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Report on the 2011 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

Determination of Aflatoxin B1 in Baby food, Maize powder, Animal feed and Test solution

Project ID: MYCO-AFLA-06

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Project ID: MYCO-AFLA-06
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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 interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). In 2011 the annual proficiency test was also open to EU official control laboratories falling under the responsibility of the NRLs in order to support the NRLs fulfilling their tasks according to Regulation No 882/2004.

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of aflatoxin B1 in food and feed samples.

The test materials were naturally contaminated baby food, maize powder, cereal-based animal feed, an ampouled aflatoxin B1 solution and a blank baby food material. The materials were procured at IRMM and dispatched to the participants in May 2011. Each participant received 2.5 ml of solution and four sachets containing approximately 30 g of test material each.

Sixty-nine participants from 28 countries registered for the exercise. Sixty-one sets of results were reported for the solution, 58 for the baby food, 67 for the maize powder and 62 for the animal feed. One laboratory did not report any results.

The assigned values were 12.1 µg/ml for the test solution, 0.197 µg/kg for the baby food, 3.1 µg/kg for the maize powder and 9.9 µg/kg for the animal feed. The uncertainties of the respective assigned values were 0.2 µg/ml, 0.017 µg/kg, 0.14 µg/kg and 0.66 µg/kg, respectively.

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

Laboratory results 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. No z-scores were calculated for the blank material.

In total about 90% of the attributed z-scores were below an absolute value of two, which indicated that most of the participants performed satisfactory or better.

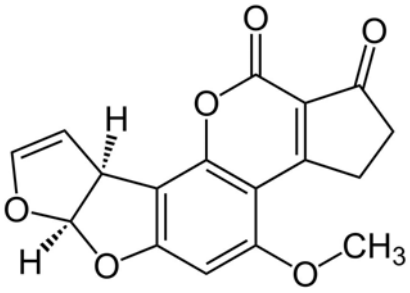
2. Introduction

Aflatoxins are mycotoxins that grow in many cereals and oilseeds but are found primarily in 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 1)* 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.

Existing methods for aflatoxin analysis in food and feed are numerous and varied. The methodologies used for the determination of aflatoxin B1 in almost all food and feed matrices range from high-performance liquid-chromatography (HPLC) with various detection systems such as fluorescence (FLD) or mass selective detection (MSD), over thin-layer chromatography (TLC) to enzyme linked immunosorbant assays (ELISA). The most common principle in EU Member States is however HPLC with Kobra Cell derivatisation and fluorescence detection.

Figure 1: Structure of aflatoxin B1



Molecular formula: C17H12O6
CAS: 1162-65-8
Molecular weight: 312.274 g/mol

Commission Regulation (EC) No 1881/2006 lays down maximum limits for aflatoxin B1 in certain foods. For feed the guidance values are set in Directive 2002/32/EC of the European Parliament and of the Council *(Table 1)*.

Table 1: Legislation in the EU regarding aflatoxin B1 in the tested matrices in the proficiency test

Legislative reference	Matrix	Maximum limit
Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs	All cereals and all products derived from cereals, including processed cereal products, with the exception of:	2 µg/kg
	- Maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5 µg/kg
	- Processed cereal-based foods and baby foods for infants and young children	0.1 µg/kg
	- Dietary foods for special medical purposes intended specifically for infants	0.1 µg/kg
Directive 2002/32/EC of the European Parliament and of the Council	All feed materials	20 µg/kg
	Complete feedingstuffs for cattle, sheep and goats with the exception of:	20 µg/kg
	— complete feedingstuffs for dairy animals	5 µg/kg
	— complete feedingstuffs for calves and lambs	10 µg/kg
	Complete feedingstuffs for pigs and poultry (except young animals)	20 µg/kg
	Other complete feedingstuffs	10 µg/kg
	Complementary feedingstuffs for cattle, sheep and goats (except complementary feedingstuffs for dairy animals, calves and lambs)	20 µg/kg
	Complementary feedingstuffs for pigs and poultry (except young animals)	20 µg/kg
	Other complementary feedingstuffs	5 µg/kg

3. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [1], one of the core duties of the EU-RL is to organise interlaboratory comparison tests (ILCs) for the benefit of staff from NRLs. The scope of this ILC was to test the competence of the appointed NRLs to determine the amount of aflatoxin B1 in food and feed samples.

The proficiency test was also open to EU official control laboratories falling under the responsibility of the NRLs in order to support the NRLs fulfilling their tasks.

The ILC was designed and the reported data were processed along the lines of the International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories (Thompson *et al.* 2006)[2].

The assessment of the measurement results was undertaken on the basis of requirements laid down in legislation and followed administrative and logistic procedures of ISO Guide 43 [3].

3.1. Confidentiality

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

The NRLs were requested to forward the information about the possibilities that official control laboratories have, since there are two options OCL can register.

1. The NRL enrols official control laboratories and covers participation fees:
In this case the NRL submits a list of participants. The coverage of the participation fees has to be confirmed. In return the performance data of the respective official control laboratories will be disclosed to the NRL.

2. The official control laboratory enrols itself and covers the participation fee:
In this case the NRL will get access to the performance data of the official control laboratory only upon providing a letter of consent by the participating lab."

4. Time frame

The ILC was agreed upon by the NRL network at the fifth EU-RL Mycotoxins workshop held on 10-11 March 2010. Specific details of the exercise were refined during the sixth EU-RL Mycotoxins workshop held on 7 April 2011. Invitation letters were sent to the participants on 25 March 2011 *(Annex 13.3)* and the planned ILC was published on the IRMM web page [4]. The opening of registration was on 4 May 2011 *(Annex 13.4)*. The samples were dispatched to the participants on 30 May 2011. Reporting deadline was 1 July 2011 which was postponed by a week.

5. Material

5.1. Preparation

The test materials were naturally contaminated cereal-based baby food, maize powder and cereal-based animal feed test samples from various sources.

Six kilos of baby food, 4 kg of maize powder and 5 kg of animal feed were stored at IRMM at -20 °C waiting for processing. The materials were individually homogenized for 2 hours in Lödige

laboratory mixer (Model L20, Paderborn, Germany). Thereafter, about 120-120 vacuum sealed packages were produced at room temperature. The amount of material in each sachet was about 30 g.

A test solution was also prepared, which contained Aflatoxin B1 (obtained from Sigma, code A-6636, 10 mg, Lot 56H4027) in a mixture of toluene and acetonitrile (both supplied by VWR) 98:2 (v/v).
About 150 ampoules were filled under inert atmosphere, each with 2.5 ml of solution and flame sealed.

All the test materials were stored under -18 °C until dispatch.

5.2. Homogeneity

Sufficient homogeneity was assumed for the test solution after mixing.

Homogeneities of the contaminated test materials were evaluated according to chapter 3.11.2 of the Harmonized Protocol [2]. The contents of 10 randomly selected test sample sachets were analysed in duplicate by liquid chromatography with fluorescent detection (HPLC-FLD) and Kobra Cell derivatisation. [5]

All analyses complied with the provisions given by the Harmonized Protocol. Hence it was concluded that the test materials were sufficiently homogeneous. *(Annex 13.1)*

5.3. Stability

The amount of aflatoxin B1 in the test materials was monitored at the beginning of the study, during the study as well as after receipt of the results of the participants as it is suggested in the Harmonized Protocol. Statistically significant differences of the results of analysis obtained on the three mentioned dates were not found. *(Annex 13.2)*

5.4. Distribution

All samples were packed in cardboard boxes and sent via express mail. One set of material was sent to every participant. The test materials were dispatched to the participants by IRMM on 30 May 2011. The samples were mostly received within 24 hours after dispatch.

Each participant received:
a) four packages containing approximately 30 g of test materials (3 contaminated and 1 blank),
b) one ampoule containing the aflatoxin B1 solution,
c) an accompanying letter with instructions on sample handling and reporting *(Annex 13.5)*,
d) a sample receipt form *(Annex 13.6)* and
e) a registration key for the reporting interface.

The materials were shipped at room temperature; storage however 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, for storage above -18° C over a longer period of time no stability information is available.

6. Instructions to participants

The laboratories were asked to report the recovery corrected value in µg/kg, including the recovery in % and measurement uncertainty plus coverage factor. For recovery experiments they had the chance to use the material containers marked as "Baby food - Blank".

Another aim was to assess the content of aflatoxin B1 in solution by spectrophotometer. The laboratories were asked to report the value in µg/ml.

The results were to be reported in a special on-line form for which each participant received an individual access code. A specific questionnaire was attached to this on-line form. The questionnaire was intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in *Annex 13.7*.

7. Reference values and their uncertainties

For the test solution the consensus value of the reported results was used.

Assigned values and their uncertainties for the aflatoxin B1 content for baby food, maize powder and animal feed were established by "Exact-matching Double Isotope Dilution Mass Spectrometry". This methodology is considered to be a primary ratio method with a direct link to SI units. [6]

More information about the assigned values is presented in *Annex 13.9.*

8. Evaluation of results

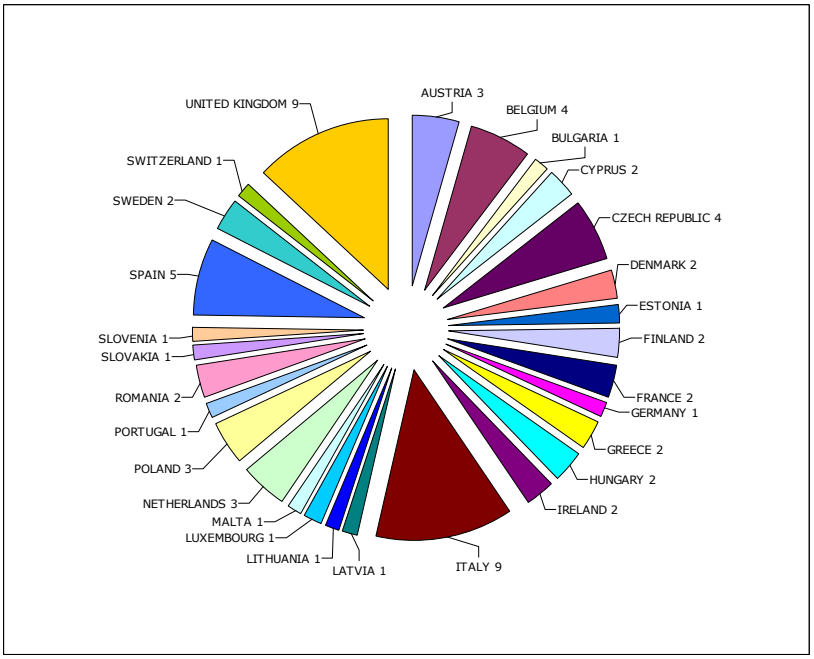
8.1. General observations

Sixty-nine participants from twenty-eight countries registered to the PT *(Figure 2)* and sixty-eight sent back results. Lab 102 couldn't submit their results before the deadline because they had a problem with their instrument.

Sixty-one sets of results were reported for the aflatoxin B1 solution, 58 for baby food, 67 for maize powder and 62 for animal feed. Thirty-two laboratories reported uncertainties for aflatoxin B1 in solution, 52 for baby food, 61 for maize powder and 57 for animal feed.

All member states of the European Union and Switzerland participated in the study. 33 out of 69 were official control laboratories.

Figure 2: Participating countries, number of laboratories



8.2. Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta (ζ) scores in accordance with ISO 13528 [7] and the International Harmonised Protocol [2].

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

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{lab} + u^2_{ref}}}$$ **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 by the Horwitz equation:

- for analyte concentrations < 120 ppb *(baby food, maize powder, animal feed)*

$$\sigma_p = 0.22 \cdot c$$ **Equation 3.**

- for analyte concentrations ≥ 120 ppb ≤ 13.8% *(test solution)*

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

where:
c = concentration of the 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 |ζ|-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 |ζ|-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 and ProLab software [8].

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528 [7] by application of a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [9]. The representative figures are tabulated for each test sample in the following sections of the report.

Table 2: Summary statistics for the test solution

Number of results		61
Range of results	µg/ml	10.3-15
Median of results of participants	µg/ml	12.0
Mean of results of participants	µg/ml	12.1
Robust mean of results of participants	µg/ml	12.1
Assigned value (consensus value of participants' results)	µg/ml	**12.1**
Expanded uncertainty (k=2) of the assigned value	µg/ml	0.2
Robust standard deviation ($\hat{\sigma}$)	µg/ml	0.3
Target standard deviation (fitness for purpose, RSD_R= 11 %)	µg/ml	1.3
Number (percentage) of results of |z| > 2.0		1 (2%)

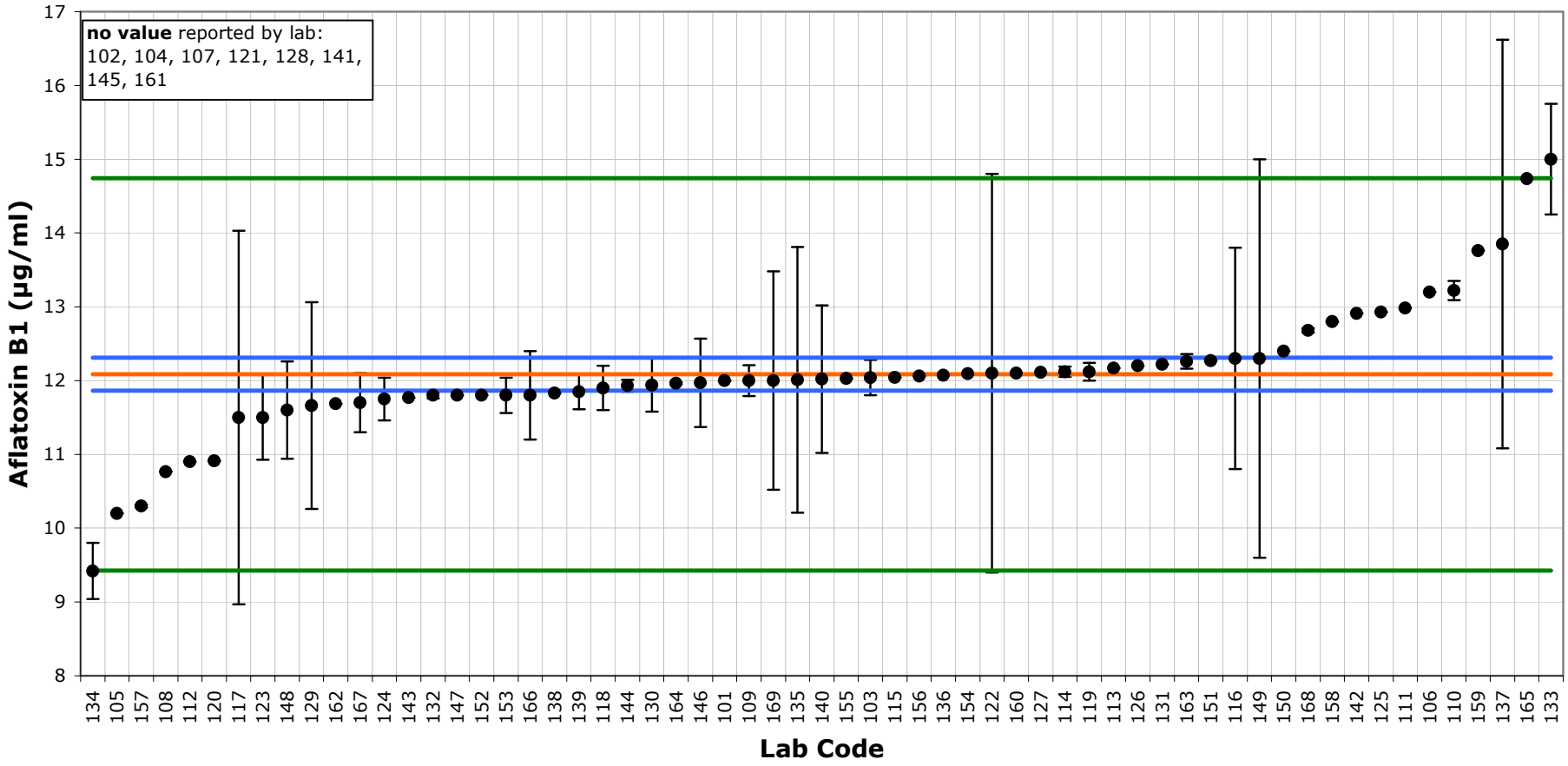
Table 3: Results of analysis and z-scores for the test solution
(The meaning of colours: green – satisfactory, yellow – questionable result)

Lab Code	Result [µg/ml]	z-score	Lab Code	Result [µg/ml]	z-score
101	**12**	-0,1	**136**	**12.07**	0,0
103	**12.04**	0,0	**137**	**13.85**	1,3
104	*No result*	-	**138**	**11.83**	-0,2
105	**10.2**	-1,4	**139**	**11.85**	-0,2
106	**13.2**	0,8	**140**	**12.02**	0,0
107	*No result*	-	141	*No result*	-
108	**10.766**	-1,0	**142**	**12.91**	0,6
109	**12**	-0,1	**143**	**11.77**	-0,2
110	**13.22**	0,9	**144**	**11.93**	-0,1
111	**12.9811**	0,7	145	*No result*	-
112	**10.9**	-0,9	**146**	**11.97**	-0,1
113	**12.17**	0,1	**147**	**11.8**	-0,2
114	**12.12**	0,0	**148**	**11.6**	-0,4
115	**12.044**	0,0	**149**	**12.3**	0,2
116	**12.3**	0,2	**150**	**12.4**	0,2
117	**11.5**	-0,4	**151**	**12.27**	0,1
118	**11.9**	-0,1	**152**	**11.8**	-0,2
119	**12.12**	0,0	**153**	**11.8**	-0,2
120	**10.912**	-0,9	**154**	**12.092**	0,0
121	*No result*	-	**155**	**12.027**	0,0
122	**12.1**	0,0	**156**	**12.06**	0,0
123	**11.5**	-0,4	**157**	**10.3**	-1,3
124	**11.75**	-0,3	**158**	**12.8**	0,5
125	**12.93**	0,6	**159**	**13.76**	1,3
126	**12.2**	0,1	**160**	**12.1**	0,0
127	**12.11**	0,0	161	*No result*	-
128	*No result*	-	**162**	**11.688**	-0,3
129	**11.66**	-0,3	**163**	**12.26**	0,1
130	**11.94**	-0,1	**164**	**11.963**	-0,1
131	**12.22**	0,1	**165**	**14.736**	2,0
132	**11.8**	-0,2	**166**	**11.8**	-0,2
133	**15**	2,2	**167**	**11.7**	-0,3
134	**9.42**	-2,0	**168**	**12.68**	0,4
135	**12.01**	-0,1	**169**	**12**	-0,1

The results are written as reported by the laboratories.

Figure 3: EU-RL Mycotoxins PT 2011: Aflatoxin B1 in test solution

Certified value: Xref = 12,1 µg/ml; Uref = 0,2 µg/ml (k=2); σ = 1,3 µg/ml



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 4: Summary statistics for the baby food

Number of results		58
Range of results	µg/kg	0.1 – 3.27
Median of results of participants	µg/kg	0.21
Mean of results of participants	µg/kg	0.30
Robust mean of results of participants	µg/kg	0.202
Assigned value (isotope dilution LC-MS/MS)	µg/kg	**0.197**
Expanded uncertainty (k=2) of the assigned value	µg/kg	0.017
Robust standard deviation ($\hat{\sigma}$)	µg/kg	0.044
Target standard deviation (fitness for purpose, RSD_R= 22 %)	µg/kg	0.04
Number (percentage) of results of |z| > 2.0		8 (14%)
Number (percentage) of results of |ζ| > 2.0		19 (33%)

