetrahydrofuran - Water 21:79
LC0031	IAC	R-Biopharm	No	60:40 % v/v Water:Methanol 119mg/L KBr & 350uL/L 4M Nitric acid
LC0032	IAC	R-Biopharm	No	Water : acetonitrile : methanol, 56 : 30: 14
LC0033	GPC, SX-3		No	water/methanol/acetonitrile : 65/17/18
LC0034			No	
LC0035	IAC	R-Biopharm	No	Water:acetonitrile:methanol
LC0036	IAC	Vicam	No	Water/Methanol/Acetonitrile
LC0037	Mycosep		No	mobile A: water, 0.1 % HCOOH, 5mM NH4OOCH mobile B: methanol, 0.1 % HCOOH, 5mM NH4OOCH
LC0038		R-Biopharm	No	Water, methanol, acetonitrile, acetic acid
LC0039	IAC	R-Biopharm	No	H2O:MeOH:ACN 54:29:17
LC0040	IAC	R-Biopharm	No	
LC0041	IAC	Romer	No	MeOH/ACN/Water/HNO3/KBr
LC0042	IAC	Vicam	No	methanol/water with ammonium acetate and acetic acid
LC0043	IAC	Mycosep	No	410 grammes de méthanol + 265 grammes d'acétonitrile + 1200 grammes d'eau + 700 µl d'acide nitrique 4M + 240 mg de bromure de potassium.
LC0044	IAC	R-Biopharm	No	water:acetonitrile:methanol 60:20:20
LC0045	IAC	R-Biopharm	No	water/ACN/MeOH 570:140:290
LC0046	IAC	Vicam	No	acetonitrile:water
LC0047	IAC	LC Tech	No	560ml water, 25mg KBr, 280ml methanol, 160ml acetonitrile, 100ul conc. nitric acid
LC0048	IAC	Romer Labs Diagnostic GmbH	No	Methanole/Acetonitrile/Water with KBr and HNO3
LC0049	IAC	Vicam	After elution of aflatoxins from IAC , samples were blown to dryness and stored in the freezer overnight.	Solvent A:Methanol: Water 20:80 and Solvent B: Methanol: Water 80:20
LC0050	IAC	R-Biopharm	All samples, stored in fridge overnight prior to running on HPLC	62:19:19 Water:Methanol:Acetonitrile + 119mg KBr + 350ul 4M nitric acid
LC0051	IAC	R-Biopharm	No	0.2 % acetic acid/ : MeOH/AcN(260/110)

Lab Code	What type of sample clean-up did you use?	Brand immuno affinity column	Did you need to include an overnight stop?	What mobile phase did you used?
LC0052	IAC	R-Biopharm	For the all samples: one day extraction and the second day passing through IAC and HPLC-FLD	KBr/HNO3 solution/ACN/MeOH: 6/2/3
LC0053	QuEChERS		No	water / MeOH 10mMammonium formate / 0.01 % (v/v) formic acid
LC0054	IAC	R-Biopharm	No	Water, Methanol, Acetonitrile, Nitric Acide, Potassium bromide,
LC0055	IAC	R-Biopharm	No	Water/Acetonitrile/Methanol with KBr
LC0056	IAC	Protealmmun	No	Water:MeCN:MeOH=3:1:1
LC0057	Bondesil C18		No	A: 5mM ammonium formate/0,2 %formic acid B: Methanol/0,2 %formic acid

Lab Code	What type of detection method did you use?	Emission (nm)	Excitation (nm)	Bandwidth	At what stage was the internal standard added	Which derivatisation method was applied?
LC0002	HPLC-FLD	455nm	365nm	5nm		KOBRA
LC0003	HPLC-FLD	425 nm	362 nm	18 nm		KOBRA
LC0006	HPLC-FLD	435 nm	350 nm			Iode
LC0007	HPLC-FLD	435 nm	365 nm	4nm		PBPB
LC0008	HPLC-FLD	430 nm	365 nm			KOBRA
LC0010	HPLC-FLD	435 nm	365 nm	15nm		PBPB
LC0011	HPLC-FLD	440 nm	360 nm	18		UV
LC0014	HPLC-FLD	448 nm	362 nm	15 nm		KOBRA
LC0016	HPLC-FLD	440 nm	362 nm			KOBRA
LC0017	HPLC-FLD	434 nm	364 nm	20nm		KOBRA
LC0018	HPLC-FLD	450 nm	365 nm			
LC0019	HPLC-FLD	420 nm	360 nm	20nm		Saturated Iodine Solution
LC0022	HPLC-FLD	430 nm	365 nm			KOBRA
LC0023	HPLC-FLD	430	360	15		KOBRA
LC0024	HPLC-FLD	425	362	18		KOBRA
LC0025	HPLC-FLD	440	365	18		KOBRA
LC0026	HPLC-FLD	430 nm	360 nm			KOBRA
LC0027	HPLC-FLD					PBPB
LC0028	HPLC-FLD	455 nm	365 nm	1		KOBRA

