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Report on the 2013 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

Determination of Fumonisin B_1, Deoxynivalenol and Aflatoxin B_1 in Cereals

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Report on the 2013 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

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1. Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the European Union Reference Laboratory (EU-RL) 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 of the EU-RL for Mycotoxins which focused on the determination of fumonisin B1 (FB1), deoxynivalenol (DON) and aflatoxin B1 (Afla B1) in cereal samples.

The two test items were naturally contaminated maize flour. The two materials were prepared at IRMM and dispatched to the participants end of July 2013. Each participant received two containers of approximately 80 g per test material.

Seventy one participants from 31 countries registered for the exercise. Fifty-nine sets of results were reported for FB1 in both test items, 69 for DON and 70 for Afla B1.

The assigned values, established by exact-matching double isotope dilution mass spectrometry, were 4.26 mg/kg (Sample A) and 31.2 mg/kg (Sample B) for FB1, 1.10 and 2.29 mg/kg for DON, and 8.90 and 18.4 µg/kg for Afla B1. The uncertainties of the respective assigned values were 0.24 and 1.2 mg/kg, 0.13 and 0.22 mg/kg, and 0.75 and 2.2 µg/kg.

Participants were invited to report the uncertainty of their measurements. This was done by the majority of laboratories.

Laboratory results for FB1, DON and Afla B1 were rated with z-scores and zeta-scores in accordance with ISO 13528 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, 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 of underperformance. In total about 70 % of the attributed z-scores were below an absolute value of 2, which indicated that most of the participants performed satisfactorily. The conducted PT revealed that the biggest challenge was the accurate determination of FB1 at higher concentration levels.

2. Introduction

Aflatoxins are mycotoxins that are found on many cereals and oilseeds, primarily on maize and peanuts. They are produced by strains of Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. Aspergillus flavus produces B aflatoxins only, while the other species produce both B and G ones.

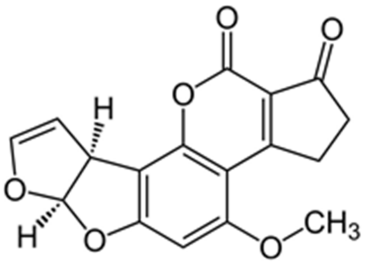
Toxic effects of aflatoxins include carcinogenic, mutagenic, teratogenic, and immunosuppressive activity. Aflatoxin B1 [Figure 1a] is the most potent hepatocarcinogen known in mammals and it is classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen [1].

Fusarium species produce a heterogeneous variety of mycotoxins such as trichothecenes and fumonisins. They are mainly contaminating cereals like wheat, barley and maize used as food and feed.

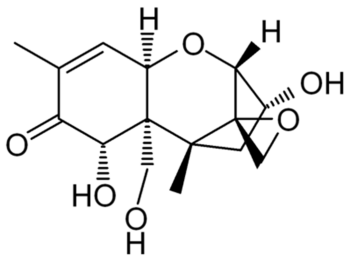
The most abundant type B trichothecene is deoxynivalenol (DON, vomitoxin) [Figure 1b], produced by F. graminearum and F. culmorum. Emesis, reduced weight gain and other gastrointestinal disorders are the most sensitive functional manifestations of the type B trichothecenes [2],[3]. DON is ordered in category 3 (not classified relating to carcinogenicity for humans) by the IARC [1].

Fumonisin B1 [Figure 1c], mainly produced by F. verticillioides (F. moniliforme) is known to be nephrotoxic and hepatotoxic in animals. FB1 has been classified by the IARC as carcinogenic to animals and as possibly carcinogenic to humans (Group 2B) [1].

a) Afla B1



b) DON



c) FB1

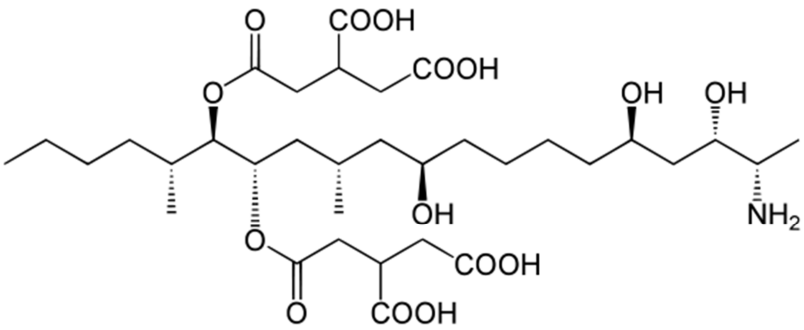


Figure 1: Chemical structures of the analytes in the proficiency test

Commission Regulation (EC) No. 1881/2006 [**4**] lays down maximum limits for FB1, DON and Aflatoxin B1 in cereal grains and cereal-based products intended for human consumption. The European Commission also sets guideline limits for FB1, DON and Afla B1 in animal feed in Commission Recommendations (2006/576/EC and 2002/32/EC) [**5**], [**6**], [**7**]

3. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [**8**], one of the core duties of the EURL is to organise proficiency test (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 FB1, DON and Afla B1 in cereal samples.

The EU-RL for mycotoxins organised a PT on DON in 2008 and 2012 [**9,10**] and on Afla B1 in 2011 [**11**] in cereal products. This year's PT was the first one also covering the determination of FB1.

All invited laboratories were free to use their method of choice. The methodologies used for the determination of these mycotoxins range from high-performance liquid chromatography (HPLC) with various detection systems, over gas chromatography and enzyme linked immunosorbent assays (ELISA). The two equally common approaches in EU member states are HPLC with either ultra-violet (UV), fluorescence detection (FL) or mass selective (MS) detection, with slight variations in frequency towards one or the other, depending on the analyte.

The ILC was designed and the reported data were processed in line with the International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories [**12**].

Accredited according to ISO 17043 the EU-RL Mycotoxins performed the assessment of the measurement results on the basis of requirements laid down in legislation and followed administrative and logistic procedures of ISO 17043 [**13**].

3.1. Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed.

4. Time frame

The ILC was discussed and agreed upon by the NRL network at the seventh EU-RL Mycotoxins workshop held on 26-27 April 2012. Specific details of the exercise were refined during the eighth EURL Mycotoxins workshop held on 10-11 June 2013 and the planned PT was published on the EU-RL web page [**14**]. The exercise was opened for registration on 25 June 2013 [**Annex 13.1**]. The samples were dispatched to the participants on 29-31 July 2013 [**Annex 13.2**]. Reporting deadline was 25 September 2013.

5. Material

5.1. Preparation

The test materials (maize samples) used in this study were purchased from Trilogy, Washington, MO, USA and further processeded by the EU-RL by re-milling to a particle size < 500 µm with a high speed centrifugal mill and homogenisation in a tumble mixer. Processed material was then packed in plastic screw-cap containers, taking portions from different places of the lot at random, and making up to a total sample size of at least 80 g (usually ca. 81 g).

5.2. Homogeneity

To verify the homogeneity of the test materials 10 units per material (Sample A and Sample B) were selected at random. Two independent determinations per unit were performed with an LC-MS/MS based method, which has been validated in-house. The measurement batch order was randomised. Homogeneity was evaluated according to ISO 13528:2005 [**15**].

The material proved to be adequately homogeneous. The details of the procedure and results are listed in **Annex 13.3**

5.3. Stability

The amount of FB1, DON and Afla B1 in the test materials were monitored (n=4) over a period of eight months (from March 2013 until October 2013) with an isochronous stability test as published by Lamberty, Schimmel & Pauwels [**16**]. No indication of degradation was found over the whole period at 4 °C and up to 5 months at room temperature. It was therefore concluded that the materials are sufficiently stable when stored below 4 °C as it was applied prior dispatch and requested after shipment. Moderate exposures to room temperatures also did not influence the stability, provided the sample was protected from direct sunlight.

5.4. Distribution

All samples were packed in cardboard boxes and sent to the participant via DHL express mail. One set of material was sent to every participant. The test materials were dispatched to the participants between 29-31 July 2013. The samples were mostly received within 24 hours after dispatch.

Each participant received:
a) two units containing approximately 80 g of test materials,
b) an accompanying letter with instructions on sample handling and reporting [**Annex 13.2**],
c) a sample receipt form [**Annex 13.4**] and
d) a registration key for the reporting interface.

The materials were shipped at room temperature; storage upon arrival was required to be at -18 °C until the analysis was performed. Based on previous experience a short period of 1-2 days without cooling imposes no harm for the material; storage at 4 °C over a longer period of time was also indicated as acceptable.

6. Instructions to participants

The laboratories were asked to report the recovery corrected value and the measurement uncertainty in µg/kg (for Afla B1) and/or mg/kg (for DON and FB1), the coverage factor used and the recovery in %.

Results were reported in a special online form for which each participant received an individual access code. A specific questionnaire was attached to this online form. The questionnaire was intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in **Annex 13.5**.

7. Reference values and their uncertainties

Assigned values and their uncertainties for the test samples were established by "Exact-matching Double Isotope Dilution Mass Spectrometry" at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [**17**]. The details of the procedure can be found in the report of the NRL PT from 2011 [**11**].

8. Evaluation of results

8.1. General observations

Seventy-one laboratories participated in this PT: NRLs from twenty-eight Member States (two different NRLs for food and feed in eleven Member States), four NRLs from 3rd countries, and 28 appointed Official Control Laboratories (OLC) from 9 Member States registered to the PT. All laboratories reported results.

Fifty-nine sets of results were reported for FB1 for both test samples, 69 for DON and 70 for Afla B1.

8.2. Scores and evaluation criteria

Individual laboratory performance was assessed in terms of z and zeta (ζ) scores in accordance with ISO 13528 [**15**] and the International Harmonised Protocol [**12**].

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

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{lab} + u^2_{ref}}} \qquad \textbf{Equation 2.}$$

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 [**18**] (for analyte concentrations < 120 ppb):

- for analyte concentrations < 120 ppb *(Afla B1 Sample A, Afla B1 Sample B)*

$$\sigma_p = 0.22 \cdot c \qquad \textbf{Equation 3.}$$

- for analyte concentrations ≥ 120 ppb *(DON Sample A, DON Sample B, FB1 Sample A, FB1 Sample B)*

$$\sigma_p = 0.02 \cdot c^{0.8495} \qquad \textbf{Equation 4.}$$

where:
c = concentration of the measurand (assigned value, $X_{ref,}$) expressed as a dimensionless mass ratio, e.g. 1 ppb = 10^{-9}, 1 ppm = 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:

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

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 zeta-score is similar to the interpretation of the z-score:

$\|\zeta\| \leq 2$	satisfactory result

$2 < \|\zeta\| \leq 3$	questionable result
$\|\zeta\| > 3$	unsatisfactory result

An unsatisfactory $|\zeta|$-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory $|\zeta|$-score indicated an uncertainty which is not consistent with the laboratory's deviation from the reference value.

8.3. Laboratory results and scoring

Statistical evaluation of the results was performed using MS Excel.

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528 [**15**] by application of a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [**19**].

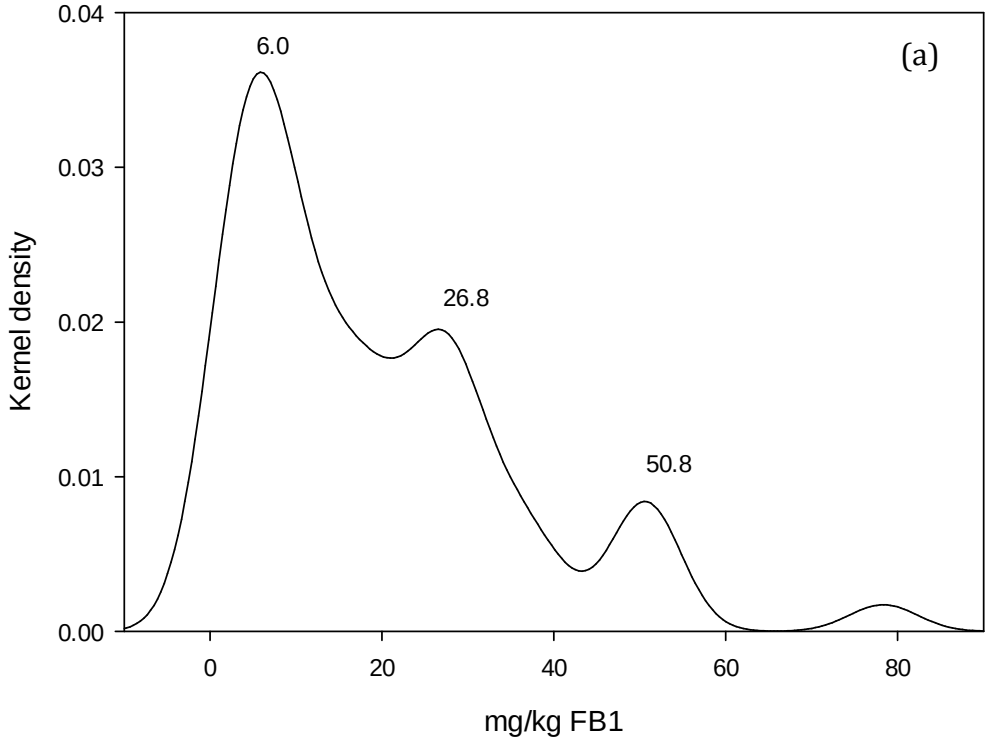
z-scoring and zeta-scoring was done for FB1, DON and Afla B1. However, only unsatisfactory z-scores will result in the request for corrective actions for these three mycotoxins.

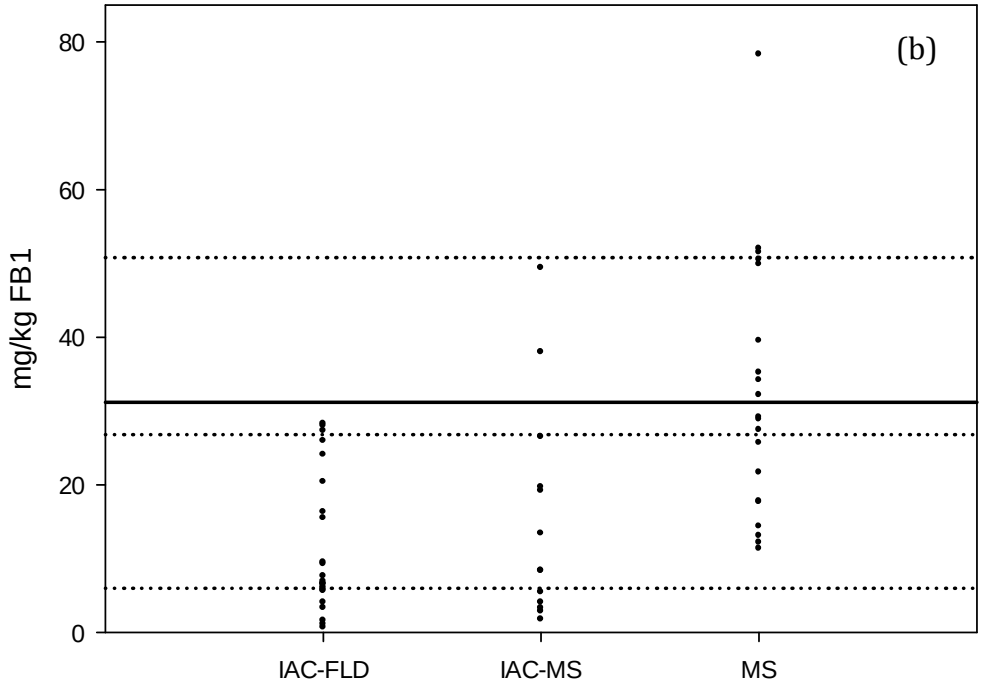
The results from the FB1 measurements at high level (sample B) are distributed over a wide range spanning two orders of magnitude (from ca. 0.7 to ca. 78 mg/kg). This rather wide distribution of results indicates that a systematic investigation of the methodologies would be needed to identify possible reasons for the scatter observed.

Plotting results for sample B for kernel density revealed a clear multimodality of results (Fig. 2a). Exploratory plots for the different methodologies used, classified for clean-up and detection methods, indicate a methodological influence on the distribution of results.

As it can be seen in Fig. 2b, the three maxima, one at 6.0 mg/kg a 2nd one at 26.8 mg/kg and a 3rd at 50.8 mg/kg all shown with dotted lines, can be attributed to the different classes of methodologies used. The 1st maximum has its main contribution from methodologies using IAC clean-up.
The 2nd maximum lays close to the assigned value. Contributors to this maximum mainly come from labs using either IAC clean-up with fluorescence detection or mass spectrometry without immunoaffinity clean-up. The 3rd maximum has contributions only from MS-based methodologies.