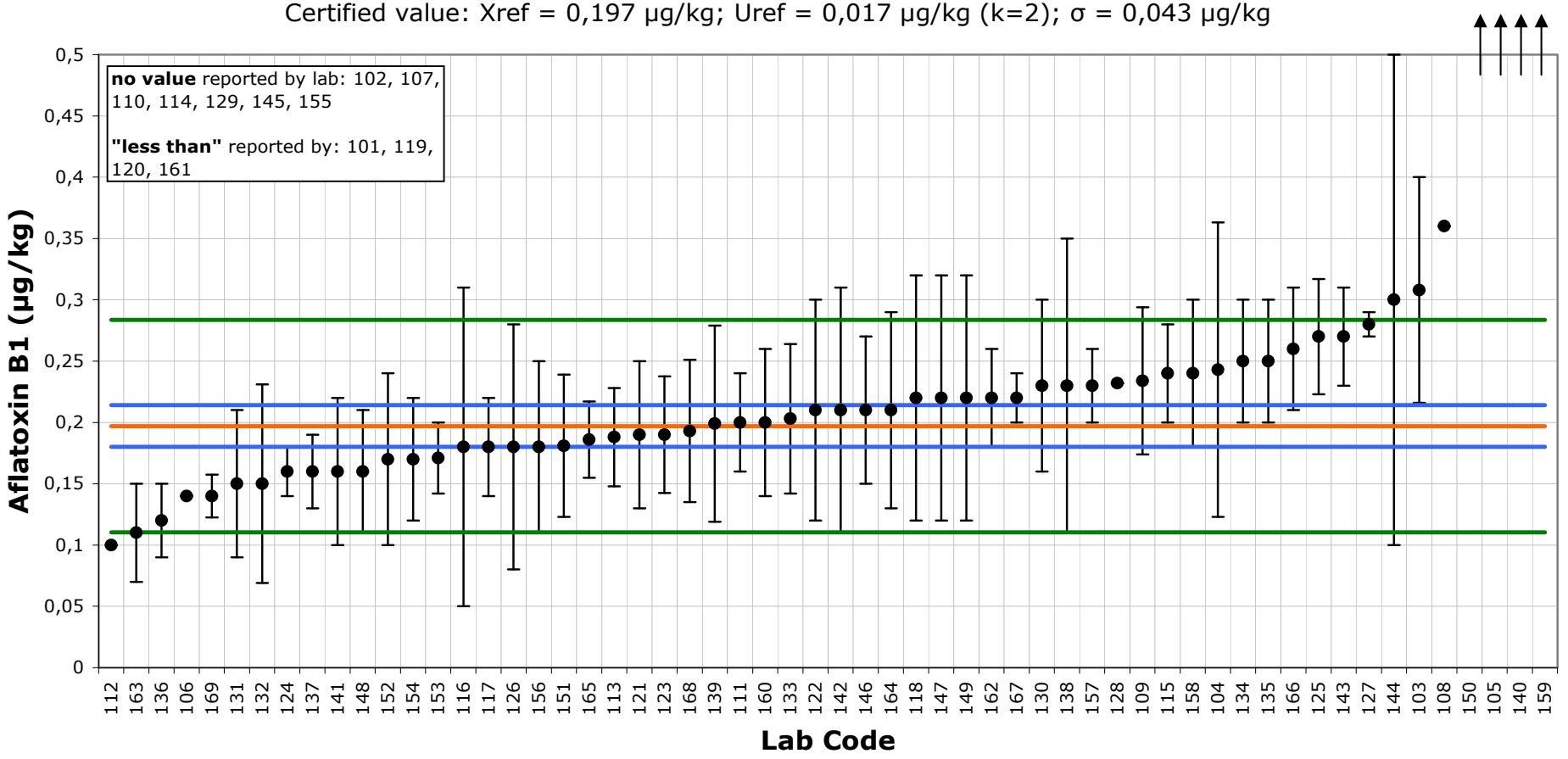
Table 5: Results of analysis and z-scores for the baby food
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	Result [µg/kg]	z-score	ζ-score	Lab Code	Result [µg/kg]	z-score	ζ-score
101	<0.3	-	-	**136**	**0.12**	-1.8	-4.5
103	**0.308**	2.6	2.4	**137**	**0.16**	-0.9	-2.1
104	**0.243**	1.1	0.8	**138**	**0.23**	0.8	0.5
105	**1.1**	20.8	106.2	**139**	**0.199**	0.0	0.0
106	**0.14**	-1.3	-6.7	**140**	**1.11**	21.1	4.3
107	*No result*	-	-	**141**	**0.16**	-0.9	-1.2
108	**0.36**	3.8	19.2	**142**	**0.21**	0.3	0.3
109	**0.234**	0.9	1.2	**143**	**0.27**	1.7	3.4
110	*No result*	-	-	**144**	**0.3**	2.4	1.0
111	**0.2**	0.1	0.1	145	*No result*	-	-
112	**0.1**	-2.2	-11.4	**146**	**0.21**	0.3	0.4
113	**0.188**	-0.2	-0.4	**147**	**0.22**	0.5	0.5
114	*No result*	-	-	**148**	**0.16**	-0.9	-1.4
115	**0.24**	1.0	2.0	**149**	**0.22**	0.5	0.5
116	**0.18**	-0.4	-0.3	**150**	**0.56**	8.4	13.7
117	**0.18**	-0.4	-0.4	**151**	**0.181**	-0.4	-0.5
118	**0.22**	0.5	0.5	**152**	**0.17**	-0.6	-0.7
119	<0.3	-	-	**153**	**0.171**	-0.6	-1.5
120	<1	-	-	**154**	**0.17**	-0.6	-1.0
121	**0.19**	-0.2	-0.8	155	*No result*	-	-
122	**0.21**	0.3	0.3	**156**	**0.18**	-0.4	-0.5
123	**0.19**	-0.2	-0.8	**157**	**0.23**	0.8	1.9
124	**0.16**	-0.9	-2.8	**158**	**0.24**	1.0	1.4
125	**0.27**	1.7	2.9	**159**	**3.27**	70.9	361.5
126	**0.18**	-0.4	-0.3	**160**	**0.2**	0.1	0.1
127	**0.28**	1.9	9.8	161	<0.3	-	-
128	**0.232**	0.8	4.1	**162**	**0.22**	0.5	1.1
129	*No result*	-	-	**163**	**0.11**	-2.0	-4.0
130	**0.23**	0.8	0.9	**164**	**0.21**	0.3	0.3
131	**0.15**	-1.1	-1.5	**165**	**0.186**	-0.3	-0.6
132	**0.15**	-1.1	-1.1	**166**	**0.26**	1.5	2.4
133	**0.203**	0.1	0.2	**167**	**0.22**	0.5	2.1
134	**0.25**	1.2	2.0	**168**	**0.193**	-0.1	-0.1
135	**0.25**	1.2	2.0	**169**	**0.14**	-1.3	-4.7

The results are written as reported by the laboratories.

Figure 4: EU-RL Mycotoxins PT 2011: Aflatoxin B1 in baby food

Certified value: Xref = 0,197 µg/kg; Uref = 0,017 µg/kg (k=2); σ = 0,043 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)**.

Table 6: Summary statistics for the maize powder

Number of results		67
Range of results	µg/kg	0.165 - 44.7
Median of results of participants	µg/kg	2.74
Mean of results of participants	µg/kg	3.35
Robust mean of results of participants	µg/kg	2.77
Assigned value (isotope dilution LC-MS/MS)	µg/kg	**3.1**
Expanded uncertainty (k=2) of the assigned value	µg/kg	0.14
Robust standard deviation ($\hat{\sigma}$)	µg/kg	0.68
Target standard deviation (fitness for purpose, RSD_R= 22 %)	µg/kg	0.68
Number (percentage) of results of |z| > 2.0		7 (10%)
Number (percentage) of results of |ζ| > 2.0		34 (51%)

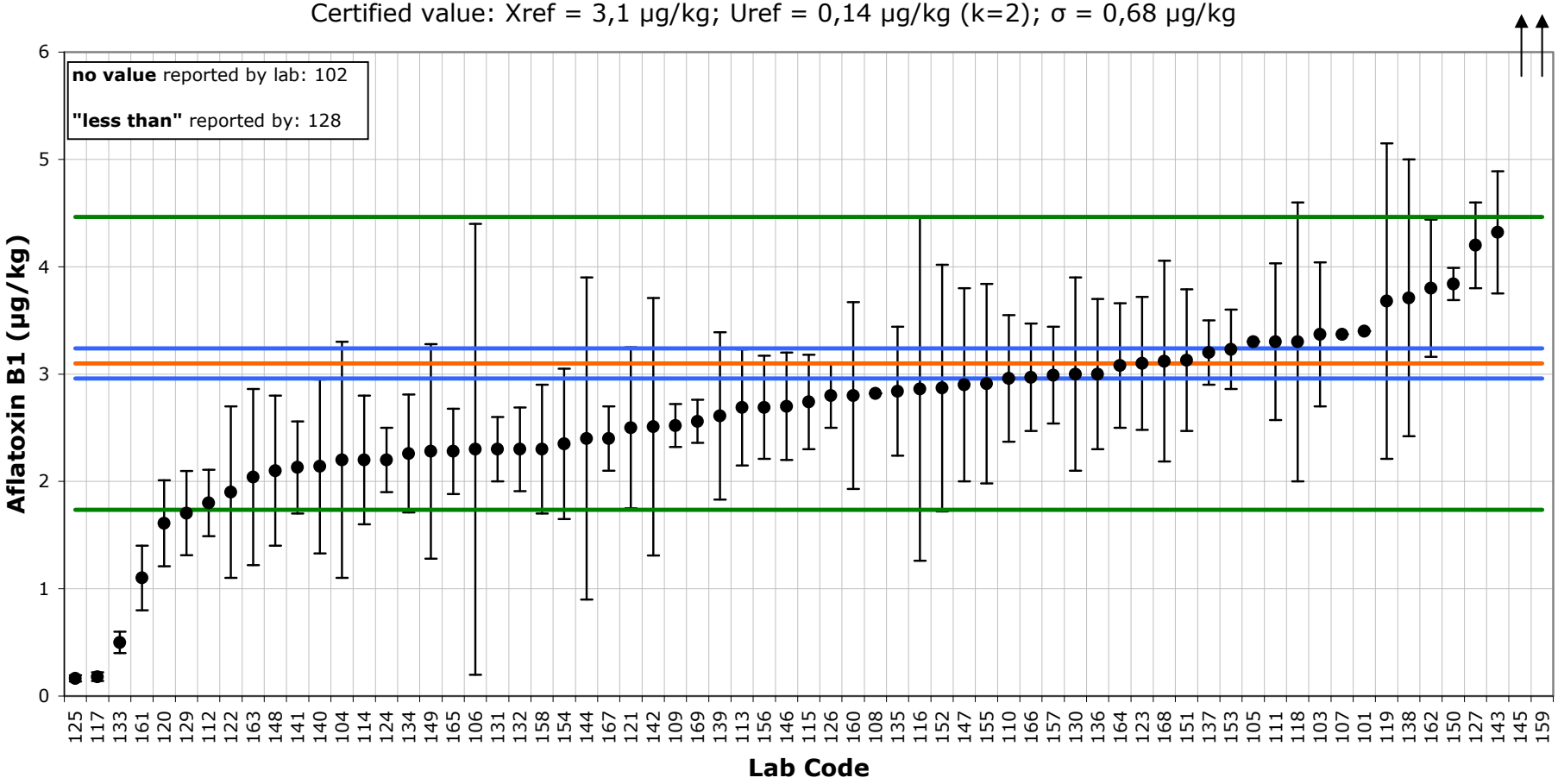
Table 7: Results of analysis and z-scores for the maize powder
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	Result [µg/kg]	z-score	ζ-score	Lab Code	Result [µg/kg]	z-score	ζ-score
101	**3.4**	0.4	4.3	**136**	**3**	-0.1	-0.3
103	**3.37**	0.4	0.8	**137**	**3.2**	0.1	0.6
104	**2.2**	-1.3	-1.6	**138**	**3.71**	0.9	0.9
105	**3.3**	0.3	2.9	**139**	**2.61**	-0.7	-1.2
106	**2.3**	-1.2	-0.8	**140**	**2.14**	-1.4	-2.3
107	**3.37**	0.4	3.9	**141**	**2.13**	-1.4	-4.3
108	**2.82**	-0.4	-4.0	**142**	**2.51**	-0.9	-1.0
109	**2.52**	-0.9	-4.8	**143**	**4.32**	1.8	4.2
110	**2.96**	-0.2	-0.5	**144**	**2.4**	-1.0	-0.9
111	**3.3**	0.3	0.5	**145**	**8.7**	8.2	80.0
112	**1.8**	-1.9	-7.6	**146**	**2.7**	-0.6	-1.5
113	**2.689**	-0.6	-1.5	**147**	**2.9**	-0.3	-0.4
114	**2.2**	-1.3	-2.9	**148**	**2.1**	-1.5	-2.8
115	**2.74**	-0.5	-1.6	**149**	**2.28**	-1.2	-1.6
116	**2.86**	-0.4	-0.3	**150**	**3.84**	1.1	7.2
117	**0.18**	-4.3	-36.2	**151**	**3.13**	0.0	0.1
118	**3.3**	0.3	0.3	**152**	**2.87**	-0.3	-0.4
119	**3.68**	0.9	0.8	**153**	**3.23**	0.2	0.7
120	**1.61**	-2.2	-7.0	**154**	**2.35**	-1.1	-2.1
121	**2.5**	-0.9	-2.3	**155**	**2.91**	-0.3	-0.4
122	**1.9**	-1.8	-3.0	**156**	**2.69**	-0.6	-1.6
123	**3.1**	0.0	0.0	**157**	**2.99**	-0.2	-0.5
124	**2.2**	-1.3	-5.4	**158**	**2.3**	-1.2	-2.6
125	**0.165**	-4.3	-41.1	**159**	**44.77**	61.1	595.3
126	**2.8**	-0.4	-1.8	**160**	**2.8**	-0.4	-0.7
127	**4.2**	1.6	15.7	**161**	**1.1**	-2.9	-12.1
128	<8	-	-	**162**	**3.8**	1.0	2.1
129	**1.704**	-2.0	-6.7	**163**	**2.04**	-1.6	-2.5
130	**3**	-0.1	-0.2	**164**	**3.08**	0.0	-0.1
131	**2.3**	-1.2	-4.8	**165**	**2.28**	-1.2	-3.9
132	**2.3**	-1.2	-3.9	**166**	**2.97**	-0.2	-0.5
133	**0.5**	-3.8	-30.2	**167**	**2.4**	-1.0	-7.1
134	**2.26**	-1.2	-3.0	**168**	**3.12**	0.0	0.0
135	**2.84**	-0.4	-0.8	**169**	**2.56**	-0.8	-4.4

The results are written as reported by the laboratories.

Figure 5: EU-RL Mycotoxins PT 2011: Aflatoxin B1 in maize powder

Certified value: Xref = 3,1 µg/kg; Uref = 0,14 µg/kg (k=2); σ = 0,68 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)**.

Table 8: Summary statistics for the animal feed

Number of results		62
Range of results	µg/kg	3.72 - 45
Median of results of participants	µg/kg	8.81
Mean of results of participants	µg/kg	9.28
Robust mean of results of participants	µg/kg	8.63
Assigned value (isotope dilution LC-MS/MS)	µg/kg	**9.9**
Expanded uncertainty (k=2) of the assigned value	µg/kg	0.66
Robust standard deviation ($\hat{\sigma}$)	µg/kg	1.74
Target standard deviation (fitness for purpose, RSD_R= 22 %)	µg/kg	2.2
Number (percentage) of results of |z| > 2.0		3 (5%)
Number (percentage) of results of |ζ| > 2.0		19 (31%)

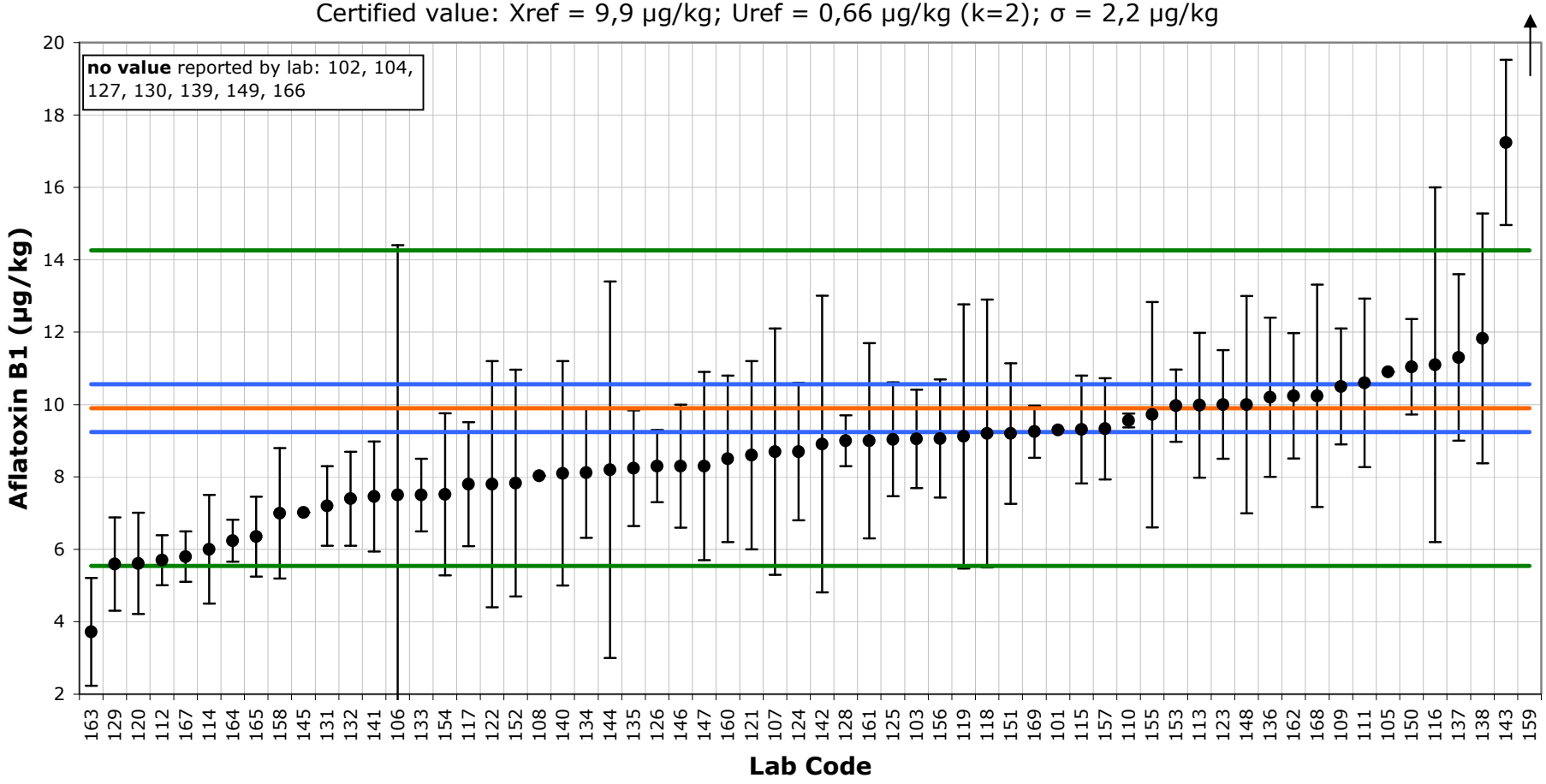
Table 9: Results of analysis and z-scores for the animal feed
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	Result [µg/kg]	z-score	ζ-score	Lab Code	Result [µg/kg]	z-score	ζ-score
101	**9.3**	-0,3	-1,8	**136**	**10.2**	0,1	0,3
103	**9.05**	-0,4	-1,1	**137**	**11.3**	0,6	1,2
104	*No result*	-	-	**138**	**11.83**	0,9	1,1
105	**10.9**	0,5	3,0	139	*No result*	-	-
106	**7.5**	-1,1	-0,7	**140**	**8.1**	-0,8	-1,1
107	**8.7**	-0,6	-0,7	**141**	**7.46**	-1,1	-2,9
108	**8.03**	-0,9	-5,7	**142**	**8.91**	-0,5	-0,5
109	**10.5**	0,3	0,7	**143**	**17.24**	3,4	6,2
110	**9.56**	-0,2	-1,0	**144**	**8.2**	-0,8	-0,6
111	**10.6**	0,3	0,6	**145**	**7.02**	-1,3	-8,7
112	**5.7**	-1,9	-8,8	**146**	**8.3**	-0,7	-1,8
113	**9.981**	0,0	0,1	**147**	**8.3**	-0,7	-1,2
114	**6**	-1,8	-4,8	**148**	**10**	0,0	0,1
115	**9.31**	-0,3	-0,7	149	*No result*	-	-
116	**11.1**	0,6	0,5	**150**	**11.04**	0,5	1,5
117	**7.8**	-1,0	-1,2	**151**	**9.2**	-0,3	-0,7
118	**9.2**	-0,3	-0,4	**152**	**7.83**	-1,0	-1,3
119	**9.12**	-0,4	-0,4	**153**	**9.97**	0,0	0,1
120	**5.61**	-2,0	-5,5	**154**	**7.52**	-1,1	-2,0
121	**8.6**	-0,6	-1,4	**155**	**9.72**	-0,1	-0,1
122	**7.8**	-1,0	-1,2	**156**	**9.06**	-0,4	-1,0
123	**10**	0,0	0,3	**157**	**9.33**	-0,3	-0,7
124	**8.7**	-0,6	-1,2	**158**	**7**	-1,3	-3,0
125	**9.04**	-0,4	-1,0	**159**	**45**	16,1	106,4
126	**8.3**	-0,7	-2,7	**160**	**8.5**	-0,6	-1,2
127	*No result*	-	-	**161**	**9**	-0,4	-0,6
128	**9**	-0,4	-2,0	**162**	**10.24**	0,2	0,4
129	**5.597**	-2,0	-6,0	**163**	**3.72**	-2,8	-7,6
130	*No result*	-	-	**164**	**6.24**	-1,7	-8,3
131	**7.2**	-1,2	-4,2	**165**	**6.35**	-1,6	-5,5
132	**7.4**	-1,1	-3,4	166	*No result*	-	-
133	**7.5**	-1,1	-4,0	**167**	**5.8**	-1,9	-11,1
134	**8.12**	-0,8	-1,9	**168**	**10.24**	0,2	0,2
135	**8.24**	-0,8	-1,9	**169**	**9.25**	-0,3	-1,3

The results are written as reported by the laboratories.

Figure 6: EU-RL Mycotoxins PT 2011: Aflatoxin B1 in animal feed

Certified value: Xref = 9,9 µg/kg; Uref = 0,66 µg/kg (k=2); σ = 2,2 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

Sixty-one laboratories analyzed the aflatoxin B1 solution. Even though the EU-RL asked in the accompanying letter to do the analysis with a spectrophotometer, two laboratories analyzed the test solution with LC-MS/MS and three with HPLC-FLD technique.

For the recovery estimation nearly all of the participants used a "standard spiked to blank" method.

Sixty-three laboratories used immunoaffinity columns (IAC) as a clean up methodology. The manufacturers and the number of the labs using them are the following: R-Biopharm (37), Vicam (17), Romer Labs (5), Neogen (2), LC Tech (2).

Forty-four percent of the participants used acid washed glassware during the analyses and 88 % reported that protection against daylight was applied.

Ninety-two percent of the participants found the instructions distributed adequate and regarding the registering-reporting interface the EU-RL received mostly good reviews.

Details on the spectrophotometer conditions, samples preparation and HPLC analyses etc can be found in *Annex 13.8*.

No statistically relevant information could be obtained that linked performance results with answers on methodology, overnight step, calibrant control etc.

9. Conclusions

Sixty-nine participants from twenty-eight countries registered to the interlaboratory comparison for aflatoxin B1 of which 61 sets of results were reported for the test solution, 58 for baby food, 67 for maize powder and 62 for animal feed. One laboratory did not report any results.

The performance of most participants was outstanding compared with PTs of previous years organized be the EU-RL. In total about 90% of the attributed z-scores were below an absolute value of two, which indicated that most of the participants performed satisfactory or better than the minimal performance criteria required. The analysis of all data revealed that laboratory performance was not linked to any analytical technique or sample preparation method used. The great majority of laboratories in this interlaboratory comparison applied analytical methods which, with the regard to performance characteristics, were compliant with EU legislation.

Zeta-scores were not as good as the z-scores, which indicates that the respective participants should review their uncertainty estimation.

Only z-scores were used for benchmarking and NRLs with unsatisfactory z-scores will be invited for a corrective action.

10. Acknowledgements

The organisers of the study would like to thank *Franz Ulberth*, *Beatriz de la Calle, Ines Baer* and *Donata Lerda* for their support and the Reference Materials Unit at IRMM, in particular *Håkan Emteborg*, for ampouling of the test samples.

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The laboratories participating in this exercise, listed in *Table 10*, are also kindly acknowledged.