Lab Code	What type of detection method did you use?	Emission (nm)	Excitation (nm)	Bandwidth	At what stage was the internal standard added	Which derivatisation method was applied?
LC0029	HPLC-FLD	435 nm	365 nm	15nm		KOBRA
LC0030	HPLC-FLD	454	362			Saturated iodine solution
LC0031	HPLC-FLD	455	362	peakwidth? >0.2min		KOBRA
LC0032	HPLC-FLD	434 nm	364 nm	2nm		
LC0033	HPLC-FLD	425 nm	365 nm	4 nm		online derivatisation Pickering
LC0035	HPLC-FLD	435 nm	362 nm	Agilent 1100		KOBRA
LC0036	HPLC-FLD	435	365	-		PBPB
LC0038	HPLC-FLD	440 nm	360 nm	20 nm		PBPB
LC0039	HPLC-FLD	435	365	18		PBPB
LC0040	HPLC-FLD	420	360			
LC0041	HPLC-FLD	440 nm	362 nm	10		KOBRA
LC0043	HPLC-FLD	435 nm	365 nm	15 nm		KOBRA
LC0044	HPLC-FLD	435 nm	365 nm	15 nm		TFA
LC0045	HPLC-FLD	435	365	18		PBPB
LC0046	HPLC-FLD	450 nm	365 nm			precolumn derivatisation with TFA
LC0047	HPLC-FLD	435	366	gain 4		KOBRA
LC0048	HPLC-FLD	438 nm	368 nm		before extraction	KOBRA
LC0050	HPLC-FLD	425nm	362nm			KOBRA
LC0052	HPLC-FLD	435 nm	365 nm	>0.2 min		KOBRA
LC0054	HPLC-FLD	435 nm	465 nm	-		KOBRA
LC0055	HPLC-FLD	430	365			KOBRA
LC0056	HPLC-FLD	435nm	365nm		before extraction	

Lab Code	What type of detection method did you use?	If you used ELISA, please specify the brand	At what stage was the internal standard added
LC0021	ELISA	Neogen Veratox Aflatoxin HS	before extraction
LC0034	ELISA	R-Biopharm	

Lab Code	What type of detection method did you use?	Ionisation mode & transitions	Did you use a stable isotope labelled internal standard?	At what stage was the internal standard added
LC0004	LC-MS	ESI+. AFG2: 331/201, AFG1: 329/243, AFB2: 315/259, AFB1: 313/241	no	before extraction
LC0005	LC-MS		aflatoxin B1 aflatoxin B2 aflatoxin G1 aflatoxin G2	after extraction
LC0009	LC-MS	Aflatoxin B1 (ql)- ESI+ m/z 313>128.1 Aflatoxin B1 (qn)- ESI+ m/z 313>285.2 Aflatoxin B2 (ql)- ESI+ m/z 315.1>259.2 Aflatoxin B2 (qn)- ESI+ m/z 315.1>287.2 Aflatoxin G1 (ql)- ESI+ m/z 329>200 Aflatoxin G1 (qn)- ESI+ m/z 329>243.2 Aflatoxin G2 (ql)- ESI+ m/z 331.1>245.2 Aflatoxin G2 (qn)- ESI+ m/z 331.1>313.2	no	
LC0012	LC-MS	B1 (ESI +) 313.1>285.0 and 313.1>241.0 B2 (ESI +) 315.1>287.0 and 315.1>259.0 G1 (ESI +) 329.1>243.1 and 329.1>311.0 G2 (ESI +) 331.1>313.1 and 331.1>245.1	no	
LC0015	LC MSMS	Precursor Product 1 Product2 Mode AFB1 313.0 285.2 128.1 Po AFB2 315.1 287.2 259.5 Po AFG1 329.0 243.2 200.0 Po AFG2 331.1 313.2 245.2 Po	aflatoxin B1 aflatoxin B2 aflatoxin G1 aflatoxin G2	after extraction
LC0037	LC-MS	AFB1 312.2 (Mw) 313.1 (Precursor) [M+H]+ (adduct) 285.0/241.0 (Products) 21/41 (CE) 3/3 (CAV) positiv (polarity) 5.80 (retention) AFB2 314.2 (Mw) 315.1 (Precursor) [M+H]+ (adduct) 287.0/258.9 (Products) 21/29 (CE) 3/3 (CAV) positiv (polarity) 5.60 (retention) AFG1 328.2 (Mw) 329.1 (Precursor) [M+H]+ (adduct) 243.0/200.1 (Products) 25/41 (CE) 3/3 (CAV) positiv (polarity) 5.40 (retention) AFG2 330.2 (Mw) 331.1 (Precursor) [M+H]+ (adduct) 313.0/245.1 (Products)(Products) 21/25 (CE) 3/3 (CAV) positiv (polarity) 5.20 (retention)	aflatoxin B1 aflatoxin B2 aflatoxin G1 aflatoxin G2	after extraction
LC0042	LC-MS	ESI positive AfB1: 313.0>285.2 AfB2: 315.0>287.1 AfG1: 329.0>311.1 AfG2: 331.0>313.1 13CAfB1: 330.1>301.1	aflatoxin B1	after extraction

