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

Determination of Patulin in Apple Juice

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Determination of Patulin in Apple Juice

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Project ID: MYCO-PT-2013-PAT
PT coordinator: Katy Kroeger-Negoita

September 2013

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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 of the EURL for Mycotoxins which focused on the determination of patulin in apple juice. Patulin is a mycotoxin produced by a number of fungi, such as brown rot in apples. The main source of dietary exposure to patulin is apple juice. It has been shown that patulin causes immunotoxic effects and is neurotoxic in animals. Therefore, EU legislation sets a maximum limit of 50 µg patulin/kg of fruit juices.

The test items for this PT were two naturally contaminated clear apple juice samples. These materials were produced by the IRMM and dispatched to the participants in April 2013. Each participant received one container per test material containing approximately 45 g each.

Fifty-one participants from 27 countries (among them 30 NRLs and 21 official food control laboratories) registered for the exercise and 50 sets (Sample A and B) of results were reported.

The assigned values were 39.1 (Sample A) and 60.5 µg/kg (Sample B) for patulin established by an exact-matching double isotope dilution mass spectrometric technique used by the EURL Mycotoxins. The uncertainties of the assigned values were 1.5 and 2.0 µg/kg, respectively.

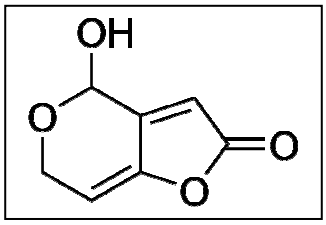
Participants' results were rated with z-scores and zeta-scores in accordance with ISO 13528:2005 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Those scores were used for evaluating the performance of the individual laboratories in the PT. The standard deviation for proficiency assessment (target standard deviation) was set to 22 % of the assigned value. To be able to calculate zeta-scores participants were invited to report the uncertainty of their measurements. This was done by the majority of laboratories.

Only z-scores were used for the evaluation whether an individual laboratory underperformed. In total, 88 % 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

Patulin [**Figure 1**] is a mycotoxin produced by several fungal species of *Penicillium*, *Aspergillus* and *Byssochlamys*. This toxic metabolite has been found in fruits, vegetables, grains and silage but the main foods contaminated with patulin are apples and their products such as apple juice [1].

Figure 1: Chemical structure of patulin



Several acute (e.g. gastrointestinal hyperaemia, distension, haemorrhage and ulceration) and chronic immunotoxic and neutrotoxic effects caused by patulin were observed in different studies [1].

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

Commission Regulation (EC) No. 1881/2006 [3] sets a maximum level of 50 µg/kg for Patulin in fruit juices, concentrated fruit juices as reconstituted and fruit nectars.

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 patulin in apple juice.

All invited laboratories were allowed to use their method of choice. The methodologies used for the determination of patulin were mainly high-performance liquid chromatography (HPLC) with ultra-violet or mass selective detection systems.

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

IRMM is an accredited PT provider according to ISO 17043:2010, and administrative and logistic procedures of ISO 17043:2010 [6] 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 12th February 2013 and the planned PT was published on the IRMM web page [7]. The exercise was opened for registration on 18th March 2013 [**Annex 1**]. The samples were dispatched to the participants on 23rd April 2013 [**Annex 2**]. Reporting deadline was 4th June 2013.

5 Material

5.1 Preparation

The test materials used for this study were a blend of naturally contaminated clear apple juice with blank clear apple juice.

Prior blending the naturally contaminated apple juice was filter-sterilised with a 0.2 µm filter. The blank and the contaminated apple juice were homogenised with a magnetic stirrer for 2 hours at room temperature. About 5 l of each of the two test materials were available for filling. A total of 115 Sample A and 113 Sample B units were produced with approximately 45 g per unit filled in 50 ml – screw-cap plastic containers and stored at -18°C until dispatch.

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 applying the method EN 15890:2010 [8]. Homogeneity was evaluated according to ISO 13528:2005 [9].

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

5.3 Stability

The stability study was conducted following an isochronous experimental design [10]. Based on previous experience -18 °C was chosen as temperature at which patulin does not decay during sample storage. The study was carried out at 4 °C for 3 days, 4 weeks and 8 weeks and at 25 °C for 3 days to mimic the worst case scenario during transport.

Stability was evaluated according to the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

The materials proved to be adequately stable for 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 dry ice, on 23rd April 2013.

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

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 on dry ice and that upon arrival the materials needed to be stored immediately at -18 °C until the analysis is performed.

7 Reference values and their uncertainties

The assigned values were 39.1 (Sample A) and 60.5 µg/kg (Sample B) for patulin. The expanded measurement uncertainties (k=2) of the respective assigned values were 1.5 and 2.0 µg/kg.

Assigned values and their uncertainties for the test samples were established by "Exact-matching Double Isotope Dilution Mass Spectrometry" at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [11]. The details of the procedure can be found in the report of the NRL PT from 2011 [12].

8 Evaluation of results

8.1 General observations

Fifty-one participants from 27 countries (among them 30 NRLs and 21 official food control laboratories) registered to the exercise [**Table 3**] and 50 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 [9] and the International Harmonised Protocol [5].

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

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

where:

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

σ_p was calculated using the Horwitz equation modified by Thompson [13] (for analyte concentrations < 120 ppb):

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

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 uncertainty is consistent with the observed deviation from the assigned value. The ζ-score is the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value, its uncertainty as well as the uncertainty of the assigned values.

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

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

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

8.3 Laboratory results and scoring

Statistical evaluation of the results was performed using MS Excel.

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528:2005 [9] by application of a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [14].

As a result z-scoring and zeta-scoring was done, but the EURL will only require corrective actions being taken by participants that earned unsatisfactory z-scores.

Two laboratories (123 and 138) did not report a value for uncertainty and therefore no zeta-score was calculated. One laboratory (150) reported a value for uncertainty, but did not report their coverage factor (k). As it was asked in the instructions to use a coverage factor of 2, the zeta-score was calculated with k=2.

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 laboratories values and their uncertainty as reported.