Table 10: Participating laboratories (Entries in italic are official control laboratories)

Organisation	Country
Institut für Umwelt und Lebensmittelsicherheit	*Austria*
Eurofins-ofi Lebensmittelanalytik GmbH	*Austria*
AGES GmbH	Austria
Fytolab	*Belgium*
Coda-Cerva (VAR)	Belgium
Federal Laboratory for the Safety of the Food Chain	*Belgium*
Oleotest n.v.	*Belgium*
NDRVMI	Bulgaria
Department Of Agriculture	Cyprus
State General Laboratory	Cyprus
Institute of Chemical Technology in Prague	*Czech Republic*
Czech Agriculture and Food Inspection Authority	Czech Republic
UKZUZ (Central Institute for Supervising and Testing in Agriculture)	Czech Republic
State Veterinary Institute Prague	*Czech Republic*
The Danish Plant Directorate	Denmark
National Food Institute	Denmark
Agricultural Research Centre	Estonia
Finnish Food Safety Authority Evira	Finland
Finnish Customs Laboratory	Finland
LDA 22	*France*
Laboratoire SCL de Rennes	France
Federal institute for risk assessment -BfR	Germany
General Chemical State Laboratory	Greece
General Chemical State Laboratory	*Greece*
Central Agricultural Office, Food and Feed Safety Directorate – Feed NRL	Hungary
Central Agricultural Office, Food and Feed Safety Directorate – Food NRL	Hungary
Dublin Public Analyst's Laboratory	Ireland
The State Laboratory	*Ireland*
Istituto Zooprofilattico Sperimentale delle Venezie	*Italy*
Istituto Zooprofilattico Sperimentale regioni Lazio e Toscana	*Italy*
ARPA Piemonte	*Italy*
Istituto Zooprofilattico Sperimentale Umbria Marche	*Italy*
Istituto Zooprofilattico Sperimentale LER	*Italy*
Istituto Zooprofilattico Puglia Basilicata Foggia	*Italy*
ARPAL	*Italy*
Istituto Superiore di Sanità	Italy
Istituto Zooprofilattico Sperimentale	*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
Food and Consumer Product Safety Authority (nVWA)	*Netherlands*
Silliker Netherlands BV - Dr. A. Verwey	*Netherlands*
RIKILT	Netherlands
WSSE Katowice	*Poland*
National Veterinary Research Institute	Poland
National Institute of Public Health - National Institute of Hygiene	Poland
INRB, IP - Laboratório Nacional de Investigação Veterinária	Portugal
Sanitary Veterinary And Food Safety Directorate	Romania
Hygiene Institute of Veterinary Public Health	Romania
State Veterinary and Food Institute	Slovakia
University in Ljubljana, Veterinary Faculty-National Veterinary Institute	Slovenia
Centro Nacional De Alimentacion (Spanish Food Safety and Nutrition Agency)	Spain
Servicio Territorial de Sanidad y B. Social de Soria-Junta de Castilla y León	*Spain*
Laboratorio Normativo de Salud Pública	*Spain*

Organisation	Country
Centro de Salud Pública de Valencia	*Spain*
CNTA	*Spain*
National Food Administration	Sweden
National Veterinary Institute (SVA)	Sweden
Kantonales Laboratorium Basel-Landschaft	Switzerland
Leicestershire and Staffordshire Scientific Services	*United Kingdom*
The City of Edinburgh Council	*United Kingdom*
Lancashire County Laboratory	*United Kingdom*
Minton, Treharne & Davies Ltd.	*United Kingdom*
Kent County Council	*United Kingdom*
Worcestershire Scientific Services	*United Kingdom*
Food and Environment Research Agency	United Kingdom
Cardiff Scientific Services	*United Kingdom*
Somerset County Council	*United Kingdom*

11. Abbreviations

ANOVA	Analysis of variance
CEN	European Committee for Standardisation
EC	European Commission
ELISA	Enzyme linked immunosorbant assays
EU	European Union
EU-RL	European Reference Laboratory
FLD	Fluorescent detection
HPLC	High-performance liquid chromatography
IAC	Immunoaffinity column
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
OCL	Official Control Laboratory
PT	Proficiency Test
TLC	Thin-layer chromatography

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13. Annexes

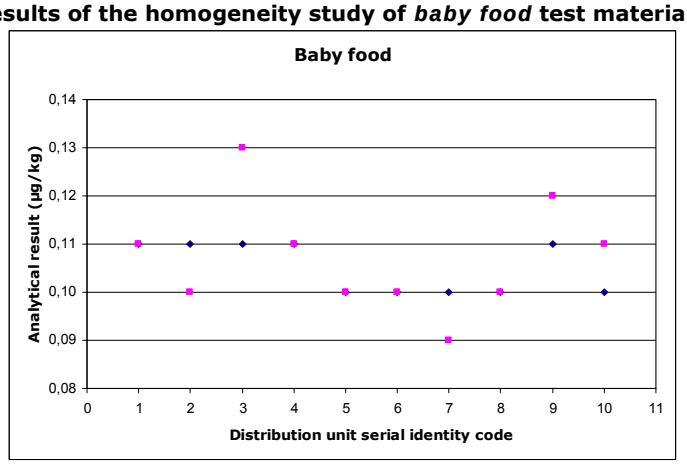
13.1. Homogeneity tests

Homogeneities of the contaminated baby food, maize power and animal feed materials were evaluated according to chapter 3.11.2 of the Harmonized Protocol.

Table 11: Duplicated results for 10 distribution units of baby food analysed for aflatoxin B1 (µg/kg), together with some intermediate stages of the ANOVA calculation

Sample	Result *a*	Result *b*	$D = a - b$	$S = a + b$	$D^2 = (a - b)^2$
1	0,11	0,11	0,00	0,22	0,00
2	0,11	0,10	0,01	0,21	0,00
3	0,11	0,13	-0,02	0,24	0,00
4	0,11	0,11	0,00	0,22	0,00
5	0,10	0,10	0,00	0,20	0,00
6	0,10	0,10	0,00	0,20	0,00
7	0,10	0,09	0,01	0,19	0,00
8	0,10	0,10	0,00	0,20	0,00
9	0,11	0,12	-0,01	0,23	0,00
10	0,10	0,11	-0,01	0,21	0,00

Figure 7: Analytical results of the homogeneity study of *baby food* test material



The data are presented visually above, and show no suspect features such as discordant duplicated results, outlying samples, trends, discontinuities, or any other systematic effects.

Cochran's test: The largest value of D^2 is 0.0004 and the sum of D^2 is 0.0008, so the Cochran test statistic is 0.0004/0.0008=0.5. This is less than the 5% critical value of 0.602 for this type of test, so there is no evidence for analytical outliers and we proceed with the complete data set.

Homogeneity test

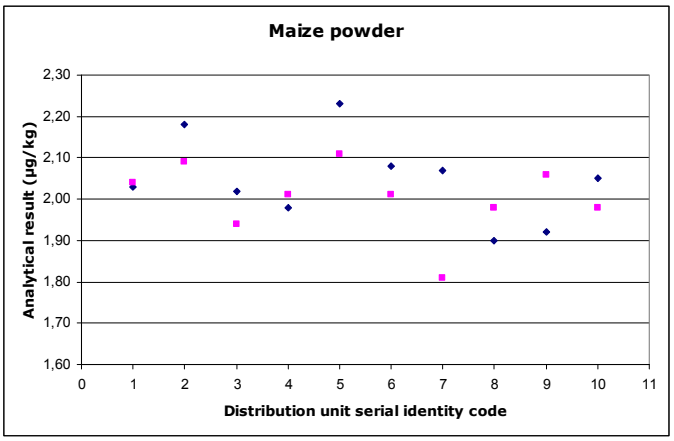
- Analytical variance: $s_{an}^2 = \sum D^2/2m = 0.0008/20 = 0.00004$
- Between-sample variance: the variance of the sums S = a + b is 0.00024, so $s_{sam}^2 = (V_s/2 - s_{an}^2)/2 = (0.00024/2 - 0.00004)/2 = 0.00004$
- Acceptable between-sample variance: the target standard deviation is 0.02332 µg/kg, so the allowable between-sample variance is $\sigma_{all}^2 = (0.3\sigma_p)^2 = (0.3 \text{ X } 0.02332)^2 = 0.000049$
- Critical value: The critical value for the test is $1.88\ \sigma_{all}^2 + 1.01\ s_{an}^2 = 1.88 \text{ X } 0.000049 + 1.01 \text{ X } 0.00004 = 0.00013$

Since $s_{sam}^2 = 0.00004 < 0.00013$, passed and the baby food material is sufficiently homogeneous.

Table 12: Duplicated results for 10 distribution units of maize powder analysed for aflatoxin B1 (µg/kg), together with some intermediate stages of the ANOVA calculation

Sample	Result *a*	Result *b*	$D = a - b$	$S = a + b$	$D^2 = (a - b)^2$
1	2,03	2,04	-0,01	4,07	0,00
2	2,18	2,09	0,09	4,27	0,01
3	2,02	1,94	0,08	3,96	0,01
4	1,98	2,01	-0,03	3,99	0,00
5	2,23	2,11	0,12	4,34	0,01
6	2,08	2,01	0,07	4,09	0,00
7	2,07	1,81	0,26	3,88	0,07
8	1,90	1,98	-0,08	3,88	0,01
9	1,92	2,06	-0,14	3,98	0,02
10	2,05	1,98	0,07	4,03	0,00

Figure 8: Analytical results of the homogeneity study of *maize powder* test material



The data are presented visually above, and show no suspect features such as discordant duplicated results, outlying samples, trends, discontinuities, or any other systematic effects.

Cochran's test: The largest value of D^2 is 0.07 and the sum of D^2 is 0.13, so the Cochran test statistic is 0.07/0.13=0.538. This is less than the 5% critical value of 0.602 for this type of test, so there is no evidence for analytical outliers and we proceed with the complete data set.

Homogeneity test

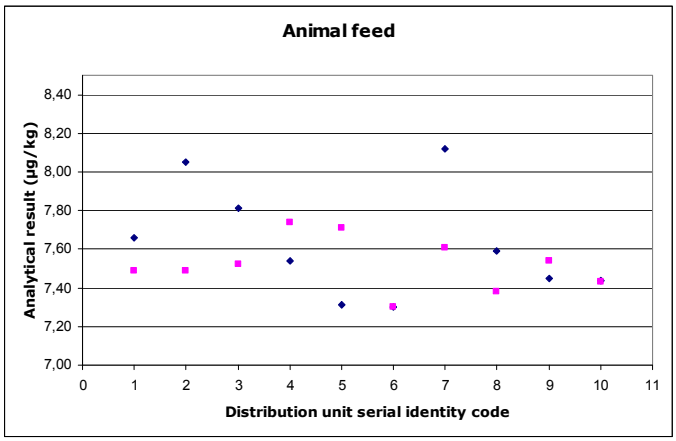
- Analytical variance: $s_{an}^2 = \Sigma D^2/2m = 0.13/20 = 0.0065$
- Between-sample variance: the variance of the sums S = a + b is 0.02, so $s_{sam}^2 = (V_s/2 - s_{an}^2)/2 = (0.02/2 - 0.0065)/2 = 0.00175$
- Acceptable between-sample variance: the target standard deviation is 0.45 µg/kg, so the allowable between-sample variance is $\sigma_{all}^2 = (0.3\sigma_p)^2 = (0.3 \text{ X } 0.45)^2 = 0.018$
- Critical value: The critical value for the test is $1.88\ \sigma_{all}^2 + 1.01\ s_{an}^2 = 1.88 \text{ X } 0.018 + 1.01 \text{ X } 0.0065 = 0.0404$

Since $s_{sam}^2 = 0.00175 < 0.0404$, passed and the maize powder material is sufficiently homogeneous.

Table 13: Duplicated results for 10 distribution units of animal feed analysed for aflatoxin B1 (µg/kg), together with some intermediate stages of the ANOVA calculation

Sample	Result *a*	Result *b*	$D = a - b$	$S = a + b$	$D^2 = (a - b)^2$
1	7,66	7,49	0,2	15,2	0,03
2	8,05	7,49	0,6	15,5	0,31
3	7,81	7,52	0,3	15,3	0,08
4	7,54	7,74	-0,2	15,3	0,04
5	7,31	7,71	-0,4	15,0	0,16
6	7,30	7,30	0,0	14,6	0,00
7	8,12	7,61	0,5	15,7	0,26
8	7,59	7,38	0,2	15,0	0,04
9	7,45	7,54	-0,1	15,0	0,01
10	7,44	7,43	0,0	14,9	0,00

Figure 9: Analytical results of the homogeneity study of *animal feed* test material



The data are presented visually above, and show no suspect features such as discordant duplicated results, outlying samples, trends, discontinuities, or any other systematic effects.

Cochran's test: The largest value of D^2 is 0.31 and the sum of D^2 is 0.94, so the Cochran test statistic is 0.31/0.94=0.330. This is less than the 5% critical value of 0.602 for this type of test, so there is no evidence for analytical outliers and we proceed with the complete data set.

Homogeneity test

- Analytical variance: $s_{an}^2 = \Sigma D^2/2m = 0.94/20 = 0.047$
- Between-sample variance: the variance of the sums S = a + b is 0.11, so $s_{sam}^2 = (V_s/2 - s_{an}^2)/2 = (0.11/2 - 0.047)/2 = 0.004$
- Acceptable between-sample variance: the target standard deviation is 1.67 µg/kg, so the allowable between-sample variance is $\sigma_{all}^2 = (0.3\sigma_p)^2 = (0.3 \text{ X } 1.67)^2 = 0.251$
- Critical value: The critical value for the test is $1.88\ \sigma_{all}^2 + 1.01\ s_{an}^2 = 1.88 \text{ X } 0.251 + 1.01 \text{ X } 0.047 = 0.519$

Since $s_{sam}^2 = 0.004 < 0.519$, passed and the animal feed material is sufficiently homogeneous.

13.2. Stability tests

Stabilities of the contaminated baby food, maize power and animal feed materials were evaluated according to chapter 3.11.5 of the Harmonized Protocol.

Table 14: Stability study for aflatoxin B1 (µg/kg) in baby food

sample	15/04/2011	20/06/2011	6/10/2011
1	0,13	0,15	0,17
2	0,14	0,14	0,15
3	0,14	0,17	0,13
mean	0,14	0,15	0,15
difference		0,01	0,01

Table 15: Stability study for aflatoxin B1 (µg/kg) in maize powder

	5/05/2011	20/06/2011	6/10/2011
sample 1	2,28	2,28	2,45
sample 2	2,33	2,32	2,43
sample 3	2,40	2,45	2,31
mean	2,34	2,35	2,40
difference		0,01	0,06

Table 16: Stability study for aflatoxin B1 (µg/kg) in animal feed

	10/05/2011	20/06/2011	6/10/2011
sample 1	7,81	7,75	6,89
sample 2	7,45	7,79	8,16
sample 3	7,71	7,77	8,08
mean	7,66	7,77	7,71
difference		0,11	0,05

The differences due to instability were smaller than the desired limit of $0.1 * \sigma_p$ according to the Harmonized Protocol, so there are no consequential instabilities and the materials are suitable for use.

13.3. Invitation letter to laboratories

KUNSAGI Zoltan (JRC-GEEL)

From: KUNSAGI Zoltan (JRC-GEEL) on behalf of JRC IRMM CRL MYCOTOX
Sent: vendredi 25 mars 2011 10:03
Cc: ULBERTH Franz (JRC-GEEL); DE LA CALLE GUNTINAS Maria Beatriz (JRC-GEEL); STROKA Joerg (JRC-GEEL); LERDA Donata (JRC-GEEL)
Subject: ARES(2011)328720: Participation offer for the official control labs in the next Mycotoxin PT

Importance: High

Follow Up Flag: Follow up
Flag Status: Red

ARES(2011)328720

Dear colleagues,

This year the EU-RL Mycotoxins will organize its annual proficiency test (PT) on the determination of Aflatoxin B1 in baby food, animal feed, a coconut product and in a test solution.

This PT will be open to EU official control laboratories falling under the responsibility of the NRLs in order to support the NRLs fulfilling their tasks according to Regulation No 882/2004.
The participation fee for official control laboratories will be 250 EUR per participation.

The NRLs are kindly requested to forward this information to those official control laboratories they wish to participate in the study.

There will be two options:

1) The NRL enrols official control laboratories and covers participation fees:

In this case the NRL submits to the EU-RL a list of participants. The coverage of the participation fees has to be confirmed and details for invoicing (e.g. order number) have to be provided. It shall be made clear, that the full participation fee is payable upon dispatch of the test samples. In return the performance data of the respective official control laboratories will be disclosed to the NRL.

2) The official control laboratory enrols itself in the inter-laboratory comparison and covers the participation fee:

In this case the NRL will get access to the performance data of the official control laboratory only upon providing a letter of consent by the participating lab.

Please inform us by **15 April 2011** which laboratories will participate in this study and submit the following information:

Name of laboratory:
Address (no post box):
Contact person:
E-mail:
Telephone:

Lastly I would like to inform you that participation in this proficiency test remains obligatory and is free of charge for NRLs.

Kind regards,
Zoltan Kunsagi

Zoltan Kunsagi
Institute for Reference Materials and Measurements (IRMM)
Food Safety and Quality Unit
Retieseweg 111

1

13.4. Opening of registration

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 4 May 2011
JRC.DDG.D.6/JS/bk/ARES(2011)490161

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Madame/Sir,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of aflatoxin B_1 in:

- cereal-based babyfood
- maize,
- animal feed (cereal-based) and
- a test solution.

This proficiency test (PT) was announced during the last EU-RL Mycotoxins workshop. As already mentioned during the workshop, the comprehension of challenging matrices such as copra or spices would depend on their availability. Up to now no suitable material could be acquired in sufficient amounts. It was therefore agreed with DG SANCO that a follow-up PT will be organised with such challenging matrices upon availability for the National Reference Laboratories (NRLs).

The 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 National Reference Laboratories the participation is mandatory and free of charge.

- The participation fee for official food control laboratories is 250 Euro per participant[1]. The full participation fee is payable upon dispatch of the test samples. Our institute will contact them about the details of the payment.

Confidentiality of the participants and their results are granted.

[1] The fee covers the participation to both parts of the PT; the current one and the prospective one with more challenging matrices.

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-crl-mycotox@ec.europa.eu

Registration of participants is open until to midnight of 16th May, 2011.

Dispatch of the PT materials is foreseen to be at the end of May and will be announced in advance.

In order to register, laboratories must:

1. Enter the details online:

   https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=700

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:

Zoltan KUNSAGI
Tel: +32 14 571 313
Fax: +32 14 571 783

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

Deadline for reporting will be the 28th June. 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.

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-crl-mycotox@ec.europa.eu

Please contact us at the mail address:

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

With kind regards,

Joerg Stroka

(Operating Manager of the EU-RL Mycotoxins)

Cc: Franz Verstraete, Franz Ulberth, Beatriz De La Calle, Zoltan Kunsagi

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-crl-mycotox@ec.europa.eu

13.5. Accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 30 May 2011
ARES(2011)578552

Ref: 2011 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on aflatoxin B_1 in baby food, maize powder, animal feed (maize-based) and test solution

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 2011 PT aims to:

1. Assess the content in three contaminated test samples (marked as "Baby food - contaminated", "Maize powder - contaminated", "Animal feed - contaminated"). You will be asked to report the **recovery corrected value** in µg/kg, including your **recovery** in % and **measurement uncertainty** for a coverage factor of 2 (k=2).

For recovery experiments for baby food you can choose to use the material marked as "Baby food - blank".

2. Assess **by spectrophotometer** the content of aflatoxin B_1 in solution. For this, an ampoule of solution containing aflatoxin B_1 in 98 parts per volume of toluene and 2 parts per volume of acetonitrile is supplied. The aflatoxin B_1 content is in the range of 5-30 µg/mL. Report the value in exactly this unit [µg/mL].

Please calculate the concentration of aflatoxin B_1 according to the below mentioned procedure. This procedure refers to CEN EN 14123 *(Foodstuffs - Determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder - High performance liquid chromatographic method with post-column derivatisation and immunoaffinity column cleanup).*

WARNING – Aflatoxins are carcinogenic to humans. Gloves and safety glasses should be worn at all times and all standard and sample preparation stages should be carried out in a fume cupboard.
Protect aflatoxin containing solutions and test materials from light as much as possible (keep in dark, use aluminium foll or ambercoloured glassware).