A much better agreement of the methodologies used is present for sample A (Fig. 2c) and such effects as were found for sample B (higher concentration) (Fig. 2b) could not be confirmed.


(a)
6.0
26.8
50.8
Kernel density
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
mg/kg FB1


(b)
mg/kg FB1
80
60
40
20
0
IAC-FLD
IAC-MS
MS

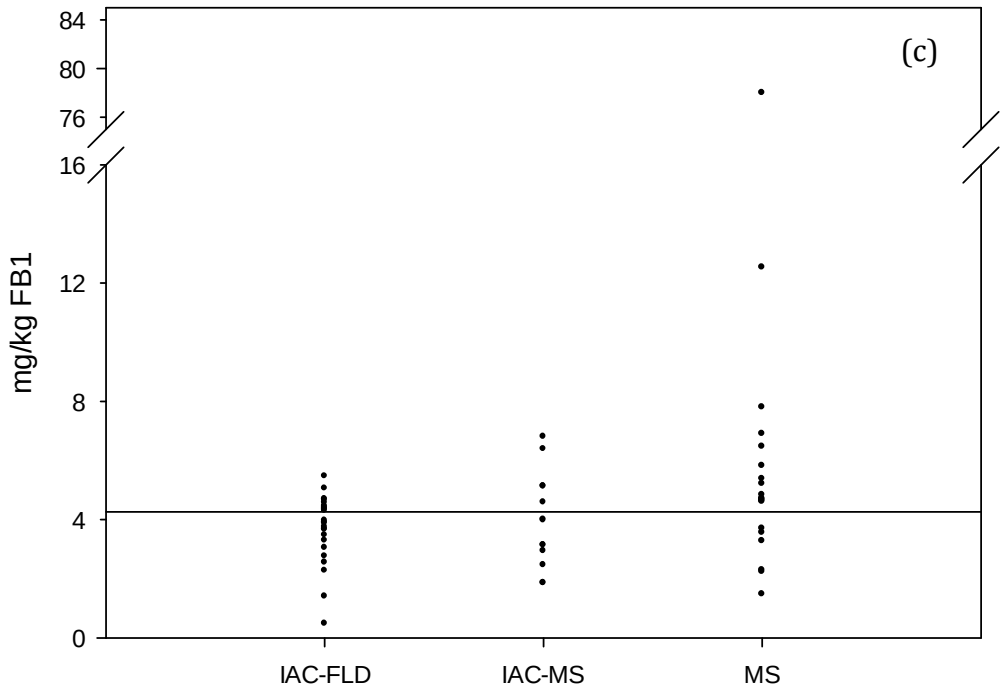


Figure 2: (a) Kernel density plot (smoothing parameter h=4) (sample B) and exploratory plots of the results of FB1 analysis ((b), (c) - sample B and A, respectively) with distinction of clean-up and detection techniques. Solid lines represent reference values, dotted lines represent levels at density maximas.

Further statements on other relevant parameters and possible combinations thereof can, however, only be made after full evaluation of the reported parameters from the questionnaire. This will be discussed at the next EU-RL/NRL network meeting.

The results as reported by the participants are summarised in **Tables 2, 4 and 6** together with the z-scores and zeta-scores. Summaries of the statistical evaluation for each analyte and test sample are presented in **Tables 1, 3 and 5.**

Figures 3-8 provide for each analyte/matrix combinations the individual laboratory values and their uncertainty as reported.

Table 1: Summary statistics for deoxynivalenol (DON)

		Sample A	Sample B
Number of results		67	67
Range of results	mg/kg	0.537-3.007	0.345-3.24
Median of results of participants	mg/kg	1.12	2.197
Mean of results of participants	mg/kg	1.153	2.133
Robust mean of results of participants	mg/kg	1.110	2.159
Assigned value	mg/kg	**1.10**	**2.29**
Expanded uncertainty (k=2) of the assigned value	mg/kg	0.13	0.22
Robust standard deviation ($\hat{\sigma}$)	mg/kg	0.22	0.58
Target standard deviation (fitness for purpose)	mg/kg	0.17	0.32
Number (percentage) of results of |z| > 2.0		11 (17%)	17 (25%)
Number (percentage) of results of |ζ| > 2.0		18 (27%)	19 (28%)

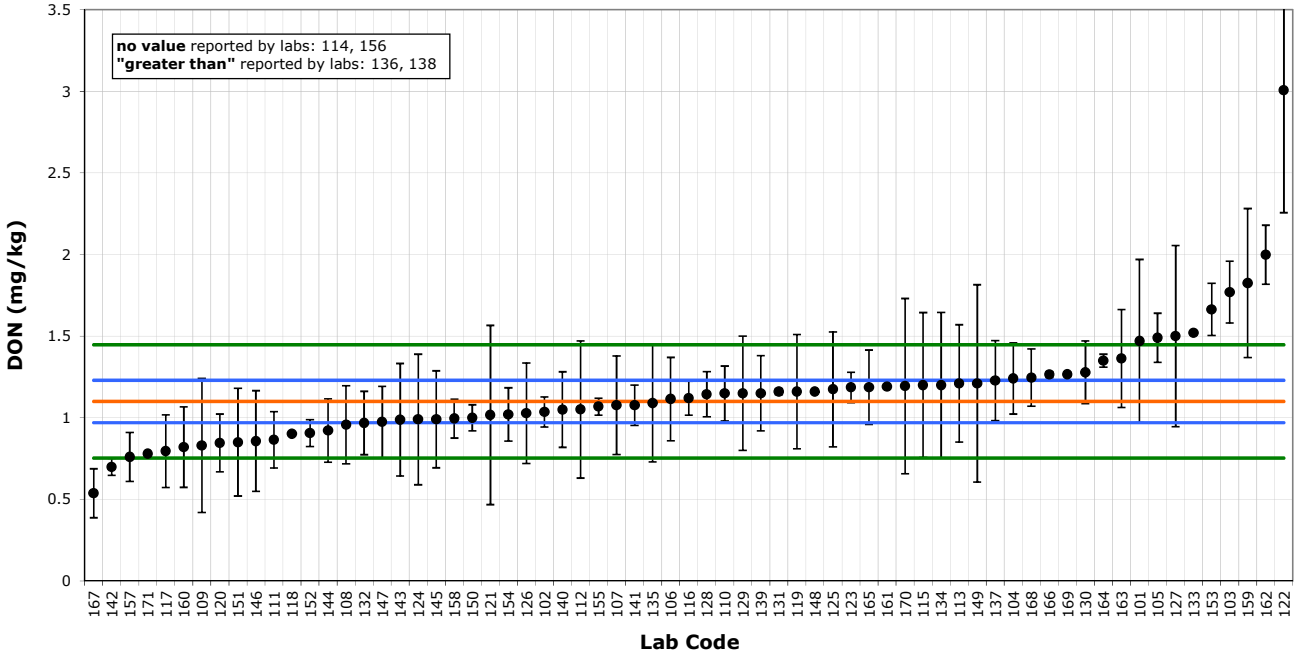
Table 2: Results of analysis, z-scores and zeta-scores for deoxynivalenol (DON)
(The meaning of colors: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [mg/kg]	z-score	zeta-score	Result [mg/kg]	z-score	zeta-score
101	1.47	2.1	1.4	2.86	1.8	1.1
102	1.035	-0.4	-0.8	2.097	-0.6	-1.3
103	1.77	3.9	5.8	3.15	2.7	4.2
104	1.241	0.8	1.1	2.502	0.7	0.9
105	1.49	2.2	3.9	2.91	1.9	3.3
106	1.11452	0.1	0.1	2.19554	-0.3	-0.3
107	1.077	-0.1	-0.1	2.197	-0.3	-0.3
108	0.957	-0.8	-1.1	1.778	-1.6	-2.1
109	0.83	-1.6	-1.3	1.66	-1.9	-1.5
110	1.149	0.3	0.5	1.48	-2.5	-5.4
111	0.865	-1.4	-2.2	1.8946	-1.2	-1.8
112	1.051	-0.3	-0.2	2.238	-0.2	-0.1
113	1.21	0.6	0.6	2.43	0.4	0.4
114	*No result*			*No result*		
115	1.2	0.6	0.4	2.5	0.6	0.4
116	1.12	0.1	0.2	1.469	-2.5	-6.5
117	0.795	-1.8	-2.4	1.566	-2.2	-3.0
118	0.902	-1.1	-3.0	2.484	0.6	1.8
119	1.16	0.3	0.3	2.33	0.1	0.2
120	0.846	-1.5	-2.3	1.424	-2.7	-4.7
121	1.017	-0.5	-0.3	1.949	-1.1	-0.6
122	3.007	11.0	5.0	1.275	-3.1	-5.2
123	1.1859	0.5	1.1	1.4832	-2.5	-6.5
124	0.99	-0.6	-0.5	2.78	1.5	0.9
125	1.1741	0.4	0.4	2.1754	-0.4	-0.3
126	1.027	-0.4	-0.4	1.972	-1.0	-1.0
127	1.5	2.3	1.4	2.7	1.3	0.8
128	1.144	0.3	0.5	2.33	0.1	0.2
129	1.15	0.3	0.3	2.4	0.3	0.3
130	1.278	1.0	1.5	2.868	1.8	2.4
131	1.15956	0.3	0.9	2.46442	0.5	0.5
132	0.968	-0.8	-1.1	2.06	-0.7	-1.0
133	1.52	2.4		3.24	2.9	
134	1.2	0.6	0.4	2.2	-0.3	-0.2
135	1.09	-0.1	-0.1	2.87	1.8	1.2

Lab Code	SAMPLE A			SAMPLE B		
	Result [mg/kg]	z-score	zeta-score	Result [mg/kg]	z-score	zeta-score
136	>1			>3.6		
137	1.2277	0.7	0.9	2.374	0.3	0.3
138	>1.5			>1.5		
139	1.15	0.3	0.4	2.32	0.1	0.1
140	1.05	-0.3	-0.4	2.03	-0.8	-1.0
141	1.077	-0.1	-0.3	2.102	-0.6	-1.1
142	0.69895	-2.3	-5.7	1.03591	-3.9	-10.9
143	0.988	-0.6	-0.6	1.988	-0.9	-0.8
144	0.922	-1.0	-1.5	1.848	-1.4	-2.0
145	0.9906	-0.6	-0.7	1.993	-0.9	-0.9
146	0.857	-1.4	-1.4	1.971	-1.0	-0.9
147	0.9739	-0.7	-1.0	1.9159	-1.2	-1.7
148	1.16	0.3		2.98	2.1	
149	1.21	0.6	0.4	2.73	1.4	0.6
150	1	-0.6	-1.3	1.7	-1.8	-3.8
151	0.85	-1.4	-1.4	1.71	-1.8	-1.7
152	0.9065	-1.1	-2.5	2.4545	0.5	1.4
153	1.664	3.3	5.5	3.019	2.3	4.0
154	1.02	-0.5	-0.8	2.042	-0.8	-1.3
155	1.068	-0.2	-0.5	2.25942	-0.1	-0.3
156	*No result*			*No result*		
157	0.76	-2.0	-3.4	1.66	-1.9	-3.2
158	0.995	-0.6	-1.2	2.34	0.2	0.2
159	1.825	4.2	3.1	0.905	-4.3	-8.8
160	0.82	-1.6	-2.0	1.146	-3.5	-5.6
161	1.19	0.5		2.51	0.7	
162	1.99882	5.2	8.1	1.0081	-4.0	-10.8
163	1.363	1.5	1.6	2.711	1.3	1.3
164	1.35	1.4	3.7	3.09	2.5	5.1
165	1.187	0.5	0.7	2.27	-0.1	-0.1
166	1.264	0.9	2.5	2.5	0.6	1.9
167	0.537	-3.2	-5.7	1.212	-3.3	-5.3
168	1.246	0.8	1.3	2.328	0.1	0.2
169	1.266	1.0	2.5	2.116	-0.5	-1.5
170	1.194	0.5	0.3	2.37	0.2	0.1
171	0.780	-1.8		0.345	-6.0	

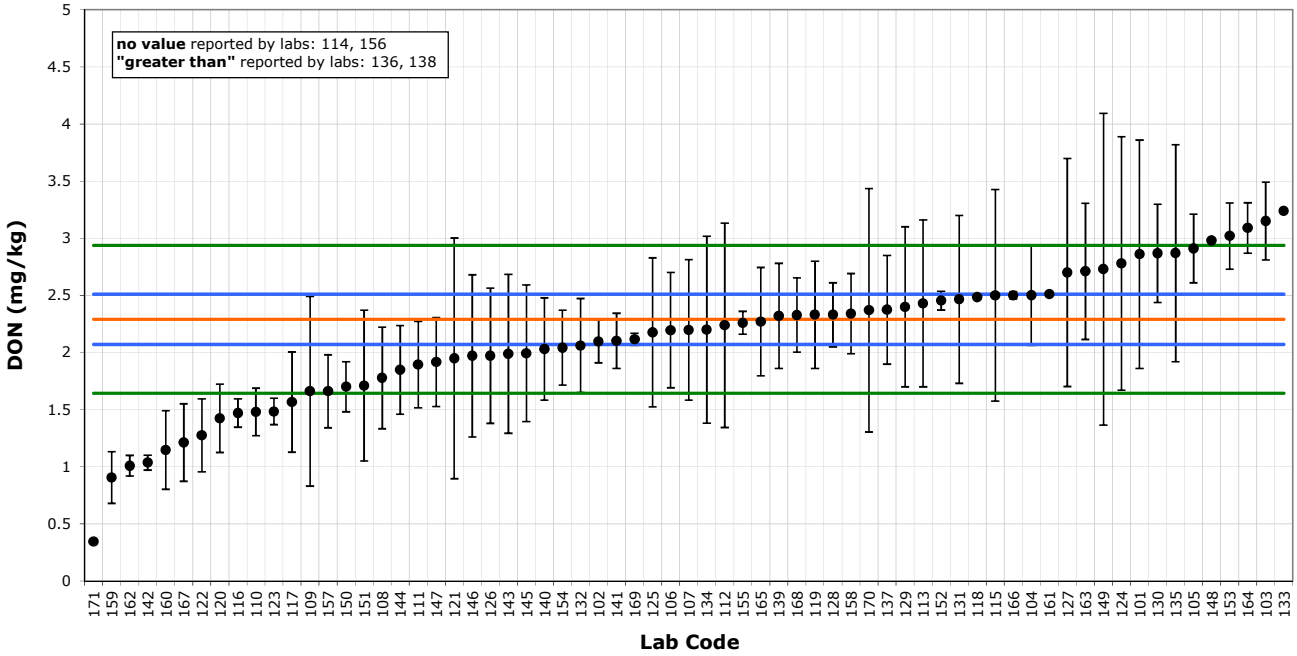
Results as reported by the laboratories.

