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Report on the 2017 Proficiency Test of the European Union Reference Laboratory for Mycotoxins

Determination of deoxynivalenol in wheat

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European Union Reference Laboratory
Mycotoxins

EURL-MYCO-PT-2017 Proficiency Test Report

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Belgian Accreditation Body (BELAC)

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Executive Summary

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

This report presents the results of the PT on the determination of deoxynivalenol in wheat.

The test materials for this PT were four naturally contaminated wheat materials. The test items were dispatched to the participants at the end of April 2017. Each participant received one test item per material containing approximately 55 g each.

Fifty-nine participants from 32 countries (among them 41 NRLs and 18 official food control laboratories-OCLs) registered for the exercise and 59 sets of results for test items A, B, C and D) were reported.

The assigned values, established by an exact-matching double isotope dilution mass spectrometric technique (EMD-IDMS), were 551 µg/kg (± 37 µg/kg) deoxynivalenol for material A, 1556 µg/kg (± 83 µg/kg) for material B, 4405 µg/kg (± 265 µg/kg) for material C and 1160 µg/kg (± 60 µg/kg) for material D.

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

Only z-scores were used for the evaluation of whether an individual laboratory underperformed. In total, 93 % of the attributed z-scores were below an absolute value of two for test items A and C, 95 % for test item B and 92 % for test item D. This indicates that most of the participants performed satisfactorily. One NRL had a z-score above an absolute value of 3 and will have to investigate the reasons for the deviation (root-cause analysis) and report the planned corrective actions to the EURL.

Participants were requested to assess the compliance of the sample against legislative limits. Eighty-five percent to 100 % of the participants assessed the compliance/non-compliance of the test materials A, C and D correctly. Only 36 % of the laboratories assessed correctly the non-compliance of Material B, and 56 % classified the test material as compliant providing a proper justification (taking into account their measurement result and reported uncertainty).

Acknowledgements

The organizers of the study would like to thank the JRC colleagues involved in the project for their support. The laboratories participating in this exercise, listed in Table 1, are also kindly acknowledged.

Table 1: Participating laboratories

Organisation	Country
AGES GmbH	Austria
LVA GmbH	Austria
CODA-CERVA	Belgium
Central Laboratory for Chemical Testing and Control, BFSA	Bulgaria
E.C. Inspekt d.o.o.	Croatia
Agrokontrola d.o.o.	Croatia
Department of Agriculture, Analytical Laboratories	Cyprus
State General Laboratory	Cyprus
UKZUZ - Central Institute for Supervising and Testing in Agriculture	Czech Republic
Czech Agriculture and Food Inspection Authority (CAFIA)	Czech Republic
Danish Veterinary and Food Administration	Denmark
Agricultural Research Centre, laboratory for Residues and Contaminants	Estonia
Finnish Customs Laboratory	Finland
Finish Food Safety Authority Evira	Finland
Laboratoire SCL de Rennes	France
Laboratoire des Pyrénées et des Landes	France
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei MV	Germany
LAVES, Futtermittelinstitut Stade	Germany
CVUA Sigmaringen	Germany
Federal Institute for Risk Assessment (BfR)	Germany
Chemical State Laboratory, Division of Piraeus and Aegean	Greece
National Food Chain Safety Office, Food And Feed Safety Directorate, Food Toxicological NRL	Hungary
National Food Chain Safety Office, Food and Feed Safety Directorate, Feed Investigation NRL	Hungary
Matis, Research and Innovation	Iceland
The State Laboratory	Ireland
Public Analyst's Laboratory	Ireland
Azienda USL Toscana Centro, Laboratorio Sanità Pubblica di Firenze	Italy
ATS Città Metropolitana di Milano, Laboratorio di Prevenzione	Italy
IZSLER	Italy
ARPA PUGLIA	Italy
IZS Sardegna	Italy
ARPAM	Italy
IZSLT	Italy
Istituto Zooprofilattico Sperimentale del Mezzogiorno	Italy
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
Faculty of Veterinary medicine, Food Institute	former Yugoslav Republic of Macedonia
Public Health Laboratory	Malta
RIKILT - Wageningen UR	The Netherlands
The Norwegian Veterinary Institute	Norway
National Institute of Public Health - National Institute of Hygiene	Poland

