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2014 Proficiency Test of the European Union Reference Laboratory for Mycotoxins for the Network of National Reference Laboratories

Determination of Zearalenone in Maize Oil

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Abstract

This report presents the results of the PT of the EURL for Mycotoxins which focused on the determination of zearalenone in maize oil.

Forty-eight participants from thirty countries (among them 32 NRLs, 2 Non-EU Reference Laboratories and 13 official food control laboratories) registered for the exercise and 46 sets (Sample A and B) of results were reported.

Only z-scores were used for the evaluation whether an individual laboratory underperformed. In total, 87 % of the attributed z scores were below an absolute value of two, which indicates that most of the participants performed satisfactorily.

Report on the 2014 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

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Project ID: MYCO-PT-2014-ZON
PT coordinator: Katy Kroeger-Negoita

September 2014

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1 Executive 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 (EURL) for Mycotoxins. One of its core tasks is to organise proficiency tests (PT) among appointed National Reference Laboratories (NRLs).

This report presents the results of the PT on the determination of zearalenone in maize oil. Zearalenone is a mycotoxin produced by *Fusarium* species. The main source of dietary exposure to zearalenone is wheat, rye, oats, maize and products thereof. It has been shown that zeralenone has an influence on the reproductive system and causes genotoxic, immunotoxic, hepatotoxic and haematotoxic effects. Therefore, EU legislation sets a maximum limit of 400 µg/kg zearalenone in refined maize oil.

The test items for this PT were two contaminated maize oil samples. These materials were produced by the IRMM and dispatched to the participants in May 2014. Each participant received one bottle per test material containing approximately 45 g each.

Forty-eight participants from thirty countries (among them 32 NRLs, 2 Non-EU Reference Laboratories and 13 official food control laboratories) registered for the exercise and 46 sets (Sample A and B) of results were reported.

The assigned values were 437 (Sample A) and 514 µg/kg (Sample B) for zearalenone established by an exact-matching double isotope dilution mass spectrometric technique used by the EURL Mycotoxins. The expanded measurement uncertainties of the assigned values were 26 and 31 µg/kg, respectively.

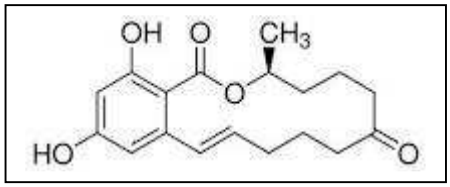
Participants' results were rated with z-scores and zeta-scores in accordance with ISO 13528:2005 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the PT, whereas the zeta-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value.

Only z-scores were used for the evaluation whether an individual laboratory underperformed. In total, 87 % of the attributed z-scores were below an absolute value of two, which indicates that most of the participants performed satisfactorily. The few participants that had z-scores above an absolute value of two will have to investigate the reasons for the deviation (root-cause analysis) and report the planned corrective actions to the EURL.

2 Introduction

Zearalenone [**Figure 1**] is a non-steroidal oestrogenic mycotoxin produced by several *Fusarium* species. This metabolite has been mainly found in maize but also in wheat, rye, oats, barley, sorghum, millet, rice and products thereof [1].

Figure 1: Chemical structure zearalenone



While having a relatively low acute toxicity after oral or intraperitoneal administration in rodents, zearalenone is often associated with reproductive disorders of farm animals and sometimes with hyperoestrogenic syndroms in humans. Furthermore genotoxic, immunotoxic, hepatotoxic and haematotoxic effects caused by zearalenone were observed in different studies [1].

Zearalenone has been classified as category 3 agent (evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals) by the International Agency for Research on Cancer (IARC) [2].

Commission Regulation (EC) No. 1881/2006 [3] sets a maximum level of 400 µg/kg for zearalenone in refined maize oil.

3 Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [4], one of the core duties of the EURL is to organise proficiency tests (PTs) for the benefit of staff from NRLs. The scope of this PT was to test the competence of the appointed NRLs and selected food control laboratories to determine the amount of zearalenone in maize oil.

All invited laboratories were allowed to use their method of choice.

The PT was designed and the reported data were processed according to the provisions of ISO 13528:2005 [5] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories [6].

IRMM is an ISO 17043:2010 [7] accredited PT provider , and the respective administrative and logistic procedures were adhered to in this PT.

3.1 Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed by non-disclosing the identity of participants to third-parties, transmission of data through a dedicated web-based interface and a secure databank hosted by JRC. European Commission rules on data protection were strictly followed as well.

4 Time frame

The PT was announced to the NRL network on 5th February 2014 and the planned PT was announced on the IRMM web page [8]. The exercise was opened for registration on 18th March 2014 [**Annex 1**]. The samples were dispatched to the participants on 19th and 20th May 2014 [**Annex 2**]. Reporting deadline was 1st July 2014.

5 Material

5.1 Preparation

Commercially obtained blank maize oil was fortified with zearalenone. The fortification levels were targeted to be 400 µg/kg for sample A and 500 µg/kg for sample B.

5.2 Homogeneity

The homogeneity was verified by a random selection of 10 units per test material (Sample A and B). Two independent determinations per unit were performed by a single-laboratory validated method based on solid-phase extraction (SPE) clean-up and HPLC with fluorescence detection. Homogeneity was evaluated according to ISO 13528:2005 [5].

The material proved to be adequately homogeneous. The details of the homogeneity study are listed in **Annex 5**.

5.3 Stability

The stability study was conducted following an isochronous experimental design [9]. Based on previous experience -18 °C was chosen as reference temperature at which zearalenone does not decay during sample storage. The study was carried out at 4 °C and 25 °C for 8 weeks.

Stability was evaluated according to ISO 13528:2005 [5].

The materials proved to be adequately stable at the tested temperatures for a period of 8 weeks, which covers the period between dispatch and the deadline for submission of results. The details of the study are listed in **Annex 6**.

5.4 Distribution

The test materials were dispatched in polystyrene boxes, containing freeze packs, on 19th and 20th May 2014.

Each participant received one box containing:

- One bottle with approximately 45 g of Sample A
- One bottle with approximately 45 g of Sample B
- The "Sample accompanying letter" [**Annex 2**]
- The "Materials Receipt form" [**Annex 3**]
- Password key (for the online reporting interface) and laboratory code

6 Instructions to participants

The participants received an individual password key to access the online reporting interface to report their measurement results and complete the related questionnaire.

The laboratories were asked to report the recovery corrected value of their results in µg/kg, the expanded measurement uncertainty in µg/kg, the coverage factor and the recovery in %.

A questionnaire was distributed to the participants to collect further information on the analytical methods used. A copy of the questionnaire is presented in **Annex 4**.

Participants received the information that the materials were shipped with freeze packs and that upon arrival the materials had to be stored immediately at -18 °C until the analysis was performed.

7 Reference values and their uncertainties

The assigned values were 437 (Sample A) and 514 µg/kg (Sample B) for zearalenone. The expanded measurement uncertainties (k=2) of the respective assigned values were 26 and 31 µg/kg.

Assigned values and their uncertainties for the test samples were established by "Exact-matching Double Isotope Dilution Mass Spectrometry" (IDMS) at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [10]. The details of the procedure can be found in the report of the NRL PT from 2011 [11].