Figure 3: EU-RL Mycotoxins PT 2013: Deoxynivalenol in cereals - Sample A
Certified value: Xref = 1.10 mg/kg; Uref = 0.13 mg/kg (k=2); σ = 0.173 mg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Figure 4: EU-RL Mycotoxins PT 2013: Deoxynivalenol in cereals - Sample B
Certified value: Xref = 2.29 mg/kg; Uref = 0.22 mg/kg (k=2); σ = 0.323 mg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Table 3: Summary statistics for the fumonisin B1 (FB1)

		Sample A	Sample B
Number of results		59	58
Range of results	mg/kg	0.477-78	0.704-78.33
Median of results of participants	mg/kg	4.3	14.94
Mean of results of participants	mg/kg	5.45	19.29
Robust mean of results of participants	mg/kg	4.12	17.65
Assigned value	mg/kg	**4.26**	**31.2**
Expanded uncertainty (k=2) of the assigned value	mg/kg	0.24	1.2
Robust standard deviation ($\hat{\sigma}$)	mg/kg	1.48	14.63
Target standard deviation (fitness for purpose)	mg/kg	0.55	2.97
Number (percentage) of results of |z| > 2.0		26 (44%)	46 (79%)
Number (percentage) of results of |ζ| > 2.0		27 (47%)	42 (75%)

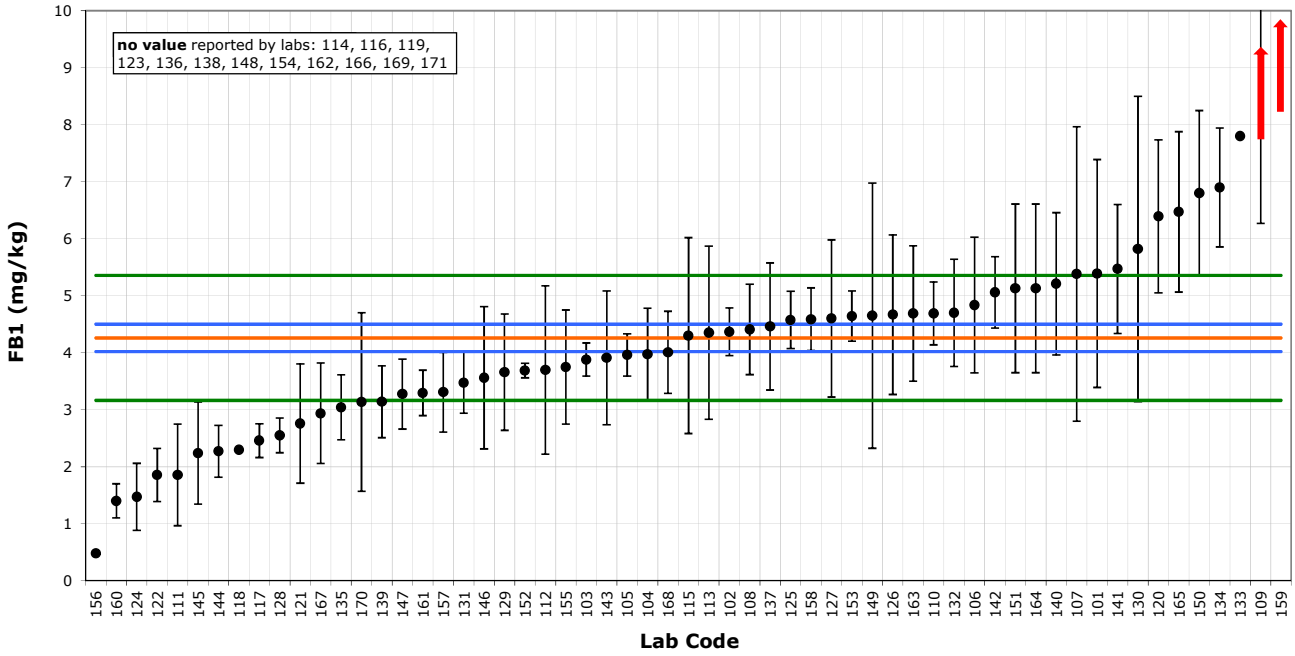
Table 4: Results of analysis, z-scores and zeta-scores for fumonisin B1 (FB1)
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [mg/kg]	z-score	zeta-score	Result [mg/kg]	z-score	zeta-score
101	5.39	2.1	1.1	>6		
102	4.367	0.2	0.4	25.972	-1.8	-3.8
103	3.88	-0.7	-2.0	24.1	-2.4	-6.3
104	3.972	-0.5	-0.7	26.494	-1.6	-1.7
105	3.96	-0.5	-1.4	7.64	-7.9	-33.7
106	4.83579	1.1	0.9	29.17404	-0.7	-0.6
107	5.381	2.0	0.9	28.909	-0.8	-0.3
108	4.408	0.3	0.4	5.625	-8.6	-32.6
109	12.54	15.1	2.6	78.33	15.8	2.4
110	4.689	0.8	1.4	32.163	0.3	0.6
111	1.8563	-4.4	-5.2	4.073	-9.1	-23.7
112	3.697	-1.0	-0.8	21.706	-3.2	-2.2
113	4.35	0.2	0.1	28	-1.1	-0.6
114	*No result*			*No result*		
115	4.3	0.1	0.0	9.5	-7.3	-10.9
116	*No result*			*No result*		
117	2.457	-3.3	-9.5	2.87	-9.5	-45.4
118	2.294	-3.6	-16.4	17.677	-4.5	-22.5
119	*No result*			*No result*		
120	6.392	3.9	3.1	37.984	2.3	1.7
121	2.758	-2.7	-2.8	1.109	-10.1	-47.3
122	1.855	-4.4	-9.2	1.776	-9.9	-46.0
123	*No result*			*No result*		
124	1.47	-5.1	-8.8	12.2	-6.4	-7.6
125	4.576	0.6	1.1	28.298	-1.0	-1.7
126	4.669	0.7	0.6	27.338	-1.3	-0.9
127	4.6	0.6	0.5	49.9	6.3	3.1
128	2.55	-3.1	-8.8	5.744	-8.6	-36.8
129	3.66	-1.1	-1.1	15.5	-5.3	-6.5
130	5.82	2.8	1.2	50.527	6.5	1.7
131	3.47416	-1.4	-2.7	20.39242	-3.6	-3.8
132	4.7	0.8	0.9	35.2	1.3	1.1
133	7.8	6.5		51.5	6.8	
134	6.9	4.8	4.9	52.0	7.0	5.2

Lab Code	SAMPLE A			SAMPLE B		
	Result [mg/kg]	z-score	zeta-score	Result [mg/kg]	z-score	zeta-score
135	3.04	-2.2	-3.9	6.60	-8.3	-28.5
136	*No result*			*No result*		
137	4.4605	0.4	0.4	6.5775	-8.3	-24.2
138	*No result*			*No result*		
139	3.14	-2.0	-3.3	19.7	-3.9	-7.1
140	5.21	1.7	1.5	34.2	1.0	0.7
141	5.471	2.2	2.1	4.085	-9.1	-36.9
142	5.0592	1.5	2.4	6.5357	-8.3	-38.4
143	3.911	-0.6	-0.6	6.178	-8.4	-22.7
144	2.272	-3.6	-7.7	16.336	-5.0	-8.5
145	2.238	-3.7	-4.4	14.387	-5.7	-5.7
146	3.561	-1.3	-1.1	27.442	-1.3	-0.8
147	3.275	-1.8	-3.0	17.795	-4.5	-9.4
148	*No result*			*No result*		
149	4.65	0.7	0.3	25.7	-1.8	-0.8
150	6.8	4.6	3.5	49.4	6.1	3.5
151	5.13	1.6	1.2	8.38	-7.7	-17.0
152	3.687	-1.0	-4.2	3.339	-9.4	-46.2
153	4.643	0.7	1.5	11.36	-6.7	-24.6
154	*No result*			*No result*		
155	3.75	-0.9	-1.0	5.93	-8.5	-32.4
156	0.4773	-6.9		0.7035	-10.3	
157	3.31	-1.7	-2.6	2.24	-9.7	-45.8
158	4.588	0.6	1.1	5.491	-8.6	-37.6
159	78	134.6	6.3	13.1	-6.1	-8.8
160	1.4	-5.2	-14.9	1.6	-10.0	-46.8
161	3.295	-1.8	-4.1	6.954	-8.2	-33.2
162	*No result*			*No result*		
163	4.689	0.8	0.7	9.294	-7.4	-18.0
164	5.13	1.6	1.2	8.38	-7.7	-17.0
165	6.471	4.0	3.1	39.531	2.8	1.5
166	*No result*			*No result*		
167	2.937	-2.4	-2.9	3.297	-9.4	-35.9
168	4.007	-0.5	-0.7	13.416	-6.0	-13.2
169	*No result*			*No result*		
170	3.135	-2.1	-1.4	19.21	-4.0	-2.5
171	*No result*			*No result*		

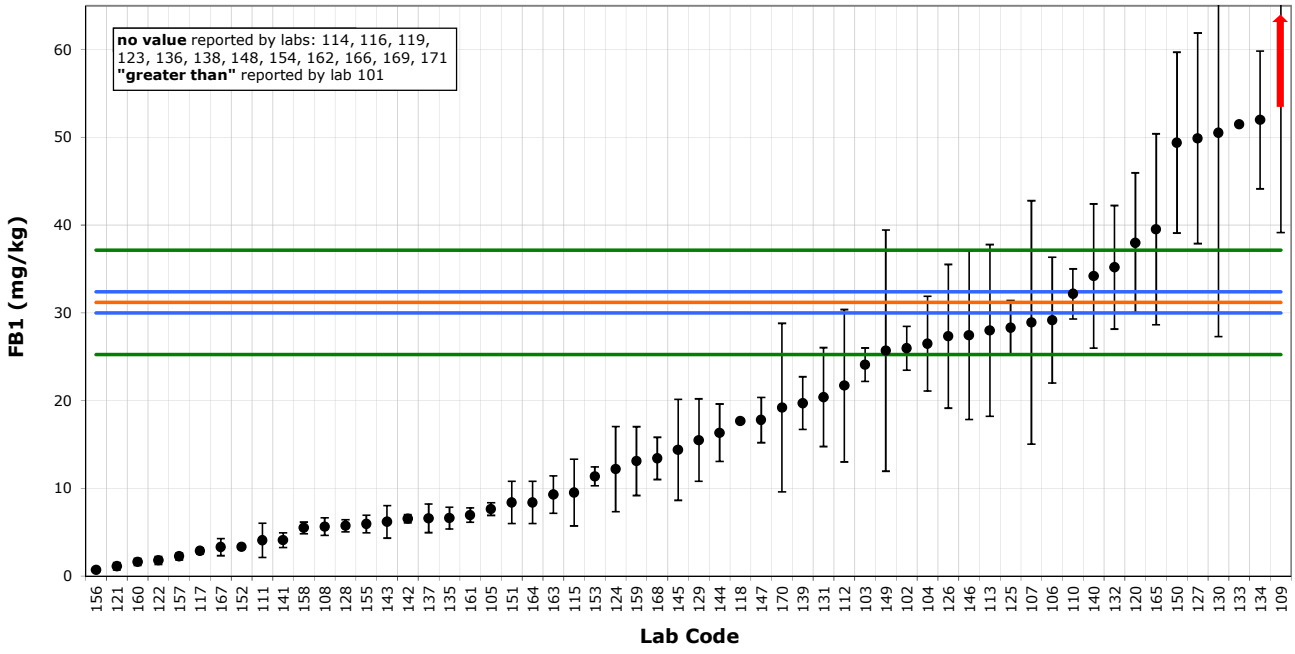
Results as reported by the laboratories.

Figure 5: EU-RL Mycotoxins PT 2013: Fumonisin B1 in cereals - Sample A
Certified value: Xref = 4.26 mg/kg; Uref = 0.24 mg/kg (k=2); σ = 0.548 mg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Figure 6: EU-RL Mycotoxins PT 2013: Fumonisin B1 in cereals - Sample B
Certified value: Xref = 31.2 mg/kg; Uref = 1.2 mg/kg (k=2); σ = 2.97 mg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Table 5: Summary statistics for the aflatoxin B1 (AfB1)

		Sample A	Sample B
Number of results		69	68
Range of results	µg/kg	0.35-22	2-43.5
Median of results of participants	µg/kg	8.90	18.9
Mean of results of participants	µg/kg	8.83	19.0
Robust mean of results of participants	µg/kg	8.86	19.1
Assigned value	µg/kg	**8.90**	**18.4**
Expanded uncertainty (k=2) of the assigned value	µg/kg	0.75	2.2
Robust standard deviation ($\hat{\sigma}$)	µg/kg	2.24	5.07
Target standard deviation (fitness for purpose)	µg/kg	1.96	4.05
Number (percentage) of results of |z| > 2.0		8 (12%)	12 (18%)
Number (percentage) of results of |ζ| > 2.0		21 (32%)	21 (32%)

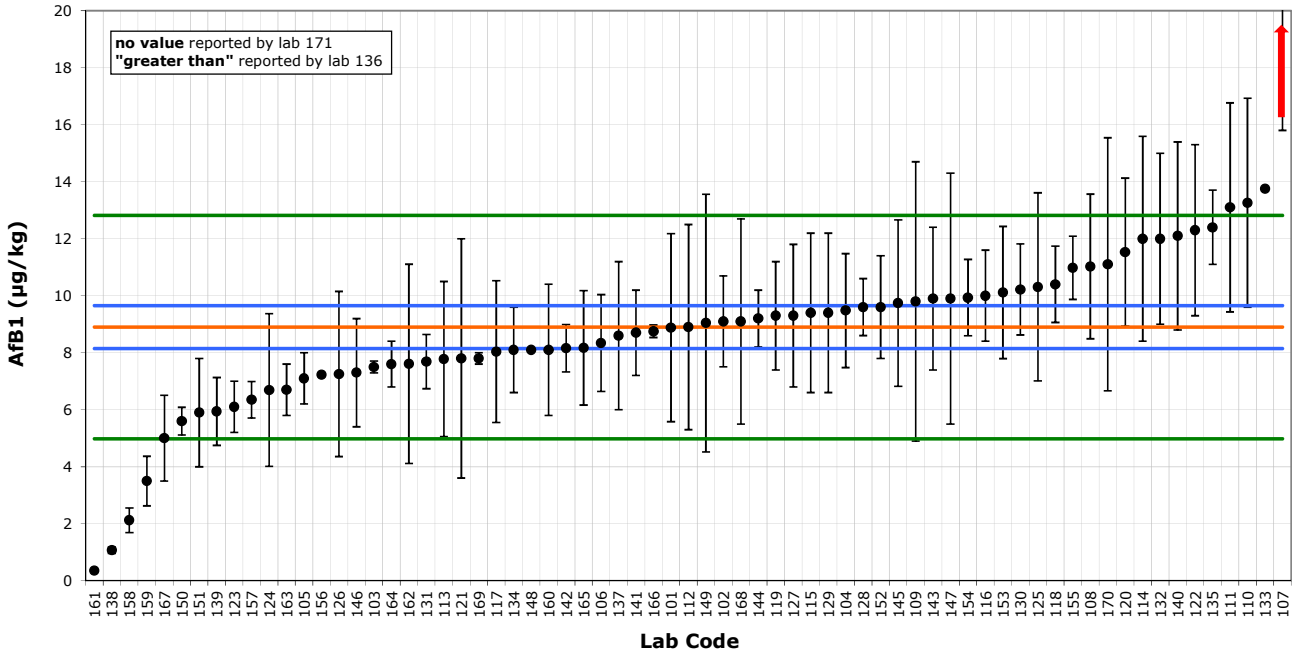
Table 6: Results of analysis, z-scores and zeta-scores for aflatoxin B1 (Afla B1)
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [µg/kg]	z-score	zeta-score	Result [µg/kg]	z-score	zeta-score
101	8.88	0.0	0.0	18.13	-0.1	-0.1
102	9.1	0.1	0.2	20.1	0.4	0.8
103	7.5	-0.7	-3.6	21.3	0.7	2.2
104	9.48	0.3	0.5	27.2	2.2	2.9
105	7.1	-0.9	-3.1	14.1	-1.1	-3.0
106	8.34	-0.3	-0.6	18.59	0.0	0.1
107	22	6.7	4.2	43.5	6.2	4.0
108	11.03	1.1	1.6	24.18	1.4	1.9
109	9.8	0.5	0.4	18.9	0.1	0.1
110	13.26	2.2	2.3	20.96	0.6	0.9
111	13.1	2.1	2.2	29.1	2.6	2.5
112	8.9	0.0	0.0	18.1	-0.1	-0.1
113	7.78	-0.6	-0.8	17.24	-0.3	-0.4
114	12	1.6	1.7	23	1.1	1.2
115	9.4	0.3	0.3	19	0.1	0.1
116	10	0.6	1.2	17.1	-0.3	-0.9
117	8.04	-0.4	-0.7	14.6	-0.9	-1.5
118	10.4	0.8	2.0	19.9	0.4	1.2
119	9.3	0.2	0.4	17.6	-0.2	-0.4
120	11.53	1.3	1.9	26.79	2.1	2.6
121	7.8	-0.6	-0.5	18.3	0.0	0.0
122	12.3	1.7	2.2	26.3	2.0	2.3
123	6.1	-1.4	-4.8	9.8	-2.1	-6.5
124	6.69	-1.1	-1.6	22.02	0.9	0.8
125	10.31	0.7	0.8	21.48	0.8	0.9
126	7.25	-0.8	-1.1	15.47	-0.7	-1.5
127	9.3	0.2	0.3	15	-0.8	-1.5
128	9.6	0.4	1.1	16.3	-0.5	-1.5
129	9.4	0.3	0.3	21	0.6	0.8
130	10.22	0.7	1.5	20.27	0.5	1.0
131	7.69	-0.6	-2.0	18.48	0.0	0.1
132	12	1.6	2.0	23.1	1.2	1.5
133	13.75	2.5		31.2	3.2	
134	8.1	-0.4	-1.0	17.6	-0.2	-0.4
135	12.4	1.8	4.7	23	1.1	2.8
136	>8.7			>19.8		