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-crl-mycotox@ec.europa.eu

To determine the exact concentration, record the absorption curve between a wavelength of 330 nm and 370 nm in a 1 cm quartz cells in a spectrometer with solvent mixture (98 parts per volume of toluene and 2 parts per volume of acetonitrile) in the reference path. Identify the wavelength for maximum absorption. Calculate the mass concentration of aflatoxin B_1, ρ in micrograms per millilitre using equation:

$$\rho = \frac{A_{max} \times M \times 100}{\varepsilon \times b}$$

where:

A_{max} is the absorption determined at the maximum of the absorption curve
M is the molar mass, in grams per mol, of aflatoxin B_1 (M = 312 g/mol)
ε is the molar absorption coefficient of aflatoxin B_1 in toluene/acetonitrile, in square meters per mol, (here: 1930 m^2/mol)
b is the optical path length of the cell, in centimeters

Please report the parcel's receipt by fax or e-mail immediately, by using the **"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://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do
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 **1st July 2011** to:

Zoltan Kunsagi
JRC-IRMM-FSQ
EURL Mycotoxins
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: Jrc-irmm-crl-mycotox@ec.europa.eu

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

Jörg Stroka
(Operating Manager of the Community Reference Laboratory for Mycotoxins)

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Zoltan Kunsagi

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-crl-mycotox@ec.europa.eu

13.6. Acknowledgment of receipt form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

EURL
European Union Reference Laboratory
Mycotoxins

Geel, 30th May 2011

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 the receipt	
All items have been received undamaged	YES □ / NO □
If NO, please list damaged items:	

Contents of the parcel

a) One ampoule containing a solution of aflatoxin B_1

b) Test materials for analysis:
- 1 Baby food - contaminated
- 1 Maize powder - contaminated
- 1 Animal feed – contaminated

- 1 Baby food - blank

Signature / Stamp:

Please fax or e-mail the completed form to:

Zoltan Kunsagi
JRC-IRMM-FSQ
EURL Mycotoxins
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: zoltan.kunsagi@ec.europa.eu

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-crl-mycotox@ec.europa.eu

13.7. Questionnaire

Milc questionnaire

Comparison for PT 2011 Afla B1

Please fill in your results and answers to 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-CRL-MYCOTOX@ec.europa.eu

Submission Form

1. **How many samples does your laboratory analyse for Aflatoxin B1 per year?** *

- ◯ a) < 50 samples per year
- ◯ b) 50-250 samples per year
- ◯ c) 250-1000 samples per year
- ◯ d) more than 1000 samples per year

2. **Which food or feed matrices does your laboratory analyse for Aflatoxin B1 on a routine basis the most? (maximum 3)** *

3. **Are you accredited for the determination of Aflatoxin B1?** *

- ◯ a) Yes
- ◯ b) No

3.1. If YES, please specify the scope exactly how it is mentioned in your accreditation (e.g. Aflatoxin B1 +B2+G1+G2 in roasted peanuts by RPLC-FLD in the range of 0.1-10 µg/kg OR Aflatoxins in food etc.) *

4. **Proficiency test samples: BABY FOOD**

4.1. Please indicate your recovery (%) *

4.2. Please indicate the LOD for Aflatoxin B1 of the method used (µg/kg). *

4.3. Please indicate the LOQ for Aflatoxin B1 of the method used (µg/kg). *

5. **Proficiency test samples: MAIZE POWDER**

5.1. Please indicate your recovery (%) *

5.2. Please indicate the LOD for Aflatoxin B1 of the method used (µg/kg). *

5.3. Please indicate the LOQ for Aflatoxin B1 of the method used (µg/kg). *

6. **Proficiency test samples: ANIMAL FEED (MAIZE-BASED)**

6.1. Please indicate your recovery (%) *

6.2. Please indicate the LOD for Aflatoxin B1 of the method used (µg/kg). *

6.3. Please indicate the LOQ for Aflatoxin B1 of the method used (µg/kg). *

7. **Spectrophotometric analysis**

7.1. What is the brand and model of your UV-spectrophotometer? *

7.2. Did you calibrate your UV-spectrophotometer? *

- ◯ a) Yes
- ◯ b) No

7.2.1. If YES, what procedure in short did you use (e.g. K2Cr2O7 solution or calibrated filter). *

7.3. At which wavelength did you identify the maximum for Aflatoxin B1 (nm)? *

7.4. Optical path length of the cuvette (cm): *

7.5. What was the absorbance reading you obtained with the spectrophotometer? *

7.6. Do you normally check your calibrants by UV-spectrophotometry? *

- ◯ a) Yes
- ◯ b) No

8. **How did you perform the recovery estimate?** *

- ◯ a) Internal Standard to Extract
- ◯ b) Internal Standard to Sample
- ◯ c) Standard solution to Blank
- ◯ d) other

8.1. If other please specify! *

9. **What was the solvent composition of the spiking solution (e.g. toluene, methanol etc)?**

10. **During the analysis did you need to include any over night stop?** *

- ◯ a) Yes
- ◯ b) No

10.1. If YES, please state for which samples and at what stage of the analysis. *

11. **What was the extraction solvent used?** *

12. **What was the extraction mode (e.g. blending or shaking)?** *

13. **What was the extraction time?** *

14. **What was the solvent to sample ratio used during extraction (in mL/g)? Please specify it for the different PT materials!** *

15. **Were any extraction aids added (e.g. NaCl)?** *

- ◯ a) Yes
- ◯ b) No

15.1. If YES, please state what and in which quantity. *

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

17. **If you used immunoaffinity columns...**

17.1. ... please specify the manufacturer of the immunoaffinity columns you used during the analysis!

17.2. ... please specify the brand and the production code of the immunoaffinity column!

18. **What type of detection method did you use?** *

- a) HPLC-FLD
- b) LC-MS/MS
- c) ELISA
- d) other

18.1. If HPLC-FLD, please specify your method (type of column, injection volume, mobile phase etc.)! *

18.2. If LC-MS/MS, please specify your method! *

18.3. If ELISA, please specify the method! *

18.4. If other, please specify the type of your method! *

19. **Which derivatisation method was applied (e.g. Kobra cell)** *

20. **How did you integrate the signals?**

- Automatic
- Manual

20.1. If automatic, did you confirm the integration correctness visually? *

- a) Yes
- b) No

21. **Did you use acid washed glassware?** *

- a) Yes
- b) No

22. **Was protection against daylight applied?** *

- ○ a) Yes
- ○ b) No

23. **Did you encounter any problems during the analysis?** *

- ○ a) Yes
- ○ b) No

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

24. **Did you notice any unusual observations which, however, did not seem to have any effect on the results?** *

- ○ a) Yes
- ○ b) No

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

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

- ○ a) Yes
- ○ b) No

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

26. **What is your opinion about the registering / reporting format of this interface?** *

27. **Any other comments you wish to address?**

13.8. Experimental details

Table 17: Results and method performance characteristics (test solution)

Lab Code	Technique	Result [µg/ml]	Uncertainty value [µg/ml]	Coverage factor
101	Spectrophometer	**12**		
103	Spectrophometer	**12.04**	0.24	2
104		*No result*		
105	LC-MS/MS	**10.2**		
106	LC-MS/MS	**13.2**		
107		*No result*		
108	Spectrophometer	**10.766**		
109	Spectrophometer	**12**	0.21	2
110	HPLC-FLD	**13.22**	0.13	2
111	Spectrophometer	**12.9811**		
112	Spectrophometer	**10.9**		
113	Spectrophometer	**12.17**		
114	Spectrophometer	**12.12**	0.07	2
115	Spectrophometer	**12.044**		
116	Spectrophometer	**12.3**	1.5	2
117	Spectrophometer	**11.5**	22	1
118	Spectrophometer	**11.9**	0.3	2
119	Spectrophometer	**12.12**	0.12	2
120	Spectrophometer	**10.912**		
121		*No result*		
122	Spectrophometer	**12.1**	2.7	2
123	HPLC-FLD	**11.5**	5	
124	Spectrophometer	**11.75**	0.29	2
125	Spectrophometer	**12.93**		
126	Spectrophometer	**12.2**		
127	Spectrophometer	**12.11**		
128		*No result*		
129	Spectrophometer	**11.66**	1.4	2
130	Spectrophometer	**11.94**	0.36	2
131	Spectrophometer	**12.22**		
132	Spectrophometer	**11.8**	0.04	2
133	HPLC-FLD	**15**	0.75	2
134	Spectrophometer	**9.42**	0.38	2
135	Spectrophometer	**12.01**	1.8	2
136	Spectrophometer	**12.07**		
137	Spectrophometer	**13.85**	2.77	2
138	Spectrophometer	**11.83**		
139	Spectrophometer	**11.85**	0.24	2
140	Spectrophometer	**12.02**	1	2
141		*No result*		
142	Spectrophometer	**12.91**		
143	Spectrophometer	**11.77**		
144	Spectrophometer	**11.93**	0.08	2
145		*No result*		
146	Spectrophometer	**11.97**	0.6	2
147	Spectrophometer	**11.8**		2
148	Spectrophometer	**11.6**	0.66	2

Lab Code	Technique	Result [µg/ml]	Uncertainty value [µg/ml]	Coverage factor
149	Spectrophometer	**12.3**	2.7	2
150	Spectrophometer	**12.4**		
151	Spectrophometer	**12.27**		
152	Spectrophometer	**11.8**		
153	Spectrophometer	**11.8**	0.24	2
154	Spectrophometer	**12.092**		
155	Spectrophometer	**12.027**	0	0
156	Spectrophometer	**12.06**		
157	Spectrophometer	**10.3**	0.02	2
158	Spectrophometer	**12.8**		
159	Spectrophometer	**13.76**		
160	Spectrophometer	**12.1**		
161		*No result*		
162	Spectrophometer	**11.688**		
163	Spectrophometer	**12.26**	0.1	2
164	Spectrophometer	**11.963**	0.01	2
165	Spectrophometer	**14.736**		
166	Spectrophometer	**11.8**	0.6	2
167	Spectrophometer	**11.7**	0.4	4.303
168	Spectrophometer	**12.68**	0.03	2
169	Spectrophometer	**12**	1.48	2

Table 18: Results and method performance characteristics (baby food)

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
101	HPLC-FLD	<0.3			99	0.1	0.3
103	HPLC-FLD	**0.308**	0.092	2	61.0	0.01	0.05
104	LC-MS/MS	**0.243**	0.12	2	79	0.004	0.008
105	LC-MS/MS	**1.1**			82	0.03	0.1
106	LC-MS/MS	**0.14**			95	0.05	0.1
107		*No result*			-	-	-
108	HPLC-FLD	**0.36**			114.3	0.3	0.8
109	HPLC-FLD	**0.234**	0.06	2	103.7	0.070	0.100
110		*No result*			35	0.03	0.1
111	HPLC-FLD	**0.2**	0.04	2	82-109	0.001	0.1
112	HPLC-FLD	**0.1**			67	0.02	0.05
113	HPLC-FLD	**0.188**	0.04	2	89.66	0.1	0.25
114		*No result*			-	-	-
115	HPLC-FLD	**0.24**	0.04	2	69	0.01	0.05
116	HPLC-FLD	**0.18**	0.13	2	70.9	0.02	0.05
117	HPLC-FLD	**0.18**	22	1	86.3	0.10	0.10
118	LC-MS/MS	**0.22**	0.1	2	106	0.03	0.08
119	HPLC-FLD	<0.3			107	0.3	0.3
120	LC-MS/MS	<1			70	0.3	1.0
121	HPLC-FLD	**0.19**	0.06		100	0.003	0.01
122	HPLC-FLD	**0.21**	0.09	2	84	0.08	0.08
123	HPLC-FLD	**0.19**	25		101	0.07	0.2
124	HPLC-FLD	**0.16**	0.02	2	77	0.008	0.015
125	HPLC-FLD	**0.27**	0.047	2	83.4	0.15	0.5
126	HPLC-FLD	**0.18**	0.1	2	100	0.06	0.1
127	HPLC-FLD	**0.28**	0.01		69	0.02	0.05
128	HPLC-FLD	**0.232**			56	0.025	0.050
129		*No result*			55	0.1	0.1
130	HPLC-FLD	**0.23**	0.07	2	49	0.024	0.032
131	HPLC-FLD	**0.15**	0.06	2	79	0.02	0.05
132	HPLC-FLD	**0.15**	0.081	2	60.8	0.15	0.4
133	HPLC-FLD	**0.203**	0.061	2	91.2	0.02	0.04
134	HPLC-FLD	**0.25**	0.05	2	60	0.05	0.08
135	HPLC-FLD	**0.25**	0.05	2	94.2	0.05	0.2
136	HPLC-FLD	**0.12**	0.03	2	133.7	0.02	0.1
137	HPLC-FLD	**0.16**	0.03	2	81.0	0.025	0.075
138	HPLC-FLD	**0.23**	0.12	2	73.9	0.002	0.004
139	HPLC-FLD	**0.199**	0.08	2	80	0.02	0.05
140	HPLC-FLD	**1.11**	0.42	2	72.0	0.5	1.5
141	LC-MS/MS	**0.16**	0.06	2	80	0.03	0.05
142	HPLC-FLD	**0.21**	0.1	2	91.35	0.2	0.5
143	HPLC-FLD	**0.27**	0.04	2	93.3	-	0.03
144	HPLC-FLD	**0.3**	0.2	2	125	0.08	0.1
145		*No result*			-	-	-
146	HPLC-FLD	**0.21**	0.06	2	93.4	0.018	0.035
147	HPLC-FLD	**0.22**	0.1	2	33	0.0035	0.007
148	HPLC-FLD	**0.16**	0.05	2	100	0.02	0.06
149	HPLC-FLD	**0.22**	0.1	2	80.4	0.007	0.1
150	HPLC-FLD	**0.56**	0.05	2	60	0.012	0.014

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
151	HPLC-FLD	**0.181**	0.058	2	100	0.10	0.10
152	HPLC-FLD	**0.17**	0.07	2	87	0.02	0.05
153	HPLC-FLD	**0.171**	0.029	2	77	0.05	0.15
154	HPLC-FLD	**0.17**	0.05	2	86	0.03	0.09
155		*No result*			95	0.02	0.1
156	HPLC-FLD	**0.18**	0.07	2	85	0.025	0.05
157	HPLC-FLD	**0.23**	0.03	2	98	0.05	0.06
158	LC-MS/MS	**0.24**	0.06	2	108	0.02	0.05
159	ELISA	**3.27**			4	1.7	1
160	HPLC-FLD	**0.2**	0.06	2	76.7	0.03	0.1
161	LC-MS/MS	<0.3			125	0.15	0.3
162	HPLC-FLD	**0.22**	0.04	2	77.5	0.25	0.5
163	HPLC-FLD	**0.11**	0.04	2	86	0.02	0.05
164	HPLC-FLD	**0.21**	0.08	2	74.6	0.02	0.08
165	HPLC-FLD	**0.186**	0.031	2	120	0.2	0.24
166	HPLC-FLD	**0.26**	0.05	2	97.4	0.2	0.4
167	LC-MS/MS	**0.22**	0.02	2.776	90.0	0.02	0.06
168	LC-MS/MS	**0.193**	0.058	2	78	0.02	0.06
169	HPLC-FLD	**0.14**	0.0174	2	105	0.05	0.1

Table 19: Results and method performance characteristics (maize powder)

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
101	HPLC-FLD	**3.4**			92	0.1	0.3
103	HPLC-FLD	**3.37**	0,67	2	94.0	0.04	0.20
104	LC-MS/MS	**2.2**	1.1	2	100	0.5	1.0
105	LC-MS/MS	**3.3**			79	0.03	0.1
106	LC-MS/MS	**2.3**	2.1	2	80	0.5	1
107	HPLC-FLD	**3.37**			82	n.d.	0.8
108	HPLC-FLD	**2.82**			110	0.3	0.8
109	HPLC-FLD	**2.52**	0,2	2	94.2	0.07	0.50
110	HPLC-FLD	**2.96**	0,59	2	102.3	0.03	0.1
111	HPLC-FLD	**3.3**	0,73	2	82-109	0.001	0.1
112	HPLC-FLD	**1.8**	0,31	2	81	0.2	0.37
113	HPLC-FLD	**2.689**	0.54	2	93.28	0.1	0.25
114	HPLC-FLD	**2.2**	0,6	2	-	-	-
115	HPLC-FLD	**2.74**	0,44	2	82.6	0.17	0.2
116	HPLC-FLD	**2.86**	1,6	2	65.1	0.02	0.05
117	HPLC-FLD	**0.18**	22	1	90.5	0.10	0.10
118	LC-MS/MS	**3.3**	1,3	2	108	0.3	1
119	HPLC-FLD	**3.68**	1,47	2	107	0.3	0.3
120	LC-MS/MS	**1.61**	0,4	2	71	0.3	1.0
121	HPLC-FLD	**2.5**	0,75	3	98	0.03	0.1
122	HPLC-FLD	**1.9**	0,8	2	84	0.08	0.08
123	HPLC-FLD	**3.1**	20		107	0.07	0.2
124	HPLC-FLD	**2.2**	0,3	2	71	0.1	0.5
125	HPLC-FLD	**0.165**	0,029	2	-	0.15	0.5
126	HPLC-FLD	**2.8**	0,3	2	87	0.06	0.1
127	HPLC-FLD	**4.2**	0,4		91	0.02	0.05
128	HPLC-FLD	<8			100	2.0	8.0
129	HPLC-FLD	**1.704**	0,392	2	88	0.1	0.1
130	HPLC-FLD	**3**	0.9	2	56	0.006	0.011
131	HPLC-FLD	**2.3**	0,3	2	105	0.2	0.5
132	HPLC-FLD	**2.3**	0,39	2	85	0.15	0.4
133	HPLC-FLD	**0.5**	0,1	2	83.9	0.2	0.4
134	HPLC-FLD	**2.26**	0,55	2	97	0.4	1
135	HPLC-FLD	**2.84**	0,6	2	93.7	0.1	0.3
136	HPLC-FLD	**3**	0,7	2	99.9	0.02	0.1
137	HPLC-FLD	**3.2**	0,3	2	80.9	0.2	0.6
138	HPLC-FLD	**3.71**	1,29	2	90.7	0.002	0.004
139	HPLC-FLD	**2.61**	0,78	2	92	0.1	0.2
140	HPLC-FLD	**2.14**	0,81	2	72.0	0.5	1.5
141	LC-MS/MS	**2.13**	0.43	2	75	0.3	0.5
142	HPLC-FLD	**2.51**	1.2	2	103	0.2	0.5
143	HPLC-FLD	**4.32**	0,57	2	89.3	-	0.03
144	HPLC-FLD	**2.4**	1,5	2	107	0.08	0.1
145	ELISA	**8.7**			82	0.5	-
146	HPLC-FLD	**2.7**	0,5	2	93.0	0.018	0.035
147	HPLC-FLD	**2.9**	0,9	2	85	0.09	0.2
148	HPLC-FLD	**2.1**	0,7	2	103	0.2	0.6
149	HPLC-FLD	**2.28**	1	2	87.5	0.007	0.1
150	HPLC-FLD	**3.84**	0,15	2	80	0.25	0.29

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
151	LC-MS/MS	**3.13**	0,66	2	100	1.0	2.0
152	HPLC-FLD	**2.87**	1.15	2	99	0.1	0.5
153	HPLC-FLD	**3.23**	0,37	2	50	0.2	0.6
154	HPLC-FLD	**2.35**	0,7	2	86	0.03	0.09
155	HPLC-FLD	**2.91**	0,93	2	96	0.02	0.1
156	HPLC-FLD	**2.69**	0,48	2	92	0.025	0.05
157	HPLC-FLD	**2.99**	0,45	2	93	0.05	0.06
158	LC-MS/MS	**2.3**	0,6	2	95	0.2	0.6
159	ELISA	**44.77**			1	1.7	1
160	HPLC-FLD	**2.8**	0,87	2	77.5	0.07	0.2
161	LC-MS/MS	**1.1**	0,3	2	-	0.15	0.3
162	HPLC-FLD	**3.8**	0,64	2	61.6	0.25	0.5
163	HPLC-FLD	**2.04**	0,82	2	71	0.1	0.2
164	HPLC-FLD	**3.08**	0,58	2	96.1	0.1	0.5
165	HPLC-FLD	**2.28**	0,396	2	120	0.2	0.24
166	HPLC-FLD	**2.97**	0,5	2	97.7	0.025	0.05
167	LC-MS/MS	**2.4**	0,3	4,303	71.0	0.02	0.06
168	LC-MS/MS	**3.12**	0,936	2	98	0.2	0.6
169	HPLC-FLD	**2.56**	0,2	2	83.4	0.05	0.1

Table 20: Results and method performance characteristics (animal feed)

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
101	HPLC-FLD	**9.3**			92	0.1	0.3
103	HPLC-FLD	**9.05**	1.36	2	103.0	0.04	0.20
104		*No result*			-	-	-
105	LC-MS/MS	**10.9**			76	0.03	0.1
106	LC-MS/MS	**7.5**	6.9	2	80	0.5	1
107	HPLC-FLD	**8.7**	3.4	2	109.45	n.a.	>0.5
108	HPLC-FLD	**8.03**			105.5	0.3	0.8
109	HPLC-FLD	**10.5**	1.6	2	108.6	0.071	0.25
110	HPLC-FLD	**9.56**	0.19	2	102.2	0.03	0.1
111	HPLC-FLD	**10.6**	2.33	2	82-109	0.001	0.1
112	HPLC-FLD	**5.7**	0.69	2	81	0.2	1.4
113	HPLC-FLD	**9.981**	2.0	2	88.29	0.1	0.25
114	HPLC-FLD	**6**	1.5	2	86	0.5	2.0
115	HPLC-FLD	**9.31**	1.49	2	82.6	0.17	0.2
116	HPLC-FLD	**11.1**	4.9	2	70.9	0.02	0.05
117	HPLC-FLD	**7.8**	22	1	91.3	0.10	0.10
118	LC-MS/MS	**9.2**	3.7	2	118	0.3	1
119	HPLC-FLD	**9.12**	3.65	2	107	0.3	0.3
120	LC-MS/MS	**5.61**	1.4	2	73	0.3	1.0
121	HPLC-FLD	**8.6**	2.6	3	98	0.03	0.1
122	HPLC-FLD	**7.8**	3.4	2	84	0.2	0.2
123	HPLC-FLD	**10**	15		103	0.07	0.2
124	HPLC-FLD	**8.7**	1.9	2	89	0.1	0.2
125	HPLC-FLD	**9.04**	1.57	2	-	0.15	0.5
126	HPLC-FLD	**8.3**	1	2	87	0.06	0.1
127		*No result*			-	-	-
128	HPLC-FLD	**9**	0.7	2,31	101	0.5	2.0
129	HPLC-FLD	**5.597**	1.287	2	88	0.1	0.1
130		*No result*			-	-	-
131	HPLC-FLD	**7.2**	1.1	2	105	0.5	1
132	HPLC-FLD	**7.4**	1.3	2	85	0.15	0.4
133	HPLC-FLD	**7.5**	1	2	84.0	0.2	0.4
134	HPLC-FLD	**8.12**	1.8	2	94	0.4	1
135	HPLC-FLD	**8.24**	1.6	2	96.1	0.1	0.3
136	HPLC-FLD	**10.2**	2.2	2	97.2	0.02	0.1
137	HPLC-FLD	**11.3**	2.3	2	80.9	0.2	0.6
138	HPLC-FLD	**11.83**	3.45	2	93.1	0.002	0.004
139		*No result*			-	-	-
140	HPLC-FLD	**8.1**	3.1	2	72.0	0.5	1.5
141	LC-MS/MS	**7.46**	1.52	2	75	0.3	0.5
142	HPLC-FLD	**8.91**	4.2	2	103	0.2	0.5
143	HPLC-FLD	**17.24**	2.28	2	71.0	-	0.03
144	HPLC-FLD	**8.2**	5.2	2	93	0.08	0.1
145	ELISA	**7.02**			82	0.5	-
146	HPLC-FLD	**8.3**	1.7	2	93.0	0.018	0.035
147	HPLC-FLD	**8.3**	2.6	2	85	0.09	0.2
148	HPLC-FLD	**10**	3	2	89	0.1	1
149		*No result*			-	-	-
150	HPLC-FLD	**11.04**	1.32	2	76	0.25	0.29