Table 1: Summary statistics for Patulin

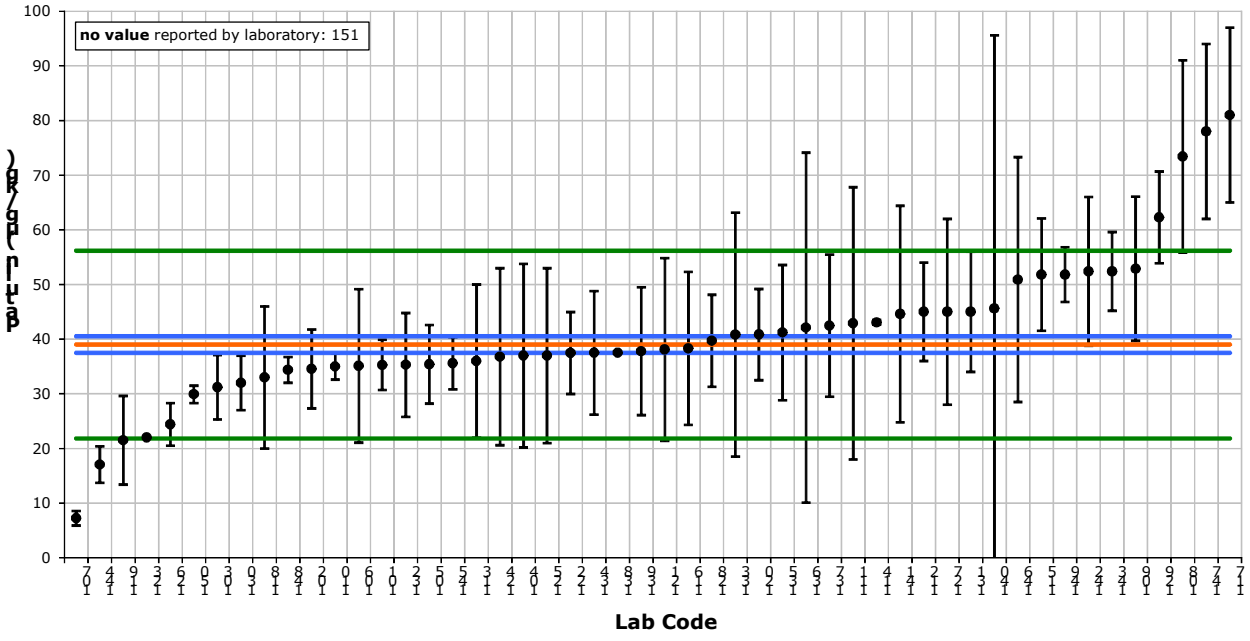
		Sample A	Sample B
Number of results		50	50
Range of results	µg/kg	7.23 - 81	15.77 - 140
Median of results of participants	µg/kg	38.0	63.7
Mean of results of participants	µg/kg	40.8	66.2
Robust mean of results of participants	µg/kg	39.9	64.8
Assigned value	µg/kg	**39.1**	**60.5**
Expanded uncertainty (k=2) of the assigned value	µg/kg	1.5	2.0
Robust standard deviation ($\hat{\sigma}$)	µg/kg	9.2	13.8
Target standard deviation (fitness for purpose)	µg/kg	8.6	13.2
Number (percentage) of results of $\|z\| > 2.0$		6 (12 %)	6 (12 %)
Number (percentage) of results of $\|\zeta\| > 2.0$		18 (36 %)	18 (36 %)

Table 2: Results of analysis, z-scores and zeta-scores for patulin
(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	35.28	-0.4	-1.5	54.93	-0.4	-1.0
102	34.55	-0.5	-1.3	59.17	-0.1	-0.1
103	31.2	-0.9	-2.6	58.9	-0.1	-0.2
104	37	-0.2	-0.2	60	0.0	0.0
105	35.4	-0.4	-1.0	82.2	1.7	2.6
106	35.1	-0.5	-0.6	70.7	0.8	0.8
107	7.23	-3.7	-31.8	15.77	-3.4	-38.4
108	73.4	4.0	3.9	69.3	0.7	1.1
109	52.9	1.6	2.1	73.3	1.0	1.5
110	35	-0.5	-2.8	55.8	-0.3	-1.3
111	42.9	0.5	0.3	73.8	1.0	0.6
112	45	0.7	1.3	84	1.8	2.8
113	36	-0.3	-0.4	54	-0.5	-0.5
114	43.06	0.5	5.2	67.93	0.6	3.0
115	51.8	1.5	2.5	74.3	1.1	1.9
116	38.3	-0.1	-0.1	67.3	0.6	0.6
117	81	4.9	5.2	140	6.1	5.7
118	33	-0.7	-0.9	54	-0.5	-0.5
119	21.5	-2.0	-4.2	49.9	-0.8	-1.3
120	40.85	0.2	0.4	95.6	2.7	3.5
121	38.1	-0.1	-0.1	74.6	1.1	0.9
122	37.47	-0.2	-0.4	63.49	0.3	0.5
123	22.005	-2.0		49.845	-0.8	
124	36.8	-0.3	-0.3	61.8	0.1	0.1
125	37	-0.2	-0.2	60	0.0	0.0
126	24.4	-1.7	-7.0	43.6	-1.2	-4.6
127	45	0.7	0.7	71	0.8	0.8
128	39.7	0.1	0.2	66.7	0.5	2.2
129	62.28	2.7	5.5	91.99	2.4	5.1
130	32	-0.8	-2.7	56	-0.3	-1.5
131	45	0.7	1.1	35	-1.9	-5.4
132	35.3	-0.4	-0.8	60.6	0.0	0.1
133	40.83	0.2	0.2	60.08	0.0	0.0
134	37.5	-0.2	-0.3	61.9	0.1	0.2
135	41.2	0.3	0.4	63.9	0.3	0.4
136	42.1	0.4	0.2	66.5	0.5	0.4
137	42.46	0.4	0.5	67.07	0.5	0.7
138	37.5	-0.2		63.1	0.2	
139	37.8	-0.1	-0.2	44.6	-1.2	-2.2
140	45.6	0.8	0.3	71.7	0.9	0.5
141	44.6	0.7	0.6	65.4	0.4	0.4
142	52.4	1.6	2.0	77.1	1.3	1.8
143	52.4	1.6	3.6	87.4	2.1	7.3
144	17.04	-2.6	-12.0	48.65	-0.9	-2.3
145	35.6	-0.4	-1.4	53.75	-0.5	-2.3
146	50.9	1.4	1.1	81.8	1.7	1.2
147	78	4.5	4.9	122	4.7	5.1
148	34.37	-0.5	-3.3	55.47	-0.3	-2.1
149	51.8	1.5	4.9	74.2	1.1	5.3
150	29.9	-1.1	-8.3	50.6	-0.7	-7.3
151	No result			No result		