Organisation	Country
ASAE - DRAL – LFQ	Portugal
Sanitary Veterinary and Food Safety Directorate Bucharest	Romania
DSVSA GALATI – LSVSA	Romania
Veterinary Laboratory and Food Safety, Control Bureau Residues and Contaminants	Romania
Hygiene and Veterinary Public Health Institute	Romania
Directia Sanitara Veterinara Si Pentru Siguranta Alimentelor Calarasi	Romania
SP Laboratorija A.D.	Serbia
Regional Public Health Authority - RUVZ so sídlom v Poprade	Slovakia
State veterinary and food institute Dolný Kubín, Veterinary and food institute in Košice	Slovakia
University of Ljubljana, Veterinary Faculty, National Veterinary Institute	Slovenia
National laboratory for health, environment and food	Slovenia
Laboratorios ECOSUR, s.a.	Spain
National Centre for food – Spanish consuming, food safety and nutrition agency	Spain
National Food Agency	Sweden
National Veterinary Institute, SVA	Sweden
Fera Science Ltd.	UK

List of abbreviations

DON	Deoxynivalenol
ELISA	Enzyme-Linked Immunosorbent Assay
EC	European Commission
EMD-IDMS	Exact-Matching Double Isotope Dilution Mass Spectrometry
EN	European Standard
EU	European Union
EURL	European Union Reference Laboratory
FLD	Fluorescence detector
HPLC	High-Performance Liquid Chromatography
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC-ID-MS/MS	Liquid Chromatography Isotope Dilution tandem Mass Spectrometry detection
LC-MS	Liquid Chromatography-tandem Mass Spectrometry
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PT	Proficiency Test
U_{ref}	Expanded uncertainty of the reference value
X_{ref}	Reference value
σ_{pt}	standard deviation for proficiency assessment

1 Introduction

Deoxynivalenol (DON, vomitoxin) is a type B trichothecene mycotoxin produced by *Fusarium graminearum* and *F. culmorum*. It is the most prevalent of the trichothecenes detected in cereal crops, contaminating cereal-based food and feed. DON can cause acute nausea, vomiting and diarrhea in animals and humans, as well as abdominal pain, headache, dizziness, and fever. In order to protect human and animal health, maximum values for DON in food and feed have been set at EU level with Regulation (EC) No 1881/2006 [1].

The most frequently used method for DON determination is liquid chromatography and gas chromatography coupled to mass spectrometry (LC-MS and GC-MS), and high-performance liquid chromatography coupled to an ultraviolet detector (HPLC-UV) or a fluorescence detector (HPLC-FLD). Enzyme-linked immunosorbent assays (ELISA) are also a commonly used method for DON analysis, but involve multiple steps and time-consuming procedures. In response to the demand for rapid screening for mycotoxins, an extensive number of immunological techniques (screening methods) have been developed for quantitation of DON in cereals in the form of lateral-flow test kits [2].

2 Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [3], one of the core duties of the EURL is to organise PTs for the benefit of the NRLs. The scope of this PT was to test the competence of the appointed NRLs to determine the amount of deoxynivalenol in wheat. All invited laboratories were allowed to use their method of choice, but participants were encouraged to use screening methods according to Commission Regulation (EU) No 519/2014 [4]. The materials had to be classified as 'compliant' or 'non-compliant' assuming the material fell into category 2.4.1 of the Annex in Commission Regulation (EC) No 1881/2006 (1250 µg/kg DON) [1].

The EURL Mycotoxins assessed the measurement results based on the requirements laid down in legislation and followed the procedures of ISO/IEC 17043:2010 [5]. The JRC Unit managing the EURL Mycotoxins is an ISO/IEC 17043:2010 accredited PT provider.

2.1 Confidentiality

The procedures used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 [5] and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the NRLs will be disclosed to DG SANTE upon request for performance assessments.

3 Time Frame

The PT was opened for registration on the 1st of March 2017, and the deadline for registration was 12th of April 2017 (Annex 1). The test items were dispatched to the participants on the 25th and 26th of April 2017 (Annexes 2 and 3). The deadline for reporting the results was the 12th of June 2017.

4 Material

4.1 Preparation

The test materials used in this study were stemming from two naturally contaminated wheat materials (test items A and C). Test items B and D were prepared by mixing small portions of test material C into a blank wheat material. All materials were sieved with a 0.5 mm sieve, cryo-milled and then homogenised in a tumble mixer.

All the test items were then packed in plastic containers, taking portions from different places of the lot at random and stored at -18 °C until dispatch. The total sample size was ca. 55 g.

4.2 Homogeneity

To verify the homogeneity of the test materials, 10 test items per material (A-D) were randomly selected. Two independent determinations per test item were performed using liquid chromatography isotope dilution tandem mass spectrometry detection (LC-ID-MS/MS). The order of measurements of the batch was randomised. Homogeneity was evaluated according to ISO 13528:2015 [6]. The materials proved to be adequately homogeneous (Annex 4).