Lab Code	Technique	Result [µg/kg]	Uncertainty value [µg/kg]	Coverage factor	Recovery [%]	LoD [µg/kg]	LoQ [µg/kg]
151	LC-MS/MS	**9.2**	1.94	2	100	1.0	2.0
152	HPLC-FLD	**7.83**	3.13	2	99	0.1	0.5
153	HPLC-FLD	**9.97**	1	2	50	0.2	0.6
154	HPLC-FLD	**7.52**	2.24	2	86	0.03	0.09
155	HPLC-FLD	**9.72**	3.11	2	95	0.02	0.1
156	HPLC-FLD	**9.06**	1.63	2	92	0.025	0.05
157	HPLC-FLD	**9.33**	1.4	2	93	0.05	0.06
158	LC-MS/MS	**7**	1.8	2	95	0.2	0.6
159	ELISA	**45**			2	1.7	1
160	HPLC-FLD	**8.5**	2.3	2	69.6	0.13	0.4
161	LC-MS/MS	**9**	2.7	2	-	0.15	0.3
162	HPLC-FLD	**10.24**	1.73	2	70.5	0.25	0.5
163	HPLC-FLD	**3.72**	1.49	2	100	0.1	0.2
164	HPLC-FLD	**6.24**	0.58	2	119.4	0.5	1.0
165	HPLC-FLD	**6.35**	1.1	2	120	0.2	0.24
166		*No result*			-	-	-
167	LC-MS/MS	**5.8**	0.7	4.303	87.5	0.02	0.06
168	LC-MS/MS	**10.24**	3.07	2	98	0.2	0.6
169	HPLC-FLD	**9.25**	0.72	2	85	0.05	0.1

Table 21: Number of samples analysed by laboratories per year for aflatoxin B1

Lab Code	Number of samples per year			
	< 50	50 – 250	251 – 1000	1000 <
101			X	
103		X		
104				X
105	X			
106				X
107			X	
108			X	
109		X		
110	X			
111		X		
112		X		
113		X		
114	X			
115		X		
116			X	
117				X
118	X			
119		X		
120	X			
121				X
122			X	
123			X	
124	X			
125		X		
126			X	
127		X		
128		X		
129			X	
130	X			
131			X	
132			X	
133		X		
134	X			
135		X		
136		X		
137			X	
138		X		
139			X	
140		X		
141			X	
142		X		
143	X			
144	X			
145			X	
146			X	
147				X
148			X	
149			X	
150			X	
151			X	
152		X		
153		X		
154		X		
155			X	
156		X		
157		X		
158		X		
159		X		
160		X		
161			X	
162		X		
163			X	

Lab Code	Number of samples per year			
	< 50	50 – 250	251 – 1000	1000 <
164	X			
165		X		
166		X		
167	X			
168				X
169	X			

Table 22: Matrices analysed on routine basis, accreditation

Lab Code	Which food or feed matrices does your laboratory analyse for aflatoxin B1 on a routine basis the most?	Are you accredited for the determination of aflatoxin B1?	If YES, please specify the scope exactly how it is mentioned in your accreditation
101	maize, corn gluten, soya	No	
103	pig feed, poultry feed, soy been etc.	Yes	Determination of total aflatoxins (B1, B2, G1, G2) in feeding stuffs and foodstuffs by HPLC method
104	all kinds of nuts and spices	Yes	Mycotoxins in food
105	cereals - barley	Yes	Aflatoxins B1, B2, G1, G2 in cereals by UPLC-MS/MS
106	wheat – flour - barley	Yes	RES44 nivalenol, 3-Ac DON, aflaB1,aflaB2,aflaG1,aflaG2,fumonisinB1,HT2,T2, zearalenone, aochratoxinA,cytohalasinE for cereals and cereal products/dried fruit/nuts/babyfood with UPLC-MS/MS
107	maize; complete animal feed various matrices based	Yes	Aflatoxin B1 in animal feed by HPLC-FLD - UNI EN ISO 17375:2006 at concentration in the sample > 0.5 ug/kg// Aflatoxin B1 in raw materials for livestock by HPLC-FLD in the range 0.010-0.030 ug/kg // Method not accredited, used for maize powder: Aflatoxin B1+B2+G1+G2 in cereals based food by HPLC-FLD in the range 0.4-4 ug/kg for aflatoxin B1
108	feed and feedstuffs matrices	No	
109	nuts	Yes	Mycotoxins in foodstuffs by HPLC-FL/UV
110	hazelnuts	Yes	nuts, spice, cereal
111	peanuts, animal feeds	Yes	Determination of aflatoxins B1, B2, G1 and G2 in foods such as peanut butter, pistachio paste, fig paste and paprika powder and for the determination of aflatoxin B1 in baby food adn animal feeding stuffs.
112	complementary and complete feedingstuffs, maize, food (no baby food)	Yes	Aflatoxin B1
113	animal feed, nuts, nut products	Yes	Aflatoxins B1 B2 G1 G2 in animal feeding stuffs, nuts & nut products using immunoaffinity column/HPLC/fluorescence detector
114	feed, peanuts	No	
115	cereals, nuts, spices	Yes	Aflatoxins B1, B2, G1, G2 and Total Aflatoxins in following ranges: Seeds: Cereals: nut products: dried fruit and dried fruit products: 0.2 - 20(µg/kg), Shelled nuts: 0.2 - 25 (µg/kg), Nuts and groundnuts in shell: 0.2 - 40 (µg/kg), Spices: 0.2 - 30 (µg/kg), aflatoxin B1 in Babyfood: 0.05 - 20 (µg/kg)
116	food matrices - nuts, peanuts, dried fruit, spices, cereals, baby food	Yes	Aflatoxin B1+B2+G1+G2 in nuts, peanuts, cocoa, coffee, dried fruit, spices, cereals, baby food by HPLC-FLD
117	peanuts, spices and vegetable oils	Yes	Bepalen van het gehalte aan aflatoxine B1, B2, G1 en G2; ImmunoAffiniteit clean-up en detectie mbv HPLC en Fluorescentie detectie. Scope noten, kopra, vijgen, pindakaas, specerijen, kruiden, plantaardige olien, mengvoeders en diervoeders
118	cereals	No	
119	feed, nuts	Yes	The determination of aflatoxins B1, G1, B2, G2 in nuts, nut products, pulses, cereal products, spices and animal feeding stuufs by immunoaffinity column separation and HPLC
120	cereals, animal feeds	Yes	Aflatoxins in feed by LC-MS/MS
121	hazelnuts cocoa nuts	Yes	aflatoxin in food
122	nuts, dried fruit, cereals powder	Yes	Aflatoxin B1, B2, G1, G2 and sum in food
123	animal feed, human food ,	Yes	aflatoxin B1+B2+G1+G2 in animal feed and human food
124	dried fruit and nuts	No	
125	cereal feeds, nuts, spices	No	
126	nuts, spices, rice	Yes	The Determination of Aflatoxins in Food and Feeding Stuffs by Immunoaffinity Column and High Performance Liquid Chromatography
127	spices, cereals, peanuts	Yes	C-P 215 Aflatoxine B & G und Ochratoxin A über IAC mittels HPLC und Nachsäulenderivatisierung (Coring-Zelle)
128	maize, bovine feed, chicken feed	Yes	Aflatoxin B1 in animal feed by HPLC-FLD and IAC VICAM AOZ in the range 0.0020 - 0.040 mg/Kg

Lab Code	Which food or feed matrices does your laboratory analyse for aflatoxin B1 on a routine basis the most?	Are you accredited for the determination of aflatoxin B1?	If YES, please specify the scope exactly how it is mentioned in your accreditation
129	animal feed	Yes	Aflatoxin B1 in animal feed in the range 0.1 -20 µg/kg
130	we do not analyse on routine basis	Yes	Aflatoxin B1+B2+G1+G2 in figs, peanutbutter, pistachio and other nuts by RPLC-FLD
131	nuts, animal feed	Yes	Aflatoxin B1 in feed and babyfood, aflatoxin B1, B2, G1, G2 in cereals, nuts and derived products
132	cereals, nuts, dried fruits and their products	Yes	Determination of Aflatoxins (B1,B2,G1,G2) in nuts,dried fruits,cereals and their products by HPLC-FLD
133	peanuts, almonds, dried figs	Yes	peanuts,fruits,cereals,maize,seasonings by HPLC:Aflatoxin B1+G1 in the range of 0.4-180 µg/kg;AflatoxinB2+G2 in the range of 0.2-45 µg/kg
134	animal feed	No	
135	peanut, baby food	Yes	Aflatoxin B1,B2,G1,G2 in in food of plant origin by HPLC
136	nuts, rice, dried fruit	Yes	Aflatoxin B1, B2, G1 and G2 in food, 0,1-120 µg/kg, HPLC-fluorescence
137	nuts, cereals, dried fruits	Yes	Determination of aflatoxins B1, B2, G1, G2 in nuts, dried fruits and cereals
138	peanuts pistachios rice	Yes	aflatoxins B1 B2 G1 G2 in nuts, ground nuts, spices and cereals by HPLC-FLD
139	nuts, almonds, nut-cereal-products	Yes	Sum of aflatoxins B1, B2, G1 and G2 in food
140	animal feeds, spices, nuts	Yes	Aflatoxins G1,G2,B1,B2 in animal feeds, cereals, dried fruit, spices and herbs using Rhone Diagnostics Easi Extract columns and HPLC with detection by Kobra
141	baby food, food for human use	Yes	Aflatoxin B1+B2+G1+G2 in food for human use and B1 in baby food
142	animal feeds & compound animal feedstuffs	Yes	Aflatoxin B1 in straight & compound animal feedstuffs by RP HPLC-FLD in the range of 0.5-50µg/kg
143	paprika, chili, nutmeg	No	
144	maize	Yes	Aflatoxins B and G in food by IAC/HPLC-FLD
145	feed for dairy cows and cereals	Yes	Aflatoxin B1 in food and cereals
146	nuts, animal feed, spices	Yes	Aflatoxin B1 - Aflatoxins B1,B2,G1 and G2 in Feed and feedstuffs (incl. nuts and spices) - Own method (performance characteristics of regulation (EC) 2006/401) - HPLC after immuno-affinity clean-up
147	baby food, cereal, animal feed	Yes	Aflatoxins B1, B2, G1 + G2 using automated immunoaffinity column clean up & HPLC with post column derivatisation
148	oilseeds, cereals for food an feed, spices	Yes	Aflatoxin in animals feed, extraction/cleaning by solvant and SPE, ISO 14718
149	peanut, hazelnut, rice	Yes	METHOD N.1 : Determination of aflatoxin B1 and the sum of aflatoxin B1,B2,G1 and G2 in hazelnuts, peanuts, pistachios, figs and paprika powder. METHOD N.2 : Determination of total aflatoxins (B1,B2,G1,B2) with HPLC.
150	food	Yes	Food and animal feed. Aflatoxin B1,B2,G1,G2 by HPLC-FLD using IAC clean-up.
151	feed and feed ingredients in the broadest thinkable range	Yes	Aflatoxin B1 in Feed & Feed Ingredients (as part of a multimycotoxin method)
152	mixed animal feed, nuts, rice	Yes	Food/animal feed-Analyse of Aflatoxin B1, B2, G1 and G2 in cereals, cereal products, nuts etc., spices, oils and meat
153	feed, animal liver, eggs	Yes	Aflatoxin B1 in feed and feed material by HPLC-FLD, 0.2-7.5 ug/kg
154	nut products, spices	Yes	Aflatoxin B1,B2,G1,G2 in Food and Animal Feeding Stuffs
155	nuts, feed materials, figs	Yes	Aflatoxins in food (except baby food), feed materials and compound feed
156	cereals, animal feed, liver	Yes	Aflatoxin B1+B2+G1+G2 in food and feed
157	pistachios, animal feed, maize	Yes	Aflatoxin B1 in maize (0.06-17.57ug/kg) and animal feed (0.51-21.07ug/kg); Aflatoxin B1,B2,G1,G2 in peanut butter and pistachios
158	cereals, dried fruit, nuts	Yes	Aflatoxin B1+B2+G1+G2 in cereals, dried fruit by UPLC-MS/MS in the range 0.6,1.2,0.9,1.2-9 µg/kg ; Aflatoxin B1+B2+G1+G2 in nuts by UPLC-MS/MS in the range 0.3-9 µg/kg.

Lab Code	Which food or feed matrices does your laboratory analyse for aflatoxin B1 on a routine basis the most?	Are you accredited for the determination of aflatoxin B1?	If YES, please specify the scope exactly how it is mentioned in your accreditation
159	only feed	No	
160	cereals, premixes,	Yes	Aflatoxin B1 and Aflatoxin B1+B2+G1+G2 in cereals, nuts, spices, dried fruits and vegetables, dried figs, cocoa, cocoa products, cereals products, feedstuff
161	wheat, flour, malt	No	
162	compound animal feeds	Yes	Aflatoxins B1,B2,G1,G2 in Animal Feeds and Foods, General
163	nuts, feed, grain	Yes	Aflatoxins in vegetal origin food products and feedstuff by HPLC-FLD
164	nuts, spices, cereals based feeds	Yes	Aflatoxins in food, Aflatoxin B1 in feed
165	cereals	Yes	Aflatoxin B1 and Aflatoxin B1+B2+G1+G2 in nonanimal products By HPLC-FLD
166	herbs	Yes	mycotoxins in food by HPLC
167	baby food	No	
168	cereal, feed	No	
169	animal feed, fish meat; spices	Yes	Afl B1 in feed SR EN ISO 17375:2006; Afl B1 and sum of B1, B2, G1, G2 in food SR EN 16050/2007; SR EN 14123/2008

Table 23: Spectrophotometric analysis I.

Lab Code	What is the brand and model of your UV-spectrophotometer?	Do you normally check your calibrants by UV-spectrophotometry?	At which wavelength did you identify the maximum for aflatoxin B1 (nm)?
101	Shimadzu UV-1700 Pharma Spec	No	347.3
103	Thermo Electron Corp, Nicolet Evolution 300	No	348.3
104	Perkin-Elmer, Lambda Bio	Yes	366
105	not applied	No	
106	-	Yes	
107	not tested.	No	
108	Hitachi U-2000 spectrophotometer	Yes	363
109	SHIMADZU UV-160	Yes	348
110	-	No	
111	Cary UV300	No	349
112	Perkin Elmer Lambda BIO20	No	348
113	Varian Cary 50 Bio	No	347.6
114	Thermo Spectronic HeliosB	No	348
115	Shimadzu, UV-Vis 2450	Yes	348
116	Analytic Jena, Specord 210	Yes	348
117	Thermo	Yes	360
118	Thermo Scientific Genesys 6	No	348
119	Unicam alpha helios	No	349
120	VARIAN CARY 50 Win UV	Yes	358 and 349
121	We do not have a UV-spectrophotometer	No	
122	Shimadzu UV 2401PC	No	348
123	perkin elmer - lamda 12	Yes	360
124	GBC UV/VIS 911A	Yes	348
125	Thermo Unicam Helios alpha	No	360
126	Thermo Evolution 100	Yes	348
127	VARIAN Cary 3	Yes	349
128	n.a.	No	
129	UV/VIS Lamba 12	Yes	355
130	Perkin-Elmer Lambda 10	Yes	347
131	Unicam UV/Vis Spectrometer 2	No	348
132	UV-1700, Pharma Spec, Shimadzu	Yes	347.2
133	-	No	
134	ThermoSpectronic Helios Epsilon 9423UUE1000E	No	365
135	Beckman	Yes	347
136	Unicam UV2-100	Yes	348
137	Shimadzu UV-1700 Pharma Spec	No	347.7
138	Perkin Elmer lambda 400	No	348
139	Varian Cary 1E UV-VIS	Yes	348
140	Varian Cary 50 Solascreen	No	349.5

Lab Code	What is the brand and model of your UV-spectrophotometer?	Do you normally check your calibrants by UV-spectrophotometry?	At which wavelength did you identify the maximum for aflatoxin B1 (nm)?
141	--	No	
142	Brand: PerkinElmer, Model: Lambada 35 UV/Vis spectrophotometer	No	347.4
143	Perkin-Elmer Lambda 25	Yes	348
144	Thermo Spectronic UV 500	Yes	350
145	-	No	
146	SHIMADZU UV 1601	Yes	348.2
147	Hitachi U2000	Yes	348.2
148	SHIMADZU UV-1800	Yes	348.3
149	Perkin Elmer UV/VIS Spectrometer Lambda 2	No	347.8
150	Perkin Elmer Lambda 25	No	348
151	Hitachi	No	346.96
152	Shimadzu UV-1602	No	348
153	Beckman DU-62	No	350
154	Varian, Cary 300	Yes	348
155	Ultrospec 2100 pro	Yes	348
156	Cecil CE7400	No	347.8
157	BECKMAN - DU 640	No	365
158	Shimadzu UV-160 A	No	349.5
159	lary 50 Scan (Varian)	No	350
160	Agilent 8453	Yes	348
161	It isn't realized by technical problems	No	
162	Cecil CE1021	No	349.3
163	PerkinElmer Lambda 35 UV/VIS	No	347
164	Shimadzu UV-1700	Yes	348
165	GBC, Cintra 10e	No	346
166	Hitachi U1800	Yes	348
167	SHIMADZU UV-1601	No	348.2
168	JASCO V530	No	347.3
169	GBC CINTRA-10	No	330-400

Table 24: Spectrophotometric analysis II.

Lab Code	Did you calibrate your UV-spectrophotometer?	If YES, what procedure in short did you use (e.g. $K_2Cr_2O_7$ solution or calibrated filter)	Optical path length of the cuvette (cm):	What was the absorbance reading you obtained with the spectrophotometer?
101	Yes	calibrated filters	1	0.745
103	No		1	0.745
104	Yes	K2Cr2O7 and Holmium filter	1	none, because was not available in june 2011 (see remarks)
105	No	not applied		not applied
106	Yes	-		-
107	No	not tested		not tested
108	No		1	0.666
109	Yes	K2Cr2O7 solution	1	0.741 / 0.736 / 0.749
110	No			no
111	Yes	Calibrated filters	1	0.803
112	Yes	Calibrated filter	1	0.674
113	Yes	Calibrated Filter	1	0.753
114	No		1	0.750
115	Yes	K2Cr2O7 solutions used to check on absorbance accuracy and Holmium perchlorate to check on wavelength calibration	1	0.745
116	Yes	K2Cr2O7, calibrated filter	1	0.7606
117	Yes	Filter Holmium / Didyrum	1	0.714
118	No		1	0.735
119	No		1	0.75
120	Yes	calibrated filter	1	0.675 and 0.672
121	No			
122	Yes	K2Cr2O7 and calibrated filter	1	0.749
123	Yes	calibrated filter		
124	Yes	K2Cr2O7	1	0.727
125	No		1	0.160 on a 5 fold dilution of solution supplied
126	Yes	K2Cr2O7 and Holmium Perchlorate	1	0.753
127	No		1	0.749
128	No			
129	No		1	0.721
130	Yes	K2Cr2O7 solution	1	0.758
131	Yes	1x per year calibrated filter	1	0.756
132	Yes	K2Cr2O7 solution, calibrated filters, dihymium and holmium glass filters	1	0.732
133	No			
134	Yes	K2Cr2O7	1	0.583
135	Yes	Toluene:acetonitrile 98:2	1	0.743
136	No		1	0.746
137	Yes	K2Cr2O7 solution	1	0.857

Lab Code	Did you calibrate your UV-spectrophotometer?	If YES, what procedure in short did you use (e.g. $K_2Cr_2O_7$ solution or calibrated filter)	Optical path length of the cuvette (cm):	What was the absorbance reading you obtained with the spectrophotometer?
138	Yes	calibrated filter (Holmium oxyde)	1	0.7315
139	No		1	0.733
140	Yes	K2Cr2O7	1	0.7439
141	No	--		--
142	No		1	0.7983
143	Yes	Calibrated filter (14-02-2011)	1	0.7279
144	Yes	K2Cr2O7	1	0.738
145	No	-		-
146	Yes	calibrated filter	1	0.7402
147	Yes	K2Cr2O7 + Calibrated Filters	1	0.73
148	Yes	Aqueous solutions Co Ni Ref NIST SRM931g-LGC Pr, holmium oxyde 4% in HCLO4 10% Ref RM-HL n° 11989	1	0.718
149	No		1	0.76
150	No		1	0.76722
151	No		1	0.759
152	No		1	0.73
153	No		1	0.146
154	Yes	calibrated filter	1	0.748
155	Yes	Calibrated regularly by a service company	1	0.744
156	No		1	0.746
157	Yes	K2Cr2O7 solution	1	0.6371 (mean value)
158	Yes	calibrated filter	1	0.792
159	No		1	0.851
160	Yes	K2Cr2O7 solution	1	0.748538
161	Yes	K2Cr2O7	1	x
162	Yes	Calibrated Dimydium and Holmium filters	1	0.723
163	Yes	K2Cr2O7	1	0.7546; 0.7574; 0.7596; 0.7616
164	Yes	calibrated filters (checked each year by eichamt)	1	0.74
165	No		1	0.9116
166	No		1	0.731
167	No		1	0.723
168	Yes	calibration glass filter	1	0.78432
169	No		1	0.1361

Table 25: Recovery estimate, overnight stop

Lab Code	How did you perform the recovery estimate?	If other please specify!	What was the solvent composition of the spiking solution?	During the analysis did you need to include any over night stop?	If YES, please state for which samples and at what stage of the analysis.
101	Standard solution to Blank		methanol	No	
103	Standard solution to Blank		methanol	No	
104	Standard solution to Blank		methanol	No	
105	Standard solution to Blank		acetonitrile	No	
106	other	C13-labelled internal standards	methanol	No	
107	other	Animal feed: recovery from official method. // Maize powder: recovery from daily analysis of standard solution added to blank matrices	methanol	No	
108	Internal Standard to Sample		toluene-acetonitrile (98:2)	No	
109	Standard solution to Blank		benzene / acetonitrile (98:2)	No	
110	Internal Standard to Sample		acetonitrile	No	
111	Internal Standard to Sample		methanol	Yes	All samples prior to immuno-affinity column cleanup
112	Standard solution to Blank		benzene, acetonitrile 98:2	No	
113	other	Standard solution to sample for maize & animal feed. Standard solution to blank for baby food	50% methanol	Yes	Spike sample overnight at 4°C
114	Standard solution to Blank		Methanol	No	
115	other	Blank matrix spiked with analyte and analysed using entire method.	methanol	Yes	With the Maize and Animal feed. Extracts stored in freezer overnight.
116	Standard solution to Blank		water/methanol /acetonitrile (56:22:22)	Yes	all samples, between extraction and clean-up
117	Standard solution to Blank		methanol	No	
118	Internal Standard to Extract		acetonitrile	Yes	Sample (matrix) is spiked rougly 24 hours before extraction to ensure the analyte has properly entered the sample matrix
119	Standard solution to Blank		25% methanol, 75% acetonitrile	No	
120	other	IS to immunoaffinity columns	toluene	No	
121	Standard solution to Blank	methanol		No	
122	Standard solution to Blank		methanol, H_2O	Yes	just before hplc injection
123	Standard solution to Blank		methanol/H_2O	No	
124	other	Sample material spiked with a known standard solution	methanol	No	
125	other	Standard addition to	methanol/water (1:1)	No	