8 Evaluation of results

8.1 General observations

Forty-eight participants from thirty countries (among them 32 NRLs, 2 Non-EU Reference Laboratories and 13 official food control laboratories) registered to the exercise [**Table 3**] and 46 sets of results were reported.

8.2 Scores and evaluation criteria

Individual laboratory performance was expressed in terms of z- and zeta (ζ)-scores in accordance with ISO 13528:2005 [5] and the International Harmonised Protocol [6].

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

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

where:

x_{lab} is the measurement result reported by a participant
X_{ref} is the reference value (assigned value)
u_{lab} is the standard measurement uncertainty reported by a participant
u_{ref} is the standard measurement uncertainty of the reference value
σ_p is the standard deviation for proficiency assessment (target standard deviation)

σ_p was calculated using the Horwitz equation (for analyte concentrations ≥ 120 ppb ≤ 13.8%) [12]:

$$\sigma_p = 0.02 \cdot c^{0.8495} \qquad \textbf{Equation 3}$$

where:

c = concentration of the measurand (assigned value, $X_{ref,}$) expressed as a dimensionless mass ratio, e.g. 1 ppb = 10^{-9}, 1 ppm = 10^{-6}

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

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

The zeta (ζ)-score provides an indication of whether the participant's estimate of measurement 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 both. A laboratory with an unsatisfactory |ζ|-score indicates an uncertainty which is not consistent with the laboratory's deviation from the reference value.

8.3 Laboratory results and scoring

The methodologies used for the determination of zearalenone were mainly high-performance liquid chromatography (HPLC) with fluorescence or mass selective detection systems.

Statistical evaluation of the results was performed using MS Excel®.

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528:2005 [5] by application of an MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [13].

The EURL will only require corrective actions being taken by participants that earned unsatisfactory z-scores.

Three laboratories (123, 127 and 133) did not report a value for their measurement uncertainty and therefore no zeta-score was calculated.

A summary of the statistical evaluation for each test sample is presented in **Table 1**. The results, as reported by the participants, are summarised in **Table 2** together with the z-scores and zeta-scores.

Figures 2 and 3 provide the individual laboratory values and their uncertainty as reported.

Table 1: Summary statistics for zearalenone

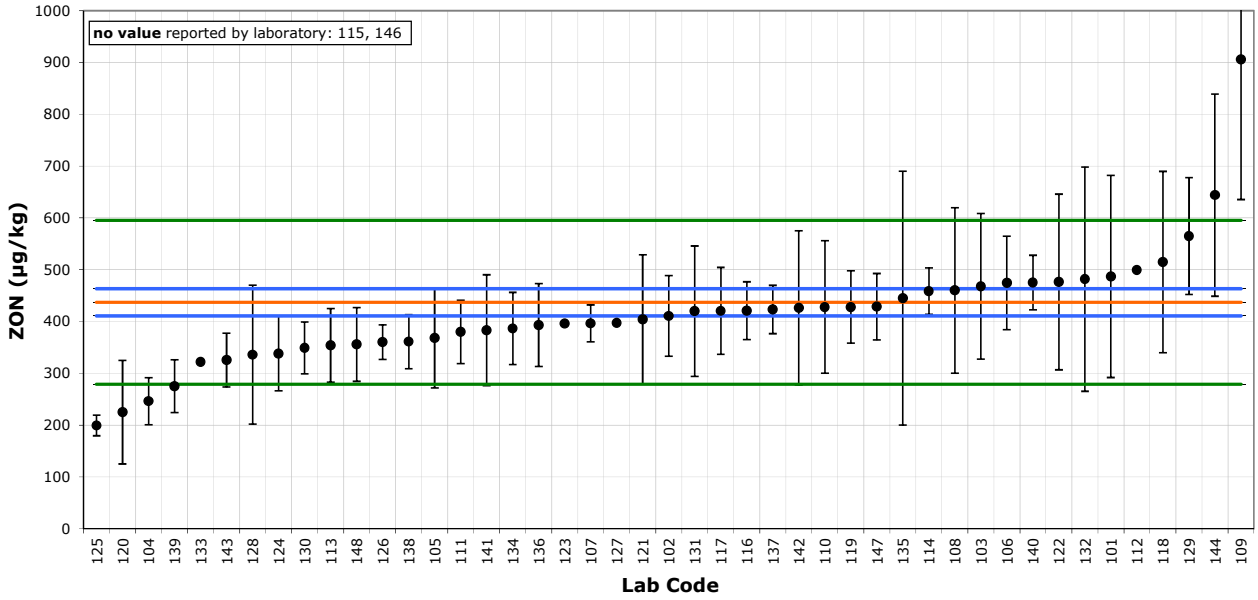
		Sample A	**Sample B**
Number of results		45	47
Range of results	µg/kg	199.1 – 905.73	232.2 – 1003.15
Median of results of participants	µg/kg	411	467
Mean of results of participants	µg/kg	416	485
Robust mean of results of participants	µg/kg	409	476
Assigned value	µg/kg	**437**	**514**
Expanded uncertainty (k=2) of the assigned value	µg/kg	26	31
Robust standard deviation ($\hat{\sigma}$)	µg/kg	73	102
Target standard deviation (fitness for purpose)	µg/kg	79	91
Number (percentage) of results of |z| > 2.0		5 (11 %)	7 (15 %)
Number (percentage) of results of |ζ| > 2.0		15 (33 %)	14 (30 %)

Table 2: Results of analysis (as reported by participants), z-scores and zeta-scores for zearalenone
(green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [µg/kg]	z-score	zeta-score	Result [µg/kg]	z-score	zeta-score
101	486.9	0.6	0.5	589.6	0.8	0.6
102	410.7	-0.3	-0.6	405.9	-1.2	-2.6
103	468	0.4	0.4	511	0.0	0.0
104	246.1	-2.4	-7.3	437.3	-0.8	-1.9
105	368	-0.9	-1.4	404	-1.2	-2.0
106	474.6	0.5	0.8	599.7	0.9	1.5
107	396.42	-0.5	-1.8	472.83	-0.5	-1.6
108	460	0.3	0.3	570	0.6	0.6
109	905.73	5.9	3.5	1003.15	5.4	3.3
110	428	-0.1	-0.1	612	1.1	1.1
111	379.95	-0.7	-1.7	619.27	1.2	2.0
112	499.5	0.8	4.8	600.5	1.0	5.6
113	354	-1.0	-2.2	428	-0.9	-1.9
114	458.60	0.3	0.8	519.94	0.1	0.3
115	No result			No result		
116	420.9	-0.2	-0.5	515.01	0.0	0.0
117	420.5	-0.2	-0.4	546.5	0.4	0.6
118	514.82	1.0	0.9	550.56	0.4	0.4
119	428	-0.1	-0.2	505	-0.1	-0.2
120	225	-2.7	-4.1	258	-2.8	-4.5
121	404	-0.4	-0.5	440	-0.8	-1.1
122	476.3	0.5	0.5	493.9	-0.2	-0.2
123	395.8	-0.5		393.7	-1.3	
124	338	-1.3	-2.6	467	-0.5	-0.9
125	199.1	-3.0	-14.5	232.2	-3.1	-14.6
126	360.24	-1.0	-3.6	460.82	-0.6	-3.1
127	397	-0.5		436	-0.9	
128	336	-1.3	-1.5	480	-0.4	-0.3
129	565	1.6	2.2	298	-2.4	-6.4
130	349	-1.1	-3.1	391	-1.4	-4.2
131	420	-0.2	-0.3	500	-0.2	-0.2
132	481.8	0.6	0.4	762.1	2.7	1.4
133	322	-1.5		347	-1.8	
134	386.5	-0.6	-1.4	491	-0.3	-0.5
135	445	0.1	0.1	507	-0.1	0.0
136	393	-0.6	-1.0	450	-0.7	-1.3
137	423.16	-0.2	-0.5	529.66	0.2	0.5
138	361	-1.0	-2.6	444	-0.8	-2.0
139	275	-2.0	-5.7	328	-2.0	-5.6
140	475.2	0.5	1.3	576.7	0.7	3.1
141	383	-0.7	-1.0	455	-0.6	-0.9
142	426.2	-0.1	-0.1	443.7	-0.8	-0.9
143	325.4	-1.4	-3.8	414.4	-1.1	-2.7
144	644	2.6	2.1	726	2.3	1.9
145	No result			316	-2.2	-6.7
145	No result			409	-1.2	-3.6
146	No result			No result		
147	428.5	-0.1	-0.2	460.1	-0.6	-1.4
148	355.8	-1.0	-2.1	392.6	-1.3	-2.9