4.3 Stability

The stability study was conducted following an isochronous experimental design [7]; -18 °C was chosen as the reference temperature for sample storage. The stability was checked at room temperature (≈20 °C) and at 4 °C. The time periods considered in this study were: 14, 28 and 55 days. Stability was evaluated according to ISO 13528:2015 [6]. A linear regression

was drawn for each tested temperature over the duration of the study, and the significance of the slope departure from zero at 95 % confidence level was verified. The materials proved to be adequately stable room temperature and at 4 °C for the period between dispatch and the submission date of the last results (55 days). The results of the study are listed in Annex 5.

4.4 Distribution

The test items were dispatched with cooling packs on the 25th and 26th of April 2017, and they were mostly received within 24 hours after dispatch.

Each participant received:

a) four test items containing approximately 55 g of each test material

b) an accompanying letter with instructions on sample handling and reporting (Annex 2)

c) a material receipt form (Annex 3)

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

5 Instruction to participants

The laboratories were asked to report the recovery corrected levels as well as their expanded measurement uncertainty in µg/kg (coverage factor k=2). If the laboratories used a screening method, they were asked to report the cut-off value of the method, as well as the reading given and the units.

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

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

6 Assigned values and their uncertainties

The assigned values of the DON contents and their uncertainties for the test materials were established by Exact-Matching Double Isotope Dilution Mass Spectrometry (EMD-IDMS) at the JRC. This methodology is considered to provide the highest degree of accuracy of the measurement results. The assigned values ± the expanded uncertainties (k=2) were 551 ± 37 µg/kg (material A), 1556 ± 83 µg/kg (material B), 4405 ± 265 µg/kg (material C) and 1160 ± 60 µg/kg (material D).

7 Evaluation of the results

7.1 General observations

Fifty-nine participants from 32 countries (among them 41 NRLs and 18 OCLs) registered for the exercise (Table 1) and 59 sets of results were reported.

The laboratories were free to use their method of choice. Twenty-seven laboratories analysed the samples with LC-MS, 25 laboratories used HPLC-UV, 4 used ELISA, 1 used HPLC with Diode-Array Detection (HPLC-DAD), 1 used GC-MS, and 1 laboratory used lateral flow strips (screening test). One of the laboratories that used LC-MS also reported results using ELISA, but these have not been used for the evaluation of the laboratory performance (LC0018, see Annex 10).

7.2 Scores and evaluation criteria

Individual laboratory performance was assessed in terms of z and zeta (ζ) scores in accordance with ISO 13528:2005 [6]. The following formulas were used:

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

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

where:

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

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

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

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

σ_{pt} is the standard deviation for proficiency assessment (target standard deviation)

σ_{pt} was calculated as 22 % of the assigned value. This derived from the Horwitz equation for a mass fraction of 120 µg/kg ($\sigma_{pt} = 0.22\,C$) was applied regardless of the magnitude of the mass fraction of the analyte in each given material.

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

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

The zeta (ζ)-score indicates whether the participant's estimate of measurement uncertainty is consistent with the observed deviation from the assigned value. The ζ score is a very 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:

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

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

7.3 Laboratory results and scoring

The statistical evaluation of the results was performed using ProLab software [8]. Z- and ζ-scoring was based on the reference values (and respective uncertainties) assigned by EMD-IDMS. The robust mean of participant's results was computed according to Algorithm A of ISO 13528:2015, and is given just for information purposes [6] (Table 2).

The calculated z- and ζ-scores are presented in Table 3. All z- and ζ-scores in the satisfactory performance range are shown on a green background; those in the questionable range are displayed on a yellow background and scores indicating unsatisfactory performance are presented on a light-red background. Ninety-three percent of the results reported by the participants were rated with satisfactory z-scores ($|z| \leq 2$) for materials A and C, 95 % for material B and 92 % for material D. A total of 3.4 % of the results reported by the participants were rated with unsatisfactory z-scores ($|z| \geq 3$) for materials A and C and 1.7 % for materials B and D. Materials A and C had the lowest (551 µg/kg) and the highest (4405 µg/kg) levels of DON, respectively.

Only 1 NRL reported a result with an unsatisfactory z-score and 34 laboratories (out of 59) reported results that were rated satisfactorily for all four test items.

Figure 1 presents an overview of the individual z-scores assigned to the results provided by each laboratory. The longer the triangles, the larger were the differences to the assigned values. Blue triangles represent z-scores in the satisfactory range, yellow triangles in the questionable range and red triangles in the unsatisfactory performance range. The unsatisfactory scores are shown next to the red triangles.