Lab Code	How did you perform the recovery estimate?	If other please specify!	What was the solvent composition of the spiking solution?	During the analysis did you need to include any over night stop?	If YES, please state for which samples and at what stage of the analysis.
		portion of sample			
126	Standard solution to Blank		methanol	No	
127	Standard solution to Blank		toluene	No	
128	other	ERM-376		No	
129	Standard solution to Blank		chloroform	No	
130	Standard solution to Blank		toluene : acetonitrile = 98:2	Yes	WE did stop after IAC elution and before HPLC/FLD
131	Standard solution to Blank		acetonitrile	No	
132	Standard solution to Blank		toluene: acetonitrile	No	
133	Standard solution to Blank		methanol, water	No	
134	Standard solution to Blank		acetonitrile	No	
135	Standard solution to Blank		acetonitrile	No	
136	other	Baby Food, Maize std to blank sample, Animal Feed std to EURL-contam. sample	benzene:acetonitrile 98:2	Yes	Clean-up 1 day eluates frozen, HPLC analysis next day
137	Standard solution to Blank		[methanol - (benzene 98/acetonitrile 2)]=[87.15-12.85]	No	
138	other	standard solution to sample	methanol/water 50/50% V/V	Yes	after IAC purification
139	Standard solution to Blank		methanol-water	No	
140	Internal Standard to Sample		methanol	No	
141	Standard solution to Blank		methanol-water	No	
142	other	result obtained from a known concentration spiked blank sample	methanol	No	
143	Internal Standard to Extract		methanol	Yes	For all samples
144	Standard solution to Blank		methanol	No	
145	Internal Standard to Sample		methanol	No	
146	Standard solution to Blank		methanol	No	
147	Standard solution to Blank		acetonitrile	No	
148	Standard solution to Blank		chloroform	No	
149	Standard solution to Blank		methanol	No	
150	Standard solution to Blank		acetonitrile	Yes	after evaporation
151	other	Check versus assigned value of Fapas CRM (baby food); Standard addition procedure for maize powder & animal feed (maize-based)	methanol/water	No	Samples were analysed overnight
152	Standard solution to Blank		acetonitrile	No	
153	Standard solution to		methanol:water	No	

Lab Code	How did you perform the recovery estimate?	If other please specify!	What was the solvent composition of the spiking solution?	During the analysis did you need to include any over night stop?	If YES, please state for which samples and at what stage of the analysis.
	Blank		(1+1)		
154	Standard solution to Blank		methanol	No	
155	Internal Standard to Sample		methanol	No	
156	other	standard solution spike to matrix	methanol	No	
157	Standard solution to Blank			No	
158	Standard solution to Blank		acetonitrile	Yes	Baby Food, between extraction and purification
159	Internal Standard to Sample			No	
160	Standard solution to Blank		toluene	No	
161	Standard solution to Blank		acetonitrile	No	
162	other	Standard solution to sample	methanol	No	
163	Standard solution to Blank		methanol	Yes	For all samples one day sample preparation and the second day HPLC analysis
164	Standard solution to Blank		acetonitrile	No	
165	Standard solution to Blank		acetonitrile	No	
166	Standard solution to Blank		methanol	No	
167	Standard solution to Blank		methanol	No	
168	Standard solution to Blank		acetonitrile	No	
169	Internal Standard to Sample		acetonitrile	Yes	All samples were left at the room temperature till next day for defrosting

Table 26: Extraction mode

Lab Code	What was the extraction solvent used?	What was the extraction mode (e.g. blending or shaking)?	What was the extraction time?	What was the extraction solvent to sample ratio used during extraction (in ml/g)?	Were any extraction aids added?	If YES, please state what and in which quantity.
101	85:15 acetone:water	shaking	30mins	1:5	No	
103	acetone/water	shaking	60 min	5ml / 1g	No	
104	acetonitrile-water (80-20)	shaking	120 min	maize powder 4 ml/g, babyfood 4 ml/g	Yes	for babyfood 0.3 g NaHCO3 per 25 g sample
105	methanol, water	shaking	60 min	25/5	Yes	NaCl, 0.4g
106	methanol-water	ultra-turrax	15 minutes	60/10	Yes	defatting with hexane
107	animal feed: acetone/water, 85/15% v/v. // maize powder: methanol/water, 70/30% v/v	Animal feed: magnetic stirrer. // Maize powder: Ultra-turrax	Animal feed: 30 min. // Maize powder: 3 min	Animal feed: 1:5. // Maize powder: 1:5	Yes	Maize powder: NaCl, 10g
108	pure acetone and HPLC grade water (85:15)	shaking	30 minutes	5 ml/g for all materials	No	
109	methanol / water (70:30) for babyfood and maize. dichloromethane for feed	blending	2 minutes	5	Yes	Na Cl 1 g for babyfood and maize, Celite 6.25 g for feed
110	methanol/water (80/20)	blending	3 minutes	10 ml/g	No	
111	maize and animal feed 85:15 acetone/water. baby food 80:20 methanol/water	shaking	30 minutes	5	Yes	Only for baby food 5 grams sodium chloride
112	methanol-water 80:20	shaking	10 min	4 ml/g	No	
113	70% methanol	blending	1 minute	Baby food 50/10 Animal feed & maize 50/5	Yes	NaCl 5g
114	methanol:H_2O (60:40)	shaking	30 min	Sample 5 grams:extraction solvent 25 ml. Final ratio was 5g/50ml liquid, because 25 ml of water was added before filtering.	Yes	0,4 gram
115	80:20 methanol : water	shaking	30 minutes	6	Yes	50ml Cyclohexane for Maize and Animal feed samples, 2.5g NaCL in all samples.
116	80 % methanol	blending (ultra-turrax)	3 min	5:1	Yes	NaCl - 5 g
117	methanol / water	blending	3 min.	150 ml solvent / 15g sample	Yes	NaCl 0.5g
118	Step1: 49% ACN / 50 % H_2O / 1% HaC | Step2 (additive): 99% ACN / 1% HaC = (final ACN: 79%)	shaking	2 times 60 minutes	Step1: 1 g sample + 3.35 ml 49% ACN / 50 % H20 / 1% HaC | Step 2: + 4.65 ml 99% ACN / 1% HaC final = 1 g sample + 8 ml solvent	Yes	NaCl : 1 g sample + 0.2 g NaCl
119	60% acetonitrile 40% water	blending	2 minutes	6.25 for 12 gram sample weight. 12.5 for 5 gram sample weight.	No	
120	methanol:water 60:40	shaking, ultrasonic	70 min	baby food, maize 3.3 ml/g , animal feed 5 ml/g	Yes	1.5 g
121	methanol, water	shaking	30 min	10	Yes	NaCl, 5g

Lab Code	What was the extraction solvent used?	What was the extraction mode (e.g. blending or shaking)?	What was the extraction time?	What was the extraction solvent to sample ratio used during extraction (in ml/g)?	Were any extraction aids added?	If YES, please state what and in which quantity.
122	methanol water (80:20)	blending	30 min	baby food 6ml/g - maize powder 2ml/g - feed 5ml/g	Yes	feed
123	H_2O et chloroforme	blending and shaking	2 mn and 1 hour shaking	137,5ml/25g	No	
124	methanol/water for baby food and maize ; acetone for feed	shaking for baby food and animal feed ; blending for maize	30 minutes for Baby Food and Animal feed ; 2 minutes for Maize	5 ml/g for Baby Food ; 5 ml/g for Animal feed ; 5 ml/g for Maize	Yes	5g NaCl for Baby Food and Maize ; No extraction aid for Animal Feed
125	methanol/water (3:2)	blending	2 minutes	250/25	Yes	2g NaCl
126	70% methanol in water	blending	1 minute	5ml/g for all	Yes	5g of NaCl
127	methanol 4 volume parts + water 1 volume part	blending	3 minutes	20ml/g	No	
128	acetonitrile/water 60/40 (v/v)	blending	30 min	40 ml / 10 g	No	
129	acetone/vand (85/15 v/v)	shaking	30 min	50 ml /10 g	No	
130	80 % MeOH	shaking	15 min	baby food and maize : 20 ml/5g	Yes	NaCl
131	babyfood: methanol/water 80/20 v/v, other: chlorophorm	shaking	30 minutes	5 ml per g for all	Yes	baby food: NaCl 5g
132	methanol:water	shaking	30 min	250ml/50g	Yes	5g NaCl
133	methanol, water	shaking	30 min	baby food: 25 g in 250 ml; maize powder: 25g in 125 ml; animal feed: 25g in 100ml	Yes	NaCl: baby food-2.5 g; maize powder and animal feed- 5 g
134	acetonitrile+ water, 60:40, v/v	shaking	60 min	4:1	No	
135	methanol:water 70:30	shaking	30 min	4/1	No	
136	baby food: 60 % acetonitril, maize, animal feed: 84 % acetonitril	shaking	30 minutes	Baby Food: 4 Maize: 4 Animal Feed: 4	No	
137	baby food: (methanol/water)=(80/20), animal feed: (methanol/water)=(80/20), maize: (methanol/water)=(62.5/37.5)	shaking	30 minutes	baby food:4.0, animal feed:6.0, maize:4.0	Yes	NaCl, baby food: 2.50g , animal feed: 6.84g, maize: 4.13g
138	methanol/water 80/20%V/V	blending+ shaking	30 minutes	4 ml/g for all matrixes	Yes	25g NaCl/l
139	methanol-water (80-20)	baby food shaking, maize flour blending	30-45 min, 3 min	BabyFood 5 ml/g, Maize Flour 4 ml/g	Yes	NaCl 1 g/ 10 g of sample
140	methanol :water 80:20	blending	2 minutes	2	No	
141	methanol-water PBS buffer	shaking	30 min	1:40	No	
142	chloroform & water	shaking	30mins	125ml/25g=chloroform, 12.5ml/25g= Water	Yes	Diatomaceous earth, 12.5g
143	methanol	shaking	60 min	4 ml/g for all materials	Yes	NaCl (5 g)
144	methanol:water	blending	2 min	4	Yes	5g NaCl
145		shaking	15 min		No	
146	methanol-water 80/20	shaking	30 minutes	4 ml/g for each material	Yes	4 g NaCl / 50 g
147	maize & animal feed acetonitrile:water, 6:4, v/v. baby food MeOH:water, 1:1, v/v	blending for maize & animal feed, shaking for baby food	Blending 4 minutes, Shaking 2 hours	Maize 20g / 100ml, Animal feed 20g/ 100ml, baby food 20g/ 100ml	No	

Lab Code	What was the extraction solvent used?	What was the extraction mode (e.g. blending or shaking)?	What was the extraction time?	What was the extraction solvent to sample ratio used during extraction (in ml/g)?	Were any extraction aids added?	If YES, please state what and in which quantity.
148	chloroform for animals feed, methanol water (80/20) for maize powder and baby food	shaking	30 min	animals feeds:5, maize powder and baby food:4	Yes	animals feeds: hyflosupercel 0.5g/g, maize powder and baby food: 0.1g/g
149	methanol/water 60:40	shaking	30 minutes	100ml/25g for each PT material	No	
150	MeOH/H_2O	shaking	60 min	25g/100ml	Yes	2.5g
151	acetonitrile/water/formic acid (84/16/1) for maize powder & animal feed (maize-based); chloroform for baby food	shaking for both	2 hr	10/2.5 for MAIZE POWDER & ANIMAL FEED (MAIZE-BASED); 100/10 for BABY FOOD	No	
152	Baby food: methanol/water 80/20; animal feed, maize: acetone/water 85/15	shaking	30 min	babyfood: 10; animalfeed, maize: 8	Yes	NaCl 5g/50g weight of sample
153	methanol-water (1+1)	shaking	1 hour	10 ml/g	Yes	0.5 g / 6 g of sample
154	70% methanol 30% water	blending	1 minute	5	Yes	NaCl 20% of test sample weight
155	acetonitrile + water (4:1)	shaking	45 min	5:1	No	
156	mixture of methanol+acetonitrile+water (35:35:30)	blending	1 minute	50ml/4g for animal feed, 50ml/10g for maize powder, 50ml/20g for baby food	Yes	NaCl 2.5g
157	MeOH:H_2O 80:20 v:v	blending	3 minutes	5 for all the PT materials	Yes	NaCl 10% of the sample weight
158	acetonitrile:water (80:20)	shaking	90 min	4 ml/g	No	
159	methanol	shaking	3 min	25 ml/5 g	No	
160	methanol:water	blending	3 min	80:20(v:v)	Yes	2.5 g
161	methanol/water, 70/30	Shaking	20 minutes	5 ml/g	No	
162	70% methanol / water	blending	1 minute	5	Yes	NaCl 0.1g/g
163	60% acetonitrile (water) for feed and 80% methanol (water) for baby food and maize powder samples	shaking	2 hours	10 ml/5g for feed, 20 ml/5g for maize, 20 ml/10g for baby food samples	No	
164	aceton/water (85 + 15) for maize (+ salt) and feed; methanol/water (80+20) for baby food	overhead shaking	30 min	maize sample (6 ml/g;25 g + 150 extr. solv), feed sample (5ml/g; 25 g + 125 extr. solv), baby sample (5ml/g; 25 g + 125 extr. solv)	Yes	NaCl: maize sample (5g), feed sample (no salt), baby sample (2.5g)
165	acetonitril-Water (60:40, v/v)	shaking	30 minute	4ml/g	No	
166	methanol /water	shaking	30 min	10 ml/g sample (baby food and maize)	Yes	2, 5 g /25 g samples, before shaking
167	methanol-water	blending, shaking	3 min (blending), 30 min (shaking)	250 ml / 50 g	Yes	NaCl, 2 g
168	acetonitrile:water=84:16	shaking	60 min	4:1 (for baby food) ; 5:1 (for maize and feed)	No	
169	acetonitrile 60%(animal feed);	blending	2-3 minutes	4ml/g(animal feed); 2 ml/g(baby	No	

Lab Code	What was the extraction solvent used?	What was the extraction mode (e.g. blending or shaking)?	What was the extraction time?	What was the extraction solvent to sample ratio used during extraction (in ml/g)?	Were any extraction aids added?	If YES, please state what and in which quantity.
	methanol 80%(maize and baby food)			food and maize)		

Table 27: Immunoaffinity column

Lab Code	What type of clean up methodology was used (e.g. immunoaffinity column)?	Please specify the manufacturer of the immunoaffinity columns you used during the analysis	Please specify the brand and the production code of the immunoaffinity column!
101	immunoaffinity column	LC Tech	Aflaclean 12058
103	immunoaffinity column	Romer Labs.	AflaStar R COIAC 1004
104	dilution with water prior to injection in LC-MS/MS		
105	immunoaffinity column	R-Biopharm	Aflaprep
106	immunoaffinity column	Vicam	AOZ HPLC (G1031)
107	Animal feed: immunoaffinity column // Maize powder: immunoaffinity column	Animal feed: R-Biopharm. // Maize powder: Vicam	Animal feed: Aflaprep, cod. P07. // Maize powder: Aflatest WB SR, cod. G1068
108	immunoaffinity column	Vicam Afla B (TM)	VICAM afla (B) G1003; production code 143
109	immunoaffinity column for babyfood and maize	R-Biopharm Rhône LTD.	Easi Extract Aflatoxins RP7 0N
110	immunoaffinity column	R-Biopharm	r-biopharm ee afla 50 xh 172/50
111	immunoaffinity column	Romer	Aflastar IAC column Lot No. AF1019-1102
112	immunoaffinity column	Vicam	G1031
113	immunoaffinity column	R-Biopharm	Aflaprep Batch no : YD299
114	immunoaffinity column. Sample volume 10 ml, water for washing 10 ml, methanol for extraction 3 ml. Volumetric flask of 5ml was filled with water.	R-Biopharm Rhone Ltd	Aflaprep, product code P07
115	immunoaffinity column	Vicam	Aflatest G1010
116	immunoaffinity column	R-Biopharm	Aflaprep, RBR P07
117	immunoaffinity column	Rhone Biopharm Ltd	batch VJ 664/50 Product number PO7
118	none (evaporate and shoot)	NA	NA
119	immunoaffinity column	R Biopharm	
120	immunoaffinity column	R- Biopharm Rhone Ltd	Aflaprep P07
121	immunoaffinity column	Vicam	VICAM, GXP 18.10.2012
122	immunoaffinity column	Vicam	Vicam Aflatest 12022
123	immunoaffinity column	R-biopharm	
124	immunoaffinity column	R-Biopharm Rhone Ltd	AflaPrep (50) P07
125	immunoaffinity column	r-Biopharm	Aflaprep P07
126	immunoaffinity column	R-Biopharm	Aflaprep YD299
127	immunoaffinity column	LC-Tech combined IAC for aflatoxins and ochratoxin A	Lot 709 expiry date 11-2012
128	immunoaffinity column	Vicam	AOZ G1031
129	immunoaffinity column	Aflaprep R-Biopharm	R-Biopharm YA 245/50
130	immunoaffinity column	R-Biopharm Rhone	Easi-Extract aflatoxin; YE 307/10
131	babyfood: immunoaffinity column, other: SPE (florisil + C18)	R-Biopharm	Easi-Extract aflatoxin XA 941/50
132	immunoaffinity column	Vicam Wide Bore	Aflatest WB,LOT 1791
133	immunoaffinity column	Vicam	VICAM G1024
134	immunoaffinity column	Vicam	G1024
135	immunoaffinity column	R-Biopharm Rhone LTD	Easi-Extract Aflatoxin, RP71/RP70N
136	MultiSep-columns 226 Afla ZON+ Romer Labs		
137	immunoaffinity column	Vicam	Aflaprep Lot 1769
138	immunoaffinity column	R-BioPharm	Aflaprep P07 production code: XL236/50
139	immunoaffinity column	R-Biopharm-Rhone	Easi-Ectract Aflatoxin RP71/RP70N
140	immunoaffinity column	R-Biopharm Rhone	Easi Extract
141	immunoaffinity column	R-Biopharm-Orsell	
142	immunoaffinity column	R-Biopharm, Rhone LTD	Aflaprep, Code:P07
143	immunoaffinity column	Biopharm-Rhône	Aflaprep: PC P07
144	immunoaffinity column	R-Biopharm Rhône	Aflaprep 50
145	-		
146	immunoaffinity column	Vicam	Aflatest WB Super Recovery (G1068)

Lab Code	What type of clean up methodology was used (e.g. immunoaffinity column)?	Please specify the manufacturer of the immunoaffinity columns you used during the analysis	Please specify the brand and the production code of the immunoaffinity column!
147	immunoaffinity column	R-Biopharm Rhone	Aflaprep
148	animals feed: SPE forisil and C18, maize powder and baby food: immunoaffinity column	Neogen	for aflatoxin 8043
149	immunoaffinity column	Romer	Aflastar fit COIAC1001
150	immunoaffinity column	Romer Labs	AflaStar IAC Order No. COIAC1004
151	maize powder & animal feed (maize-based): none; baby food: immunoaffinity column	R-Biopharm	AflaPrep WH 887/50
152	immunoaffinity column	R-Biopharm	Aflaprep RBRP07
153	immunoaffinity column	R-Biopharm Rhone	Aflaprep, RBRP07
154	immunoaffinity column	Neogen	Neocolumn aflatoxin- narrow bore. Product code 8040
155	immunoaffinity column	Vicam	AflaTest WB, Reorder #G1025
156	immunoaffinity column	Vicam	AflaTest, product code G1010
157	immunoaffinity column	R-Biopharm	Aflaprep 50 P07 Bx:YA 246/50
158	immunoaffinity column for baby food ; 80% acetonitrile extract diluted with water (1:1) to corn and feed	R-Biopharm Rhone	Aflaprep P07
159	No used		
160	immunoaffinity column	R-Biopharm	
161	immunoaffinity column	R-Biopharm	RIDA aflatoxin column, art. nº R5002, lot. 14490
162	immunoaffinity column	R-Biopharm Rhone Ltd	Aflaprep 50 XH174/50
163	immunoaffinity column	R-Biopharm Rhone Ltd	Easi-Extract® Aflatoxin RP71
164	immunoaffinity column	Vicam	Aflatest widebore
165	immunoaffinity column	Romer	AflaStar
166	immunoaffinity column	Vicam	AflaTest WB; lot 1802
167	immunoaffinity column	R-Biopharm, Rhone	Aflaprep P07
168	Mycosep 226		
169	immunoaffinity column	R-Biopharm-Rhone	Easi-extract aflatoxin EE AFLA 50/BX YB 268/50