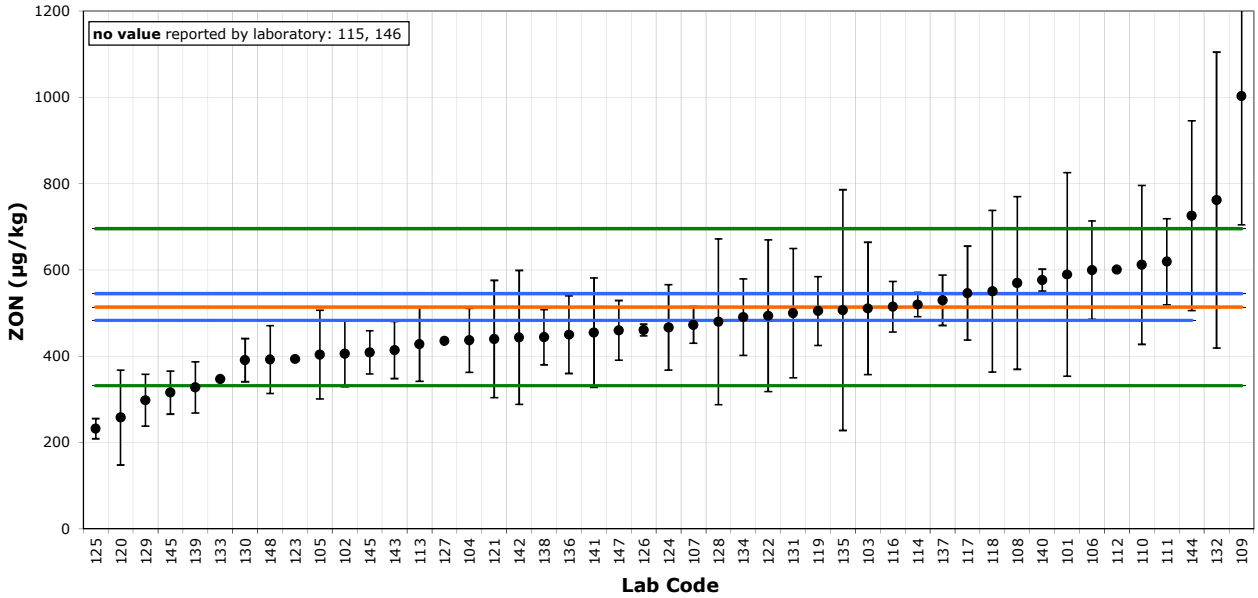
The results are written as reported by the laboratories.

Figure 2: EU-RL Mycotoxins PT 2014: Zearalenone in maize oil - Sample A
Certified value: Xref = 437 µg/kg; Uref = 26 µg/kg (k=2); σ = 79 µg/kg



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

Figure 3: EU-RL Mycotoxins PT 2014: Zearalenone in maize oil - Sample B
Certified value: Xref = 514 µg/kg; Uref = 31 µg/kg (k=2); σ = 91 µg/kg



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

8.4 Evaluation of the questionnaire

All 46 laboratories that reported results supplied the filled in questionnaire. The summary of the answers are presented in **Annex 7**.

The main techniques used to determine zearalenone were HPLC-FLD (59 %) and LC-MS (33 %). The remaining four laboratories indicated ELISA as their method of choice. The limit of detection was for the majority of the methods 10 µg/kg or even below and the limit of quantification between 10 to 30 µg/kg. Fifty-two percent of the laboratories were accredited for the determination of zearalenone.

Most of the laboratories analyse 20 to 200 samples per year. The main matrices are cereals and cereal-based products for human or animal consumption. Three participants mentioned maize or edible oil specifically as matrix.

For recovery estimation the majority of the participants added zearalenone standard solution to a blank sample.

Details about the applied methodology – extraction, clean up, overnight stop, etc. - are presented in **Annex 7**.

Two participants had comments related to the provided instructions. The other 44 participants found the instructions adequate.

9 Conclusions

This was the first EURL/NRL PT conducted for the determination of zearalenone in maize oil and most of the participants (87 %) earned satisfactory z-scores.

In line with observations of previous PTs organised by the EURL for Mycotoxins, zeta-scores were not as satisfactory as the z-scores, which indicate that the respective participants should review their uncertainty estimation.

Acknowledgements

The organizers of the study would like to thank Franz Ulberth and Beatriz de la Calle for their support.

The laboratories participating in this exercise, listed in **Table 3**, are also kindly acknowledged.