Lab Code	What type of detection method did you use?	Ionisation mode & transitions	Did you use a stable isotope labelled internal standard?	At what stage was the internal standard added
LC0049	LC-MS		no	
LC0051	LC-MS	ESI+ 313; 315; 329;330	no	
LC0053	LC-MS	ESI+ AfB1: 313 > 241 AfG1: 329.1 > 242.9 AfB2: 315.1 > 258.9 AfG2: 331.1 > 217	aflatoxin B1 aflatoxin B2 aflatoxin G1 aflatoxin G2	after extraction
LC0057	LC-MS	ESI+ AFB1: 313/285, 313/241 AFB2: 315/287, 315/259 AFG1: 329/311, 329/115 AFG2: 331/313, 331/115	aflatoxin B1 aflatoxin B2 aflatoxin G1 aflatoxin G2	after extraction

Lab Code	Did you use acid washed glassware?	Was protection against daylight applied?	Did you encounter any problems during the analysis?
LC0002	Yes	Yes	No
LC0003	Yes	Yes	No
LC0004	No	Yes	No
LC0005	No	Yes	No
LC0006	No	Yes	No
LC0007	Yes	Yes	No
LC0008	Yes	Yes	No
LC0009	No	No	No
LC0010	No	Yes	No
LC0011	Yes	Yes	No
LC0012	Yes	Yes	No
LC0014	Yes	Yes	No
LC0015	No	No	No
LC0016	Yes	No	when adding PBS, the solution got cloudy, so we had to dilute with water instead

Lab Code	Did you use acid washed glassware?	Was protection against daylight applied?	Did you encounter any problems during the analysis?
LC0017	No	Yes	We were unable to use our accredited method extraction of 60 % acetonitrile as two layers were formed on centrifuging. Further portions of the samples (A and B) were obtained and a modified procedure used (extraction solvents as above). It was then necessary to filter the extract again before IA column.
LC0018	Yes	Yes	No
LC0019	No	Yes	No
LC0021		Yes	No
LC0022	Yes	Yes	No
LC0023	No	Yes	No
LC0024	No	Yes	No
LC0025	No	Yes	sample extracts were turbid and after had problems with SPE
LC0026	No	Yes	No
LC0027	No	Yes	
LC0028	Yes	Yes	No
LC0029	No	Yes	No
LC0030	No	Yes	No
LC0031	Yes	Yes	Peak shape of stored standard deteriorated badly. Kobra cell membrane was used up during analysis
LC0032	Yes	Yes	No
LC0033	No	Yes	No
LC0034	No	Yes	No
LC0035	No	Yes	No
LC0036	No	Yes	No
LC0037	No	Yes	No
LC0038	No	Yes	Set 3 standard: injection of the standard at the 3. round gave of unknown reason splitting peaks. No other samples are affected. Reanalysis of the standard did not solve the problem. No results are therefore entered.
LC0039	Yes	Yes	No
LC0040			
LC0041	Yes	Yes	No
LC0042	No	Yes	No
LC0043	Yes	Yes	No
LC0044	No	Yes	No
LC0045	Yes	Yes	No
LC0046	No	No	No
LC0047	No	Yes	No

Lab Code	Did you use acid washed glassware?	Was protection against daylight applied?	Did you encounter any problems during the analysis?
LC0048	No	Yes	No
LC0049	No	No	We have had exceptionally low recoveries for all four aflatoxins. we have repeated 3 times extraction method. Usually our acceptable recoveries between 70-110 percent. But this time they have been recoveries between 50 to 30 percent on all three occasions. The reported results are corrected for recovery
LC0050	No	Yes	No
LC0051	No	No	Unusual behaviour of that kind of sample...we have got very dirty extract
LC0052	Yes	Yes	No
LC0053	No	Yes	No
LC0054	No	Yes	No
LC0055	No	Yes	No
LC0056	No	Yes	No
LC0057	Yes	Yes	No