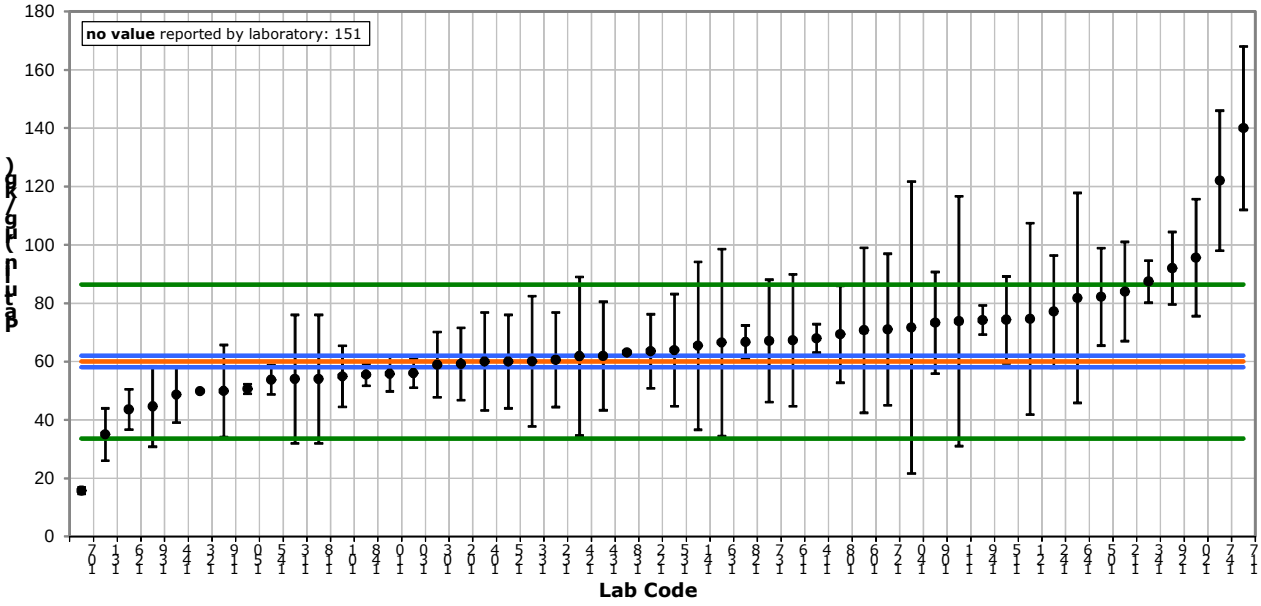
The results are written as reported by the laboratories.

Figure 2: EU-RL Mycotoxins PT 2013: Patulin in apple juice - Sample A
Certified value: Xref = 39.1 µg/kg; Uref = 1.5 µg/kg (k=2); s = 8.6 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 ± 2s)**.

Figure 3: EU-RL Mycotoxins PT 2013: Patulin in apple juice - Sample B
Certified value: Xref = 60.5 µg/kg; Uref = 2.0 µg/kg (k=2); s = 13.2 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 ± 2s)**.

8.4 Evaluation of the questionnaire

All 50 laboratories that reported results supplied the filled in questionnaire. The summary of the answers are presented in **Annex 7**.

The main techniques indicated to determine patulin were HPLC-UV (74 %) and LC-MS (20 %). The remaing laboratories either indicated another technique (GC-MS) or nothing. The limit of detection is for the majority of the methods below 2 µg/kg and the limit of quantification between 2 to 5 µg/kg. Forty-four percent of the laboratories are accredited for the determination of patulin.

Most of the laboratories analyse 10 to 50 samples per year. The main matrices are apple juice and puree. Some participants analyse additionally baby food and other fruit beverages and purees.

Forty-two percent of the laboratories confirmed the mass concentration of the standard used for calibration by UV-spectrometry. For recovery estimation the majority of the participants added patulin standard solution to a blank apple juice sample.

Four laboratories (122, 129, 143 and 147) reported that they encountered unexpectedly low recovery values during the analysis. This indicates the reason for the rather high z-scores obtained by three of those laboratories.

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

All participants found the provided instructions adequate.

9 Conclusions

This was the first EURL/NRL PT conducted for the determination of patulin in apple juice and most of the participants (88 %) earned satisfactory z-scores.

In line with observations of previous PTs organised by the EURL for Mycotoxins, zeta-scores were not as good 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
LFSAL	BELGIUM
CODA-CERVA	BELGIUM
Fytolab cvba	BELGIUM
BFSA, Central Laboratory for Chemical Testing and Control	BULGARIA
State General Laboratory	CYPRUS
Czech Agriculture and Food Inspection Authority	CZECH REPUBLIC
Central Institute for Supervising and Testing in Agriculture (UKZUZ)	CZECH REPUBLIC
National Food Institute	DENMARK
Agricultural Research Centre	ESTONIA
Finnish Food Safety Authority Evira	FINLAND
Finnish Customs Laboratory	FINLAND
SCL Laboratoire De Bordeaux / Pessac	FRANCE
Laboratoire SCL de Rennes	FRANCE
LDA 22	FRANCE
Laboratoire SCL de La Reunion	FRANCE
Federal Institute for Risk Assessment (BfR)	GERMANY
Landesbetrieb Hessisches Landeslabor	GERMANY
General Chemical State Laboratory of Greece	GREECE
National Food Chain Safety Office,Food And Feed Safety Directorate	HUNGARY
Public Analyst's Laboratory	IRELAND
Istituto Superiore di Sanità	ITALY
Istituto Zooprofilattico Sperimentale Lombardia Emilia romagna	ITALY
Laboratorio di Sanità Pubblica	ITALY
Arpa Piemonte	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	PORTUGAL
Sanitary Veterinary and Food Safety Directorate Bucharest	ROMANIA
State veterinary and food institute Košice	SLOVAKIA
Zavod za zdravstveno varstvo Maribor	SLOVENIA
University in Ljubljana, Veterinary Faculty - National Veterinary Institute	SLOVENIA
National centre for Food (Spanish Food Safety and Nutrition Agency)	SPAIN
Centro de Salud Pública	SPAIN
Laboratorio de Salud Pública de Galicia	SPAIN
National Food Agency	SWEDEN
Public Analyst Scientific Services (Norwich)	UNITED KINGDOM
City of Edinburgh Council	UNITED KINGDOM
Kent County Council	UNITED KINGDOM
Staffordshire County Council	UNITED KINGDOM
Tayside Scientific Services	UNITED KINGDOM
Minton Treharne & Davies Ltd	UNITED KINGDOM
West Yorkshire Analytical Services	UNITED KINGDOM
Food and Environment Research Agency	UNITED KINGDOM
Glasgow Scientific Services	UNITED KINGDOM
Worcestershire Scientific Services	UNITED KINGDOM