Table 3: Participating laboratories

Organisation	Country
AGES GmbH	AUSTRIA
LVA GmbH	AUSTRIA
CODA-CERVA	BELGIUM
OLEOTEST NV	BELGIUM
Fytolab cvba	BELGIUM
Laboratory of SGS Bulgaria	BULGARIA
Bulgarian Food Safety Agency	BULGARIA
Institute of Public Health Dr. Andrija Štampar	CROATIA
Department of Agriculture	CYPRUS
State General Laboratory	CYPRUS
Czech Agriculture and Food Inspection Authority (CAFIA)	CZECH REPUBLIC
Central Institute for Supervising and Testing in Agriculture (UKZUZ)	CZECH REPUBLIC
National Food Institute	DENMARK
Danish Veterinary and Food Administration	DENMARK
Agricultural Research Centre	ESTONIA
Finnish Customs Laboratory	FINLAND
Laboratoire SCL-35	FRANCE
Federal Inst. for Risk Assessment	GERMANY
General Chemical State Laboratory	GREECE
National Food Chain Safety Office, Food And Feed Safety Directorate	HUNGARY
Public Analyst's Laboratory	IRELAND
Istituto Superiore di Sanità	ITALY
Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
Laboratoire national de santé	LUXEMBOURG
Public Health Laboratory	MALTA
RIKILT	NETHERLANDS
NVWA - Netherlands Food and Consumer Product Safety Authority	NETHERLANDS
National Institute of Public Health - National Institute of Hygiene	POLAND
ASAE - LFQ	PORTUGAL
Service Commun Des Laboratoires	REUNION
Sanitary Veterinary and Food Safety Laboratory Brasov	ROMANIA
Sanitary Veterinary and Food Safety Laboratory Bucharest	ROMANIA
Sanitary Veterinary and Food Safety Laboratory Constanta	ROMANIA
Sanitary Veterinary and Food Safety Laboratory Dolj	ROMANIA
Sanitary Veterinary and Food Safety Laboratory Galati	ROMANIA
University of Novi Sad, Faculty of Technology	SERBIA
Health Sciences Authority	SINGAPORE
State Veterinary and Food Institute, Veterinary and food institute in Košice	SLOVAKIA
University of Ljubljana, Veterinary Faculty, National Veterinary Institute	SLOVENIA
National Laboratory of Health, Environment and Food	SLOVENIA
National Center for Food	SPAIN
National Food Agency	SWEDEN
Food & Environment Research Agency	UNITED KINGDOM
Staffordshire County Council	UNITED KINGDOM
The City of Edinburgh Council	UNITED KINGDOM
Kent County Council	UNITED KINGDOM

Abbreviations

AMC	Analytical Methods Committee
EC	European Commission
ELISA	Enzyme-Linked Immunosorbant Assay
EU	European Union
EURL	European Union Reference Laboratory
FLD	Fluorescence Detection
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IDMS	Isotope Dilution Mass Spectrometry
ILC	Interlaboratory Comparison
IRMM	Institute for Reference Materials and Measurements
ISO	International Organisation for Standardisation
JRC	Joint Research Centre
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MS	Mass Spectrometry
NRL	National Reference Laboratory
PT	Proficiency Test
SPE	Solid-Phase Extraction

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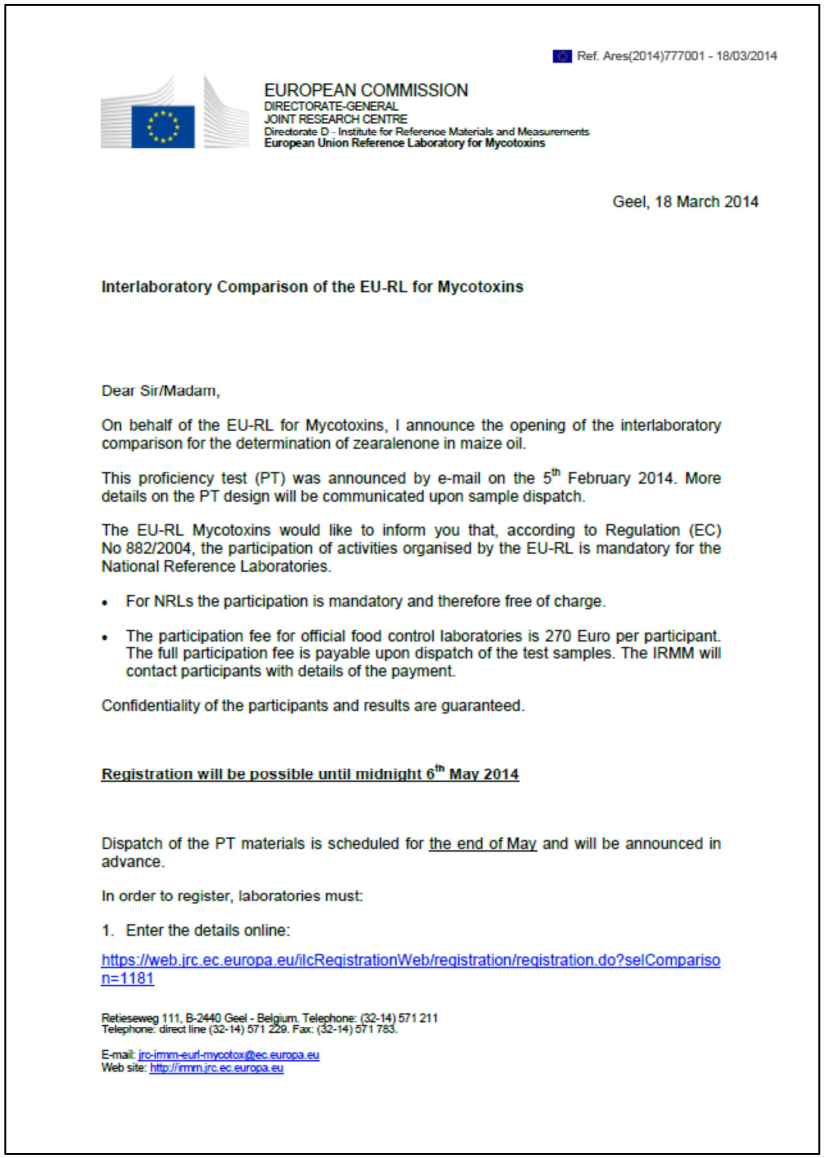
Annexes

Annex 1: Announcement letter – Opening of registration



Ref. Ares(2014)777001 - 18/03/2014

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

Geel, 18 March 2014

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Sir/Madam,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of zearalenone in maize oil.

This proficiency test (PT) was announced by e-mail on the 5th February 2014. More details on the PT design will be communicated upon sample dispatch.

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

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

Confidentiality of the participants and results are guaranteed.

Registration will be possible until midnight 6th May 2014

Dispatch of the PT materials is scheduled for the end of May and will be announced in advance.

In order to register, laboratories must:

1. Enter the details online:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1181

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

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

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 PT coordinator of the EU-RL Mycotoxins indicated below:

Katy Kroeger-Negoita

Tel: +32 14 571 523
Fax: +32 14 571 783

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

Deadline for reporting will be the 1st July 2014. 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. Please do not hesitate to contact us if you require further clarification.

Please contact us at the e-mail address:

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

With kind regards,

Katy Kroeger-Negoita

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

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

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

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

Annex 2: Sample accompanying letter

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

Ref. Ares(2014)1537382 - 14/05/2014

Geel, 19 May 2014

2014 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on the determination of zearalenone in maize oil

Dear Participant,

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

The 2014 PT aims to assess the zearalenone content in two contaminated test samples (marked as "Sample A", "Sample B"). You will be asked to report the **recovery corrected value** (µg/kg), together with your **recovery** (%) and **measurement uncertainty** (µg/kg) for a coverage factor of 2 (k=2).

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

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

Please report all requested results and answer the questionnaire at:
https://web.jrc.ec.europa.eu/ilcReportingWeb

The password key for this interface is included in the parcel with the test materials. When you enter the code please pay attention to the capital letters!