Lab Code	SAMPLE A			SAMPLE B		
	Result [µg/kg]	z-score	zeta-score	Result [µg/kg]	z-score	zeta-score
137	8.6	-0.2	-0.2	20.7	0.6	0.7
138	1.07	-4.0	-20.7	2.36	-4.0	-14.5
139	5.94	-1.5	-4.2	18.5	0.0	0.0
140	12.1	1.6	1.9	17.9	-0.1	-0.2
141	8.7	-0.1	-0.2	16.7	-0.4	-1.0
142	8.16	-0.4	-1.3	17.06	-0.3	-0.6
143	9.9	0.5	0.8	22	0.9	1.2
144	9.2	0.2	0.5	20.5	0.5	1.3
145	9.742	0.4	0.6	22.45	1.0	1.1
146	7.3	-0.8	-1.6	19.8	0.3	0.5
147	9.9	0.5	0.4	23.4	1.2	1.1
148	8.1	-0.4		13	-1.3	
149	9.04	0.1	0.1	28.9	2.6	1.4
150	5.6	-1.7	-7.4	10.7	-1.9	-6.4
151	5.9	-1.5	-2.9	18.9	0.1	0.2
152	9.6	0.4	0.7	25.6	1.8	5.1
153	10.11	0.6	1.0	21.06	0.7	1.0
154	9.93	0.5	1.3	19.23	0.2	0.5
155	10.98	1.1	3.1	22.82	1.1	2.5
156	7.23	-0.9		17.68	-0.2	
157	6.35	-1.3	-5.2	12.1	-1.6	-5.0
158	2.12	-3.5	-15.7	4.91	-3.3	-10.3
159	3.5	-2.8	-9.4	2	-4.1	-14.5
160	8.1	-0.4	-0.7	17.7	-0.2	-0.3
161	0.35	-4.4		<0.35		
162	7.61	-0.7	-0.7	16.77	-0.4	-0.4
163	6.7	-1.1	-3.8	11.7	-1.7	-4.7
164	7.6	-0.7	-2.4	26.7	2.1	2.9
165	8.17	-0.4	-0.7	15.92	-0.6	-1.0
166	8.75	-0.1	-0.4	12.65	-1.4	-5.2
167	5	-2.0	-4.7	8	-2.6	-6.4
168	9.1	0.1	0.1	20.6	0.5	0.5
169	7.8	-0.6	-2.8	13.7	-1.2	-4.2
170	11.1	1.1	1.0	20.9	0.6	0.6
171	*No result*			*No result*		

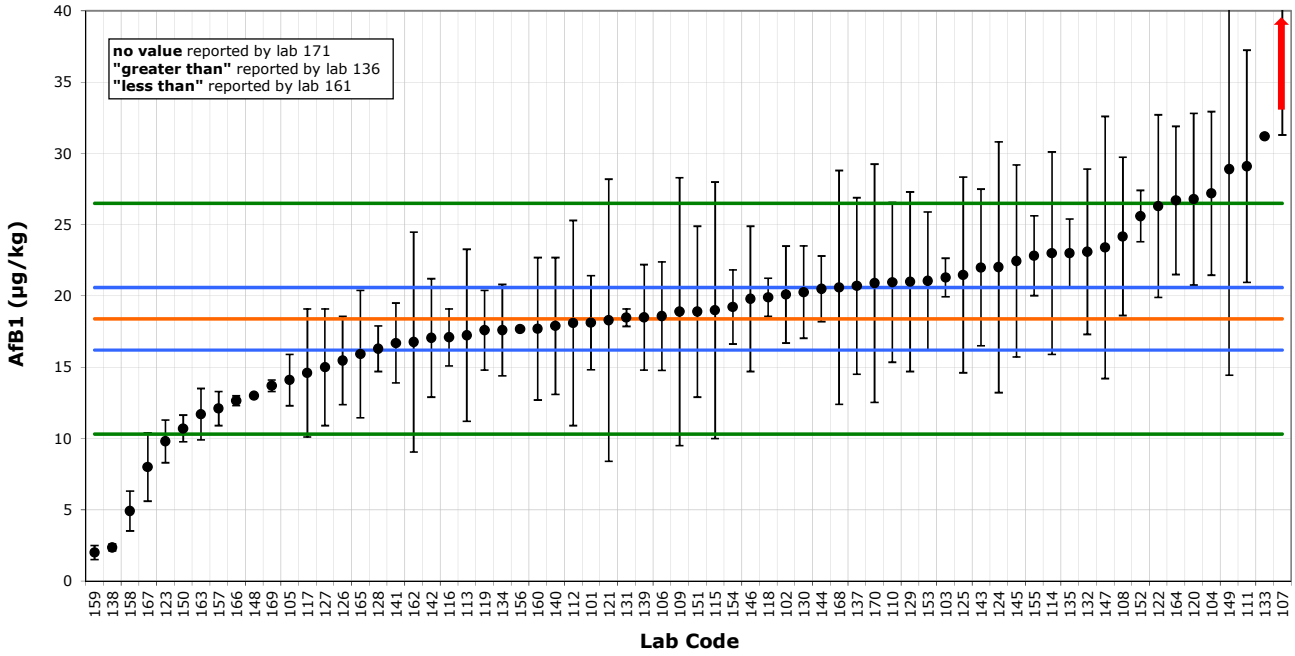
The results are written as reported by the laboratories.

Figure 7: EU-RL Mycotoxins PT 2013: Aflatoxin B1 in cereals - Sample A
Certified value: Xref = 8.90 µg/kg; Uref = 0.75 µg/kg (k=2); σ = 1.958 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Figure 8: EU-RL Mycotoxins PT 2013: Aflatoxin B1 in cereals - Sample B
Certified value: Xref = 18.4 µg/kg; Uref = 2.2 µg/kg (k=2); σ = 4.05 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

8.4. Evaluation of the questionnaire

All laboratories that reported results, in total seventy-one participants, supplied their filled in questionnaires. Experimental details along with a summary of the answers are presented in the **Annex 13.6.**

A general screening of the reported answers showed that participants used mainly three techniques – HPLC-DAD, HPLC-FLD and LC-MS/MS - for obtaining the results for different mycotoxins.

For the determination of Afla B1, most of the laboratories (60%) used HPLC-UV or FLD, whereas LC-MS was used in 35% of cases. For FB1 analysis LC-MS was applied by 54% of the participants. Regarding the analysis of DON, LC-MS was used by 50% of participants and HPLC-UV/FLD techniques were used by 41%. Single laboratories used ELISA for determinations.

Most of the laboratories analysed annually 50 samples or more for all three analytes. Seventy-six percent of the participating laboratories are accredited for the analysis of Afla B1, 68% for DON, and 48% for FB1. 24% have accredited multitoxin methods.

Most of the laboratories applied immunoaffinity clean-up of samples

For the recovery estimation the majority of the participants used a "standard solution added to blank" method.

Details about the applied methodology for different analytes – extraction, clean up, overnight stop, etc. - are presented in **Annex 13.6**. As mentioned before possible links between the reported results and answers on the used methodology will be explored and discussed in the EU-RL/NRL annual meeting 2014.

All participants found the instructions adequate and the registration-reporting interface the EU-RL received mostly positive feedback.

9. Conclusions

Fifty nine (Sample A) and 59 (Sample B) sets of results were reported for FB1, 69 sets of results each (sample A & B) for DON and 70 sets of results each (sample A & B) for Afla B1.

Most of the participants performed satisfactory or better according the evaluaton scheme used (target standard deviation computed according to Horwitz-Thompson).
Almost all laboratories had problems with determining FB1 at higher levels, what renders these determinations questionable and shows that there are significant analytical challenges for the reliable determination of FB1 at levels relevant for compliance testing.
It was noted that the consensus values and the assigned values match very well for Afla B1 and DON, and also for FB1 for sample A, but in case of results for FB1 in sample B no meaningful consensus value could be calculated using the results of participants, which stresses the importance of generating external reference value for such critical samples. The EU-RL will investigate the possible reasons for the wide scatter in the FB1 samples reported for Sample B and will suggest solutions to improve the measurements capability for the determination of FB1.

10. Acknowledgements

The organizers of the study would like to thank Andreas Breidbach, Katy Kroeger-Negoita, Katrien Bouten, Franz Ulberth and Beatriz de la Calle for their support.

The laboratories participating in this exercise, listed in [**Table 7**], are also kindly acknowledged.

Table 7: Participating laboratories

Organisation	Country
AGES GmbH	Austria
LVA GmbH	Austria
OLEOTEST N.V.	Belgium
CODA-CERVA	Belgium
FAVV	Belgium
SGS BELGIUM NV	Belgium
Fytolab cvba	Belgium
BFSA	Bulgaria
Regional Health Inspectorate - Pleven	Bulgaria
Regional Health Inspection Burgas	Bulgaria
Laboratory of SGS Bulgaria Ltd	Bulgaria
Regional Health Inspection - Varna	Bulgaria
Fytolab Bulgaria	Bulgaria
Public Health Institute "Dr.A.Štampar"	Croatia
Department of Agriculture	Cyprus
State General Laboratory	Cyprus
Czech Agriculture and Food Inspection Authority	Czech Republic
Central Institute for Supervising and Testing in Agriculture (UKZUZ)	Czech Republic
DTU Food	Denmark
Danish Veterinary and Food Administration	Denmark
Agricultural Research Centre	Estonia
Finnish Food Safety Authority Evira	Finland
Finnish Customs Laboratory	Finland
LDA 22	France
Laboratoire SCL de Rennes	France
Laboratoire des Pyrénées et des Landes (LPL)	France
Landeslbetrieb Hessisches Landeslabor	Germany
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft (BfUL)	Germany
Federal Institute for Risk Assessment (BfR)	Germany
General Chemical State Laboratory of Greece	Greece
National Food Chain Safety Office, Food and Feed Safety Directorate	Hungary
National Food Chain Safety Office, Food And Feed Safety Directorate	Hungary
Public Analyst's Laboratory	Ireland
The State Laboratory	Ireland
Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna	Italy
ISTITUTO SUPERIORE DI SANITA'	Italy
Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna Bologna	Italy
Istituto Zooprofilattico Sperimentale del Mezzogiorno	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
NVWA - Netherlands Food and Consumer Product Safety Authority	Netherlands
RIKILT	Netherlands
Nofalab	Netherlands
National Institute of Public Health - National Institute of Hygiene	Poland
National Veterinary Research Institute	Poland
ASAE	Portugal
INIAV, IP	Portugal
Sanitary Veterinary and Food Safety Directorate Bucharest	Romania
Institutul De Igiena Si Sanatate Publica Veterinara	Romania
Faculty of Technology, University of Novi Sad	Serbia
Institute of Meat Hygiene and Technology	Serbia
Health Sciences Authority	Singapore
State Veterinary and Food Institute Košice	Slovakia
Zavod za zdravstveno varstvo Maribor	Slovenia

Organisation	Country
University in Ljubljana, Veterinary Faculty - National Veterinary Institute	Slovenia
National Center for Food (Spanish Food Safety and Nutrition Agency)	Spain
Govern de les Illes Balears	Spain
ainia	Spain
Gv.Conselleria de Sanidad.Centro Salud Pública	Spain
Centro Nacional de Tecnología y Seguridad Alimentaria (CNTA)	Spain
National Veterinary Institute (SVA)	Sweden
National Food Agency	Sweden
Kantonales Laboratorium Basel-Landschaft	Switzerland
Kent County Council	United Kingdom
Food & Environment Research Agency	United Kingdom
Worcestershire Scientific Services	United Kingdom
City of Edinburgh Council	United Kingdom
Minton, Treharne & Davies	United Kingdom
Glasgow Scientific Services	United Kingdom

11. Abbreviations

Afla B1 (AfB1)	Aflatoxin B1
ANOVA	Analysis of variance
DON	Deoxynivalenol
EC	European Commission
ELISA	Enzyme linked immunosorbant assays
EU	European Union
EURL	European Reference Laboratory
FB1	Fumonisin B1
FLD	Fluorescent detection
HPLC	High-performance liquid chromatography
IAC	Immunoaffinity column
IDMS	Isotope Dilution Mass Spectrometry
ILC	Interlaboratory Comparison
IRMM	Institute for Reference Materials and Measurements
ISO	International Organisation for Standardisation
IUPAC	International Union for Pure and Applied Chemistry
JRC	Joint Research Centre
LOD	Limit of Detection
LOQ	Limit of Quantification
NRL	National Reference Laboratory
PT	Proficiency Test

12. References

[**1**]. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol.82, IARC Press, Lyon (France), 2002, p. 169.
http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf

[**2**] Bennett, J. W., & Klich, M. (2003). Mycotoxins. Clinical Microbiology Review, 16(3), 497–516.

[**3**] Desjardins, A. E., Hohn, T. M., & McCormick, S. P. (1993). Trichothecene biosynthesis in Fusarium species: Chemistry, genetics, and significance. Clinical Microbiology Reviews, 57(3), 595–604.

[**4**] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF

[**5**] 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
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF

[**6**] Lerda, D., Mycotoxins Factsheet Fourth Edition – September 2011 – Joint Research Centre
http://irmm.jrc.ec.europa.eu/EURLs/eurl_mycotoxins/Documents/Factsheet%20Mycotoxins.pdf

[**7**] Commission Directive 2002/32/EC of 7 May 2002 on undesirable substances in animal feed
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF

[**8**] Commission Regulation (EC) No 882/2004 of the European Parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
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[**13**] ISO/IEC 17043:2010 - Conformity assessment -- General requirements for proficiency testing

[**14**] IRMM. Inter-laboratory Comparisons at the Institute for Reference Materials and Measurements.; Available from:
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[**16**] Lamberty A., Schimmel H., Pauwels J., The study of the stability of reference materials by isochronous measurements, Fresenius Journal of Analytical Chemistry 36093-40:359-361
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13. Annexes

13.1. Opening of registration

Ref. Ares(2013)2485240 - 25/06/2013

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 25 June 2013

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Sir/Madam,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of

- aflatoxin B1 (Afla B1)
- deoxynivalenol (DON),
- fumonisin B1 (FB1).

This proficiency test (PT) was announced beginning of this year, and more recently by e-mail dated 28th May 2013. More details on the PT design will be communicated upon sample dispatch.

The EU-RL Mycotoxins would like to inform you that, according to Regulation (EC) No 882/2004, the participation of activities organised by the EU-RL is mandatory for the NRLs.

- For NRLs the participation is mandatory and therefore free of charge.
- The participation fee for Official Control laboratories is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. The IRMM will contact participants with details of the payment.

Confidentiality of the participants and their results are granted.

Registration of participants is open until midnight of 17th July, 2013.

Dispatch of the PT materials is foreseen to be 23rd July and will be announced in advance.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 849. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

In order to register, laboratories must:

1. Enter the details online:

   https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1101

2. Print the completed form (approved and confirmed version) when the system asks to do so, sign it and stamp it with your company stamp

3. Send it to the EU-RL Mycotoxins members indicated below:

   The PT coordinator is:

   Maciej KUJAWSKI
   Tel: +32 14 571 849
   Fax: +32 14 571 783

   Email: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

Deadline for reporting will be the 18th September. You will receive the link for entering the results upon reception of the PT samples.

A detailed outline of the PT will accompany the PT sample parcel; anyhow we would like to encourage you to contact us in case you seek further clarification.

Please contact us at the mail address:

JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

With kind regards,

Maciej Kujawski

(on behalf of the Operating Manager of the EU-RL Mycotoxins)

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Joerg Stroka

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 849. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

13.2. Accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 24 July 2013

Ref: 2013 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on Afla B1, DON and FB1 in cereal samples

Dear Participant,

Please read the following information carefully before starting any analysis. If there are additional questions, do not hesitate to contact us by either phone or email (see details below).

The 2013 PT aims to:

Assess the content in two naturally contaminated test samples (marked as "Sample A", "Sample B"). You will be asked to report the **recovery corrected value** (µg/kg), including your **recovery** (%) and **measurement uncertainty** (µg/kg) for a coverage factor of 2 (k=2).

Please confirm the parcel's receipt by fax or e-mail immediately, by using the "**Materials receipt form**". If any material is damaged, please request new material immediately.

The materials are shipped at room temperature; storage however should be at -18° C until the analysis is performed. A short period of 1-2 days without cooling is no harm for the material, but a longer period of storage above -18° C shall be avoided.