Abbreviations

AMC	Analytical Methods Committee
EC	European Commission
EN	European Standard
EU	European Union
EURL	European Union Reference Laboratory
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
IUPAC	International Union for Pure and Applied Chemistry
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
UV	Ultra-Violet

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Annexes

Annex 1: Announcement letter – Opening of registration

Ref. Ares(2013)381443 - 18/03/2013

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
European Union Reference Laboratory for Heavy Metals

Geel, 18 March 2013

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Sir/Madam,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of patulin in apple juice.

This proficiency test (PT) was announced by e-mail on the 12th February 2013. More details on the PT design will be communicated upon sample dispatch.

The EU-RL Mycotoxins would like to inform you that, according to Regulation (EC) No 882/2004, the participation of activities organised by the EU-RL is mandatory for the 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 15th April 2013

Dispatch of the PT materials is scheduled for the end of April 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=1080

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

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

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-CRL-MYCOTOX@ec.europa.eu

Deadline for reporting will be the 4th 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. Please do not hesitate to contact us if you require further clarification.

Please contact us at the e-mail address:

JRC-IRMM-CRL-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-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

Annex 2: Sample accompanying letter

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

Ref. Ares(2013)713414 - 17/04/2013

Geel, 23 April 2013

2013 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on the determination of patulin in apple juice

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 email (see details below).

The 2013 PT aims to assess the content in two naturally contaminated test samples (marked as "Sample A", "Sample B"). You will be asked to report the **recovery corrected value** (µg/kg), including your **recovery** (%) and **measurement uncertainty** (µg/kg) for a coverage factor of 2 (k=2).

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

The materials are shipped in dry ice. 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 4th June 2013 to:

Katy Kroeger-Negoita

Tel: +32-14-571 523
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.

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

Annex 3: Materials receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 23 April 2013

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Address:
Member State:

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

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

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

Contents of the parcel:

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

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

Signature / Stamp:

Please fax or e-mail the completed form to:

Katy Kroeger-Negoita

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

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

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

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

Annex 4: Questionnaire

Milc questionnaire

Comparison for PT 2013 Patulin

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-CRL-MYCOTOX@ec.europa.eu.

Submission Form

1. **Which matrices does your laboratory analyse for Patulin on a routine basis?** *

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

3. **Please specify the reference (e.g. EN 15890) 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 Patulin of the method used [µg/kg]. *

4.2. Please indicate the LOQ for Patulin 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) Patulin Standard 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.** *

6.1. Did you confirm the mass concentration of your calibrant standard by spectrophotometer?

- ◯ a) Yes
- ◯ b) No

6.1.1. If Yes, how long ago was the confirmation done prior analysis? *

7. **Please indicate the sample amount (in grams) 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) Liquid/liquid extraction
- ◯ 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 other, please specify. *

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

- ◯ a) Yes
- ◯ b) No

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

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

- ◯ a) Yes
- ◯ b) No

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

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

- ◯ a) Yes
- ◯ b) No

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

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

Annex 5: Homogeneity study

Homogeneity study - Sample A

Container	Patulin [µg/kg]	
A 05	40.9	43.0
A 29	41.8	41.5
A 31	40.9	41.4
A 32	37.5	36.2
A 47	40.5	42.8
A 49	41.8	43.4
A 59	41.7	43.4
A 88	40.2	43.4
A 104	41.3	42.5
A 112	42.3	44.0

Homogeneity according to ISO 13528:2005 [9]	[µg/kg]
Mean	41.5
$\hat{\sigma}$	6.2 (15 %)
0.3 $\hat{\sigma}$ (critical value)	1.9
S_X (standard deviation of sample averages)	1.8
S_W (within-sample standard deviation)	1.3
S_S (between-sample standard deviation)	1.5
$S_S < 0.3\ \hat{\sigma}$	**Passed**

Homogeneity study - Sample B

Container	Patulin [µg/kg]	
B 13	59.7	57.3
B 14	55.9	61.6
B 36	63.2	62.8
B 42	63.9	60.3
B 43	62.1	63.4
B 60	65.1	64.1
B 68	65.0	64.3
B 71	63.0	62.3
B 86	64.2	62.0
B 93	61.7	64.4

Homogeneity according to ISO 13528:2005 [9]	[µg/kg]
Mean	62.3
$\hat{\sigma}$	9.3 (15 %)
0.3 $\hat{\sigma}$ (critical value)	2.8
S_X (standard deviation of sample averages)	2.1
S_W (within-sample standard deviation)	1.8
S_S (between-sample standard deviation)	1.7
$S_S < 0.3\ \hat{\sigma}$	**Passed**

Annex 6: Stability study

Stability study - Sample A

Date	Time	- 18°C (reference)		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
26/04/2013	3 days				37.6	36.6	**37.1**	-0.3	36.7	37.1	**36.9**	0.8
14/05/2013	4 weeks				36.8	34.6	**35.7**	-0.3				
07/06/2013	8 weeks	36.6	35.4	**36.0**	36.4	33.7	**35.1**	0.9				

Stability study - Sample B

Date	Time	- 18°C		mean	4°C		mean	t calc	RT (~25°C)		mean	t calc
26/04/2013	3 days				58.5	57.9	**58.2**	0.4	60.8	60.5	**60.7**	1.7
14/05/2013	4 weeks				59.2	57.8	**58.5**	0.5				
07/06/2013	8 weeks	58.1	56.9	**57.5**	60	57.5	**58.8**	1.7				

Taking into account the repeatability of the method (4.7%) obtained during the homogeneity study, all the mean values for Sample A as well as for Sample B at the tested temperature/time conditions were not statistically different than the respective mean value at the reference temperature (-18 °C) - t critical of two-side t-test = 2.26 (0.05, 9).