Print out the final pdf and return the signed and stamped report sheet NOT later than 1st July 2014 to:

Katy Kroeger-Negoita

Tel: +32-14-571 523
FAX: +32-14-571 783
E-mail: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

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

With kind regards,

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

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

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

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

Annex 3: Materials receipt form

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

Geel, 19 May 2014

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Member State:

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

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

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

Contents of the parcel:

a) 2 test materials for analysis:
- Sample A
- Sample B

b) 1 envelope with documents:
- Material receipt form
- Copy of instructions
- Password key and laboratory code

Signature / Stamp:

Please fax or e-mail the completed form to:

Katy Kroeger-Negoita

Tel: +32-14-571 523
FAX: +32-14-571 783

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

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

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

Annex 4: Questionnaire

Milc questionnaire

Comparison for PT 2014 Zearalenone

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

Submission Form

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

2. How many samples does your laboratory approximately analyse for Zearalenone per year? *

3. Please specify the reference (e.g. modified EN 15850) of the analytical method used. *

3.1. Is your method accredited? *

- a) Yes
- b) No

4. Proficiency test samples:

4.1. Please indicate the LOD for Zearalenone of the method used [µg/kg]. *

4.2. Please indicate the LOQ for Zearalenone of the method used [µg/kg]. *

5. What is your main procedure for recovery estimation? *

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

5.1. If you used an Internal Standard, please specify.

5.2. If other, please specify. *

6. Please specify the source of the standard used for calibration. *

7. Please indicate the sample amount (in g) for extraction. *

8. What was the extraction solvent used? *

9. What was the extraction solvent to sample ratio used during extraction (in ml/g)? *

10. What was the extraction mode (e.g. shaking)? *

11. What was the extraction time? *

12. What kind of sample clean-up did you apply? *

- a) Solid phase extraction (SPE)
- b) Immunoaffinity column (IAC)
- c) None
- d) Other

12.1. If you used solid phase extraction, please specify the sorbent (e.g. silica), the form factor (e.g. 500mg/3ml) and the brand of the SPE column. *

12.2. If you used an immunoaffinity column, please specify the brand of the IAC. *

12.3. If other, please specify. *

13. For methods with MS detection only:

13.1. Did you use a stable isotope labelled internal standard?

- a) Yes
- b) No

13.1.1. If Yes, was the internal standard added:

- ◯ a) before extraction
- ◯ b) after extraction

13.2. Please state the injection volume (in µl).

13.3. What was the mass fraction of test portion in the injection solution (e.g. 0.16 g test portion per 1 ml injection solution)?

13.4. Please state the ionisation mode and transitions used for the analysis.

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

- ◯ a) Yes
- ◯ b) No

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

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

- ◯ a) Yes
- ◯ b) No

15.1. If Yes, what were the specific problems and to which sample do they apply? *

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

- ◯ a) Yes
- ◯ b) No

16.1. If No, which parts do you think can be improved? *

17. **Are there any other comments you wish to make?**

Annex 5: Homogeneity study

Homogeneity study - Sample A

Bottle	Zearalenone [µg/kg]	
A 11	346	353
A 19	348	342
A 23	346	343
A 40	355	287
A 42	335	347
A 47	352	348
A 61	353	350
A 90	330	337
A 96	346	337
A 111	333	277
Homogeneity according to ISO 13528:2005 [9]		**[µg/kg]**
Mean		338.3
$\hat{\sigma}$		60.9 (18 %)
0.3 $\hat{\sigma}$ (critical value)		18.3
S_X (standard deviation of sample averages)		14.8
S_W (within-sample standard deviation)		20.2
S_S (between-sample standard deviation)		3.9
$S_S < 0.3\ \hat{\sigma}$		**Passed**

Homogeneity study - Sample B

Bottle	Zearalenone [µg/kg]	
B 23	459	433
B 38	453	459
B 71	468	455
B 87	468	460
B 96	461	466
B 101	454	442
B 104	457	469
B 107	466	450
B 116	431	431
B 117	465	459
Homogeneity according to ISO 13528:2005 [9]		**[µg/kg]**
Mean		455.3
$\hat{\sigma}$		82.0 (18 %)
0.3 $\hat{\sigma}$ (critical value)		24.6
S_X (standard deviation of sample averages)		10.7
S_W (within-sample standard deviation)		8.8
S_S (between-sample standard deviation)		8.7
$S_S < 0.3\ \hat{\sigma}$		**Passed**

Annex 6: Stability study

Stability study – Sample A

Date	Time	- 18 °C (Reference)		4 °C		25 °C	
17/03/2014	3 days			348	341	370	345
09/04/2014	4 weeks			359	359	377	346
06/05/2014	8 weeks	359	361	366	349	355	346
Slope of linear regression significantly <> 0 (95 %)				**No**		**No**	

Stability study – Sample B

Date	Time	- 18 °C (Reference)		4 °C		25 °C	
17/03/2014	3 days			452	452	471	449
09/04/2014	4 weeks			453	442	466	465
06/05/2014	8 weeks	456	477	437	448	449	458
Slope of linear regression significantly <> 0 (95 %)				**No**		**No**	

Annex 7: Experimental details

Lab Code	Technique	Sample A			Sample B			Coverage factor	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Recovery [%]	Result [µg/kg]	Uncertainty [µg/kg]	Recovery [%]			
101	LC-MS/MS	486.9	195	85	589.6	236	85	2	10	25
102	HPLC-FLD	410.7	78	91.9	405.9	77	105.4	2	10	20
103	HPLC-FLD	468	140.4	90	511	153.3	90	2	10	20
104		246.1	45.4	89.3	437.3	74.1	78.3	2	2.5	10
105	LC-MS	368	96	86	404	103	86	2	2	10
106	HPLC-FLD	474.6	90.2	96	599.7	113.9	92	2	0.6	1.8
107	HPLC-FLD	396.42	35.757	103.36	472.83	42.649	103.36	2	12.5	25.0
108	LC-MS	460	160	92	570	200	92	2	20	50
109	UHPLC-MS/MS	905.73	270.27	53.44	1003.15	298.34	53.44	2	0.059	0.2
110	LC-MS	428	128	119	612	184	119	2	Never analysed ZON in oil, therefore no value for LOD and LOQ	
111	Elisa method.	379.95	61.09	89	619.27	99.58	89	2	1.867	3.733
112	HPLC-FLD	499.5	0.166	96.6	600.5	0.166	96.6	2	5	20
113	LC-MS	354	71	94	428	86	94	2	0.5	1.5
114	HPLC-FLD	458.60	44.94	87.3	519.94	28.08	87.3	2	1.52	47.74
115		No result			No result					
116	HPLC-FLD and LC-MS/MS	420.9	55.7	100	515.01	58.6	100	2	HPLC/FLD: 11.3	HPLC/FLD: 34
117	ELISA	420.5	84.1	89	546.5	109	90	2	1.85	2.32
118	HPLC-FLD	514.82	175.04	98.80	550.56	187.19	98.80	2	6	19
119	HPLC-FLD	428	70	93	505	80	93	2	5	10
120	HPLC-FLD	225	100	91	258	110	91	2	25	50
121	LC-MSMS(QQQ)	404	125	96	440	136	96	2	5	10
122	HPLC-FLD	476.3	169.6	80.2	493.9	175.8	90.7	2	10	50
123	LC-MS	395.8			393.7				10	20
124	HPLC-FLD	338	72	68	467	99	68	2	10	21
125	HPLC-FLD	199.1	19.9	114	232.2	23.2	117	2	10	20
126	HPLC-FLD	360.24	33.5	108	460.82	13.6	108	2	2.5	8
127	HPLC-FLD	397		70	436		70		-	-
128	HPLC-FLD	336	134	78.1	480	192	78.1	2	10	30
129	LC-MS	565	113	85	298	60	85	2	5	10
130	LC-MS	349	50	100	391	50	100	2	5	40
131	HPLC-FLD	420	126	91	500	150	91	2	10	30
132	HPLC-FLD	481.8	216.6	85	762.1	343	85	2	3	10
133	ELISA	322			347				17	50
134	HPLC-FLD	386.5	69.83	45	491	88.58	45	2	12	24
135	HPLC-FLD	445	245	110.1	507	279	110.1	2	2	4
136	HPLC-FLD	393	80	79.5	450	90	79.5	2	20	50
137	HPLC-FLD	423.16	46.55	91.62	529.66	58.35	91.62	2	3.13	10.43
138	LC-MS	361	52	99	444	64	99	2	5.8	19
139	HPLC-FLD	275	51	99.6	328	59	99.6	2	8	24