Lab Code	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	Did you find the instructions distributed for this PT adequate? Which parts can be improved?	Comments
LC0002	No	yes	No
LC0003	No	yes	
LC0004	No	Instructions for handling with 1 mL standard solution in case of LC-MS.	Thank you for the opportunity to participate
LC0005	No	It was not clear from the instruction sheet, what to do with the standard solution measuring LC-MS. I had to check this separately by e-mail. Furthermore it is unclear if to enter the data with dot or comma. We used comma!	
LC0006	No	yes	set 1 analysed 08/09/2016 set 2 analysed 07/10/2016 set 3 analysed 21/11/2016 The reported data for set 1, 2 and 3 were multiplied by 5 to take in account an extra dilution 1/5 to get our calibration range
LC0007	It took longer than usual for both extracted solutions to pass through the IAC columns	yes	

Lab Code	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	Did you find the instructions distributed for this PT adequate? Which parts can be improved?	Comments
LC0008	No	yes	The vial with standard was not injected upon arrival but was diluted and run September 21st (together with the EURL PT corn sample) and then run again November 1st (together with there two PT samples). The results are corrected for recovery, 92 % B1, 93 % B2, 91 % G1, 90 % G2.
LC0009	No	The purpose of the standard solution was not completely clear for LC-MS users.	
LC0010	Centrifugation and filtration using GFA filter paper was required following dilution of extract with PBS, otherwise sample would not pass through IAC column	yes	The unit for the standard is in ng/ml. The edit feature for the unit tab for the standard injections was not enabled hence unit could not be inserted
LC0011	No	yes	
LC0012	No	yes	
LC0014	No	yes	
LC0015		yes	
LC0016	No	I'm not sure I have correctly understood the procedure to follow for the standard injections.....	
LC0017	See above, do not know if there was any effect on results. Applied to both sample A and B	yes	We have analysed defatted peanut powder in the past without the problems which we encountered. was it totally defatted?
LC0018	No	yes	The EURL-std solution was diluted 1/2 before injection. (Calibration curve std max 50 ng/ml)
LC0019	No	yes	
LC0021	sample was defatted which we do not make with routine samples	parts for reporting Total aflatoxin amount	Our recovery factor was 103 % obtained with ELISA kit (Aflatoxin Veratox HS, LOT:227494) for sum of total aflatoksin (B1+B2+G1+G2) with the determined cross - reactivity of a test (B1- 100 %, B2-18 %, G1-13 % and G2-1 %).Out results of recovery corrected results for sum of aflatoxins in sample named "A" =4,14 µg/kg "B" =5,10 µg/kg LOD=0,5 µg/kg LOQ=1,0 µg/kg Linearity area=1-8 µg/kg
LC0022	No	yes	No
LC0023	No	yes	
LC0024	No	yes	
LC0025	No	yes	
LC0026	Problems when using KOBRA CELL	yes	
LC0027	No	yes	

Lab Code	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	Did you find the instructions distributed for this PT adequate? Which parts can be improved?	Comments
LC0028	No	yes	
LC0029	No	yes	
LC0030	No	yes	
LC0031	No	yes	The stored standard deteriorated badly under conditions set, we would not have accepted data generated by it. Lack of sample meant sample could not be repeated as would be standard practice following an analysis run where the kobra membrane has been depleted during run.
LC0032	No		
LC0033	No	yes	
LC0034	No	yes	The analysis method is ELISA and the result is the sum of B1+B2+G1+G2 (not just AFLA
LC0035	No	yes	
LC0036	Probably due to the fact that the samples are defatted, we had to make some modifications in the slurry preparation. Both samples	yes	
LC0037	No	yes	
LC0038	No	yes	You may in your description of the use of RingDat mention, that after finishing there will be a possibility to print out the input data
LC0039	No	yes	
LC0040	No	yes	
LC0041	No	yes	The results are calculated from three replicates' results and corrected with recoveries.
LC0042	No	yes	For sample A: AfB2 result below LOQ (without Uc), AfG1 and AfG2 both below LOD (without Uc)
LC0043	No	yes	
LC0044	No	yes	
LC0045	No	Otherwise well instructed but some more advice with data input would have been helpful (such as how many decimals were needed, if use comma or column as decimal separator etc.)	
LC0046	No	yes	