The instability differences were, therefore, not significant at the 95 % level of confidence following the approach of the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

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	HPLC-UV	35.28	4.59	97.13	54.93	10.44	97.13	2	0.9	3
102	HPLC-UV	34.55	7.2	72	59.17	12.4	72	2.2	N\A	5
103	HPLC-DAD	31.2	5.9	86	58.9	11.2	86	2	0.4	1.3
104	LC-MS	37	16.8	100	60	16.8	100	2	5	10
105	HPLC-UV	35.4	7.2	92	82.2	16.7	92	2	1	2
106	HPLC-UV	35.1	14.04	87	70.7	28.28	87	2	3	8
107	HPLC-UV	7.23	1.32	149	15.77	1.14	149	2	50	100
108	HPLC-UV	73.4	17.6	73	69.3	16.6	73	2	0.1	1
109	LC-MS	52.9	13.2	66.9	73.3	17.4	77.6	2	0.5	2.5
110	HPLC-UV	35	2.4	100.5	55.8	6	100.5	2	0.1	3.97
111	HPLC-UV	42.9	24.9	78	73.8	42.8	78	2	2	5
112	LC-MS	45	9	100	84	17	100	2	2	25
113	LC-MS	36	14	101	54	22	101	2	2	5
114	HPLC-UV	43.06	0.44	87.93	67.93	4.93	87.93	2	5	15
115	HPLC-UV	51.8	10.3	85	74.3	14.9	85	2	1.3	4
116	HPLC-UV	38.3	14	101	67.3	22.6	101	2	1	3
117	LC-MS	81	16		140	28		2	2	5
118	LC-MS	33	13	80	54	22	80	2	1	2.5
119	HPLC-UV	21.5	8.1	106.8	49.9	15.8	90	2	0.5	2.5
120	LC-MS	40.85	8.35	100	95.6	20.08	100	2	2	6
121	HPLC-UV	38.1	16.7	99.6	74.6	32.8	99.6	2	2.5	2.5
122	HPLC-UV	37.47	7.49	60	63.49	12.7	60	2	0.65	2.18
123	HPLC-UV	22.005		97	49.845		97		2	5
124	HPLC/MS/MS	36.8	16.2	99	61.8	27.2	99	2	0.5	1.5
125	UPLC-UV	37	16	98	60	16	98	2	5	10
126	HPLC-UV	24.4	3.9	94.7	43.6	6.9	97.7	2	2.5	5
127	HPLC-UV	45	17	83	71	26	83	2	2	5
128	HPLC-UV	39.7	8.4	67	66.7	5.7	67	2	2	5
129	HPLC-UV	62.28	8.4	85.06	91.99	12.41	85.06	2	3	5.3
130	LC-MS	32	5	106	56	5	99	2	8	10
131	LC-MS	45	11	115	35	9	115	2	3	10
132	HPLC-UV	35.3	9.5	71.2	60.6	16.2	71.2	2	2	6
133	HPLC-UV	40.83	22.3	54.4	60.08	22.3	54.4	2	1	1
134	HPLC-UV	37.5	11.3	85	61.9	18.6	85	2	0.9	3
135	HPLC-UV	41.2	12.4	100	63.9	19.2	100	2	0.1	0.2
136	HPLC-UV	42.1	32	80	66.5	32	80	2	5	10
137	HPLC-UV	42.46	13	77	67.07	21	77	2	3	10
138	HPLC-UV	37.5		101.8	63.1		101.8		1	2
139	HPLC-UV	37.8	11.7	74.7	44.6	13.8	74.7	2	2	7
140	LC-MS	45.6	50	100	71.7	50	100	2	1	5

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 [%]			
141	HPLC-UV	44.6	19.8	90	65.4	28.8	90	2	0.3	5
142	HPLC-UV	52.4	13.6	75	77.1	19.3	75	2	1	2
143	HPLC-UV	52.4	7.2	56	87.4	7.2	56	2	5	15
144	HPLC-UV	17.04	3.34	91.7	48.65	9.54	74.3	2	0.5	1
145	HPLC-UV	35.6	4.8	83	53.75	5	83	2	3	9
146	HPLC-UV	50.9	22.4	81	81.8	36	81	2	4	8
147		78	16	44	122	24	44	2	1.5	5
148	HPLC-UV	34.37	2.34	87.94	55.47	3.78	87.94	2	5	10
149	HPLC-UV	51.8	5	102	74.2	5	102	2	Not available	Not available
150	GC-MS	29.9	1.6	95	50.6	1.6	95		2	2
151		No result			No result					

Lab Code	Which matrices does your laboratory analyse for Patulin on a routine basis?	How many samples does your laboratory analyse for Patulin per year?	Is your method accredited?	Reference of the analytical method used
101	Juice, puree	20-80	Yes	EN 14177
102	Apple juice	40	No	In house procedure, using molecule specific SPE column
103	beverages, processed fruits and vegetables	20	Yes	STN EN 15890
104	apple puree and baby food	500	Yes	no reference method
105	Fruit Juices	10	Yes	F/0031
106	fruit juices and fruit products (like purees)	50	Yes	EN15890
107	Apple juice	<10	No	In house based on method given for previous collaborative trial for patulin
108	Apple juice	20	No	AOAC 49.6.02
109	apple juice and apple compote	+/-50	Yes	journal of agricultural and food chemistry (2011) 59, 7659-7665
110	Apple juices, but not in routine	usually 2 (for proficiency test and internal quality control)	Yes	JAOAC Int.,(2005), vol.88, pages 518-525
111	Apple juice	20	Yes	ISO 8128-1
112	none	0	No	iso 8128-1
113	Fruit Juice, Fruit Pure	10	No	EN15890
114	no routine basis	-	No	J. of AOAC Int Vol 82, No. 5, 1999
115	apple juice, apple puree	5-10	Yes	AOAC995.10
116	apple juice, fruit baby food	50	Yes	EN 14177
117	apple juices and purees	15-30	No	The analytical method used is based on an application note of Waters
118	Juice	<10	No	NA
119	Apple juice and apple puree	<50	No	EN14177:2003
120	Apple juice, fruit juice and apple products in general	About 20	No	Based on AOAC INTERNATIONAL 995.10, Official Methods of Analysis(1995) 16th Ed. 1996 Supplement
121	fruit juices and fruit product for infants	20	Yes	method developed by laboratory
122	Any	0	No	EN 15890, but mobile phase of EN 14177
123	Apple Juice	/	No	EN 15890
124	Apple and Fruit juice, Apple mousse, Apple based baby food	30	Yes	INTERNAL METHOD BASED ON UNI EN 15662