Please report all requested results and answer the questionnaire at
https://web.jrc.ec.europa.eu/ilcReportingWeb
The password key for this interface is included in the parcel with the test materials. When you enter the code please pay attention to the capital letters!

Print out the final pdf and return the signed and stamped report sheet NOT later than **25th September 2013** to:

Maciej Kujawski

Tel: +32-14-571 849
FAX: +32-14-571 783
E-mail: JRC-IRMM-EURL-Mycotox@ec.europa.eu

In case of questions please do not hesitate to contact us.

With kind regards,

Maciej Kujawski
(on behalf of the Operating Manager of the EU-RL Mycotoxins)

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Joerg Stroka

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-eurl-mycotox@ec.europa.eu

13.3. Homogeneity test

Homogeneity according to ISO 13528:2005 [15]	**Sample A**			**Sample B**		
	Afla B1	DON	FB1	Afla B1	DON	FB1
Mean	8.65	1.10	4.24	18.16	2.33	29.87
$\hat{\sigma}$	1.9 (22%)	0.169 (15.4%)	0.547 (13%)	4.0 (22%)	0.324 (14%)	2.90 (9.7%)
0.3 $\hat{\sigma}$ (critical value)	0.571	0.051	0.164	1.199	0.097	0.869
S_X (standard deviation of sample averages)	0.514	0.024	0.049	1.706	0.053	0.691
S_W (within-sample standard deviation)	0.673	0.033	0.090	1.848	0.073	1.154
S_S (between-sample standard deviation)	0.195	0.003	0	1.096	0.010	0
$S_S < 0.3\,\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**

13.4. Stability study

18/03/2013 - 31/10/2013

Aflatoxin B1 – sample A

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	9.53	8.27	**8.90**	8.51	8.72	**8.61**	0.5	6.96	7.99	**7.48**	1.8
18/03/2013	20 weeks				8.54	8.39	**8.47**	0.7	9.91	8.85	**9.38**	-0.6
29/07/2013	13 weeks								7.10	7.77	**7.43**	2.1

Aflatoxin B1 – sample B

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	17.78	21.36	**19.57**	18.25	19.49	**18.87**	0.4	18.62	19.12	**18.87**	0.4
18/03/2013	20 weeks				19.75	19.49	**19.62**	0.0	18.84	18.77	**18.81**	0.4
29/07/2013	13 weeks								16.64	17.33	**16.99**	1.4

Deoxynivalenol – sample A

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	1.11	1.04	**1.08**	1.15	1.08	**1.11**	-0.7	1.07	0.99	**1.03**	0.8
18/03/2013	20 weeks				1.12	1.03	**1.07**	0.0	1.11	1.02	**1.06**	0.2
29/07/2013	13 weeks								1.10	1.00	**1.05**	0.4

Deoxynivalenol – sample B

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	2.13	2.02	**2.08**	2.11	1.93	**2.02**	0.5	2.14	1.98	**2.06**	0.2
18/03/2013	20 weeks				1.98	1.99	**1.98**	1.6	2.05	1.93	**1.99**	1.1
29/07/2013	13 weeks								2.10	1.97	**2.04**	0.4

Fumonisin B1 – sample A

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	4.46	4.67	**4.57**	4.45	4.67	**4.56**	0.0	4.31	4.61	**4.46**	0.6
18/03/2013	20 weeks				4.51	4.60	**4.56**	0.1	4.32	4.52	**4.42**	1.0
29/07/2013	13 weeks								4.49	4.44	**4.46**	0.9

Fumonisin B1 – sample B

Start date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
18/03/2013	31 weeks	31.6	29.3	**30.5**	30.7	28.5	**29.6**	0.5	29.3	28.1	**28.7**	1.4
18/03/2013	20 weeks				31.2	29.0	**30.1**	0.2	30.4	28.4	**29.4**	0.7
29/07/2013	13 weeks								29.9	28.3	**29.1**	1.0

Taking into account the repeatability values and the t critical value of two-side t-test obtained during the homogeneity study, t_{crit} = 2.26 (α=0.05, df=9), all the mean values for Sample A as well as for Sample B at the tested temperature/time conditions were not statistically different than the respective mean value at the reference temperature (-18 °C) ($t_{calc} < t_{crit}$).

The instability differences were, therefore, not significant at the 95 % level of confidence following the approach of the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [**12**].

13.5. Acknowledgement of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 24 July 2013

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Address:
Member State:

NOTE: STORE ALL MATERIALS 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 for analysis:
- Sample A
- Sample B

b) 1 envelope with documents:
- A copy of instructions
- Password key
- Questionnaire

Signature / Stamp:

Please fax or e-mail the completed form to:

Maciej Kujawski

Tel: +32-14-571 849
FAX: +32-14-571 783

E-mail: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

13.6. Questionnaire

Milc questionnaire

Comparison for PT EU-RL Myco 2013 multitoxin

Please fill in your results and answer the questions. Print the final pdf and return the signed and stamped copy by fax +32 14 571 783 or by e-mail to JRC-IRMM-EURL-MYCOTOX@ec.europa.eu.

Submission Form

1. **How many samples does your laboratory approximately analyse for the following mycotoxins per year?** *
See table **Please specify the amount of samples per mycotoxin.** at bottom

2. **Which food or feed matrices does your laboratory analyse most frequently for Afla B1, DON and Fum B1 on a routine basis? (maximum 3)** *
See table **Please specify the matrices.** at bottom

3. **Did you use a multitoxin method or individual methods for determination?** *
- ☐ individual methods
- ☐ multitoxin

4. **Are you accredited for the determination of these mycotoxins in maize?**

Questions/Response table	Afla B1	DON	Fum B1	Multitoxin method	None	Info
Accredited for: *	☐	☐	☐	☐	☐	

5. **Performance of the method(s)** *
See table **Please provide the LOD and LOQ.** at bottom

5.1. Please state the methodology (e.g. 10 x signal/noise) to calculate the LOQ. *

6. **What is your main procedure for recovery estimation?** *
- ○ a) Internal Standard to Extract
- ○ b) Internal Standard to Sample
- ○ c) Standard solution to Blank Sample
- ○ d) other

6.1. If other please specify! *

7. **Do you perform calibrant check on regular basis?**
- ○ a) Yes
- ○ b) No

7.1. If YES, please explain in brief how (e.g. UV-Spectrometry). *

8. **Sample preparation** *
See table **Extraction parameters** at bottom

9. **What type of clean-up methodology was used (e.g. immunoaffinity column)?** *

10. **Mention the type of column used for seperation.** *
See table **Column parameters** at bottom

11. **For LC-MS only: What is the calculated sample fraction injected onto the LC system [mg/injection]?**

12. **Did you encounter any problems during the analysis?** *
- ○ a) Yes
- ○ b) No

12.1. If YES, what were the specific problems and to which samples do they apply? *

13. **Did you notice any unusual observations which, however, did not seem to have any effect on the results?** *
- ○ a) Yes
- ○ b) No

13.1. If YES, what were these observations and to which samples do they apply? *

14. **Did you find the instructions distributed for this PT adequate?** *

- ◯ a) Yes
- ◯ b) No

14.1. If NO, which parts do you think can improve? *

15. **Any other comments you wish to make?**

Column parameters

Questions/Response table	*Stationary phase*	*Particle size [µm]*	*Inner diameter [mm]*	*Length [mm]*
Column used:				

Extraction parameters

If you use a multitoxin method it is not necessary to fill in the single analyte fields. Extraction mode e.g. shaking, blending, sonication, etc.

Questions/Response table	*Sample amount [g]*	*Extraction solvent type*	*Solvent volume [ml]*	*Extraction mode*	*Extraction time [min]*
Afla B1					
DON					
Fum B1					
Multitoxin method					

Please provide the LOD and LOQ.

Questions/Response table	*LOD [µg/kg]*	*LOQ [µg/kg]*
Afla B1		
DON		

Questions/Response table	*LOD [µg/kg]*	*LOQ [µg/kg]*
Fum B1		

Please specify the amount of samples per mycotoxin.

Questions/Response table	*null*
Afla B 1	
DON	
Fum B 1	

Please specify the matrices.

Questions/Response table	*null*
Afla B 1	
DON	
Fum B 1	

13.7. Experimental details

Results and method performance characteristics for Deoxynivalenol (DON)

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [mg/kg]	LOQ [mg/kg]
		Result [mg/kg]	Uncertainty [mg/kg]	Result [mg/kg]	Uncertainty [mg/kg]				
101	ELISA	1.47	0.5	2.86	1	2	100	-	0.2
102	HPLC-UV/FLD	1.035	0.092	2.097	0.187	2	94.75	N.D.	0.0500
103	GC	1.77	0.19	3.15	0.34	2	87		0.025
104	LC-MS/MS	1.241	0.218	2.502	0.44	2	82	0.050	0.250
105	HPLC-UV/FLD	1.49	0.15	2.91	0.3	2	75	0.05	0.10
106	LC-MS	1.11452	0.2563	2.19554	0.5049	2	100.5		
107	LC-MSMS	1.077	0.302	2.197	0.615	2	100	0.025	0.050
108	LC-MS	0.957	0.239	1.778	0.445	2	93.5	0.044	0.044
109	HPLC-MS/MS	0.83	0.41	1.66	0.83	2	105	0.025	0.030
110	UPLC-MS/MS	1.149	0.168	1.48	0.208	2	89.2	0.005	0.010
111	LC-MS	0.865	0.173	1.8946	0.3789	2	100	0.005	0.500
112	LC-MS	1.051	0.420	2.238	0.895	2	85		0.180
113	HPLC-UV/FLD	1.21	0.36	2.43	0.73	2	92	0.02	0.05
114		*No result*		*No result*					
115	GC-MS	1.200	0.444	2.500	0.925	2	83	0.005	0.010
116	HPLC-UV/FLD	1.120	0.104	1.469	0.124	2	102.6	0.020	0.060
117	LC/MS	0.795	0.223	1.566	0.438	2	0		0.115
118	UHPLC-MS/MS	0.902	0.105	2.484	0.288	2	97.65	0.01501	0.05006
119	HPLC-UV/FLD	1.160	0.350	2.330	0.470	2	104	0.050	0.100
120	HPLC-UV/FLD	0.846	0.177	1.424	0.29904	2	87.3	0.053	0.157
121	HPLC-UV/FLD	1.017	0.549	1.949	1.053	2	89	0.020	0.030
122	LC-MS	3.007	0.751	1.275	0.319	2	84.7		0.050
123	HPLC-UV/FLD	1.1859	0.0923	1.4832	0.1154	2	91.6	0.040	0.135
124	LC-MS	0.99	0.4	2.78	1.11	2	100	0.02	0.04
125	LC-MS	1.1741	0.3522	2.1754	0.6526	2	103		0.300
126	HPLC-UV/FLD	1.027	0.3081	1.972	0.5916	2	90	0.030	0.100
127	LC-MS	1.500	0.555	2.700	0.999	2	100	0.023	0.075
128	HPLC-UV/FLD	1.144	0.138	2.33	0.28	2	91.8	0.020	0.100
129	HPLC-UV/FLD	1.15	0.35	2.4	0.7	2	90	0.03	0.10
130	LC-MS	1.278	0.192	2.868	0.43	2	71	0.050	0.100
131	HPLC-UV/FLD	1.15956	0.01915	2.46442	0.73434	2	96.63	0.06038	0.1811
132	LC-MS-MS	0.968	0.194	2.06	0.412	2		0.020	0.050
133	LC-MS	1.520		3.24			100		
134	LC-MS	1.200	0.446	2.200	0.818	2	82.5	0.100	0.050
135	GC-MS	1.09	0.36	2.87	0.95	2	91	0.05	0.10
136	ELISA	>1		>3.6				0.04	0.05
137	HPLC-UV/FLD	1.2277	0.2455	2.374	0.475	2	89.3	0.0400	0.1200
138	HPLC-MS/MS	>1.500		>1.500				0.025	0.050
139	LC-MS	1.15	0.23	2.32	0.46	2	100	0.008	0.040
140	LC-MS	1.05	0.231	2.03	0.447	2	100	0.20	0.20

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [mg/kg]	LOQ [mg/kg]
		Result [mg/kg]	Uncertainty [mg/kg]	Result [mg/kg]	Uncertainty [mg/kg]				
141	HPLC-UV/FLD	1.077	0.124	2.102	0.242	2	78.7	0.030	0.060
142	LC-MS/MS	0.69895	0.05242	1.03591	0.06526	2	102	0.47	0.94
143	HPLC-UV/FLD	0.988	0.345	1.988	0.696	2	105	0.080	0.150
144	HPLC-UV/FLD	0.922	0.194	1.848	0.388	2	90	0.0023	0.0070
145	LC-MS	0.9906	0.2972	1.993	0.5979	2	88	0.017	0.050
146	LC-MS	0.857	0.309	1.971	0.710	2	89		0.05
147	LC-MS	0.9739	0.2189	1.9159	0.3889	2	81	0.025	0.085
148	HPLC-UV/FLD	1.16		2.98		2	81 / 104	0.10	0.20
149	LC-MS	1.210	0.605	2.730	1.365	2	100		0.050
150	HPLC-UV/FLD	1	0.08	1.7	0.22	3.18	70.3		0.15
151	HPLC-UV/FLD	0.85	0.33	1.71	0.66	2	77	0.059	0.100
152	HPLC-UV/FLD	0.9065	0.082	2.4545	0.082	2	96	0.025	0.050
153	LC-MS	1.664	0.160	3.019	0.290	2	90.9	0.0070	0.0240
154	HPLC-UV/FLD	1.02	0.163	2.042	0.327	2	95	0.060	0.112
155	HPLC-UV	1.068	0.0516	2.25942	0.1000	2	88.45	0.030	0.100
156		*No result*		*No result*					
157	UPLC/MS/MS	0.76	0.15	1.660	0.320	2	98.4 / 94.1	0.030	0.100
158	LC-MS	0.995	0.119	2.34	0.351	2	92	0.010	0.030
159	LC-MS	1.825	0.456	0.905	0.226	2	110	0.0030	0.0100
160	HPLC-UV/FLD	0.820	0.246	1.146	0.344	2	89	0.006	0.015
161	ELISA	1.190		2.510		2	0	0.200	
162	HPLC-UV/FLD	1.99882	0.1811	1.0081	0.09133	2	103.34	0.020	
163	HPLC-UV/FLD	1.363	0.300	2.711	0.596	2	80	0.059	0.100
164	HPLC-UV/FLD	1.35	0.04	3.090	0.220	2	87.6 / 76.4	0.025	0.050
165	LC-MS	1.187	0.228	2.270	0.474	1	106.5	0.003	0.010
166	HPLC-UV/FLD	1.264	0.019	2.500	0.032	2	53	0.003	0.009
167	LC-MS	0.537	0.15	1.212	0.339	2	72	ND	0.050
168	LC-MS	1.246	0.175	2.328	0.326	2	87	0.019	0.057
169	HPLC-UV/FLD	1.266	0.012	2.116	0.053	2	60	0.0041	0.0136
170	LC-MS	1.194	0.537	2.370	1.066	2	100	0.010	0.050
171	UPLC-MS/MS	0.780		0.345		2		0.010	0.050

Results and method performance characteristics for Aflatoxin B1 (Afla B1)