Table 28: Detection techniques, specifying the methods

Lab Code	Detection techniques	Please specify your method (type of column, injection volume, mobile phase etc.)!
101	HPLC-FLD	Symmetry C18. 100µl, 50%H2O:40%MeOH:10%CH3CN
103	HPLC-FLD	ACE C18, 5um, 150x4.6 mm, mobil phase: water/MeOH/Acn 68/16/16
104	LC-MS/MS	10 µl inj, UPLC column 100 x 2.1 mm, 1,7µm, colum temp 50 °C, flow 0.4 ml min, gradient from 90% water + 0.1% Formic acid, to 90% acetonitril + 0.1 % formic acid in 12 min. MS transitions: m/z 313 > 241 and 313 > 285
105	LC-MS/MS	HSS T3 (Waters column), 5 µl injection, 5mM
106	LC-MS/MS	multimethod mycotoxins
107	HPLC-FLD	Animal feed: C18 5 um, (250 x 4.6) mm, injection volume = 50 ul, mobile phase = H2O/acetonitrile/methanol, 6+2+3 v/v. // Maize powder: C18 5 um, (250 x 4.6) mm, injection volume = 50 ul, mobile phase = H2O/acetonitrile/methanol, 60/20/20% v/v
108	HPLC-FLD	ISO/FDIS 17375; column: LiChrospher R100 RP-18(5 um) column 25*4.6 mm EcoPack; Injection volume: 100 uL; Mobile phase: water-acetonitrile-methanol (6+2+3); flow rate: 1 ml/min for mobile phase and 0.3 ml/min for PBPB reagent; Wavelenghts: emission 435 nm and 365 for excitation
109	HPLC-FLD	Spherisorb ODS2 (250 x 4.6); 25 ul; Water / Methanol / Acetonitrile (54:28:18)
110	HPLC-FLD	column: Hypersil ODS 5µ 40°C / injection: 100 µl / eluent: water acetonitrile, with acetic acid and kaliumbromide for electochemical derivatisation / flow: 1 ml/min / FLD Ex wavelength 365nm Em wavelength 435nm
111	HPLC-FLD	25 cm x 4.6 mm id Sperisorb ODS1 5 micron, injection volume 0.1 ml, mobile phase 580 ml water + 300 ml acetonitrile + 120 ml methanol.
112	HPLC-FLD	Waters symmetry c18 15 cm, 4.6 mm, 3.5 um- 100ul - Acetic acid 0.1% Methanol Acetonitrile
113	HPLC-FLD	Sperisorb 5µ ODS(1) ; 100 µL injection ; Mobile phase H20/ACN/MeOH 65/17.5/17.5 + KBr
114	HPLC-FLD	Column: Waters Spherisorb 5µm ODS2 4.6X250mm, injection volume: 100µl, mobile phase: H2O/MEOH/ACN/THF (615/240/120/25), flow rate: 1ml/min
115	HPLC-FLD	Column (Phenomenex Kinetex 2.6u PFP, 100A), Injection Volume 200µL, Mobile phase 45:55 Methanol:water.
116	HPLC-FLD	C18 (250 mm x 4,6 mm, 5 µm), injection 100 µl, mobil phase: water/methanol /acetonitril (56:22:22), Ex. 365 nm, Em. 435 nm
117	HPLC-FLD	RP18 10 cm - 4,8 mm / Injection 100 µl / flow 0,5ml/min / Mobile phase Methanol/ACN/Water/HNO3/ KBr
118	LC-MS/MS	Waters Acquity UPLC HSS T3 2.1 x 100 mm, 1.8 µm | Micromass Quattro Premier in ESI positive | IS: 13C | Evaporate & shoot
119	HPLC-FLD	Phenomenex Spherisirb ODS1 5micron, 25 by 4.6 mm, 220 microL, Methanol: acetonitrile:water (20:20:60), Flow rate 7ml/min.
120	LC-MS/MS	Zorbax XDB C18 150x4,6mm, MP- methanol and 10mM NH4AC in water, Esquire 6000, APPI
121	HPLC-FLD	phenomenex RP18 Luna 5µµm, 250x3mm, 35°C, 0,75 ml/min inj. 200 µl
122	HPLC-FLD	C18 symmetry Waters (76mm x 4.6mm, 3.5µm) - inj 20µl - Water/methanol 60/40
123	HPLC-FLD	C 18 Waters - 20 µl - MeOH/ACN/Buffer KBr-HNO3
124	HPLC-FLD	Column: Supelcosil C18 ; Injection Volume: Maize 50ul, Feed 200ul, Baby Food 500ul ; Mobile Phase: Water/Methanol/Acetonitrile
125	HPLC-FLD	
126	HPLC-FLD	150mm Genesis C18 column, injection vol = 100µl, mobile phase = 300ml Methanol/100ml acetonitrile/600ml water/119mg KBr/350µl 4M nitric acid
127	HPLC-FLD	Luna 3umC18 Phenomenenx, 50uL injection volume, Water/acetontrile/methanol
128	HPLC-FLD	C18 (150x4,6 mm); 50 µL; MeOH, ACN, H2O, (AcOH 1%/ACN 1/1) gradient; for determination of OTA and ZON in a single run
129	HPLC-FLD	Waters Nova-Pak C 18 µm 3.6x150 mm .Inj volume 250 µl. Mobile phase water/methanol/acetonitril (540/290/200 v/v/v)
130	HPLC-FLD	Sperisorp 5 ODS-1, 250 x 4,6 mm; 25 ul
131	HPLC-FLD	injection vol.: 100 µl, column (for all materials): Novapak C18, 3,9x150 mm, babyfood: MF: 40% MeOH, flow: 1 ml/min; other: MF: 112 H2O/ 56 MeOH, 25 ACN, flow: 0,4 ml/min + 0,2 ml/min (iodine derivatisation)
132	HPLC-FLD	Phenosphere C18 5um 250x4.60mm, 200ul, Water:MeOH:ACN (60:20:20)
133	HPLC-FLD	Lichrospher C18, mobil phase: methanol:ACN:water (15:20:65) + 119 mg KBr + 100 ul HNO3;injection volume: 100ul(baby food), 50ul (maize powder, animal feed)
134	HPLC-FLD	Inertsil C8 150x4.6 mm, Varian, 50 ul, acetonitrile+methanol+water , 8: 27:65, v/v/v
135	HPLC-FLD	Thermo HPLC column ODS 250 mm × 4.6 mm × 5 µm, Acetonitrile:methanol:water:phosphoric acid 200:200:600:0.1+ 0.119 g KBr
136	HPLC-FLD	Column: ACE 3 C18 3µm 100x4.6 mm, Injection Volume: BabyFood 30µl Maize, Animal feed 20µl, Mobile phase Water:Acetonitril:Metanol 900:180:240 KBr, HNO3
137	HPLC-FLD	column: Waters Symmetry 4.6x250mm/5um/ODC(C18), inj vol=200uL, mobile phase: (THF/water)=(21.0/79.0)

Lab Code	Detection techniques	Please specify your method (type of column, injection volume, mobile phase etc.)!
138	HPLC-FLD	Lichrospher C18 250 mm 4.6 mm ID 100 microliter injection
139	HPLC-FLD	RP, NovaPak C18, injection 0.1 ml, Mobile phase methanol-acetonitrile-water(290-140-570)
140	HPLC-FLD	Pursuit XRS3 C18 150 x 4.6mm, 100ul, 60:40 water:methanol 0.9ml/min Ex 362nm Em 455nm
141	LC-MS/MS	Xterra C18 20µl Water-formic acid
142	HPLC-FLD	HPLC reverse phase C18 column, column heater 35C, 100µl injection volume,Mobile Phase: Water/MeOH/ACN (65/35/20), pump set @ 1ml/min
143	HPLC-FLD	Spherisorb 5 microm ODS2; 100 microL; methanol/water 40:60 (V/V)
144	HPLC-FLD	C18 column, 400uL, H2O:AcN:MeOH (56:15:29), 0.7 ml/min
145	ELISA	competitive assay
146	HPLC-FLD	C18 - 250 mm - 5 µm - 200 µl - water(610)/ACN(175)/methanol(215) - 1 ml/min
147	HPLC-FLD	S5-ODS1 Excel, 250mmx4.6mm, Water:Acetonitrile:MeOH, 56:30:14, with nitric acid & KBr for KOBRA cell. Injection volume 400µl
148	HPLC-FLD	Column: Lchro CART 125-4, 5µ, Injection Volume: 100µl, mobile phase: methanol, acetonitril, water (36-17-44)
149	HPLC-FLD	Column RP18e 250x4.6 mm 5 µm, injection volume 25 µl, mobile phase water/acetonitrile/methanol 66:19:15,flow rate 0.7 ml/min.
150	HPLC-FLD	Column - Phenomenex Luna C18, 20 mkl, H2O/MeOH/ KBr/ 4M HNO3
151	HPLC-FLD, LC-MS/MS	HPLC-FLD: BABY FOOD: Column: Waters Symmetry C18, 5µm, 3,0 * 150 mm; Injection volume 100 ul; Mobile phase: Water/methanol/acetonitril, 130/70/40(v/v/v) + 1 mM KBr + 1 mM HNO3 LC-MS/MS: MAIZE POWDER & ANIMAL FEED (MAIZE-BASED): Column:Restek, Ultra aqueous C18 3µm 100x2,1mm; Injection volume 5 ul; Mobile phase: Water/Methanol gradient with 1mM ammoniumformiate and 1% formic acid
152	HPLC-FLD	Zorbax Eclipse XDB-C18, 5µ, 250*4.6; injection volume: 100µl; acetonitrile/methanol/water 20/30/60; flow 1 ml/min; excitation: 362 nm, emission: 448 nm
153	HPLC-FLD	Prodigy column 5u ODS(2), 250x4.6 mm (Phenomenex), inj. volume 100 uL, mob. phase water-methanol-acetonitrile (600+200+200), λex = 362 nm, λem = 425 nm
154	HPLC-FLD	column - spherisorb 5u ODS1, injection volume 100uL, mobile phase 58 water:30 acetonitrile:12 methanol
155	HPLC-FLD	C18; 250 ul; water+methanol+acetonitrile+nitric acid+potassium bromide
156	HPLC-FLD	Waters NovaPak C18 column, 4µm 3.9x150mm, 100µl injection, 25°C, flow rate 1ml/min, mobile phase: CH3OH/H2O (with KBr and 4MHNO3)/ACN, 20:68:12
157	HPLC-FLD	C18 250x4.6mm 5micron; Vinj=150ul; MeOH:AcCN:H2O 29:17:54
158	LC-MS/MS	Column Acquity UPLC-BEH C18, 2.1x50 mm, 1.7 µm ; injection volume 10µl; mobile phase A:Water, B:Methanol+0.1% Formic Acid +0.5mM Ammonium acetate; Gradient 10-90 Methanol ; flow 0.3 ml/min
159	ELISA	Enzyme linked imunossay
160	HPLC-FLD	Inertsil ODS-2, 5 um, 150 mm x 4.6 mm; 100 ul; water:acetonitrile:methanol (60:10:30 v/v) added 119 mg KBr and 100 ul HNO3 per 1 litre
161	LC-MS/MS	Column Ascentis c18, 15cmx2.1mm, 3um. Inyection volume 20 ul. Mobile phase: A=Water/methanol, 90/10, 0.1 % acetic acid, 1mM amonium acetate, B=Methanol/water, 98/2, 0.1% acetic acid, 1mM amonium acetate. Apparatus: Quattromicro, MS method: 313.14-241 and 314.14-268.9, cone 35, collision 30
162	HPLC-FLD	C18 250x4.6mm, 100µl, water/methanol/acetonitrile 600/250/125
163	HPLC-FLD	LiChroCART 250-4 RT-18 (5µm) LiChrosper® 100; 20 µL; KBr and HNO3 solution/ acetonitrile/methanol (6/2/3)
164	HPLC-FLD	Gemini C18 (Phenomenex) 250 x 4,6 5µ, injection volume 50µl, flow: 1 ml/min,colum temp. 30°C, mobile phase: water:methanol:acetonitrile (540:290:170V/V)+ 119 mg potassium bromide + 100µl nitric acid (65%)
165	HPLC-FLD	C 18, injection volume 50 microliters, mobile phase:water 57%, Methanol 3.7%, acetonitrile 5% , 120 g potasium bromide, 350 microliters nitric acid
166	HPLC-FLD	column C-18 Symmetry Waters; inj 100 ul; MeOH:AcN:H2O - 28:19:53
167	LC-MS/MS	Flow rate=0.2 ml/min, column: hypersil gold 100x2.1, 5µm, inj. vol.: 20 µL, mobile phase: MeOH/H2O, ESI positive ion mode, precursor ion 313, products: 241, 285
168	LC-MS/MS	Column: ZORBAX SB-C18 (2.1x50 mm, 1.8 microns). Mobile phase: formic acid 0.1% and formic acid 0.1% in acetonitrile (gradient elution). Flow: 0.4 ml/min. Column temperature: 40°C. Injection volume: 10 microliters
169	HPLC-FLD	Mobile phase: 60:30:20(water:methanol:acetonitrile); 120 mg KBr, 350microl HNO3 4M, Nucleosil 100-5 C8; EC 250/4.6, flow rate 1 ml/min, injection volume 100 microl;

Table 29: Derivatisation, acid washed glassware, protection against daylight

Lab Code	Which derivatisation method was applied?	Did you use acid washed glassware?	Was protection against daylight applied?
101	Photochemical Derivatisation (UVE LCtech)	No	Yes
103	Kobra cell	Yes	Yes
104	none	Yes	Yes
105	none	No	No
106	-	No	Yes
107	UVE LC Tech	No	Yes
108	PBPB (Pyridinium hydrobromide perbromide)	Yes	Yes
109	PBPB	Yes	Yes
110	Kobra cell	No	Yes
111	Kobra cell	No	Yes
112	UVE - LC TeCK	No	No
113	Kobra Cell	No	Yes
114	Post column derivatisation with 50mg/1000mlH2O, flow rate 0.4ml/min	No	Yes
115	PHRED i.e. photochemical reactor for enhanced detection.	Yes	Yes
116	Kobra cell	Yes	Yes
117	Kobra cell	Yes	Yes
118	none	No	Yes
119	Post column derivatisation	No	Yes
120	-	No	Yes
121	-	No	No
122	Iodine	No	Yes
123	Kobra cell	Yes	Yes
124	Bromination using PBPB	No	Yes
125	Kobra cell	No	Yes
126	Kobra Cell	Yes	Yes
127	Coring-cell = Kobra cell	No	Yes
128	fotochemical UVE	No	Yes
129	post column derivatisation	Yes	Yes
130	PBPB	Yes	Yes
131	baby food: Kobra cell, other: iodine derivatisation	Yes	No
132	Post column Derivatisation with saturated iodine solution	Yes	Yes
133	Kobra cell	No	Yes
134	precolumn	Yes	Yes
135	Kobra cell	Yes	Yes
136	Kobra Cell	No	Yes
137	iodine	No	Yes
138	Kobra cell	Yes	Yes
139	Post-column with PBPB (pyridinium hydrobromide perbromide)	Yes	Yes
140	Kobra cell	No	Yes
141	--	No	Yes
142	Kobra cell, reaction tubing, minimum 34cm X 0.5mm internal diameter PTFE, current source set to 100µA	Yes	Yes
143	Kobra cell	Yes	Yes
144	Post-column derivatisation	Yes	Yes
145	-	No	Yes
146	PBPB	Yes	Yes
147	Kobra cell	Yes	Yes
148	Kobra-cell	Yes	Yes
149	post column derivatisation with iodine	No	No
150	Kobra cell	No	Yes
151	baby food: Kobra	No	Yes
152	post column with PBPB	No	Yes
153	Kobra cell	No	Yes
154	iodine saturated water	No	Yes
155	Kobra cell	Yes	Yes
156	Kobra cell	No	No

Lab Code	Which derivatisation method was applied?	Did you use acid washed glassware?	Was protection against daylight applied?
157	post column derivatisation with PBPB	Yes	Yes
158	no one	No	Yes
159	No used	No	Yes
160	Kobra cell	Yes	Yes
161	There isn´t derivatisation	Yes	No
162	Kobra cell	No	No
163	Kobra cell	Yes	Yes
164	Kobra cell	No	Yes
165	Kobra cell	No	Yes
166	second pump, PBPB	Yes	Yes
167	-	No	Yes
168	no derivatisation method was applied	Yes	Yes
169	Kobra cell	No	Yes

Table 30: Integration mode, problems during the analysis

Lab Code	How did you integrate the signals?	If automatic, did you confirm the integration correctness visually?	Did you encounter any problems during the analysis?	If YES, what were the specific problems and to which samples do they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If YES, what were these observations and to which samples do they apply?
101	Automatic	Yes	No		No	
103	Automatic	Yes	No		No	
104	Automatic	Yes	No		No	
105	Manual		No		No	
106	Automatic	Yes	No		No	
107	Manual		No		No	
108	Manual		No		No	
109	Automatic	Yes	No		No	
110	Automatic	Yes	Yes	bad recovery with baby food cont.	No	
111	Automatic	Yes	No		No	
112	Automatic	Yes	No		No	
113	Automatic	Yes	No		No	
114	Manual		No		No	
115	Automatic	Yes	No		No	
116	Manual		Yes	Samples of maize powder had to be filtered before clean-up.	No	
117	Automatic	Yes	No		No	
118	Automatic	Yes	No		Yes	The feed seemed to be particularly greasy!
119	Automatic	Yes	No		No	
120	Manual		No		No	
121	Automatic	Yes	No		No	
122	Automatic	Yes	No		No	
123	Automatic	Yes	No		No	
124	Automatic	Yes	No		No	
125	Manual		No		No	
126	Automatic	Yes	Yes	baby food extracts required centrifugation	No	
127	Automatic	Yes	No		Yes	With animal feed we had unusual low recoveries, therefore we did not commit any results
128	Manual		No		No	
129	Automatic	Yes	No		No	
130	Automatic	Yes	No		No	
131	Automatic	Yes	No		No	
132	Automatic	Yes	No		No	
133	Manual		No		No	
134	Automatic	No	No		No	
135	Automatic	Yes	No		No	
136	Automatic	Yes	No		No	
137	Manual		No		No	
138	Automatic	Yes	No		Yes	blank baby food matrix not mentioned in the delivery notice
139	Manual		Yes	Blank baby food material behaved differently compared to Baby	Yes	Not unusual but unfortunately the temperature in the lab was

Lab Code	How did you integrate the signals?	If automatic, did you confirm the integration correctness visually?	Did you encounter any problems during the analysis?	If YES, what were the specific problems and to which samples do they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If YES, what were these observations and to which samples do they apply?
				Food Test. For that reason the recovery used for result was an average of a longer time.		26-28 C during the analysis
140	Automatic	Yes	No		No	
141	Automatic	Yes	No		No	
142	Automatic	Yes	No		No	
143	Automatic	Yes	Yes	Samples´weight clearly insufficient	No	
144	Automatic	Yes	No		No	
145	Automatic	Yes	No		Yes	turbidity of the extracts
146	Automatic	Yes	No		No	
147	Automatic	Yes	Yes	Yes centrifuge normally used for baby food was broken, so had to use an alternative that is not refrigerated & has a slower speed. Extracts were dirtier than normal and much lower than expected recovery values were determined.	Yes	As above baby food extract not as clear after centrifugation as usual, and believe this led to the lower than expected recovery
148	Automatic	Yes	No		No	
149	Manual		No		No	
150	Automatic	Yes	No		No	
151	Manual		No		No	
152	Manual		Yes	unusual extraction for maize has to be applied (acetone/water instead of methanol/water): filtrate-dilution blocked IAC-column, even after filtration and centrifugation	No	
153	Automatic	Yes	No		No	
154	Automatic	Yes	No		No	
155	Automatic	Yes	No		No	
156	Automatic	Yes	No		Yes	poor filtration of baby food extract
157	Automatic	Yes	No		No	
158	Automatic	Yes	No		No	
159	Automatic	Yes	No		No	
160	Manual		No		No	
161	Manual		No		No	
162	Automatic	Yes	No		No	
163	Automatic	Yes	No		No	
164	Automatic	Yes	Yes	Baby sample: sample clumped together by adding extraction solvent.	No	
165	Automatic	Yes	No		No	
166	Automatic	Yes	No		No	
167	Automatic	Yes	No		No	

Lab Code	How did you integrate the signals?	If automatic, did you confirm the integration correctness visually?	Did you encounter any problems during the analysis?	If YES, what were the specific problems and to which samples do they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If YES, what were these observations and to which samples do they apply?
168	Automatic	Yes	No		No	
169	Manual		No		No	

Table 31: Instructions for the proficiency test

Lab Code	Did you find the instructions distributed for this PT adequate?	If NO, which parts do you think can improve?
101	Yes	
103	Yes	
104	Yes	
105	No	Instructions regarding determination of AfB1 standard solution were not absolutely clear.
106	No	instructions how to calculate the M.U. in detail are missing, since the baby food matrices are not known in detail, it's not possible to calculate a M.U. in a correct way; there's is not enough blank matrix to spike multiple times to be able to calculate a correct M.U.
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	
120	Yes	
121	Yes	
122	Yes	
123	Yes	
124	Yes	
125	No	1. More specific instruction on measuring concentration of standard solution needed 2. Should have specifically stated each sample needed spiking in introduction 3. Provide larger test portions to be sufficient to undertake spiking of all matrices
126	Yes	
127	Yes	
128	Yes	
129	Yes	
130	Yes	
131	Yes	
132	Yes	
133	Yes	
134	Yes	
135	Yes	
136	Yes	
137	Yes	
138	Yes	
139	Yes	
140	Yes	
141	Yes	
142	Yes	
143	Yes	
144	Yes	
145	Yes	
146	Yes	
147	Yes	
148	Yes	
149	Yes	
150	No	To much info requested in questionnaire.
151	Yes	
152	No	maybe unproper transportation-conditions: samples and standard-solution received uncooled and very warm (uncooled logistic-car)
153	Yes	

Lab Code	Did you find the instructions distributed for this PT adequate?	If NO, which parts do you think can improve?
154	Yes	
155	Yes	
156	Yes	
157	Yes	
158	Yes	
159	Yes	
160	Yes	
161	Yes	
162	Yes	
163	Yes	
164	Yes	
165	Yes	
166	Yes	
167	Yes	
168	Yes	
169	Yes	

Table 32: Opinions about the registering/reporting interface

Lab Code	What is your opinion about the registering / reporting format by this interface?
101	very good
103	ok
104	ok
105	organised well
106	ok
107	the format is clear, but it isn't rapid to fill in: the drop-down menus are preferable.
108	the registering and reporting interfaces are adequate and approachable.
109	Good
113	very comprehensive
115	Very good
116	OK
117	good
118	Field to report the LC-MS/MS method is small to report all details of an entire method
119	Satisfactory
120	no problem
122	good
124	Quite user-friendly and straightforward
125	The option to open PDF once results & questionnaire saved does not work.
126	Good
129	OK
130	fine
131	Ok. Possibility for comments on all questions could be advised
132	User friendly
134	O.K.
135	It is useful.
136	OK
138	it's almost OK
139	We had two different types of methods now and it's not so clear to fill all the information in one box (feed method would have been the third)
140	Satisfactory
141	very good
142	The reporting format is quite long & detailed compared to other schemes
143	normal
144	Ok
145	ok
146	good
147	OK
149	very clear
150	easy
151	the input area is rather small
152	ok
153	Proper
154	Having to print, sign and return a copy of the results causes administrative problems.
155	OK
156	no problem, simple and well-arranged
157	quite easy and quick
159	good
160	OK!
162	Good
163	better than previous
164	works very well!
165	ok
166	very well
167	very detailed
168	excellent
169	Good

Table 33: Other comments

Lab Code	Any other comments you wish to address?
104	We didn't analyse the animal feed sample, because it is out of our scope and we were not able to do the spectrophotometric analysis of the standard solution because our organisation is in the middle of a movement (instrument not available!)
106	The test solution is measured with a dilution and with LCMS-MS, since we don't use spectrophotometric analysis
107	The matrices baby food and test solution were not analyzed: no results will be sent.
108	It will be more accurate if the PT include also blanks for recoveries in maize powder and feed.
116	We consider sending of signed forms by fax or e-mail as useless, old-fashioned and redundant when are results and other forms submitted on-line.
119	A certified standard material would have been desirable for health and safety reasons.
124	It would have been preferable if more sample material was provided to perform our routine method in full
128	Data reported are only for animal feed and maize (treated as a feed material). For baby food we use a different method (not accredited yet).
132	More blank sample for baby food must be provided and the kind of baby food must be stated (e.g. cereal based baby food or infant formula)
139	We didn't analyze the feed sample. The result for baby food is given for the test material, not for dry matter.
148	One Question: why did you asked the quantification of aflatoxin B1 only?
149	The animal feed material wasn't tested because the laboratory doesn't analyze this type of material
150	Was insufficient sample amount for maize powder and animal feed to carry out routinely sample preparation. We took smaller amount of test material. It can be affected on the results.
151	When using two different methods for the samples, answering this questionnaire is not easy
160	Please, send more amount of samples the next PT. In our method the amount of sample weight is 25 g.
164	Questionnaire contains no question, if the results have been corrected for recovery! in our case: yes!