The graphical representations of the sigmoidal distribution of the results (µg/kg) for each combination of sample/analyte are given in Figures 2-5. Reported values are shown as bars. The blue line corresponds to X_{ref}; the green shadow covers the boundary of the reference interval ($X_{ref} \pm u_{ref}$), and the red lines mark the boundary of the target interval ($X_{ref} \pm 2\sigma_{pt}$). Green bars represent results with $|z\text{-score}| \leq 2$, yellow bars represent results with $2 < |z\text{-score}| < 3$, while the red bars represent results with $|z\text{-score}| \geq 3$.

The rate of the satisfactory ζ-scores is lower than the one for z-scores. The plausibility of the uncertainty statements of the laboratories was assessed by classifying every reported uncertainty into three groups (see column C, Table 3) according to the following rules:

1. The standard measurement uncertainty of a result ($u(x_i)$) is most likely to fall within a range between a minimum and a maximum uncertainty (case "a": $u_{min} \leq u(x_i) \leq u_{max}$). The minimum uncertainty (u_{min}) is set for the respective analyte to the standard uncertainty of the assigned value ($u(x_{ref})$). This is based on the assumption that it is unlikely that a laboratory carrying out the analysis on a routine basis would determine the measurand with a smaller measurement uncertainty than that achieved in the experiments for the characterisation of the test material, which was based on EMD-IDMS. The maximum uncertainty is set to the standard deviation accepted for the assessment of results (σ_{pt}). Consequently, case "a" becomes: $u(x_{ref}) \leq u(x_i) \leq \sigma_{pt}$.
2. If $u(x_i)$ is smaller than $u(x_{ref})$ (case "b": $u(x_i) < u(x_{ref})$), the laboratory might have underestimated its measurement uncertainty.

3. If $u(x_i)$ is larger than σ_{pt} (case "c": $u(x_i) > \sigma_{pt}$), the laboratory might have overestimated its measurement uncertainty or applied an analytical method that was not fit-for-purpose. Both cases require attention.

The participants in categories "b" and "c" are encouraged to assess their uncertainty estimation in line with the above observations. The uncertainty is an integral part of the measurement result and has major implications on the assessment of the compliance of food according to the European Union legislation. Annex 7 presents an overview of the individual ζ-scores.

Table 2. Summary statistics of the results for deoxynivalenol in test materials A, B, C and D.

	Units	**Material A**	**Material B**	**Material C**	**Material D**
Number of laboratories that submitted results		59	59	59	59
Assigned value (EMD-IDMS) of DON content (X_{ref})	µg/kg	551	1556	4405	1160
Expanded uncertainty (k=2) of the assigned value (U_{ref})	µg/kg	37	83	265	60
Robust mean of DON content	µg/kg	574	1536	3932	1174
Target standard deviation (σ_{pt})	µg/kg	121	342	969	255

Table 3. Analytical results, z- and zeta scores for the deoxynivalenol content in materials A, B, C and D.
Colour code: green- satisfactory performance, yellow – questionable performance, red – unsatisfactory performance.