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
101	HPLC-UV/FLD	8.88	3.3	18.13	3.3	2	71.2	0.17	0.5
102	HPLC FLD with PHRED	9.1	1.6	20.1	3.4	2	90	N.D.	0.05
103	HPLC-UV/FLD	7.5	0.21	21.3	1.36	2	113 / 94	0.1	0.3
104	LC-MS/MS	9.48	2	27.2	5.74	2	100	0.2	1
105	HPLC-UV/FLD	7.1	0.9	14.1	1.8	2	86	0.10	0.06
106	LC-MS	8.34	1.7	18.59	3.81	2	110		
107	LC-MSMS	22	6.2	43.5	12.2	2	100	0.5	1
108	HPLC-UV/FLD	11.03	2.54	24.18	5.56	2	88	0.1	0.1
109	HPLC-MS/MS	9.8	4.9	18.9	9.4	2	92	0.34	0.5
110	HPLC-UV/FLD	13.26	3.67	20.96	5.6	2	90.3	0.002	0.004
111	LC-MS	13.1	3.67	29.1	8.15	2	84.3 / 82.8	0.25	3
112	LC-MS	8.9	3.6	18.1	7.2	2	85		1
113	HPLC-UV/FLD	7.78	2.72	17.24	6.03	2	96	0.1	0.2
114	HPLC-UV/FLD	12	3.6	23	7.1	2	78	0.50	1.0
115	HPLC-UV/FLD	9.4	2.8	19	9	2	48	0.5	2
116	HPLC-UV/FLD	10	1.6	17.1	2	2	94 / 95.9	0.085	0.255
117	HPLC	8.04	2.49	14.6	4.5	2			0.2
118	UHPLC-MS/MS	10.4	1.34	19.9	1.34	2	101.05	0.10	0.33
119	HPLC-UV/FLD	9.3	1.9	17.6	2.8	2	93	0.2	0.5
120	HPLC-UV/FLD	11.53	2.6	26.79	6.03	2	98.9	0.2	0.24
121	HPLC-UV/FLD	7.8	4.2	18.3	9.9	2	99.4	0.02	0.1
122	HPLC-UV/FLD	12.3	3	26.3	6.4	2	83.8		0.5
123	HPLC-UV/FLD	6.1	0.9	9.8	1.5	2	62.7	0.3	0.7
124	LC-MS	6.69	2.68	22.02	8.8	2	100	0.1	0.2
125	HPLC-UV/FLD	10.31	3.3	21.48	6.87	2	96		0.2
126	HPLC-UV/FLD	7.25	2.9	15.47	3.094	2	99	0.02	0.06
127	LC-MS	9.3	2.5	15	4.1	2	100	0.3	1.0
128	HPLC-UV/FLD	9.6	1	16.3	1.6	2	86.6	0.1	0.2
129	HPLC-UV/FLD	9.4	2.8	21	6.3	2	85	0.1	0.3
130	HPLC-UV/FLD	10.22	1.6	20.27	3.24	2	90	0.5	1
131	HPLC-UV/FLD	7.69	0.95	18.48	0.61	2	95.02	0.06	0.2
132	LC-MS-MS	12	3	23.1	5.8	2		0.2	0.5
133	LC-MS	13.75	0	31.2	0		100		
134	LC-MS	8.1	1.5	17.6	3.2	2	92.9	1.0	0.5
135	HPLC-UV/FLD	12.4	1.3	23	2.4	2	50	0.2	0.6
136	ELISA	>8.7		>19.8			95 / 97	1	3
137	HPLC-UV/FLD	8.6	2.6	20.7	6.2	2	72.2	0.3	0.6
138	HPLC-UV/FLD	1.07	0.11	2.36	0.24	2	107.3 / 101	0.2	0.25
139	HPLC-UV/FLD	5.94	1.19	18.5	3.7	2	98	0.05	0.2
140	LC-MS	12.1	3.3	17.9	4.8	2	103	5	5
141	HPLC-UV/FLD	8.7	1.5	16.7	2.8	2	94.3	0.025	0.05
142	HPLC-UV/FLD	8.16	0.83	17.06	4.16	2	90.5	0.10	0.20
143	UHPLC-UV/FLD	9.9	2.5	22	5.5	2	95	0.2	0.5
144	HPLC-UV/FLD	9.2	1	20.5	2.3	2	85	0.06	0.18

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
145	LC-MS	9.742	2.92	22.45	6.74	2	93	0.3	1
146	LC-MS	7.3	1.9	19.8	5.1	2	97		0.6
147	LC-MS	9.9	4.4	23.4	9.2	2	84	0.1	0.9
148	LC-MS	8.1	0	13	0	2	77 / 100	0.2	0.5
149	LC-MS	9.04	4.52	28.9	14.45	2	100		1
150	HPLC-UV/FLD	5.6	0.49	10.7	0.94	2.78	81.7		0.25
151	HPLC-UV/FLD	5.9	1.9	18.9	6	2	94 / 86	0.09	0.2
152	HPLC-UV/FLD	9.6	1.8	25.6	1.8	2	72 / 83	0.3	1.0
153	LC-MS	10.11	2.32	21.06	4.84	2	93.8	0.06	0.20
154	HPLC-UV/FLD	9.93	1.34	19.23	2.6	2	95	0.05	0.2
155	HPLC-FLD	10.98	1.11	22.82	2.8	2	75.6	0.1	0.3
156	HPLC-UV/FLD	7.23	0	17.68	0	0	87.7		4.0
157	UPLC/MS/MS	6.35	0.64	12.1	1.2	2	88.4 / 98.1	0.10	0.25
158	LC-MS	2.12	0.43	4.91	1.4	2	102	0.1	0.3
159	LC-MS	3.5	0.87	2	0.5	2	94	0.3	1.0
160	HPLC-UV/FLD	8.1	2.3	17.7	5	2	95	0.06	0.2
161	HPLC-UV/FLD	0.35	0	<0.35		2	92	0.35	
162	HPLC-UV/FLD	7.61	3.5	16.77	7.71	2	102.65	0.25	
163	HPLC-UV/FLD	6.7	0.9	11.7	1.8	2	80	0.02	0.06
164	HPLC-UV/FLD	7.6	0.8	26.7	5.2	2	94.7 / 66	0.5	1
165	LC-MS	8.17	2.01	15.92	4.47	1	113.6	0.15	0.5
166	HPLC-UV/FLD	8.75	0.22	12.65	0.34	2	64	0.05	0.1
167	LC-MS	5	1.5	8	2.4	2	80	ND	0.5
168	HPLC-UV/FLD	9.1	3.6	20.6	8.2	2	86	0.05	0.15
169	HPLC-UV/FLD	7.8	0.2	13.7	0.4	2	63	0.05	0.15
170	HPLC-UV/FLD	11.1	4.44	20.9	8.36	2	78	0.3	1
171		*No result*		*No result*				0.02	0.05

Results and method performance characteristics for Fumonisin B1 (FB1)

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [mg/kg]	LOQ [mg/kg]
		Result [mg/kg]	Uncertainty [mg/kg]	Result [mg/kg]	Uncertainty [mg/kg]				
101	ELISA	5.39	2	6		2	100	-	0.22
102	HPLC/FLD with pre-column derivatisation	4.367	0.419	25.972	2.493	2	96.4	N.D.	0.0500
103	HPLC-UV/FLD	3.88	0.29	24.1	1.9	2	65 / 66		0.050
104	LC-MS/MS	3.972	0.81	26.494	5.405	2	85	0.080	0.400
105	HPLC-UV/FLD	3.96	0.37	7.64	0.72	2	90	0.180	0.180
106	LC-MS	4.83579	1.1896	29.17404	7.1768	2	99.7		
107	LC-MSMS	5.381	2.583	28.909	13.876	2	100	0.025	0.050
108	HPLC-UV/FLD	4.408	0.793	5.625	1.012	2	86	0.025	0.025
109	HPLC-MS/MS	12.54	6.27	78.33	39.17	2	100	0.005	0.025
110	UPLC-MS/MS	4.689	0.553	32.163	2.848	2	105.1	0.020	0.060
111	LC-MS	1.8563	0.891	4.073	1.955	2	100	0.0005	0.050
112	LC-MS	3.697	1.479	21.706	8.682	2	95		0.100
113	HPLC-UV/FLD	4.35	1.52	28	9.8	2	98	0.005	0.020
114		*No result*		*No result*					
115	HPLC-UV/FLD	4.3	1.72	9.5	3.8	2	65	0.150	0.450
116		*No result*		*No result*					
117	HPLC	2.457	0.295	2.87	0.344	2			0.080
118	UHPLC-MS/MS	2.294	0.177	17.677	1.366	2	112.51	0.00365	0.01217
119		*No result*		*No result*					
120	LC-MS	6.392	1.342	37.984	7.976	2	100.2	0.025	0.083
121	HPLC-UV/FLD	2.758	1.048	1.109	0.421	2	145.2	0.0020	0.0062
122	LC-MS	1.855	0.464	1.776	0.444	2	102		0.020
123		*No result*		*No result*					
124	LC-MS	1.47	0.59	12.2	4.86	2	100	0.050	0.100
125	HPLC-UV/FLD	4.576	0.503	28.298	3.113	2	91		0.100
126	HPLC-UV/FLD	4.669	1.401	27.338	8.201	2	100	0.020	0.100
127	LC-MS	4.6	1.38	49.9	12	2	100	0.023	0.075
128	HPLC-UV/FLD	2.55	0.306	5.744	0.689	2	87.1	0.006	0.019
129	HPLC-UV/FLD	3.66	1.02	15.5	4.7	2		0.020	0.060
130	LC-MS	5.82	2.677	50.527	23.242	2	64	0.050	0.100
131	HPLC-UV/FLD	3.47416	0.53723	20.39242	5.63504	2	98.98	0.07415	0.2225
132	LC-MS-MS	4.7	0.94	35.2	7.04	2		0.020	0.050
133	LC-MS	7.8		51.5		0	100		
134	LC-MS	6.9	1.042	52	7.852	2	106.7	0.060	0.030
135	HPLC-UV/FLD	3.04	0.57	6.6	1.24	2	100	0.300	1.000
136		*No result*		*No result*					
137	HPLC-UV/FLD	4.4605	1.1151	6.5775	1.6444	2	89	0.0170	0.0550
138		*No result*		*No result*					
139	LC-MS	3.14	0.63	19.7	3	2	100	0.005	0.025
140	LC-MS	5.21	1.25	34.2	8.21	2	100	0.100	0.100
141	HPLC-UV/FLD	5.471	1.132	4.085	0.846	2	85.1	0.080	0.160
142	HPLC-UV/FLD	5.0592	0.6267	6.5357	0.4575	2	92.4	0.00061	0.06000
143	HPLC-UV/FLD	3.911	1.173	6.178	1.853	2	101	0.0125	0.025
144	HPLC-UV/FLD	2.272	0.454	16.336	3.267	2	75	0.0170	0.0510

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [mg/kg]	LOQ [mg/kg]
		Result [mg/kg]	Uncertainty [mg/kg]	Result [mg/kg]	Uncertainty [mg/kg]				
145	LC-MS	2.238	0.8952	14.387	5.754	2	85	0.003	0.010
146	LC-MS	3.561	1.247	27.442	9.605	2	82		0.050
147	LC-MS	3.275	0.6136	17.795	2.5843	2	91	0.020	0.080
148		*No result*		*No result*					
149	LC-MS	4.65	2.325	25.7	13.75	2	100		0.030
150	LC-MS	6.8	1.45	49.4	10.31	3.18	91.1		
151	LC-MS	5.13	1.48	8.38	2.41	2	99.5	0.020	0.040
152	HPLC-UV/FLD	3.687	0.13	3.339	0.13	2	75	0.010	0.100
153	LC-MS	4.643	0.441	11.36	1.079	2	79.1	0.0030	0.0100
154		*No result*		*No result*					
155	HPLC-FLD	3.75	1	5.93	1	2	40	-	-
156	HPLC-UV/FLD	0.4773		0.7035			92		0.0025
157		3.31	0.7	2.24	0.4	2	98		
158	LC-MS	4.588	0.55	5.491	0.659	2	90.5	0.005	0.015
159	LC-MS	78	23.4	13.1	3.93	2	83	0.003	0.010
160	HPLC-UV/FLD	1.4	0.3	1.6	0.4	2	90	0.03	0.09
161	HPLC-UV/FLD	3.295	0.4	6.954	0.83	2	112	0.27	
162		*No result*		*No result*					
163	HPLC-UV/FLD	4.689	1.188	9.294	2.125	2	98	0.070	0.100
164	LC-MS/MS	5.13	1.48	8.38	2.41	2	99.5	0.020	0.040
165	LC-MS	6.471	1.405	39.531	10.887	1	95.17	0.015	0.050
166		*No result*		*No result*					
167	LC-MS	2.937	0.881	3.297	0.989	2	85	ND	0.025
168	LC-MS	4.007	0.72	13.416	2.415	2	48	0.040	0.120
169		*No result*		*No result*					
170	LC-MS	3.135	1.568	19.21	9.605	2	116	0.010	0.050
171		*No result*		*No result*				0.05	0.10

Please state the methodology (e.g. 10 x signal/noise) to calculate the LOQ
What is your main procedure for recovery estimation?
Do you perform calibrant check on regular basis?

Lab Code	Methodology to calculate the LOQ	Recovery estimation	Calibrant check?
101	3xLOD	Internal Standard to Sample	No
102	For AFB1 six determinations of a reference material were carried out, bias and precision was compared to that achieved at mid and high level spiking. For DON and FB1 LOQ determined to match the legislation of each parameter (see previously submitted questionnaire)	Other: Validation by spiking experiments	No
103	Afla B1 (DIN 32645:2008-11), DON and Fum B1 10 x signal/noise	Other: Standard solution to sample vs. sample without spiking	No
104	at least 10 x S/N	Other: use of certified test materials	No
105	SD of 4 X blank and 4 X low level spike. Typically 3 X signal/noise	Standard solution to Blank Sample	Yes, UV/Vis to check potency
106	not determined	Other: Spikes of matrix blank	No
107	6x S/N	Internal Standard to Sample and Standard solution to Blank Sample	No
108	based on spiked samles with low concentration	Other: standard solution to sample or blank sample	Yes, using biopure certified solution
109	10x (SD/S)	Internal Standard to Sample	No
110	LOD lowest [] where RSD <30%; LOQ lowest [] where RDS < 10%	Other: standard solution to sample	No
111	6 x signal/noise	Standard solution to Blank Sample	Yes, check of UPLC-MSMS on daily basis
112	10 * S/N on a spiked blank sample. Remark: We use the term reporting limit instead of LOD/LOQ	Standard solution to Blank Sample, We spike the sample, extract it and spike the extract with IS before injection	Yes, stock solutions versus certified reference calibrants using LC-MS
113	10 x signal/noise and spiking at level near evaluated LOQ	Standard solution to Blank Sample	Yes, UV-spectrometry and/or comparing with calibrant from Other source
114	EXPERIMENTAL	Standard solution to Blank Sample	No
115	2-10 x signal/noise	Standard solution to Blank Sample	No
116	10 x (Standard error / slope) from calibration curve	Standard solution to Blank Sample	Yes, UV-Spectrometry
117	3 x signal/noise	Internal Standard to Sample	Yes, UV-Spectrometry
118	LOQ = 10 x signal/noise	Other: fortification of PT material	No
119	6x signal/noise ratio	Internal Standard to Extract	Yes, UV-Spectrometry
120	10xsignal/noise	Standard solution to Blank Sample	No
121	10 x signal/noise	Standard solution to Blank Sample	Yes, for Afla B1 and DON: UV-Spectrometry, No for Fumo B1
122	spiked samples	Standard solution to Blank Sample	Yes, measuring standard solution by UV-spectrometry
123	10 x standard deviation	Standard solution to Blank Sample	No
124	lowest spiked and extracted concentration with satisfactory recovery	Standard solution to Blank Sample	No
125	10 x signal/noise	Standard solution to Blank Sample	No
126	in house-method, at least 3 times signal noise	Standard solution to Blank Sample	No
127	1/3 legislation limit	Other: Standard addition to quantificate, recovery not estimated	No
128	10 x signal/noise	Standard solution to Blank Sample	No
129	10 * signal / noise to calculate LOQ	Standard solution to Blank Sample	Yes, UV Spectrometry
130	spiked samples	Internal Standard to Extract and standard solution to blank samples for Afla B1	No
131	According DIN 32645	Internal Standard to Extract and use of reference materials if available	Yes, UV-Spectrometry
132	matrix spiked verification at estimated LOQ level	Other: matrix calibration with internal standard to sample -> no recovery estimation	Yes, comparison of detector response between working solution and new calibrant
133	Method currently being developed so LOD and LOQ not established yet	Standard solution to Blank Sample	Yes, comparison of old standards with new standards and participation in PT schemes