13.9. Assigned values

13.9.1. Introduction

Exact-matching, double isotope dilution mass spectrometry (EMD-IDMS), considered to be a primary ratio method, was used to determine the assigned values of the test materials. Figure 10 depicts the process of EMD-IDMS.

Sample blends (SB) are prepared by adding the spike solution, an isotopically labelled analogue of the analyte, to the test material at an amount that will result ideally in a ratio of signal analyte over signal labelled analogue of close to 1.

Calibration blends (CB) are prepared in a similar fashion. Instead of using a contaminated material an analyte-free material is fortified with the same amount of spike as in the sample blend. Additionally an amount of a reference material of the analyte (solution of analyte in pure solvent at known mass fraction and uncertainty) is added to bring the ratio close to 1 again.

Normally a few iterations of preparing SBs and CBs are needed until the right amounts of spike and reference material are fixed to obtain ratios close to 1. Once these amounts are fixed a number of SBs and CBs are prepared and measured as SB/CB pairs in direct succession. The ion ratio SB/CB is then calculated by dividing the ion ratio in the sample blend through the ion ration in the calibration blend for each of the measurement pairs.
Through this process biases caused by extraction efficiencies, chromatographic effects, mass spectrometric discrimination, etc. are effectively eliminated. And for such a bias-free process increasing the number of repetitions results in increased accuracy.

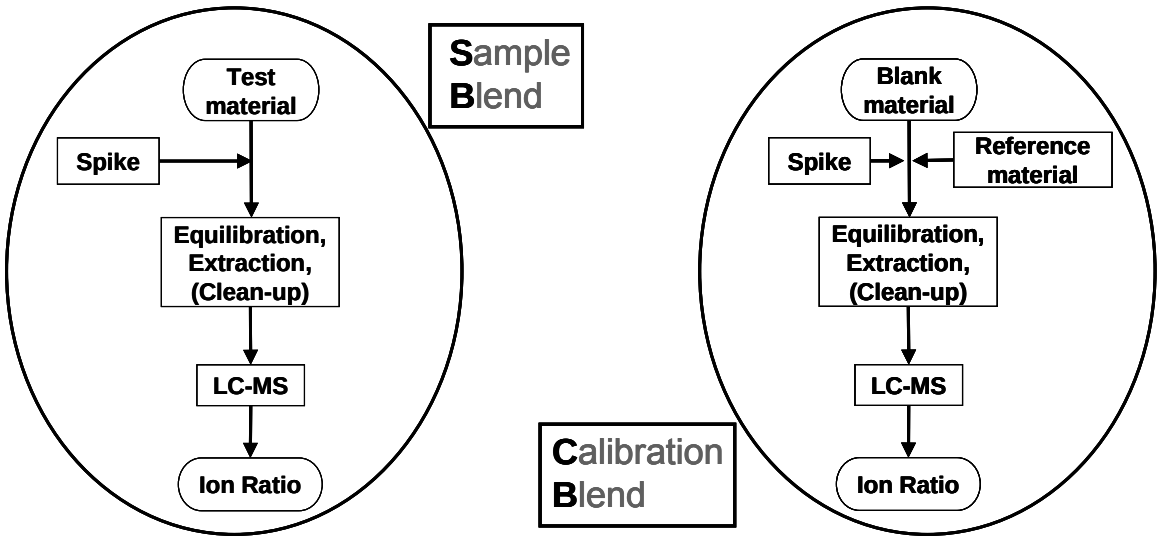


Figure 20: Depiction of the process of exact-matching, double isotope dilution mass spectrometry

13.9.2. Materials and Methods

Test Materials:

Three units each of the three test materials Baby Food, Maize, and Animal Feed were selected at random. The Aflatoxin-free Baby Food material provided with the PT was used as blank material for the Baby Food calibration blends. An Aflatoxin-free maize material was used as blank material for all other calibration blends.

Reference Materials:

The certified reference material "Aflatoxin B1 in Acetonitrile" (ERM-AC057, IRMM, from now on "AC057", 3.79±0.11 µg/g (k=2)) was used for all subsequent solutions for calibration blends. $^{13}C_{17}$ Aflatoxin B1 (Biopure, Austria, from now on "I") was used for all subsequent spike solutions. The following dilutions were prepared gravimetrically with an analytical balance (d=0.01mg):

Name	**Made from**	**Type**	**Mass fraction *w* [µg/g]**	**Standard uncertainty u(*w*) [µg/g]**
I		Spike	0.502	0.013
II	AC057	Reference	0.358	0.0052
III	I	Spike	0.0347	0.0009
IV	AC057	Reference	0.0347	0.0005
V	I	Spike	0.00219	5.7e-5
VI	AC057	Reference	0.0029	3.0e-5

Table 34: Reference and Spike solutions

Preparation of blends:

Test portions of 2 g of either test or blank material were used for the preparation of the different blends. To the test portions in a 50 ml polypropylene screw cap tube 4 ml of water were added and the whole content was fully suspended. After providing time for equilibration either spike or reference material were added and everything mixed again. The masses of the test portions, the spike, and the reference material were determined with an analytical scale (d=0.01mg) to the 5th decimal.

Then 16 ml acetonitrile were added slowly with intermediate mixing to avoid sudden precipitation of proteins in the blends which could cause loss of analyte. The blends were then agitated on an orbital shaker for a specified amount of time and briefly centrifuged at a RCF of 3200 *g.*

For the first iteration of all three test materials equilibration time was determined. To that end aliquots of 2 ml were withdrawn from the tubes after 10, 50, 100, and 1140 min. These aliquots were dried down in a stream of N_2 at 70 °C. The dry extract was reconstituted by adding 120 µl acetonitrile and vortex mixing. Then 280 µl water were added and the tube vortex mixed again. Of these solutions 20 µl were injected onto column. No significant differences between the different equilibration times could be determined. Therefore the extraction time was fixed at 30 min plus 10 min centrifugation.

The second iteration revealed an inhomogeneity within the test units at 2 g test portion size. Therefore an additional homogenization step was performed for all materials by submitting the full content of each test unit to 15 min milling in a Mortar Mill.

After the third iteration the correct amounts of spike and reference material were known for all test materials. For Baby Food and Maize two test portions each of the three test units were prepared for the final measurements. For the Animal Feed the first test unit was used up during the first three iterations and three test portions each of the remaining two test units were measured.

Measurements:

Measurements were performed on a LC-LC-MS system consisting of a binary high-pressure solvent delivery system (LC-20AD, Shimadzu), a quaternary low-pressure Accela solvent

delivery system, an Accela auto liquid sampler (Thermo Scientific), and a TSQ Quantum Ultra (Thermo Scientific). Separation was afforded in the first dimension by an Ascentis C18 column (50 x 2.1 mm, 3 µm) at a flow rate of 200 µl/min with a mobile phase of acetonitrile/water/formic acid (380/619/1,v/v/v). Elution strength was such that a retention factor of approx. 2 was obtained. The first dimension peak was trapped into a 100 µl loop installed to a 6 port, 2 way switching valve. The valve was then switched and the loop content injected onto the second dimension column.

Second dimension separation was afforded by an Ascentis Phenyl column (50 x 2.1 mm, 3 µm) at a flow rate of 200 µl/min with a mobile phase of acetonitrile/water/formic acid/ammonium formate pH 3.7 (480/519/0.5/0.5). Again, elution strength for a retention factor of approx. 2 was chosen. The 2-dimensional heart cut approach was chosen to obtain maximum resolution at high loading capacity for improved peak signal and precision while acceptable cycle times were maintained.

Electro spray ion source settings were as follows: spray voltage 2400 kV, vaporizer temperature 250 ºC, capillary temperature 320 ºC, sheath gas 30, ion sweep gas 10.0, aux gas 10 (gas pressures in arbitrary units).

In selected reaction monitoring (SRM) mode the proton-adducts of the parent compound were selected for the following transitions: 313.1->241.0, 313.1->270.0, 313.1->284.9 for Aflatoxin B1 (AFB1), and 330.1->227.2, 330.1->283.8, 330.1->301.4, 330.1->314.0 for $^{13}C_{17}$ Aflatoxin B1 (13C-AFB1). The dwell times were chosen such that about 20 scans across a peak were registered.

Batches of runs were structured such that each SB run was directly followed by a CB run. This was then repeated 10 times for each test portion. A total of 120 runs were performed for each test material

Calculation of the assigned values and their uncertainties

Since there was no significant signal of the labelled ^{13}C-AFB1 in the reference or test materials, and likewise no significant signal of the analyte AFB1 in the spike solutions the following simplified model equation was used:

$$w_{s,i} = w_{c,i} \times \overline{R} \times \frac{m_{c,i}}{m_{ISTD,CB}} \times \frac{m_{ISTD,SB}}{m_{smp,i}} \qquad \textbf{Equation 5.}$$

with

$w_{s,i}$ = mass fraction of analyte in test portion

$w_{c,i}$ = mass fraction of analyte in reference solution

$m_{c,i}$ = mass of reference solution added to CB

$m_{ISTD,CB}$ = mass of the spike added to CB

$m_{ISTD,SB}$ = mass of the spike added to SB

$m_{smp,i}$ = mass of test portion

$\overline{R}$ = Mean ion ratio SB over CB

The combined uncertainty of $w_{s,i}$ is then given by:

$$\left(\frac{u(w_{s,i})}{w_{s,i}}\right)^2 = \left(\frac{u(w_{c,i})}{w_{c,i}}\right)^2 + \left(\frac{u(m_{c,i})}{m_{c,i}}\right)^2 + \left(\frac{u(m_{ISTD,SB})}{m_{ISTD,SB}}\right)^2 + \left(\frac{u(m_{ISTD,CB})}{m_{ISTD,CB}}\right)^2 + \left(\frac{u(m_{smp,i})}{m_{smp,i}}\right)^2 + \left(\frac{u(\overline{R})}{\overline{R}}\right)^2$$

Equation 6.

The assigned value x_a was calculated as the average of all $w_{s,i}$ of the six preparations per test material:

$$x_a = \overline{w}_{s,i} \times F_{BS}$$

Equation 7.

The combined uncertainty of x_a is then given by:

$$u(x_a) = x_a \times \sqrt{\frac{\sum u^2(w_{s,i})}{nx_a^2} + \frac{u^2(F_{BS})}{x_a^2}}$$

Equation 8.

In Equation 7 the term F_{BS} has a value of 1 and accounts for the uncertainties due to the between-samples variability.

13.9.3. Results

Table 35 lists the assigned values and their uncertainties for the three materials and Figures 11, 12, and 13 depict the distribution of the six measurements per test material. The individual values for the six measurements of each test material are listed in Tables 36, 37, and 38.

Material	Assigned value x_a [µg/kg]	Expanded uncertainty u(x_a) [µg/kg]	Relative expanded uncertainty [%]	Coverage factor
Baby Food	0.197	0.017	8.9	2
Maize	3.1	0.14	4.6	2
Animal Feed	9.9	0.66	6.7	2

Table 35: Assigned values and their associated uncertainties for the three materials

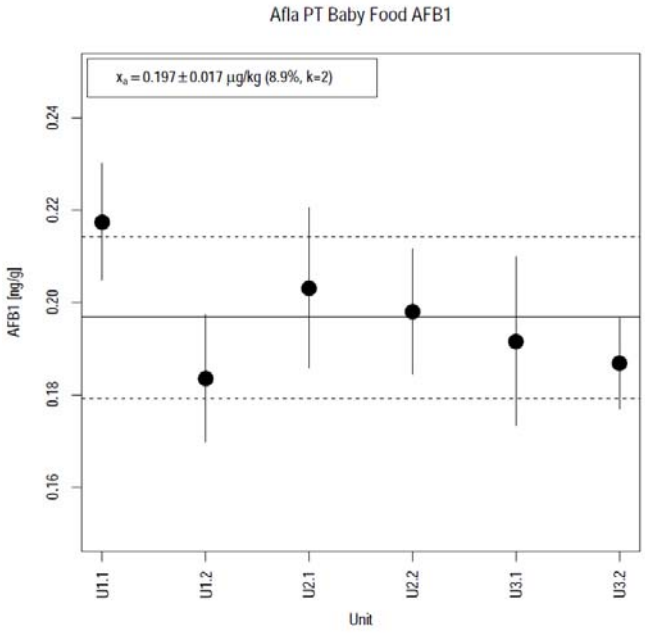


Figure 11: Distribution of the six measurements of Baby Food; the solid circles depict the measured value, the vertical lines the associated expanded uncertainties; the solid line depicts the assigned value and the broken lines the expanded uncertainty range.

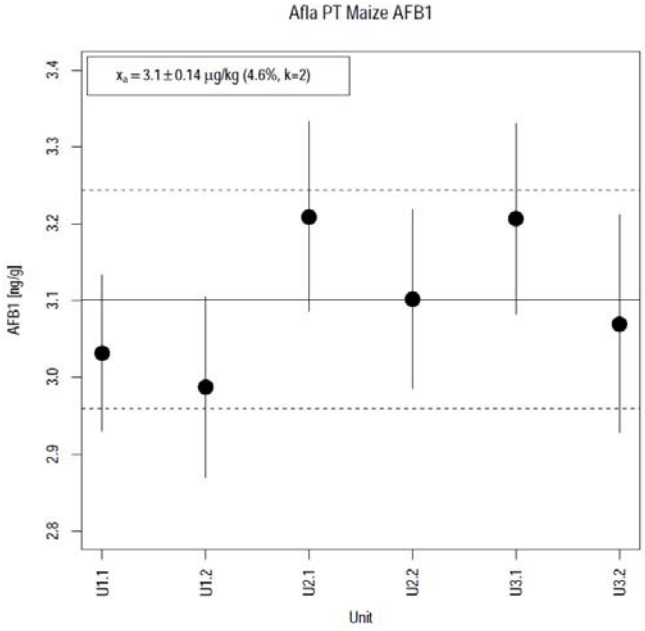


Figure 12: Distribution of the six measurements of Maize; the solid circles depict the measured value, the vertical lines the associated expanded uncertainties; the solid line depicts the assigned value and the broken lines the expanded uncertainty range.

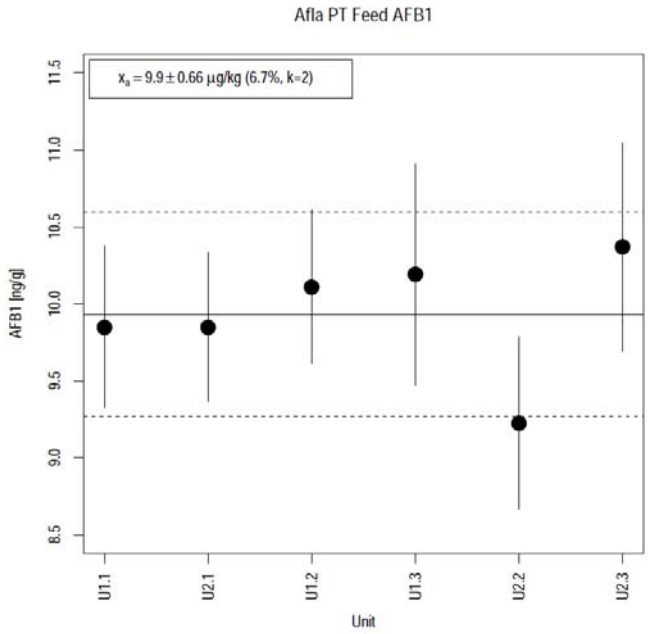


Figure 13: Distribution of the six measurements of Animal Feed; the solid circles depict the measured value, the vertical lines the associated expanded uncertainties; the solid line depicts the assigned value and the broken lines the expanded uncertainty range.

Test Unit	**Test Portion**	$w_{c,i}$ [ng/g]	$u(w_{c,i})$ [ng/g]	$m_{c,i}$ [g]	$u(m_{c,i})$ [g]	$\overline{R}$	$u(\overline{R})$	$m_{ISTD,SB}$ [g]	$u(m_{ISTD,SB})$ [g]	$m_{ISTD,CB}$ [g]	$u(m_{ISTD,CB})$ [g]	$m_{smp,i}$ [g]	$u(m_{smp,i})$ [g]
1	1	2.09090	0.03034	0.22059	0.00007	0.93662	0.02350	0.23302	0.00007	0.23065	0.00007	2.00594	0.00001
1	2	2.09090	0.03034	0.22059	0.00007	0.78196	0.02724	0.23476	0.00007	0.23065	0.00007	1.99938	0.00001
2	1	2.09090	0.03034	0.20491	0.00007	0.95870	0.03851	0.22988	0.00007	0.23246	0.00007	1.99918	0.00001
2	2	2.09090	0.03034	0.20491	0.00007	0.93204	0.02899	0.23057	0.00007	0.23246	0.00007	1.99918	0.00001
3	1	2.09090	0.03034	0.20354	0.00007	0.91353	0.04178	0.23108	0.00007	0.23358	0.00007	2.00759	0.00001
3	2	2.09090	0.03034	0.20354	0.00007	0.89419	0.01981	0.23004	0.00007	0.23358	0.00007	2.00557	0.00001

Table 36: Individual values for the six measurements of the Baby Food material; each column represents one term of either Equation 5 or Equation 6.

Test Unit	Test Portion	$w_{c,i}$ [ng/g]	$u(w_{c,i})$ [ng/g]	$m_{c,i}$ [g]	$u(m_{c,i})$ [g]	$\overline{R}$	$u(\overline{R})$	$m_{ISTD,SB}$ [g]	$u(m_{ISTD,SB})$ [g]	$m_{ISTD,CB}$ [g]	$u(m_{ISTD,CB})$ [g]	$m_{smp,i}$ [g]	$u(m_{smp,i})$ [g]
1	1	34.68843	0.50340	0.17862	0.00005	1.00752	0.00832	0.18688	0.00005	0.19160	0.00005	2.00837	0.00001
1	2	34.68843	0.50340	0.17862	0.00005	0.98658	0.01322	0.18817	0.00005	0.19160	0.00005	2.00943	0.00001
2	1	34.68843	0.50340	0.17862	0.00005	1.07440	0.01343	0.18564	0.00005	0.19160	0.00005	2.00966	0.00001
2	2	34.68843	0.50340	0.17929	0.00005	1.01424	0.01197	0.18736	0.00005	0.18962	0.00005	2.00936	0.00001
3	1	34.68843	0.50340	0.17929	0.00005	1.04011	0.01328	0.18790	0.00005	0.18962	0.00005	1.99895	0.00001
3	2	34.68843	0.50340	0.17929	0.00005	0.98913	0.01781	0.18922	0.00005	0.18962	0.00005	1.99959	0.00001

Table 37: Individual values for the six measurements of the Maize Powder material; each column represents one term of either Equation 5 or Equation 6.

Test Unit	Test Portion	$w_{c,i}$ [ng/g]	$u(w_{c,i})$ [ng/g]	$m_{c,i}$ [g]	$u(m_{c,i})$ [g]	$\overline{R}$	$u(\overline{R})$	$m_{ISTD,SB}$ [g]	$u(m_{ISTD,SB})$ [g]	$m_{ISTD,CB}$ [g]	$u(m_{ISTD,CB})$ [g]	$m_{smp,i}$ [g]	$u(m_{smp,i})$ [g]
1	1	354.72715	5.14779	0.05553	0.00002	1.02124	0.02272	0.03125	0.00002	0.03158	0.00002	2.02067	0.00002
1	2	354.72715	5.14779	0.05567	0.00002	1.04894	0.02086	0.03001	0.00002	0.03045	0.00002	2.01887	0.00002
1	3	354.72715	5.14779	0.05567	0.00002	1.06132	0.03409	0.02971	0.00002	0.03045	0.00002	2.00618	0.00002
2	1	354.72715	5.14779	0.05553	0.00002	1.02736	0.02038	0.03106	0.00002	0.03158	0.00002	2.02034	0.00002
2	2	354.72715	5.14779	0.05567	0.00002	0.98491	0.02631	0.02892	0.00002	0.03045	0.00002	2.00200	0.00002
2	3	354.72715	5.14779	0.05567	0.00002	1.06035	0.03104	0.03017	0.00002	0.03045	0.00002	2.00059	0.00002

Table 38: Individual values for the six measurements of the Animal Feed material; each column represents one term of either Equation 5 or Equation 6.

European Commission

EUR 25196 EN – Joint Research Centre – Institute for Reference Materials and Measurements
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Abstract
This report presents the results of a proficiency test of the EU-RL for Mycotoxins which focused on the determination of aflatoxin B1 in food and feed samples.

Sixty nine participants from 28 countries registered for the exercise. Sixty-one sets of results were reported for the solution, 58 for the baby food, 67 for the maize powder and 62 for the animal feed. One laboratory did not report any results.

In total about 90% of the attributed z scores were below an absolute value of two, which indicated that most of the participants performed satisfactory or better.

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