Lab Code	Technique	Sample A			Sample B			Coverage factor	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Recovery [%]	Result [µg/kg]	Uncertainty [µg/kg]	Recovery [%]			
140	LC-MS	475.2	52.5	71.0	576.7	25.3	72.0	2	1.618	4.855
141	HPLC-FLD	383	107	117	455	127	117	2	10	30
142	LC-MS	426.2	149.2	103	443.7	155.3	103	2	15	15
143	ELISA	325.4	52.0	91.52	414.4	66.3	91.52	2	0.102	0.205
144	HPLC-FLD	644	195	60	726	220	60	2	0.43	5
145	HPLC-FLD	409*	50	70	316	50	70	2	2.5	5
146		No result			No result					
147	HPLC-FLD	428.5	64.3	90	460.1	69.0	90	2	4.0	12.5
148	LC-MS	355.8	71.2	86	392.6	78.5	86	2	7.0	20.0

* Note: Sample A of laboratory 145 was contaminated with 514 ± 31 µg/kg zearalenone.

Lab Code	Which matrices does your laboratory analyse for Zearalenone on a routine basis the most?	How many samples does your laboratory analyse for Zearalenone per year?	Is your method accredited?	Reference of the analytical method used
101	Cereals	<50	No	None
102	cereals	20	No	in house method
103	mixed feed, cereals, pasta	900	Yes	EN15850, EN15792
104	cereals, snacks, bread	50-60	No	in-house based on R-Biopharm Instructions
105	maize, cereals, baby food	100	Yes	internal method
106	cereals, feeds	<25	Yes	application note EASI-EXTRACT ZEARALENONE for vegetable oil - R-BIOPHARM
107	Cereals	200	Yes	VICAM, ZearalaTest HPLC DOC# VP-1017-0
108	Feed ingredients, Cereals	550	Yes	In house method
109	wheat and maize	1	No	modified QUECHERS
110	Flour (corn flour, Wheat, etc.)	0-15	No	Food and Chemical Toxicology 62 (2013) 514-520
111	Unprocessed cereals, bread and breakfast cereal	60-70 samples	Yes	Protocol Elisa method - Enzyme immunoassay for the quantitative analysis of Zearalenone
112	Cereals, cereal-based baby foods	About 60 samples/year (all matrices)	Yes	Application Note A6-RP91.Vi R-Biopharm Rhone
113	cereal products, cereal for food and for feed	about 100	No	in-house method
114	None	5	Yes	FprEN-15850
116	edible oil, feed	<10	Yes	method draft accepted by CEN: mandate m/520 Project No. 3 "Foodstuff - Determination of zearalenone in vegetable oils including refined maize oil"
117	cereals, feed, cereals intended for direct human consumption	270	Yes	RIDASCREEN Zearalenone protocol kit
118	Feedstuff, grain, flour	130	No	R-BIOPHARM instructions
119	Raw material, feed mixture, grain	200	Yes	ISO/DIS 17372
120	Cereals	50	Yes	EN 15792 modified
121	cereals+feed	3000	Yes	in-house method
122	Cereals	<50	No	Method described in Mycotox Res (2009) 25: 117 - 121
123	cereals	1300	Yes	house method
124	maize, breakfast cereals	100	No	modified 15850
125	Wheat, baby food, feed	≤1000	No	HPLC-FLD by Majerus, P., Mycotox Res (2009), 25, 117-121
126	Baby food, cereal products, muscle (fish,poultry)	about 100	Yes	modified immunoaffinity columns guideline
127	wheat and barley	5-10	No	Application Note AFFINIMIP SPE Zearalenone Edible Corn Oil
128	animal feed, corn, wheat and other cereals	20	No	NF EN 15792

Lab Code	Which matrices does your laboratory analyse for Zearalenone on a routine basis the most?	How many samples does your laboratory analyse for Zearalenone per year?	Is your method accredited?	Reference of the analytical method used
129	Wheat, Corn	500	No	Inhouse Method
130	Cereals, Maize, nuts	2000	Yes	in house method
131	cereals, flours	40	Yes	modified EN 15850
132	Cereals	300	Yes	EN15850:2010 (cereals), Mycotox Res (2009) 25:117-121 (oils)
133	Cereals	~25	No	ELISA
134	cereals, breakfast cereals	200 samples analysed by ELISA	No	Revue Ecole Nationale Veterinaire 2007, 158, 10, 504-508; immunoaffinity column-application note
135	Cereals (wheat and oats)	50	No	For these oil samples: Gimenez et al. Food Control 34 (2013) 268-273
136	Feed	50	Yes	R-Biopharm Rhône, Instructions for Use
137	cereals, corn oil, bread	150	No	EN 15850:2010
138	Cereals and cereal products	50	No	Food Additives and Contaminants, 2008, 25(4), 472-489, Modified
139	Cereals and cereals products	30	No	internal Method
140	Cereals and cereal products	15	No	Analysis of Mycotoxins in Various Cattle Forages and Good Matrices with the TSQ Quantum Discovery Max, Thermo Scientific, Application Note 377
141	Animal feed	20	No	Method from immunoaffinity supplier
142	cereals, cereals products, feeding stuff	50 - 100	Yes	EN 15850
143	Corn, Flour, Corn oil	30-50	No	ELISA KIT Protocol
144	Animal Feeds (Feed Materials) and cereal based compound foods	50-100	Yes	ISO/CD 17372
145	flour, breakfast cereals, bread	50	Yes	rhone biopharm application note
147	cereals, maize, flour	50	Yes	EN 15850
148	cereals, comlepe feed, fodder	100 - 150	Yes	modified QuEChERS