Lab Code	Which matrices does your laboratory analyse for Patulin on a routine basis?	How many samples does your laboratory analyse for Patulin per year?	Is your method accredited?	Reference of the analytical method used
125	Patulin is not yet analyzed routinely	New method - no samples analyzed yet	No	Romer Labs: MycoSep 228 AflaPat Application Note
126	Juices, compote, baby food	60	Yes	NBN EN 15890
127	apple, pear, apricot, mango, peach juices and purees	25	Yes	EN 14177, EN 15890
128	None.	10	No	Instructions of the AC producer
129	apple puree and apple juice	100	Yes	Application Note EASIMIP PATULIN
130	juice	20	No	MycoSep 228 AflaPat Romer Labs
131	Food and Feed samples	2000	Yes	intern method by LC-MS/MS
132	apple juice, infant apple juice, apple puree, apple cider	50	No	N363 (CEN/TC 275/WG5)
133	Apple juice	10	No	Based on immunoaffinity column manufacturers instructions
134	apple juice, apple and other fruit puree for infants and young children	40	Yes	in house validated method
135	none	0	No	AOAC, 2000, vol 5, 1387
136	apple juice, apple juice concentrate, apple puree	30	No	ISO 8128
137	apple juice, purees, baby food	30	No	14177 for apple juice and 15890 for Baby food
138	Apple juice	20	No	14177:2001
139	No routine basis (some samples of apple juice)	0-5	No	Journal of AOAC, 1980, vol 63 no 5
140	apple juice and apple puree	50	Yes	in house method
141	Juice, infant foods	~100	Yes	EN 14177:2003
142	usually juices (apple clear and cloud) and baby food	100	Yes	UNI EN 14177/04
143	None	0	No	R-biopharm P250
144	apple juice	ca. 50	Yes	in-house method
145	juices, baby food, baby drink, solid apple products	97	No	Romer Labs, Application Brief: Rapid Quantitation of Patulin in various Juices by HPLC-UV
146	Apple Juice	0	No	Technical note of MycoSep Romer Labs
147	apple juices, ciders, apple purees, baby food	#200	Yes	JAOAC 79, n°5, 1107-1109
148	Juices, purees, smoothies, baby foods	Approx 30–40	No	Romer Application Brief Pat_Lc_070202_Juices_sp 2 April 2007
149	Apple Juice	2	No	Collaborative trial SMT-CT96-2045
150	Apple juice, baby food, apple puree	ca. 50-70	No	§35 LMBG L31.00-20

Lab Code	Source of the standard used for calibration	Did you confirm the mass concentration of your calibrant standard by spectrophotometer? If Yes, how long ago was the confirmation done prior analysis?	What is your main procedure for recovery estimation?
101	FLUKA	No	Patulin Standard to Blank Sample
102	Sigma	Yes (Immediately prior to analysis)	Patulin Standard to Blank Sample
103	LGC Standards	No	Patulin Standard to Blank Sample
104	Dr Ehrenstorfer	No	Other (control sample)
105	Sigma	Yes (Same day)	Patulin Standard to Blank Sample
106	Romer Labs	No	Internal Standard to Sample
107	Sigma-Aldrich std	Yes (2 days)	Patulin Standard to Blank Sample
108	Sigma	Yes (Same day)	Other (Spiked sample)
109	Coring	No	Patulin Standard to Blank Sample

Lab Code	Source of the standard used for calibration	Did you confirm the mass concentration of your calibrant standard by spectrophotometer? If Yes, how long ago was the confirmation done prior analysis?	What is your main procedure for recovery estimation?
110	SIGMA	Yes (2.5 years)	Other (Analysis of reference material from FAPAS)
111	Sigma/Aldrich	Yes (On the day of analysis)	Other (Analysis of reference (ex FAPAS) materials)
112	Sigma-Aldich, art. 34127	No	Patulin Standard to Blank Sample
113	SIGMA-ALDRICH	No	Patulin Standard to Blank Sample
114	Fa, AcrosChemicals	Yes (done on 12.3.13)	Other (Patulin to sample A)
115	Sigma-Aldrich	Yes (16.05.2013)	Patulin Standard to Blank Sample
116	Sigma Aldrich	Yes (4 weeks)	Patulin Standard to Blank Sample
117	Biopure	No	Internal Standard to Extract
118	Biopure certified reference solution	No	Patulin Standard to Blank Sample
119	LGC Promochem B-MYC 0500-1	Yes (2 days prior to analysis)	Internal Standard to Sample
120	Romer Labs	No	Patulin Standard to Blank Sample
121	supelco	No	Patulin Standard to Blank Sample
122	SIGMA P1639	Yes (3 days)	Patulin Standard to Blank Sample
123	Biopharm	Yes (/)	Patulin Standard to Blank Sample
124	BIOPURE	No	Internal Standard to Sample
125	LGC Standards	No	Patulin Standard to Blank Sample
126	Sigma aldrich (P1639)	Yes (2 weeks)	Patulin Standard to Blank Sample
127	Sigma-Aldrich	Yes (2 months)	Patulin Standard to Blank Sample
128	Romer Labs	No	Patulin Standard to Blank Sample
129	Trilogy	No	Patulin Standard to Blank Sample
130	Biopure	No	Patulin Standard to Blank Sample
131	BIOPURE	No	Internal Standard to Sample
132	Sigma Aldrich	Yes (4 years)	Patulin Standard to Blank Sample
133	R-Biopharm	No	Patulin Standard to Blank Sample
134	LGC- Standard	No	Patulin Standard to Blank Sample
135	Sigma-alderic, analytical standard	No	Patulin Standard to Blank Sample
136	LGC	No	Patulin Standard to Blank Sample
137	SIGMA	Yes (nearly one month)	Patulin Standard to Blank Sample
138	Trilogy Analytical Laboratory	No	Other (Assigned value of a proficiency test sample)
139	Makor	Yes (3 days)	Patulin Standard to Blank Sample
140	Sigma and Fermentek	Yes (+/- 1 year)	Patulin Standard to Blank Sample
141	SMT Project CT96-2045	Yes (1 week)	Patulin Standard to Blank Sample
142	SIGMA	No	Patulin Standard to Blank Sample
143	Sigma	Yes (a week)	Patulin Standard to Blank Sample
144	LGC Promochem	No	Other (Patulin standard to analyzed sample)
145	Fluka	No	Patulin Standard to Blank Sample
146	Romer Labs	No	Patulin Standard to Blank Sample
147	Biopure	No	Patulin Standard to Blank Sample
148	Biopure Product code BRM002026	No	Patulin Standard to Blank Sample
149	Aldrich	Yes (On the day of the analysis)	Internal Standard to Sample
150	Coring System Diagnostix ; Biopure	No	Patulin Standard to Blank Sample