	A					B					C					D				
Lab code	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C*	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C
LC0001	504		-0.4			1390		-0.5			3480		-1.0			1070		-0.4		
LC0002	563.3	40.0	0.1	0.4	a	1592.2	113.0	0.1	0.5	a	4305.1	305.7	-0.1	-0.5	a	1206.0	85.6	0.2	0.9	a
LC0003	619	100	0.6	1.3	a	1611	261	0.2	0.4	a	4633	752	0.2	0.6	a	1318	214	0.6	1.4	a
LC0004	421.7	125.7	-1.1	-2.0	a	1535.4	457.5	-0.1	-0.1	a	5003.5	1491	0.6	0.8	a	1241.1	369.9	0.3	0.4	a
LC0005	650		0.8			1200		-1.0			4700		0.3			1800		2.5		
LC0006	370.6	41	-1.5	-6.5	a	1585.0	107	0.1	0.4	a	3392.0	227	-1.0	-5.8	b	1213.5	83	0.2	1.0	a
LC0007	560	196	0.1	0.1	a	1498	524	-0.2	-0.2	a	3954	1384	-0.5	-0.6	a	1027	359	-0.5	-0.7	a
LC0008	529	159	-0.2	-0.3	a	1533	460	-0.1	-0.1	a	4312	1294	-0.1	-0.1	a	1125	338	-0.1	-0.2	a
LC0009	561	52	0.1	0.3	a	1464	134	-0.3	-1.2	a	4399	404	0.0	0.0	a	1092	100	-0.3	-1.2	a
LC0010	516.8	227.4	-0.3	-0.3	a	1297.7	571.0	-0.8	-0.9	a	3448.5	1517.3	-1.0	-1.2	a	974.1	428.6	-0.7	-0.9	a
LC0011	554	115	0.0	0.0	a	1580	329	0.1	0.1	a	4082	849	-0.3	-0.7	a	1175	244	0.1	0.1	a
LC0012	720	288	1.4	1.2	c	1600	640	0.1	0.1	a	4450	1780	0.0	0.1	a	1150	460	0.0	0.0	a
LC0013	566	226	0.1	0.1	a	1358	543	-0.6	-0.7	a	3555	1422	-0.9	-1.2	a	929	371	-0.9	-1.2	a
LC0014	585	30	0.3	1.4	b	1653	83	0.3	1.7	b	4416	302	0.0	0.1	a	1095	87	-0.3	-1.2	a
LC0015	550	201	0.0	0.0	a	1586	580	0.1	0.1	a	4316	1577	-0.1	-0.1	a	1166	426	0.0	0.0	a
LC0016	576	115	0.2	0.4	a	1497	299	-0.2	-0.4	a	2610	522	-1.9	-6.1	a	1221	244	0.2	0.5	a
LC0017	623	125	0.6	1.1	a	2081	416	1.5	2.5	a	4660	932	0.3	0.5	a	1427	285	1.0	1.8	a
LC0018	450	144	-0.8	-1.4	a	1400	448	-0.5	-0.7	a	3800	1216	-0.6	-1.0	a	1000	320	-0.6	-1.0	a
LC0019	635.68	93.00	0.7	1.7	a	1735.39	253.89	0.5	1.3	a	3974.05	581.40	-0.4	-1.3	a	1115.76	163.24	-0.2	-0.5	a
LC0020	430	58	-1.0	-3.5	a	1577	159	0.1	0.2	a	3819	363	-0.6	-2.6	a	1172	120	0.0	0.2	a
LC0021	737	162	1.5	2.2	a	1924	423	1.1	1.7	a	3146	692	-1.3	-3.4	a	1381	304	0.9	1.4	a
LC0022	589	24.1	0.3	1.7	b	1637	64.6	0.2	1.5	b	4399	223	0.0	0.0	b	1183	40.5	0.1	0.6	b
LC0023	554	166	0.0	0.0	a	1598	480	0.1	0.2	a	4197	1259	-0.2	-0.3	a	1208	363	0.2	0.3	a
LC0024	510	102	-0.3	-0.8	a	1576	315	0.1	0.1	a	5036	1007	0.7	1.2	a	1060	212	-0.4	-0.9	a
LC0025	577	129	0.2	0.4	a	1665	334	0.3	0.6	a	4514	845	0.1	0.2	a	1179	244	0.1	0.2	a
LC0026	570	125	0.2	0.3	a	1426	314	-0.4	-0.8	a	3830	842	-0.6	-1.3	a	1130	249	-0.1	-0.2	a