Lab Code	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	Did you find the instructions distributed for this PT adequate? Which parts can be improved?	Comments
LC0047	No	yes	Recoveries for aflatoxin B1 -92 % aflatoxin B2 - 95.2 % aflatoxin G1 -91.4 % aflatoxin G2 -102.4 %
LC0048	No	yes	
LC0049			Recovery: Aflatoxin B1 : 53 % Aflatoxin B2: 51 % Aflatoxin G1: 31 % Aflatoxin G2: 21 %
LC0050	No	yes	
LC0051	No	yes	
LC0052	No	There is no column for the recoveries.	
LC0053	No	No particular suggestions	With the samples lower than our reporting limit I reported a measurement uncertainty of 0 which is of course meaningless. If samples are considered blank we do not report a measurement uncertainty but the software refused to accept this. Our general uncertainty is 40 %, irrespective of the level.
LC0054	No	yes	-
LC0055	No	yes	Downloading the software for the results attachment was a problem.
LC0056	No	yes	
LC0057	No	Instructions regarding standard solutions were a bit misleading. Reported peak areas of aflatoxins in SET1-SET3 were obtained in our laboratory using LC-MS (NOT using FLD).	

Annex 9. Recoveries

Laboratory code	Recovery % AFLA B1	Recovery % AFLA B2	Recovery % AFLA G1	Recovery % AFLA G2	Recovery % total AFLA's	Technique
LC0002	86	86	88	73		HPLC
LC0003	98.5	95	97.8	87		HPLC
LC0004	101	86	92	92		LCMS
LC0005	95	94	96	94		LCMS
LC0006	97.4	97	100.2 / 94.9	97.7		HPLC
LC0007	100	-	-			HPLC
LC0008	92	93	91	90		HPLC
LC0009	91	93	93	95		LCMS
LC0010	77	85	84	80		HPLC
LC0011	90	93	90.1	87.2		HPLC
LC0012	88.1 / 89.4	88.7 / 92.7	97.1 / 95.1	77.2 / 67.3		LCMS
LC0013	no result	no result	no result	no result		no result
LC0014	78.1	81	77	74		HPLC
LC0015	100		80			LCMS
LC0016	81.1	80.2	80.6	62.7		HPLC
LC0017	65.7	77	71.3	77.2		HPLC
LC0018	88.4	87.4	84	86.1		HPLC
LC0019	82.3	82.8	84.9	67.2		HPLC
LC0020	no result	no result	no result	no result		no result
LC0021					103	ELISA
LC0022	92.3	92.8	87.2	78.4		HPLC
LC0023	97	97	97	97		HPLC
LC0024	65	46	57	36		HPLC
LC0025	60	65	70	75		HPLC
LC0026	95	93	87	70		HPLC
LC0027	90	93	99	76		HPLC
LC0028	84.9	84	46.2	55.8		HPLC
LC0029	84	91	84	69		HPLC

Laboratory code	Recovery % AFLA B1	Recovery % AFLA B2	Recovery % AFLA G1	Recovery % AFLA G2	Recovery % total AFLA's	Technique
LC0030	82	87.7	87.2	89.6		HPLC
LC0031	77.6	83.2	68.8	76.8		HPLC
LC0032	87	97	85	93		HPLC
LC0033	113	115	116	110		HPLC
LC0034					112	ELISA
LC0035	96	103	101	100		HPLC
LC0036	75.5	84.2	87.9	88		HPLC
LC0037	96	99	96	114		LCMS
LC0038	88	88	87	92		HPLC
LC0039	83	91	90	94		HPLC
LC0040						HPLC
LC0041	87.5	85.2	106.3	106.4		HPLC
LC0042						LCMS
LC0043	90	95	90	100		HPLC
LC0044	88 / 77	81 / 87	85 / 88	78 / 73		HPLC
LC0045	94	96	96	96		HPLC
LC0046	86	84	83	87		HPLC
LC0047	92	95.2	91.4	102.4		HPLC
LC0048	92	97	94	96		HPLC
LC0049	53	51	31	21		LCMS
LC0050	63.3	80.4	61.9	78.7		HPLC
LC0051	78.1	85.5	75.1	81		LCMS
LC0052	92 / 99	95 / 98	87 / 96	94 / 80		HPLC
LC0053	100	100	100	100		LCMS
LC0054	91.5	91.5	91.5	91.5		HPLC
LC0055	102.13	102.75	98	90.55		HPLC
LC0056	95	90	88	90		HPLC
LC0057	91	99	94	88		LCMS

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