Lab Code	Methodology to calculate the LOQ	Recovery estimation	Calibrant check?
134	Based on the standard deviation of 9 analyses in reproducibility conditions	Other: Based on validation report	No
135	estimation from the peak area	Internal Standard to Sample	No
136	cutt off value of samples response spiked on LOD value	Other: spiked sample to spiking standard	Yes, for each batch of ELISA kit
137	10 x signal/noise	Standard solution to Blank Sample	No
138	10 x signal/noise	Standard solution to Blank Sample	No
139	10 x signal/noise	Standard solution to Blank Sample	No
140	10x S/N	Other: standard addition	Yes, comparison with different batch
141	LOD=3sd; LOQ=6sd; sd for 10 measures on possible low level	Standard solution to Blank Sample	No
142	Lowest validated concentration	Standard solution to Blank Sample	Yes, UV-Spectrometry
143	6 x S/N	Standard solution to Blank Sample	No (we use certificate)
144	10 x signal/noise	Standard solution to Blank Sample	No
145	reporting limit according to DG SANCO 12495/2011	Standard solution to Blank Sample	Yes, UV-Spectrometry
146	Pre-defined target LOQ compliant with ML limits.	Standard solution to Blank Sample	No
147	3x signal/noise to calculate LOD, 10x signal/noise to calculate LOQ	Standard solution to Blank Sample	Yes, UV-Spectrometry
148	Spiked blank sample	Other: standard solution to sample	No
149	Lowest validated spike level with acceptable recovery and RSD	Standard solution to Blank Sample	Yes, UV-spectrometry and comparison old-new standard
150		Standard solution to Blank Sample	No
151	LOD = 3xs/n, LOQ = min 2xLOD, or lowest calibration standard	Standard solution to Blank Sample	Yes, UV spectrometry at least every 2 months
152	10 x signal/noise	Standard solution to Blank Sample	Yes, UV-Spectrometry
153	we spike a blank sample at low concentration	Standard solution to Blank Sample	Yes, UV-Spectrometry
154	6xstandard deviation	Internal Standard to Sample	No
155	10xsignal/noise	Other: Standard addition to sample prior to extraction.	No
156	Under validation	Standard solution to Blank Sample	Yes, UV-Spectrometry
157	10 x signal/noise	Internal Standard to Sample	No
158	10xsignal/noise	Standard solution to Blank Sample	No
159	For calculate LOQ ; graphic méthod: 10 x signal/noise and after 10 supplemented test at the estimate LOQ	Standard solution to Blank Sample	Yes, test by Others mix
160		Standard solution to Blank Sample	No
161		Other: standard solution to sample	No
162	10 * SIGNAL/NOISE	Standard solution to Blank Sample	No
163	LOQ= 10xStandard Deviation of Blank signal,then the level was validated in a nat contam sample	Other: Standard solution to a nat contam sample	No
164	Repeated analysis of fortified blank samples / repeated injections of lowest standard solution	Other: Standard solution to Sample for Afla B1 and DON / Standard Solution to Blank for Fum B1	Yes, UV-Spectrometry
165	10x signal/noise	Internal Standard to Extract	Yes,
166	10 x signal/noise	Standard solution to Blank Sample	No
167	S/N	Standard solution to Blank Sample, Fortify matrices	No
168	10 x s/n for Afla, 6 x s/n for DON and FB1	Standard solution to Blank Sample	Yes, UV-Spectrometry
169	e.g. 10 x signal/noise	Standard solution to Blank Sample	No
170	standard in matrix near LOQ, 5 replicates: criteria: recovery between 70 und 110%, st.dev.< 20%	Standard solution to Blank Sample	Yes, by a certified standard solution, which was bought
171	3 x signal/noise or lowest standard	Standard solution to Blank Sample	Yes, measurement by UV - absorbance if possible.

How many samples does your laboratory analyse for the following mycotoxins per year?
Which food or feed matrices does your laboratory analyse most frequently for Aflatoxin B1 (Afla B1), Deoxynivalenol (DON) and Fumonisin B1 (FB1) on a routine basis?
Are you accredited for the determination of these mycotoxins in maize?

Lab Code	Afla B1			DON			FB1			Multitoxin method accredited
	Samples annually	Matrices	Accredited for maize	Samples annually	Matrices	Accredited for maize	Samples annually	Matrices	Accredited for maize	
101	250	Spices soya based feed, bird feed	√	50	Grains for feed use		50	Grains for feed use		
102	100	Nuts, cereals, spices	√	50	Cereals, babyfoods, pasta	√	50	Cereals, babyfood	√	
103	300	nuts, oilseeds, feed	√	150	bakery products, feed	√	50	maize products, feed	√	
104	1000	cereals/feeds	√	500	cereals/feeds	√	500	cereals/feeds	√	
105	400	Nuts	√	50	wheat based products	√	20	maize products	√	
106	30	nut, dried fruit, cereals		30	cereals		30	cereals		√
107	3000	cereals+feed	√	3000	cereals+feed	√	2500	cereals+feed	√	√
108	100	cereals, nuts	√	400	cereals, compound feed	√	70	maize, silage maize	√	
109	>1000	nuts, wheat, spices	√	150	cereals, wheat	√	50	maize	√	√
110	90	peanuts, pistachios, rice	√	60	cereals, babyfood, biscuit	√	30	maize, baby food		
111	600	animal feed	√	100	maize	√	300	wheat		
112	50-100	Nuts / Corn		50-100	Corn / Wheat		50-100	Corn / Wheat		
113	500	nuts, oilseeds, spices	√	50	pasta, cornflakes babyfood	√	20	baby food, corn flour		
114	30	SPICES/DRIED FRUITS								
115	40	feed		80	oat, wheat	√	40	feed		
116	100	cereals, nuts, dried fruits	√	20	Cereals					
117	100	feed, nuts, spices	√	60	feed, cereals	√	10	maize	√	
118	50	wheat and maize		50	wheat and maize		25	wheat and maize		√
119	500	Nuts, Cereals, Animal fee	√	100	Cereals	√	0			
120	70	nuts, spices dried fruits	√	50	cereals	√	50 (B1+B2)	cereals (B1+B2)		
121	25	nuts		50	wheat, oats	√	<10	maize	√	
122	3000	pistachio; peanuts, almonds	√	50	wheat flour, maize		25	maize		
123	20	nuts	√	20	cereals	√				
124	500	Nuts, raisins	√	1000	Wheat, bread, bakery	√	50	maize	√	√
125	500	feed; nut; fig	√	100	cereals	√	50	maize, maize-based products	√	√
126	400	nuts, figs, mixed feed	√	1200	mixed feed, pasta, beer	√	700	mixed feed, maize, beer	√	
127	81	wheat, flour, malt	√	84	wheat, flour, malt	√	6	flour		√
128	400-500	nuts, feed, cereal	√	100	feed, cereal, flour	√	20-30	maize, feed	√	
129	500	oilseed samples	√	350	cereals and derived produ	√	150	cereals or animal feed		
130		cereals	√		cereals			cereals		
131	no routine	no routine	√	no routine	no routine	√	no routine	no routine	√	
132	250	corn, animal feed		1700	corn, animal feed, wheat	√	800	corn	√	√
133	200	feed material, compound feed		200*	feed material, compound feed		100*	feed material, compound feed		
134	20	animal feed	√	20	animal feed	√	20	animal feed	√	
135	100	feed	√	100	feed	√	50	feed	√	
136	300	feed and cereals	√	100	bakery goods, cereals	√				
137	250	nuts cereals dried fruits	√	60	cereals	√	10	maize	√	
138	>1000	equal	√	~1000	equal	√				

Lab Code	Afla B1			DON			FB1			Multitoxin method accredited
	Samples annually	Matrices	Accredited for maize	Samples annually	Matrices	Accredited for maize	Samples annually	Matrices	Accredited for maize	
139	600	food, feed, crops	√	1000	Food, feed, crops	√	150	Food, feed, crops	√	
140	700	feed	√	700	feed	√	700	feed	√	√
141	50	herbal dru, f. based cereals	√	20	flour, food based on cereals	√	20	flour, food based on cereals	√	
142	30	Almond, peanut	√	none	none		none	none	√	
143	1150	feed, nuts, spices	√	325	feed, cereals	√	350	feed, cereals, maize	√	
144	50-100	cereals, nuts	√	40-60	cereals, feed	√	10-20	maize	√	
145	100	cereals, complete feed,	√	200	cereals, complete feed,	√	150	cereals, complete feed,	√	√
146	60	Cereals, nuts and Infant		12	Cereals and Infant Food.		12	Cereals and Infant Food.		√
147	250	nuts, cereals, dried fruit	√	100	cereals	√	50	cereals	√	√
148	150	NUTS, flour, dry fruits		30	flour, maize					
149	3000	cacao, rice, cereals		2000	cereals, cacao, rice		2000	cereals, cacao, rice		√
150	50	CEREAL DRIED FRUIT	√	5	CEREALS		2	MAIS		
151	750	nuts, cereals, baby food	√	300	cereals, baby food	√	100	Cereals, baby food	√	
152	20	feed		30	feed		10	maize	√	
153	60	nuts, cereals, dried fruit	√	25	cereals, pasta, baby food			cereals, maize		
154	>200	food, feed	√	<50	food, feed	√				
155	100	peanut, figs, almond	√	35	flour, babyfood, pastry	√	0	not analysed		
156	452	feedingstuff; cereals		44	feedingstuff; cereals		23	feedingstuff; cereals		
157	2000	corn	√	500	wheat	√				
158	<5	dry s		>100	cereals, cerael products		>50	corn flakes/flour		
159	3000	FEED TMR matter first cer	√	2500	FEED TMR matter first cer	√	2500	Feed Feed TMR matter first cer	√	√
160	1000	cereals	√	10	cereals		5	cereals		
161	60	Feed		60	Feed		10	Feed		
162	50	Nuts & nut products	√	5	Animal feed		0			
163	100	FEED	√	20	FOOD		10	FOOD		
164	50-100	Dried fruit/Nuts		0-50	Cereal		<25	Cereals for infants/Maize	√	
165	300	Peanut	√	150	Flour	√	100	Corn	√	
166	30	nuts, wheat	√	5	flour	√				
167	800	Dry fruit, maize	√	500	cereals	√	500	maize derivates	√	
168	10	nuts/figs	√	20-150	wheat flour	√	10-50	maize/ corn flour		
169	87	maize, sunflower,		12	flour, forage	√				√
170	100	compound feed	√	400	cereals, maize, compound	√	50	maize	√	√
171	250	Nuts, Figs	√	100	Cereals	√	50	maize	√	√

Please indicate the sample amount (in grams) for extraction!
What was the solvent to sample ratio used during extraction (in mL/g)?
What was the extraction solvent used?
What was the extraction mode (e.g. blending or shaking)?
What was the extraction time?

Aflatoxin B1

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
101	25	MeOH 80%	50	blending	2
102	25	MeOH	150	Shaking	30
103	10	MeOH	20	Ultra Turrax	2
104	20	acetonitrile:water=84:16	100	shaking	60
105	50	methanol/water	250	blending	1
106					
107					
108	50	acetone/water	250	shaking	30
109					
110	10	MeOH/water 80/20%	40	ultraturrax + shaking	3 + 15
111					
112					
113	50	MeOH-water	200	blend	3
114	25	METHANOL:WATER	100	BLENDING	5
115	5	60% MeOH	25	shaking	30
116	10	Methano/water (75:25)	125	Blend	2
117	25	Methanol:water	100	blending	3
118					
119	50	MeOH/H2O 80/20	200	blender	2
120	5	acetonitrile:Water	20	shaking	30
121	20	Acetonitrile/water 84:16	80	Shaking	30
122	25	methanol/water	75	sonication	10
123	50	methanol	100	blending	2
124	10	ACN:H2O (84:16)	20	solid/liquid	30
125	10	C2H3N/H2O	50	shaking	45
126	25	methanol/water	150	shaking	30
127					
128	5	80% methanol/water	20	Ultra-Turrax	3
129	25	CHCl3	250	shaking	30
130	10	CH3OH/H2O	50	shaking	3
131	10	acetone/Water 85/15	50	turbulent shaking	45
132					
133					
134	5	Acetonitrile/water 80:20	40	sonication	30
135	6.25	methanol-water	62.5	shaking	60
136	5	methanol/water	25	shaking	15
137	10.0	CH3OH/H2O	80	blending	2
138	6.25	MeOH/H2O	80/20	ultrasound	20
139	25	Ac/water	125	shaking	60
140					
141	12.5	methanol/water 4+1	125	shaking	30
142	12.50	MeOH/H2O	62.50	Ultra Turrax	3
143	50	80% MeOH	250	horizontal shaker	30
144	50	MeOH/AcN + water	250 + 250	shaking	30
145					
146					
147	2	acetonitrile/formic acid	10	shaking	30
148	2	acn/water	20	shaker	120
149					
150	25	METANOLO/ACQUA	250	RIPARTIZIONE	20
151	20	ACN:H2O, 60:40	100	Blend	5
152	25	ACN-H2O	100	shaking	60
153					
154	25	MeOH 70%	125	shaking	30
155	5	MeOH:H2O (70:30)	20	shaking	30
156	50	Acetone:water	250	shaking	30
157	50	Me:H2O	100	Shaker	60
158	10	Methanol-water	50	blending	3
159	5 g	ACN/H2O	20	shaking	120
160	10	CH3CN:H2O (85:15)	50	shaker	30
161	50	ACN	250	blending	2
162	50	70% methanol	250	blending	20
163	5	water-methanol	20	shaking	45
164	25	70% Methanol	125	Blend/Filter	2
165					

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
166	25	H2O/CH3OH	100	liquid	30
167	25	acetonitrile water	100		
168	5	MeOH/H2O	25	shaking	15
169					
170	25	aceton/water=85/15	125	shaking	45
171					

Deoxynivalenol

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
101	5	water	100	shaking	3
102	25	H2O	200	Blending	2
103	10	ACN, H2O	50	Ultra Turrax	2
104	20	acetonitrile:water=84:16	100	shaking	60
105	25	water	160	shaking	30
106					
107					
108	10	acetonitril and water	100	shaking	60
109					
110	12.5	water	100	ultra-turrax	3'
111					
112					
113	25	water-PEG	200	blend	3
114					
115	25	84% ACN	100	shaking	120
116	12	water	100	Blend	2
117	25	water	200	blending	3
118					
119	25	H2O	200	blender+Turrax	2
120	5	Water	20	shaking	30
121	20	Acetonitrile/water 84:16	80	Shaking	30
122	25	ACN/water	100	sonication	10
123	25	water	200	blending	2
124	10	ACN:H2O (84:16)	20	solid/liquid	30
125					
126	25	acetonitrile/water	100	stiring	120
127					
128	5	water	40	Ultra-Turrax	3
129	25	Water	200	shaking	30
130					
131	10	Water	200	turbulent shaking	60
132					
133					
134	5	Acetonitrile/water 80:20	40	sonication	30
135	10	acetonitrile-water	100	shaking	60
136	5	methanol/water	25	shaking	15
137	10.0	H2O	100	blending	2
138	5.00	ACN/H2O	84/16	shaking on vortex	15
139	25	ACN/water	100	shaking	120
140					
141	10	water (PEG)	40	blending	3
142	5	CH3CN/H2O	20	Turrax+shaking	63
143	25	water	200	horizontal shaker	120
144	25	water	200	shaking	20
145					
146					
147	2	acetonitrile/formic acid	10	shaking	30
148	10	water	80	shaker	30
149					
150	25	ACQUA	200	RIPARTIZIONE	20
151	25	MeOH:H2O, 70:30	100	Blend	5
152	50	H2O	200	shaking	60
153					
154	25	water	200	shaking	30
155	25	H2O	200	shaking	30
156					
157	25	H2O	100	Shaker	60
158	25	water	200	blending	3
159	5 g	ACN/H2O	20	shaking	120
160	6	H2O	60	shaker	30

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
161	5	H2O	100	blending	2
162	25	Deionised water	200	blending	30
163	20	water	160	blending	3
164	5	Water	40	Blend/Centrifuge/Filter	3
165					
166	25	H2O	200	liquid	30
167					
168	5	ACN/H2O	20	shaking	120
169	50	200	H2O	liquid	1
170	10	acetonitril/water=84/16	60	shaking	90
171	2.5	Methanol 75%	10	Ultrahomogenisation	3