A						B					C					D				
Lab code	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C*	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C
LC0027	954	308	3.3	2.6	c	1642	488	0.3	0.3	a	2259	640	-2.2	-6.2	a	1423	432	1.0	1.2	a
LC0028	638	128	0.7	1.3	a	1585	317	0.1	0.2	a	4550	910	0.1	0.3	a	1215	243	0.2	0.4	a
LC0029	798.79	12.35	2.04	12.6	b	666.06	12.35	-2.6	-21.2	b	685.57	12.35	-3.8	-28.0	b	1911.26	12.35	2.9	24.5	b
LC0030	688	96	1.1	2.7	a	1985	277	1.3	3.0	a	2953	412	-1.5	-5.9	a	1406	196	1.0	2.4	a
LC0031	663.6	165.9	0.9	1.3	a	2148.8	537.2	1.7	2.2	a	4619.1	1154.8	0.2	0.4	a	1321.1	330.5	0.6	1.0	a
LC0032	656.20	55.38	0.9	3.2	a	1616.37	136.42	0.2	0.8	a	4133.69	348.88	-0.3	-1.2	a	1311.56	110.69	0.6	2.4	a
LC0033	573	90.50	0.4	1.1	a	1134	179.0	-1.1	-3.7	a	2693	425.3	-1.6	-6.3	a	943	151.6	-0.6	-1.8	a
LC0034	541	86.6	-0.1	-0.2	a	1095	175	-1.3	-4.8	a	2680	429	-1.8	-6.8	a	870	139	-1.1	-3.8	a
LC0035	1109		4.6			3593		6.0			5396		1.0			2455		5.1		
LC0036	551.8	190	0.0	0.0	a	1552.0	460	0.0	0.0	a	4136.2	1100	-0.3	-0.5	a	1111.7	350	-0.2	-0.3	a
LC0037	600	180	0.4	0.5	a	1616	485	0.2	0.2	a	4460	1338	0.1	0.1	a	1196	358	0.1	0.2	a
LC0038	271	30	-2.3	-11.7	b	635	191	-2.7	-8.8	a	1408	422	-3.1	-12.0	a	425	128	-2.9	-10.4	a
LC0039	611.9	107.6	0.5	1.1	a	1774.4	312.2	0.6	1.4	a	4665.4	821.1	0.3	0.6	a	1334.1	234.8	0.7	1.4	a
LC0040	577	140	0.2	0.4	a	2008	405	1.3	2.2	a	4217	761	-0.2	-0.5	a	1260	273	0.4	0.7	a
LC0041	495	93	-0.5	-1.1	a	1260	190	-0.9	-2.9	a	3196	646	-1.2	-3.5	a	878	112	-1.1	-4.4	a
LC0042	600	180	0.4	0.5	a	1570	470	0.0	0.1	a	4090	1230	-0.3	-0.5	a	1160	348	0.0	0.0	a
LC0043	535.1	235.456	-0.1	-0.1	a	1601	704.242	0.1	0.1	c	4515	1986.56	0.1	0.1	c	1074	472.452	-0.3	-0.4	a
LC0044	514.62		-0.3			1707.24		0.4			4791.53		0.4			1052.84		-0.4		
LC0045	557	129	0.0	0.1	a	1489	316	-0.2	-0.4	a	2851	589	-1.6	-4.8	a	1161	250	0.0	0.0	a
LC0046	577	115	0.2	0.4	a	1557	311	0.0	0.0	a	3250	650	-1.2	-3.3	a	1209	242	0.2	0.4	a
LC0047	529		-0.2			1356		-0.6			3530		-0.9			918		-0.9		
LC0048	532	160	-0.2	-0.2	a	1376	413	-0.5	-0.9	a	2888	866	-1.6	-3.4	a	1097	329	-0.2	-0.4	a
LC0049	610	130	0.5	0.9	a	1500	320	-0.2	-0.3	a	4400	920	0.0	0.0	a	1100	230	-0.2	-0.5	a
LC0050	590	236	0.3	0.3	a	1640	656	0.2	0.3	a	4550	1820	0.1	0.2	a	1210	484	0.2	0.2	a
LC0051	347	110	-1.7	-3.5	a	1380	420	-0.5	-0.8	a	5650	1700	1.3	1.4	a	1140	350	-0.1	-0.1	a
LC0052	496	99.2	-0.5	-1.0	a	1480	296	-0.2	-0.5	a	4160	832	-0.3	-0.6	a	1080	216	-0.3	-0.7	a

A						B					C					D				
Lab code	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C*	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	Z-Score	Zeta score	C
LC0053	657	309	0.9	0.7	c	1763	828	0.6	0.5	c	4806	2259	0.4	0.4	c	1281	602	0.5	0.4	c
LC0054	680	230	1.1	1.1	a	1762	520	0.6	0.8	a	2013	580	-2.5	-7.5	a	1310	400	0.6	0.7	a
LC0055	600	198	0.4	0.5	a	1300	429	-0.7	-1.2	a	2693	889	-1.8	-3.7	a	1798	593	2.5	2.1	c
LC0056	495		-0.5			975		-1.7			4830		0.4			1075		-0.3		
LC0057	610	232	0.5	0.5	a	1513	575	-0.1	-0.1	a	4103	1560	-0.3	-0.4	a	1144	434	-0.1	-0.1	a
LC0058	432.72	47.32	-1.0	-3.9	a	1152.93	169.75	-1.2	-4.3	a	3244.74	586.02	-1.2	-3.6	a	916.46	44.78	-1.0	-6.5	b
LC0059	595.65	25.58	0.4	2.0	b	1520.02	23.42	-0.1	-0.8	b	3367.45	36.08	-1.1	-7.8	b	1437.65	46.76	1.1	7.3	b