Lab Code	Extraction solvent	sample amount for extraction	solvent to sample ratio used during extraction [ml/g]	Extraction mode	Extraction time
101	Ethyl acetate	10 g	20/10	shaking	3 x 1 min.
102	N\A	2.0mL (2.1g)	N\A	N\A	N\A
103	Ethyl acetate	10 g	4	shaking	15 min
104	ethyl acetate	10 g	4/1	ultra turrax	1 min
105	ethyl acetate	20 g	30/20	shaking	1 min
106	ethylacetate/hexane 60/40 v/v	10 g	1	shaking on vortexer	3 min
107	ethyl acetate	10ml	3:1	Vortex mixing	2 min X 3 extractions
108	Ethyl acetate	10 g	5	Vortex mixing	5 min
109	ethyl acetate	10 g	2	shaking	3 x 1 min
110	ethyl acetate / hexane	10 g	2	stirring	3 min
111	2% acetic acid	2.5 g	2.5mL / 2.5g	mechanical vortex	20 sec
112	ethyl acetate	5 g	2	vortex	2
113	ETHYLACETATE:HEXANE (60:40)	10 g	1	SHAKING	5 min
114	-	5 mL	-	-	-
115	ethylacetate	10 g	150 ml solvent according to 5 g sample	shaking	30 min
116	ethyl acetate	5 g	10	shaking	3 x 3 min
117	acetonitrile	4 g	5	shaking	5 min
118	Toluene/Acetonitrile/Acetic Acid	5 g	2	Shaking	2 min
119	Ethyl acetate	10 g	6 ml/g	Shaking	3 x 1 min
120	Ethyl Acetate	10 g	25/10	vortex and centrifuge	20 min
121	ethyl acetate	30 g	3	manual shaking	3 steps
122	40/60 (V/V), hexan/ethyl acetat	10 g	10/10	shaking	6 min
123	Ethyl acetate / hexan 60/40	10 g	10 ml	shaking	6 min
124	ACETONITRILE	10 g	10/10	SHAKING	5 min
125	Acetonitrile	Sample was not weighted - 4 ml sample for extraction	4 ml sample / 21 ml ACN (0.19)	vortex	1 min
126	ethyl acetat/hexan + Na2SO4 (15g) + NaHCO3 (2g) + sea sand (2g)	9 g	ethyl acetat/hexan (60/40), 10 ml/9g	horizontal shaker with falcon tubes before centrifugate	5'
127	ethylacetate	10 g	6	shaking by hand	3
128	2 % acetic acid	2.5 mL	1/1	Vortex	20 s
129	-1.0%	2.5 g	1:1	shaking	10 min
130	ACN	2 g	0.3 g/ml	shaking	10 min
131	ACN-H2O	2 or 5 g	20 ml / 5 g	shaking	2 hours
132	ethyl acetate/n-hexane	10 g	10ml/10g	shaking	5 min
133	2 mL/100 mL acetic acid	2 g	1:1	Vortex mix	30 sec
134	ethyl acetate	10 g	60/10	shaking	3 min
135	ethyl acetate	10 g	20/10 (3 times)	shaking	1 min
136	ethyl acetate	5 g	3	shaking	5 min
137	Ethyl acetate	10 g	1/6	Ampoule	5 min x 3
138	Ethyl acetate	10 g	(3 x 20) mL/10 grams	Shaking	1 min
139	ethyl acetate	5 g	1	soft shaking by hand	3 x 2 min (3 x 5 ml)
140	ethyl acetate/hexane 60/40	10 g	1 ml/g	shaking	5 min
141	Ethyl acetate	10 g	20ml / 10 g (3 times)	Shaking	3 x 1 min

Lab Code	Extraction solvent	sample amount for extraction	solvent to sample ratio used during extraction [ml/g]	Extraction mode	Extraction time
142	ethyl acetate	10-20 g	4:1	shaking	1 min x2 times
143	-1.0%	2.5 g	-1.0%	vortex	1 min
144	Ethyl acetate / hexane (3+2 v/v)	10 g	1:1	shaking	3 min
145	Acetonitrile	4 g	4 ml sample/21 ml extractions solvent	shaking	1 min
146	acetonitrile	4 g	5.25	shaking	15 min
147	Ethyl acetate	5 g	# 10	Accelerated solvent extraction	# 20 min
148	Acetonitrile	4.2 g	1:5.25	Vortex mixer	Approx 1 min
149	Ethyl Acetate	10 g	1ml/g, 10g extracted with 3 X 10 ml	Voetex	30 sec
150	Ethyl acetate	5 g	5 mL	shaking	2 x 20 min