Fumonisin B1

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
101	5	MeOH 70%	25	shaking	3
102	25	ACN:MeOH:H2O 25:25:50	125	Blending	2
103	10	MeOH, ACN, H2O	50	Ultra Turrax	2
104	20	acetonitrile:water=84:16	100	shaking	60
105	25	methanol/acetonitrile/wat	125	blending	2
106					
107					
108	20	methanol/acetonitril/wate	200	shaking	120
109					
110	5	water/ACN/MeoH:50/25/25%	50	ultra-turrax + shaking	3 + 15
111					
112					
113	20	MeOH-ACN-water	100	blend and shake	3 + 40
114					
115	10	ACN:MeOH:H2O 25:25:50 v/v	50	shaking	120
116					
117	25	methanol:acetonitril:wate	100	blending	3
118	5	ACN/H2O/CH3COOH	20	shaking	60
119					
120	5	acetonitrile:water:methan	25	shaking	30
121	15	Citratebuffer/AcCN/water	75	Heating, Shaking	60 + 30
122	25	ACN/methanol/water	125	sonication	10
123					
124	10	ACN:H2O:HCOOH (79:21:0.1	20	solid/liquid	30
125	10	C2H3N/H2O	50	shaking	60
126	20	acetonitrile/methanol/wat	100	shaking	40
127					
128	5	ACN/MeOH/H2O (25/25/50)	25	shaking	120
129	5	PBs MeOH	150	shaking	60
130					
131	10	ACN/MeOH/Water 25/25/50	50	turbulent shaking	40
132					
133	2	acetonitrile:water	16	shaking	40
134	5	Acetonitrile/water 80:20	40	sonication	30
135	10	acetonitrile-methanol-wat	50	shaking	120
136					
137	10	CH3OH/CH3CN/H2O	50	shaking	120.0
138					
139	25	ACN/water	100	shaking	60
140					
141	10	ACN/MeOH/water 1+1+2	50	shaking	20
142	10.00	CH3CN/MeOH/H2O	50	Ultra Turrax	6
143	20	ACN/MeOH/H2O 25/25/50	100	horizontal shaker	30
144	25	AcN/MeOH/water	125	shaking	30
145					
146					
147	2	acetonitrile/formic acid	10	shaking	30
148					
149					
150	10	METANOLO/ACQUA	100	RIPARTIZIONE	20
151	25	H2O:ACN:MeOH, 50:25:25	125	Blend	5
152	20	MeOH-PBS	200	shaking	120
153					
154					
155	20	MeOH:ACN:H2O(25:25:50)	100	shaking	40

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
156	20	Acetonitrile:methanol:wat	2x50	shaking, centrifugation	2 x 20
157					
158	25	water_methanol-acetonitri	125	blending	3
159	5 g	ACN/H2O	20	shaking	120
160	5	CH3CN(25):H2)(50):MeOH(25	25	shaker	120
161	25	ACN:H2O	125	blending	2
162					
163	5	water-acetonitrile	25	shaking	45
164	25	Water/Methanol/Acetonitri	125	Blend/Filter	5
165					
166					
167					
168	15	MeOH/H2O	50	shaking	60
169					
170	10	acetonitril/methanol/wate	40	shaking	20
171					

Multitoxin methods

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
101					
102					
103					
104					
105					
106	5	Acetonitril, water	40	Sonication	30
107	10	ACN/H2O	40	Ultrathurrax	2
108					
109	10	methanol/water	60 ml	shaking	60
110					
111	10	methanol/water	60	blending with Ultra-thurrax	2
112	4	QuEChERS	17.5	Shaking	60
113					
114					
115					
116					
117					
118	5	ACN/H2O	20	shaking	60
119					
120					
121					
122					
123					
124	10	ACN:H2O:HCOOH (79:21:0.1	20	solid/liquid	30
125	10	C2H3N/H2O/HCOOH	50	shaking	60
126					
127	5	Water/Acetonitrile 50/50	20	shaking	5
128					
129					
130	10	CH3CN/H2O	40	shaking	90
131					
132	5	organic solvent/water	30	automated under-pressure	30
133	2	acetonitrile:water, 80:20	8	shaking	20
134					
135					
136					
137					
138					
139					
140	2.5	water/ACN/acetic acid	10	shaking	30
141					
142					
143					
144					
145	5	ACN+0.1% HCOOH in water	10	QuEChERS	20
146	10	ACN:H2O 80% 0.1% A.Formic	40	shaking	90
147	2	acetonitrile/formic acid	10	shaking	30
148					

Lab Code	Sample amount (g)	Extraction solvent	Solvent volume [ml]	Extraction mode	Extraction time [min]
149	5	Acetontril/ 1% acetic aci	10	shaking	1
150					
151					
152					
153	40	Acetonitrile	60	shaking	120
154					
155					
156					
157					
158					
159	5 g	ACN/H2O	20	shaking	120
160					
161					
162					
163					
164					
165	2	80% Aq ACN	8		5
166					
167					
168					
169	25	CH3OH:H2O	125	liquid	2
170	10	acetonitril/water=84/16	60	shaking	90
171					

What type of clean up methodology was used (e.g. immunoaffinity column)?
For LC-MS only: What is the calculated sample fraction injected onto the LC system [mg/injection]?
Mention the type of column used for seperation

Lab Code	Clean-up	Injected sample fraction [mg/injection]	Column used			
			Stationary phase	Length [mm]	Diameter [mm]	Particle size [µm]
101	AfB1 - immunoaffinity column		C18	150	4.6	3
102	IACs	N/A	C18	100-150	4.6	5
103	immunoaffinity comlumn		C-18	200	4.0	5
104	MYCOSEP	2.5 (Afla B1), 1 (DON), 0.02 (FB1)	ZORBAX SB-C18	50	2.1	1.8
105	immunoaffinity column in all cases		C18	150	4.6	5
106	none		C18	100	2.1	1.8
107	no cleanup	0.25	C18	100	2.1	1.7
108	immunoaffinity for Fum B1 and Afla B1 and SPE for DON	0.25 mg	Nova Pack C18, UPLCBEHC18	150 mm	3.9	
109	no clean-up	injection 0.5 ul		100	2.1	1.8
110	IAC for Afla B1 and DON, centrifugation and filtering only for FB1	12.5 (DON), 0.5 (FB1)	C18	250/50	4.6/2.1	5/1.7
111	immunoaffinity column	1.66 mg/injection	monoclonal antibody based	NOD	NOD	NOD
112	QuEChERS	2	C18	100	4.6	2.6
113	immunoaffinity column		C18	300	3.9	4
114	immunoaffinity column		-	-	-	-
115	immunoaffinity (AFB1, FB1), SPE MycoSep (DON)		C18/ODS	100/150	3/4.6	2.6/3
116	immunoaffiinity- column					
117	R-Biopharm immunoaffinity column		C18	150	4.6	5
118	None. Crude extract is used for analysis.	0,625	C18 (Hypersil GOLD TM)	50	2.1	1.9
119	immunoaffinity column		C18	250	4.6	5
120	Immunoafinity column		c18	250	4.6	5
121	Afla B1: MultiSep, DON and Fumo B1: Immunoaffinity column		C18	10/15	4.6	3/5
122	immunoaffinity column and mycosep		c-18	15	0.21	5
123	immunoaffinity column		C18	150	4.6	5
124	none	0.0019	C18	100	2.1	1.8
125	Immunoaffinity column for AFB1 and FB1; MultiSep 226 for DON	ca 1.95	C18 (AFB1, FB1) Synergi Hydro-RP (DON)	150 150	4.6 2.0	5 4
126	IAC		RP18	250	4.6	5
127	QuEChERS	10 mg	C18	100	2.1	2.7
128	immunoaffinity column		LiChrosper 100 RP 18	250	4	5
129	SPE and IAC		C18	15	4	5
130	none for Multitoxine method; immunoaffinity column for Aflat B1	1	C18	100	3	3.5
131	IAC, SPE		Nucleosil ODS; Luna P-H	150-250	4.6	5
132	none	3.3 mg/injection	C18	150	4.6	5.2
133	centrifugation	AFB 1:- 1.5, DON:- 0.5, Fum B1:- 1.5	XB-C18	50	2.1	2.6
134	filtration	50	C18	100	2.1	1.7
135	AB1, FB1-mmunoaffinity column, DON-Mycosep Trich		C18	250	4.6	5
136	filtration					
137	immunoaffinity column		C18	150	4.6	5.0
138	immunoaffinity column, Romer Labs	7.5	C18 (AflB1; DON)	150 (AFLB1); 100 (DON)	4.6 (AFLB1; DON)	5 (AFLB1); 3 (DON)
139	Afla, Fum IAC DON SPE	25	C18	150	4.6	5
140	none	0.625 mg/injection	C18	100	2	2.1
141	immunoaffinity column		C18	250	4.6	5
142	immunoaffinity column		ODS2; dC18 (DON) C18 (FB1)	250; 100 (DON); 150 (FB1)	4.6; 2.1 (DON); 4.6 (FB1)	5.6; 3 (DON); 5 (FB1)
143	IAC		RP-18 C18 (AFB1)	250 100 (AFB1)	4 2.1 (AFB1)	5 µm 1.7 (AFB1)

Lab Code	Clean-up	Injected sample fraction [mg/injection]	Column used			
			Stationary phase	Length [mm]	Diameter [mm]	Particle size [µm]
144	immunoaffinity column		C18	150	4.6	5
145	QuEChERS	0.625	C18	50	2.1	1.7
146	dilution with water	1.25	C 18	50	2.1	1.7
147		1 mg/injection	C18	150	2.1	5
148	IAC	8	C18	150	2.0	3.5
149	none	2.5 mg/10µl injection	C18	100	2.1	1.7
150	SAX-immunoaffinity column		Sinergi polar	250	4.6	4
151	Immunoaffinity columns	0.533mg	C18	250 / 100	4.6 / 2.1	5 / 1.8
152	immunoaffinity column VICAM					
153	no clean up	0.625	C18	100	2.1	1.7
154	immunoaffinity column		nucleosil C18	250	4.6	5
155	immunoaffinity column		C18	250	4.6	5
156	IAC		C18	250	4	5
157	IAC		C18	10	2.1	1.8
158	immunoaffinity column	20	C-18	100	2.1	5
159	not used					
160	Imunoaffinity Column		C18	150	4.6	5
161	Immunoaffinity columns		ODS3	150	4.6	5
162	immunoaffinity column		ODS(1)	150	4.6	5
163	immunoaffinity column		C18	150	4.6	5
164	immunoaffinity column		C18	250	4.6	5
165	none	10 microlitre of extract	C18	50	2.1	1.8
166	immunoaffinity column		dimethyl-n-octedecylsilane	150	4.6	5
167	IAC	300	C18	100	2.1	3
168	Afl: IAC; DON: mycosep 225 and FB1: SAX					
169	e.g. immunoaffinity column	-	dimethyl-n-octadecylsilane	150	4.6	5
170	immunoaffinity column for AFB1 and FB1, SPE for DON	6,6	C18	150	2	4
171	multi immunoaffinity DZT column	50 mg				

Did you encounter any problems during the analysis?
Did you notice any unusual observations which, however, did not seem to have any effect on the results?

Lab Code	Problems	Unusual observations
101	No	No
102	The IAC columns were overloaded for the fumonisin analysis	No
103	No	No
104	No	No
105	Lack of sample meant that reduced weights had to be taken for the aflatoxin analysis	No
106	No	No
107	No	No
108	No	No
109	No	No
110	LC-MS/MS (Waters) complete shut-down for 2 weeks, problems with sensitivity (DON + FB1)	No
111	No	No
112	No	No
113	No	No
114	No	No
115	No	No
116	No	No
117	No	No
118	No	No
119	No	No
120	No	No
121	During fumo-analysis there were some problems with high pressure.	No
122	No	No
123	No	No
124	No	No
125	Too high level of FB1 in the B sample required repeated measurements	No
126	No	No
127	No	No
128	No	No
129	Because of the quantity of sample we divided le sample amount	The content in fumonisin in Sample B was very high and needed a sample amount very low
130	No	No
131	No	No
132	No	No
133	No	No
134	No	No
135	No	No
136	variations ofresults forrepeated analysis of DON were uncommonly high	low correlation between values for diluted and undiluted samples for DON
137	No	No
138	Unfortunately, it wasn't possible to calculate exact DON content in two test samples: "Sample A" and"Sample B", because the method has been approved for DON detection only till 1500 µg/kg in food/feed samples.	Unfortunately, it wasn't possible to calculate exact DON content in two test samples: "Sample A" and "Sample B", because the method has been approved for DON detection only till 1500 µg/kg in food/feed samples.
139	No	No
140	No	No
141	No	No
142	Fault of the MSMS Detector in the analysis of DON	No
143	No	No
144	unexpected high level of concentration of Fum B1 in sample B	No
145	We have never met so high amount of FB1 like in sample B.	No
146	No	No
147	No	No
148	Reproducibility was poor for sample B	No
149	No	No
150	No	No
151	No	No
152	No	No
153	No	No
154	No	No
155	No	No
156	No	For fumonisin analysis we had some difficulties concerning the evaporation of the final samples A and B extracts.
157	Not enough sample size	No
158	No	No
159	No	No
160	No	No
161	I detected a peak for sample B during AFB1 analysis but it did not correlate to standard peak times and I was unsure as whether to	No

Lab Code	Problems	Unusual observations
	include it or not	
162	No	No
163	No	No
164	No	No
165	No	No
166	No	No
167	Non omogeneus results (different concentrations in replicates)	No
168	No	No
169	No	No
170	No	No
171	We could only analyse DON due to severe illness of staff members.	No

Did you find the instructions distributed for this PT adequate?
Any Other comments you wish to make?

Lab Code	Instructions adequate?	Any Other comments
101	Yes	
102	Yes	NO
103	Yes	
104	Yes	In the sample A Other mycotoxins are present: zearalenone, T-2 toxin, fumonisin B2 and B3.In the sample A Other mycotoxins are present: zearalenone, T-2 toxin, fumonisin B2 and B3. In the sample B Other mycotoxins are present: aflatoxin G1, ochratoxin A, zearalenone, T-2 toxin, HT-2 toxin, fumonisin B2 and B3.
105	Yes	NO
106	Yes	
107	Yes	
108	Yes	
109	Yes	
110	Yes	
111	Yes	
112	Yes	
113	Yes	
114	Yes	
115	Yes	
116	Yes	
117	Yes	
118	Yes	
119	Yes	NO
120	Yes	
121	Yes	Some squares in the questionnaire are too small for three parallell methods.
122	Yes	
123	Yes	
124	Yes	
125	Yes	In the Q10 data only for AFB1 and FB1; DON was analysed using Synergi Hydro-RP/4µm/2.0mm/150mm
126	Yes	
127	Yes	
128	Yes	
129	Yes	
130	Yes	
131	Yes	-
132	Yes	
133	Yes	*DON and Fumonisin B1 analyses are outsourced. Method is still being developed in our laboratory so recovery data and measurement uncertainty values not yet established
134	Yes	
135	Yes	/
136	Yes	
137	Yes	NO
138	Yes	In our opinion, the concentration of the contaminants in test samples should be at Real level in the next PT.
139	Yes	
140	Yes	
141	Yes	
142	Yes	The answer the question number 10 refers only to the analysis of aflatoxin B1. In the case of Fumonisin B1 the column parameters are the following: C18, 5 µm,4.6 x 150 mm
143	Yes	different column for AFLA (C18, 2.1 x 100 mm, 1,7 µm)
144	No, e.g you can write, that we can expect high level of concentration	
145	Yes	NO
146	Yes	
147	Yes	
148	Yes	
149	Yes	We would like to receive a blank sample to calculate matrix effect.
150	Yes	

Lab Code	Instructions adequate?	Any Other comments
151	Yes	Don't like this form, not enough space to answer the questions
152	Yes	
153	Yes	
154	Yes	Regarding determination of aflatoxin B1, for the two samples (A and B) were identified also the aflatoxins B2,G1and G2
155	Yes	
156	Yes	These methods are still in process of validation and no uncertainty values are determined. DON analysis was not performed.
157	Yes	Because of the small sample size, analyses for FUM B1 are not performed as per lab usual procedure
158	Yes	
159	Yes	
160	Yes	
161	Yes	
162	Yes	Column parameters for aflatoxin B1 only.
163	Yes	We found a little bit confounding the fact that the PT title defines the sample as cereal matrix while it was a maize sample.
164	Yes	Regarding question 4 (accreditation), Analysis of Fum B1 was carried out by proxy-NRL
165	Yes	
166	Yes	NO
167	Yes	
168	Yes	
169	no	NO
170	Yes	
171	Yes	We have analysed few fusarium toxins of the 2 samples. Sample A: HT-2 160 µg/kg, T-2 60 µg/kg, Zearalenon 285 µg/kg. Sample B: HT-2 45 µg/kg, T-2 30 µg/kg, Zearalenon 20 µg/kg. A multi immunoaffinity DZT column from r-biopharm was used for the determination of the 4 mentioned mycotoxins. The toxins were analysed by UPLC-MS/MS

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Abstract

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of aflatoxin B1 (AfB1), deoxynivalenol (DON) and fumonisin B1 (FB1) in cereal samples.

Seventy one participants from 31 countries registered for the exercise. Fifty-nine sets of results were reported for FB1 for both test samples, 69 for DON and 70 for Afla B1.

Only z-scores were used for an evaluation of an underperformance. In total about 70 % of the attributed z scores were below an absolute value of 2, which indicated that most of the participants performed satisfactory or better. The conducted PT revealed that the biggest challenge was the accurate determination of FB1 at higher concentration levels.

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