Figure 1. Individual laboratory z-scores

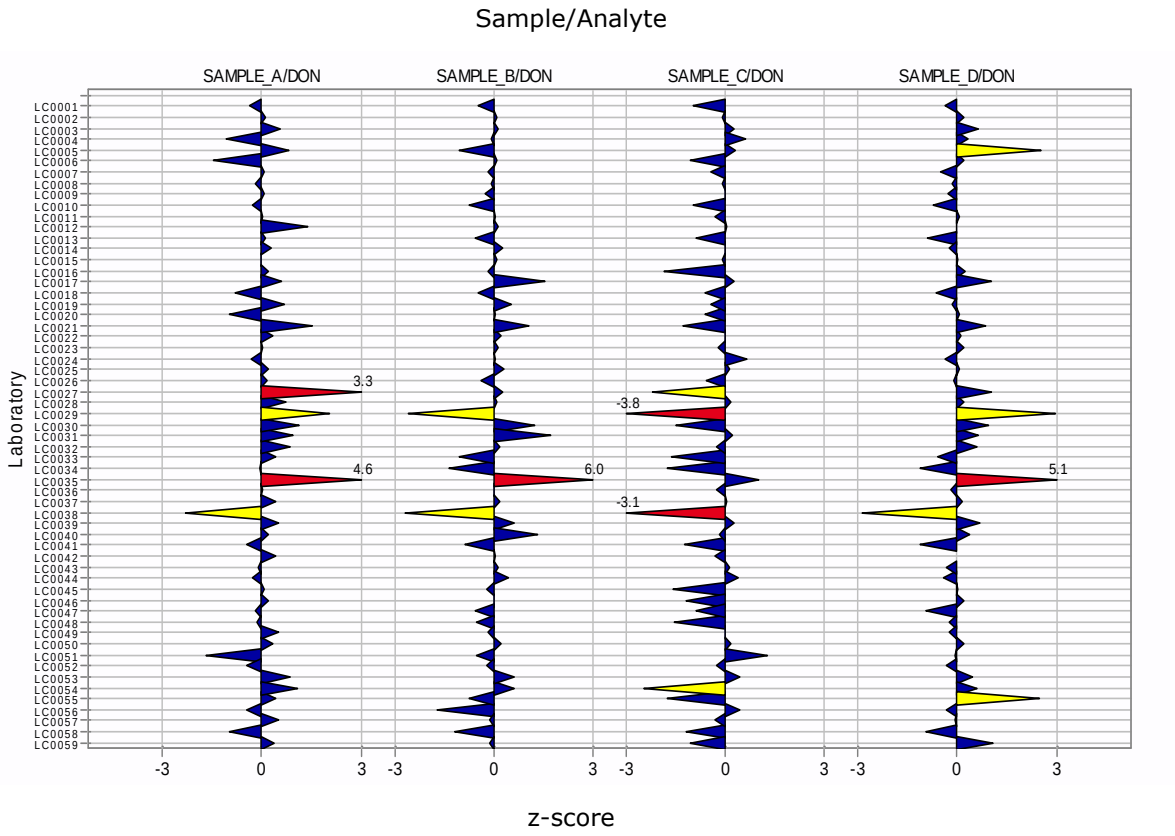


Figure 2. Sigmoidal plot of individual results reported for test Material A

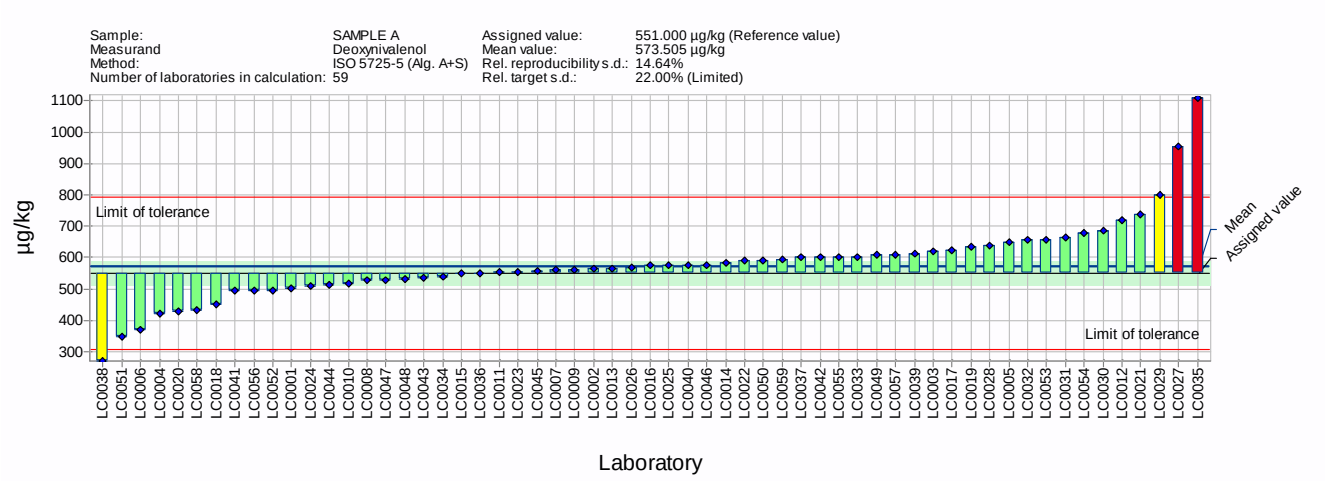