Lab Code	What is your main procedure for recovery estimation?	Source of the standard used for calibration
101	Other (Blank matrix spiked with known amount component, internal standard (U-[13C18]-Zearalenone) is added after sample extraction is completed)	Romerlabs (Biopure)
102	Other (spiked test sample to test sample)	Romer Labs Diagnostic GmbH
103	Standard solution to Blank Sample	Romer Labs
104	Standard solution to Blank Sample	Coring Diagnostics
105	Standard solution to Blank Sample	Sigma Aldrich
106	Standard solution to Blank Sample	Sigma Aldrich
107	Standard solution to Blank Sample	Sigma
108	Standard solution to Blank Sample	Sigma-Aldrich
109	Other (fortification of PT material)	FLUKA 34126-2mL 100 ug/mL
110	Standard solution to Blank Sample	External standard curve
111	Other (use a CRM)	-
112	Standard solution to Blank Sample	Biopure BRM 002029 Lot# C133244
113	Other (spiking non-contaminated sample)	SIGMA
114	Standard solution to Blank Sample	SIGMA
116	Standard solution to Blank Sample	Fluka SZBA 127 XV
117	Standard solution to Blank Sample	LGC Standards
118	Standard solution to Blank Sample	SUPELCO
119	Standard solution to Blank Sample (Zearalenone is added to the sample before extraction at a concentration of 350 µg/kg)	Sigma

Lab Code	What is your main procedure for recovery estimation?	Source of the standard used for calibration
120	Standard solution to Blank Sample	Sigma-Aldrich
121	Internal Standard to Sample (C13 ISTD)	Dr Ehrenstorfer
122	Internal Standard to Sample (Zearalenone Standard in Acetonitrile)	Biopure - Romer Labs Diagnostic GmbH
123	Standard solution to Blank Sample	RomerLabs
124	Standard solution to Blank Sample	Biopure
125	Standard solution to Blank Sample	BioPure
126	Standard solution to Blank Sample	SIGMA-ALDRICH
127	Standard solution to Blank Sample	5000 ppb
128	Other (Standard solution)	R-BIOPHARM
129	Internal Standard to Extract (c13 Zearalenone)	LGC standards
130	Standard solution to Blank Sample	Sigma
131	Standard solution to Blank Sample	Romerlabs
132	Standard solution to Blank Sample	Sigma
133	Other (Ref Material control)	KIT
134	Other (Standard solution to sample A)	Trilogy
135	Standard solution to Blank Sample	Makor
136	Internal Standard to Sample (/)	Biopure
137	Standard solution to Blank Sample	Sigma-Aldrich
138	Standard solution to Blank Sample	Sigma
139	Standard solution to Blank Sample	-
140	Standard solution to Blank Sample	Biopure
141	Standard solution to Blank Sample	R-Biopharm Trilogy
142	Standard solution to Blank Sample	Sigma-Aldrich
143	Standard solution to Blank Sample	NA
144	Standard solution to Blank Sample	Sigma Aldrich
145	Standard solution to Blank Sample	Romer labs
147	Standard solution to Blank Sample	LGC-Standards
148	Other (ZON standard to blank sample)	Sigma Aldrich

Lab Code	Extraction solvent	sample amount for extraction [g]	solvent to sample ratio used during extraction [ml/g]	Extraction mode	Extraction time
101	acetone/isopropanol/water/acetic acid, 15/5/14.9/0.1 (v/v/v/v, ml)	4	17.5 ml on 4 g	Shaking (overhead)	60 min
102	acetonitrile/water	5	4 for the first extraction and 2 for second extraction	homogenization	3 min twice
103	acetonitrile/water (75/25 ; v/v)	10	5	shaking	60 min
104	acetonitrile	10	11	ultraturrax	2 min
105	acetonitrile	2	5	shaking	30 min
106	100% acetonitrile	10	10	shaking	15 min
107	90% acetonitrile 10% ultra pure water	10 g in duplicates	1:25 and 1:50	Shaking	30 min
108	1 % Acetic acid in Acetonitrile	2.5	10	Quechers, shaking followed by partition with MgSO4	30 min

Lab Code	Extraction solvent	sample amount for extraction [g]	solvent to sample ratio used during extraction [ml/g]	Extraction mode	Extraction time
109	ACN/H2O (86:14, v/v)	3	6.67	shaking	60 min
110	acetonitrile: water(84:16)+ n-hexane	5	1:4	Ultraturrax homogenizer	3 min
111	Methanol 100%	10	10 ml sample with 10 ml of 100% methanol	Shaking	15 min
112	Acetonitrile	10	10	Blending using an Ultraturrax	2 min
113	acetonitrile-water-acetic acid 80-20-0.1	10	10	shaking	1 h
114	Acetonitrile / water	5	4	blending with ultra-turrax	2 min
116	Hexane + methanol: aqueous ammonium hydrogencarbonate solution (1 g NH4HCO3/100 mL) 9:1 v:v	2	10	horizontal shaking	20 min
117	Methanol 70%	5	25/5	shaking, centrifuge	15 min
118	Acetonitrile	2	20/2	high speed blending	2 min
119	Acetonitrile/water 90/10 (v/v)	20	10	Shaking	60 min
120	MeOH/Water 75/25	20	7.5	shaking	30 min
121	ACN+water+hexane	10	5.5	ultra turrax	2 min
122	Methanol / Ammonium Hydrogen Carbonate (9:1)	2	10	Shaking	20 min
123	Acetonitrile:Water (84:16)	10	20ml/10g	sonication and shaking	15 min sonication and 30 min shaking
124	AcCN:Water (84:16)	4	15	shaking	45 min (3 times 15 min)
125	Hexane, methanol and ammonium hydrogen carbonate mixture	2	20/2	Shaking	15 min
126	Acetonitrile/water 84/16 v/v	5	in 20 mL/5 g	shaking	30 min
127	diethyl ether	0.9	3:1	-	-
128	Methanol-Water (75/25)	20	7.5	shaking	1 h
129	Acetonitrile:H2O	40	0.8	Blending	2 min
130	Acetonitrile	1	20	shaking	2 min
131	Acetonitrile-water (75-25)	5	4	shaking	30 min
132	Hexane, methanol:10g/L ammonium bicarbonate (9:1, v/v)	2	22ml/2g	shaking	15 min
133	70:30 MeOH water	5	5	Shaking	30 min
134	Methanol	9.4 g (10 ml)	1:1	Shaking	30 min
135	Acetonitrile:water 84:16 + n-Hexane	5	6,5	Blending with Ultra-Turrax	3 min
136	Acetonitrile-water (75+25)	6.25	5	Shaking	30 min
137	Acetonitrile	5	25ml/5g	shaking, centrifugation	45 min
138	20% ACN	3	24 ml/3g	Vortex, centrifuge	3x1min
139	MeOH:Water (75:25)	20	150 ml / 20g	Shaking	1 h
140	acetonitrile / water (75/25)	25	5/1	blending	2 min
141	Acetonitrile	10	100/10	Blending	2 min
142	acetonitrile in water, 75%	25	4	blending	3 min
143	Methanol	5 ml	5/25	Shaking	10 min
144	methanol/water	20	250/10	shaking	60 min
145	75% acetonitrile	10	10:100	shaking	1 h
147	CH3CN/H2O	20	50/20	blending	2 min
148	0.1% HCOOH in H2O : ACN (QuEChERS)	2	5	shaking	20 min