Lab Code	Kind of sample clean-up	Details to SPE column and *Other kind of clean-up used*	During the analysis did you need to include any over night stop?	Did you encounter any problems during the analysis?
101	Liquid/liquid extraction		No	No
102	Solid phase extraction (SPE)	Affinimip Patulin, Polyintell	No	No
103	Liquid/liquid extraction		No	No
104	None		Yes (all samples, before extraction)	No
105	None		No	No
106	Solid phase extraction (SPE)	Supelclean LC-Si SPE Tube 3ml (Supelco; Nr.505048)	No	No
107	Liquid/liquid extraction		No	No
108	Liquid/liquid extraction		Yes (Overnight clearing with pectinase to both samples)	No
109	Liquid/liquid extraction		Yes (after extraction and before UPLC analysis)	No
110	Solid phase extraction (SPE)	silica gel; 500 mg/3 ml; SUPELCO	No	No
111	Other	easimip patulin colums Biopharm p/250/p250B	No	No
112	Liquid/liquid extraction		No	No
113	Solid phase extraction (SPE)	SILICA (40-63 µm)	No	No
114	Solid phase extraction (SPE)	HLB 3cc (60 mg), Waters	No	No
115	Solid phase extraction (SPE)	silica, 500 mg/6 ml, GRACE	Yes (all samples after liquid-liquid extraction)	No
116	Liquid/liquid extraction		No	No
117	Solid phase extraction (SPE)	Oasis HLB 3cc/60mg	No	No
118	Other	Modified QuEChERS	No	No
119	Liquid/liquid extraction		No	No
120	Liquid/liquid extraction		No	No
121	Other	*GPC*	Yes (between clean up and HPLC analysis)	No
122	Solid phase extraction (SPE)	SILICA, 500mg/3ml, APPLIED SEPARATIONS	No	Yes (unexpectedly very low recovery)
123	Solid phase extraction (SPE)	500 mg / 3 ml	Yes (Before inject in HPLC)	No
124	Other	EXTRACTION WITH QUECHERS METHOD (LIKE UNI EN 15662 FOR PESTICIDES); *PURIFICATION WITH PSA RESIN*	No	No
125	Solid phase extraction (SPE)	Romer Labs: MycoSep 228 AflaPat	No	No
126	Solid phase extraction (SPE)	spe silica (500 mg/3 ml) from Grace	No	No
127	Solid phase extraction (SPE)	Silica 2g/12ml	Yes (After the pectinase treatment and before	No

Lab Code	Kind of sample clean-up	Details to SPE column and *Other kind of clean-up used*	During the analysis did you need to include any over night stop?	Did you encounter any problems during the analysis?
			final evaporation for HPLC)	
128	Other	*Molecularly imprinted column*	No	No
129	Solid phase extraction (SPE)	Molecularly imprinted polymer, EASIMIP PATULIN, R-Biopharm	No	Yes (For both samples the recovery was very low (eg. 40%) when we spiked in sample A and sample B)
130	Other	*MycoSep 228 AflaPat*	No	No
131	None		No	No
132	Solid phase extraction (SPE)	silica,500mg/3cc	No	No
133	Other	*Molecularly imprinted polymer column*	No	No
134	Solid phase extraction (SPE)	silica 5000mg/20ml, Varian	No	No
135	Liquid/liquid extraction		Yes (after redisolvation, before HPLC analysis)	No
136	None		No	No
137	Liquid/liquid extraction	*a wash with Na2CO3*	No	No
138	Liquid/liquid extraction		No	No
139	Liquid/liquid extraction		Yes (Extracts ready for evaporation, dilution and HPLC was left over night frozen.)	No
140	Solid phase extraction (SPE)	Silica 500 mg/3ml Agilent	No	No
141	Liquid/liquid extraction		No	No
142	Liquid/liquid extraction		Yes (all samples after addiction of Pectinase enzyme)	No
143	Solid phase extraction (SPE)	R-Biopharm EASIMIP Patulin P250 - polymer	No	Yes (During the store time, the samples bottles were put in freeze on horizontal and closed but when we get them, some liquid appeared outside in the plastic pack)
144	Solid phase extraction (SPE)	Silicagel 1 g / 6 ml	No	No
145	Other	*Romer Labs: MycoSep@228 AflaPat cleanup column*	No	No
146	Other	*column MycoSep 228 AflaPat*	No	Yes (Problem with the evaporation step)
147	Solid phase extraction (SPE)	Florisil/100 mg/3mL/Agilent	Yes (All samples at 4°C overnight after extraction)	Yes (Since a few weeks, a poor recovery ratio without any explanation for all samples)
148	Solid phase extraction (SPE)	Mycosep R 228 AflaPat Kit Romer Labs.	No	No
149	Liquid/liquid extraction		No	No
150	None		No	No

Lab Code	Did you find the instructions distributed for this PT adequate?	Are there any other comments you wish to make?
101	Yes	
102	Yes	
103	Yes	
104	Yes	
105	Yes	No
106	Yes	
107	Yes	none
108	Yes	
109	Yes	

Lab Code	Did you find the instructions distributed for this PT adequate?	Are there any other comments you wish to make?
110	Yes	
111	Yes	
112	Yes	the expanded measurement uncertainty (U) is corrected for BIAS
113	Yes	NO
114	Yes	
115	Yes	
116	Yes	
117	Yes	
118	Yes	
119	Yes	None
120	Yes	
121	Yes	few quantity of sample
122	Yes	-
123	Yes	
124	Yes	
125	Yes	The method is new and just valitaded - no prior data or experience.
126	Yes	
127	Yes	
128	Yes	/
129	Yes	
130	Yes	
131	Yes	
132	Yes	The cleaning step of the samples via SPE columns doesn't give very repeatable results for the samples and the QC samples as well.
133	Yes	
134	Yes	No
135	Yes	
136	Yes	
137	Yes	
138	Yes	No
139	Yes	
140	Yes	
141	Yes	Reporting forms a little awkward
142	Yes	NO
143	Yes	It seems to us that our recovery is to much low
144	Yes	
145	Yes	
146	Yes	
147	Yes	Usually, the recovery ratio is # 70-85%.
148	Yes	The method was presented at the annual Accreditation Body visit to the laboratory and will be accredited later this year, pending clearance of non-confirmaces and subsequent approval of the INAB board.
149	Yes	Standards and samples made up in mobile phase.
150	Yes	

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Abstract

This report presents the results of the ILC of the EURL for Mycotoxins which focused on the determination of patulin in apple juice samples.

Fifty-one participants from 27 countries (among them 30 NRLs and 21 official food control laboratories) registered for the exercise and 50 sets (Sample A and B) of results were reported.

Only z-scores were used for the evaluation of an underperformance. In total 88 % of the attributed z- scores were below an absolute value of two, which indicates that most of the participants performed satisfactory.

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