Lab Code	Kind of sample clean-up	Details on sample clean-up	During the analysis did you need to include any over night stop?	Did you encounter any problems during the analysis?
101	Other	QuEChERS (MgSO4/NaCl)	No	No
102	Immunoaffinity column (IAC)	Vicam	No	No
103	Immunoaffinity column (IAC)	r-Biopharm (EasiExtract)	No	No
104	Immunoaffinity column (IAC)	R-Biopharm	Yes (after IAC-columns)	No
105	Other	partition with acetonitrile/water (1%HCOOH), addition of MgSO4 and NaCl	No	No
106	Immunoaffinity column (IAC)	EASI-EXTRACT ZEARALENONE - R-BIOPHARM	No	No
107	Immunoaffinity column (IAC)	Vicam	No	No
108	Other	filtration	Yes (All samples after filtration, waiting for LC-MS analysis)	No
109	Other	Dispersive SPE PSA/C18 SPE Clean up Tube 1, SUPELCO	No	No
110	Other	mycosep column 224	No	Yes (Injection volume reduced, due to the very high intensity signal samples by LC / MS)
111	None		No	No
112	Immunoaffinity column (IAC)	R-Biopharm Rhone Easi-Extract	No	No
113	None		No	No
114	Immunoaffinity column (IAC)	VICAM	No	No
116	None		No	No
117	None		No	No
118	Immunoaffinity column (IAC)	R-BIOPHARM EASI-EXTRACT RP91	Yes (Sample preparation one day and HPLC analysis the next day for all samples)	No
119	Immunoaffinity column (IAC)	EASI-EXTRCT from R Biopharm Rhone Ltd	No	Yes (Some problems with phase separation. Are resolved with centrifugation)
120	Immunoaffinity column (IAC)	VICAM Zearatest-WB	No	No
121	None		No	No
122	None		No	No
123	Other	Clean-up column MycoSep 226 AflaZON+ RomerLabs	No	No
124	Immunoaffinity column (IAC)	r biopharm	No	No
125	None		No	Yes (It was pretty difficult to detect the necessary pH (from pH 6 to ≤ 7.5))
126	Immunoaffinity column (IAC)	ROMER	No	No
127	Other	AFFINIMIP SPE Zearalenone cartridges	No	No
128	Immunoaffinity column (IAC)	R-BIOPHARM	No	No
129	Immunoaffinity column (IAC)	Vicam	No	No
130	None		No	No
131	Immunoaffinity column (IAC)	R-Biopharm Rhone Ltd	No	No
132	None		No	No
133	None		No	Yes (Standard procedure LISA - not used for oils before)
134	Immunoaffinity column (IAC)	Neogen	No	Yes (Very low recovery)
135	Other	MultiSep 226+ AflaZON	Yes (Sample preparation 1 day. HPLC-analysis 3 days later)	No
136	Immunoaffinity column (IAC)	Easi-Extract Zearalenone, R-Biopharm Rhone	No	No
137	Immunoaffinity column (IAC)	EASi-Extract Zearalenone R -Biopharm Rhone LTD	No	No
138	None		No	No

Lab Code	Kind of sample clean-up	Details on sample clean-up	During the analysis did you need to include any over night stop?	Did you encounter any problems during the analysis?
139	Immunoaffinity column (IAC)	Vicam	No	No
140	Immunoaffinity column (IAC)	Bio-Spectrum	No	No
141	Immunoaffinity column (IAC)	R-Biopharm	Yes (Spiked sample left overnight)	No
142	Immunoaffinity column (IAC)	R-Biopharm	No	No
143	None		No	No
144	None	R-Biopharme	No	No
145	Immunoaffinity column (IAC)	zearalenone easi extract	No	Yes (not efficient extraction)
147	Immunoaffinity column (IAC)	VICAM	No	No
148	None		No	No

For methods with MS detection only:					
Lab Code	**Did you use a stable isotope labelled internal standard?**	**Internal Standard added**	**Injection volume [µl]**	**Mass fraction of test portion in the injection solution**	**Ionisation mode and transitions**
101	Yes	after extraction	5	0.4 g/ml	ESI Pos, 319>283 / 319>187
104	No		50	0.276 g test portion per 1 ml injection solution	ES +: 319.1>283.3 (quantification) + 319.1>203.0 (qualification)
105	No		5	0.2g/1mL	ESI negative, 317.1445>131.0505, 317.1445>160.0165, 317.1445>175.0400
108	No		5	0.125 g/ml	negative ionisation mode; quantifier 317 -> 175, qualifier 317->131
109	No		10	0,0015 g test portion per 1 ml injection solution	Negative ionisation mode, 175.1/131.1
110	No		5	0,29 g	[M-H]- 317,1 m/z - FRGGMENTER 175,1 og 131,1 m/z (negative ionization, ESI)
113	Yes	after extraction	20	0.2 g/ml	ESI - 317 : 131 317 : 175
116	No		10	0.1 g per mL	ESI-, 317.14/130.8; 317.14/175; 317.14/272.8
121	Yes	after extraction	10	0.25	ESI+
123	Yes	after extraction	4		negative
129	Yes	after extraction	20	0.8	positive
130	No		2	0.05 g / ml	pos mode, 319.1>283.2
138	No		10	0.0625 g test portion per 1 ml injection solution	ESI, Parent 319.16m/z, Daughters 283.18 and 187.12 m/z
140	No		20	0.33	ESI, 319.0 - 184.8, 319.0 - 186.9
142	No		10	0.167	negative, quantifier m/z 317; qualifier m/z 353, 377
148	No		2.5	0.1 g/ml	ESI+, 319 > 187; 319 > 97

Lab Code	Did you find the instructions distributed for this PT adequate?	Are there any other comments you wish to make?
101	Yes	No
102	Yes	

Lab Code	Did you find the instructions distributed for this PT adequate?	Are there any other comments you wish to make?
103	Yes	
104	Yes	
105	Yes	
106	Yes	
107	Yes	Nil
108	Yes	
109	Yes	
110	Yes	
111	Yes	Thanks for all!!!!
112	Yes	Our method is accredited for cereals and cereal-based baby foods but not for maize oil
113	Yes	
114	Yes	We are very happy with your friendly system for reporting the results, rather than the system used for reporting the results of PT´s for PAH. Please, do not change it.
116	Yes	
117	Yes	
118	Yes	
119	No (In the letter from 19 may 2014 it was written, that we will be asked about "Recovery corrected Sample" together with "Recovery". The only value asked was "recovery %".)	
120	Yes	
121	Yes	
122	Yes	None
123	Yes	
124	Yes	Strangely enough, the recovery experiments performed better in lower values (100ppb>200ppb>400ppb)
125	Yes	
126	Yes	
127	Yes	
128	Yes	
129	Yes	
130	Yes	
131	Yes	Zearalenone in vegetable oils is not included in the scope of accreditation. Our laboratory had no experience of analysing vegetable oils for zearalenone.
132	Yes	
133	No (Documented procedure for HPLC assay received too late)	
134	Yes	The method used for this PT (HPLC-FLD) is not validated.
135	Yes	
136	Yes	/
137	Yes	I like to have an workshop in your laboratory.
138	Yes	
139	Yes	
140	a) Yes	
141	a) Yes	
142	a) Yes	
143	a) Yes	No
144	a) Yes	

Lab Code	Did you find the instructions distributed for this PT adequate?	Are there any other comments you wish to make?
145	a) Yes	we were not satisfy with results
147	a) Yes	no
148	a) Yes	We have analysed this type of matrice for the first time.

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