Figure 3. Sigmoidal plot of individual results reported for test Material B

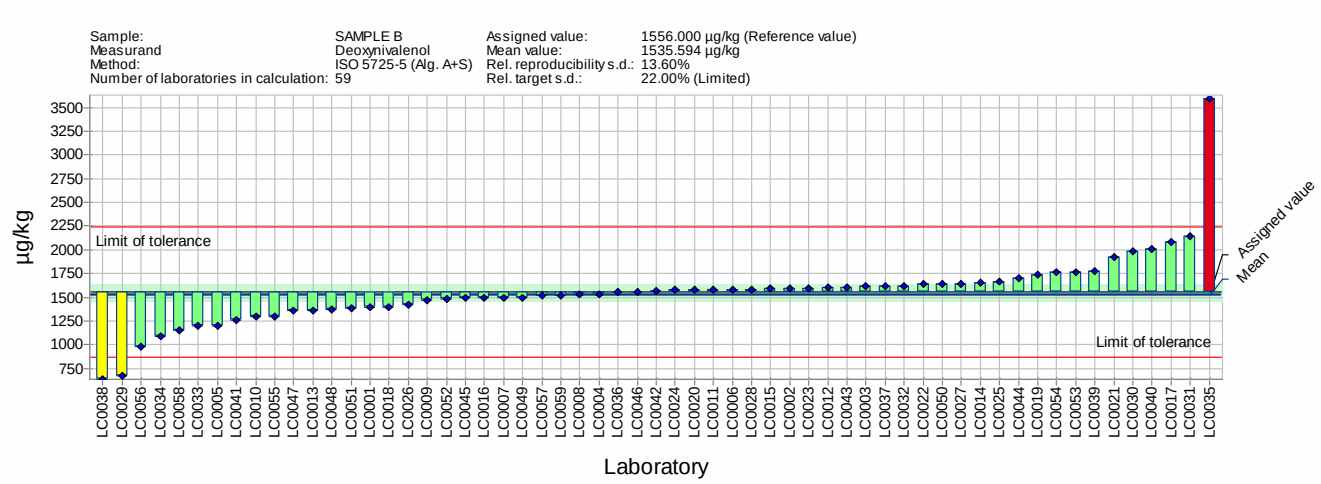


Figure 4. Sigmoidal plot of individual results reported for test Material C

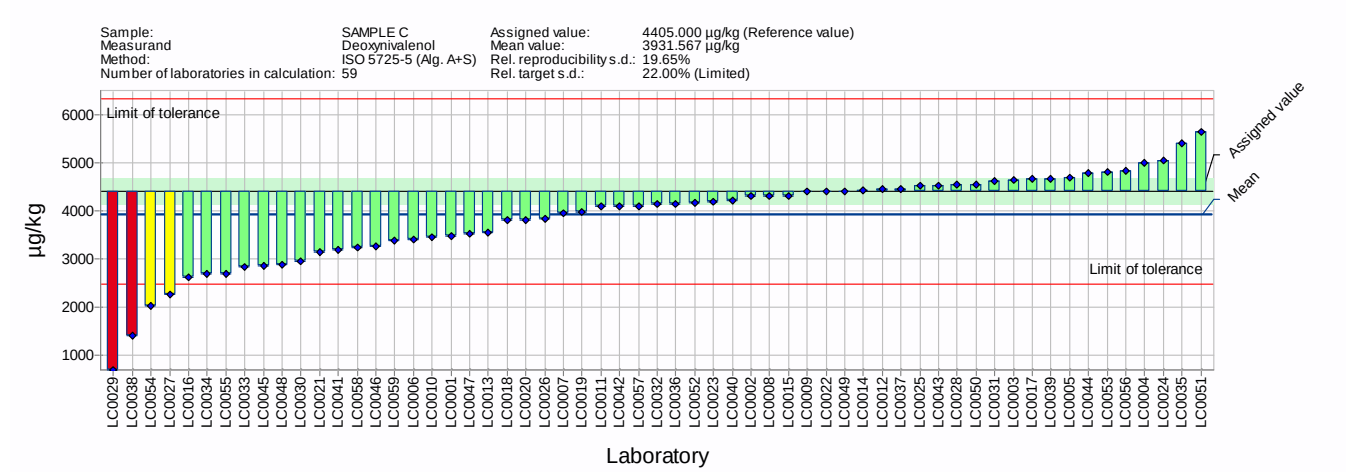
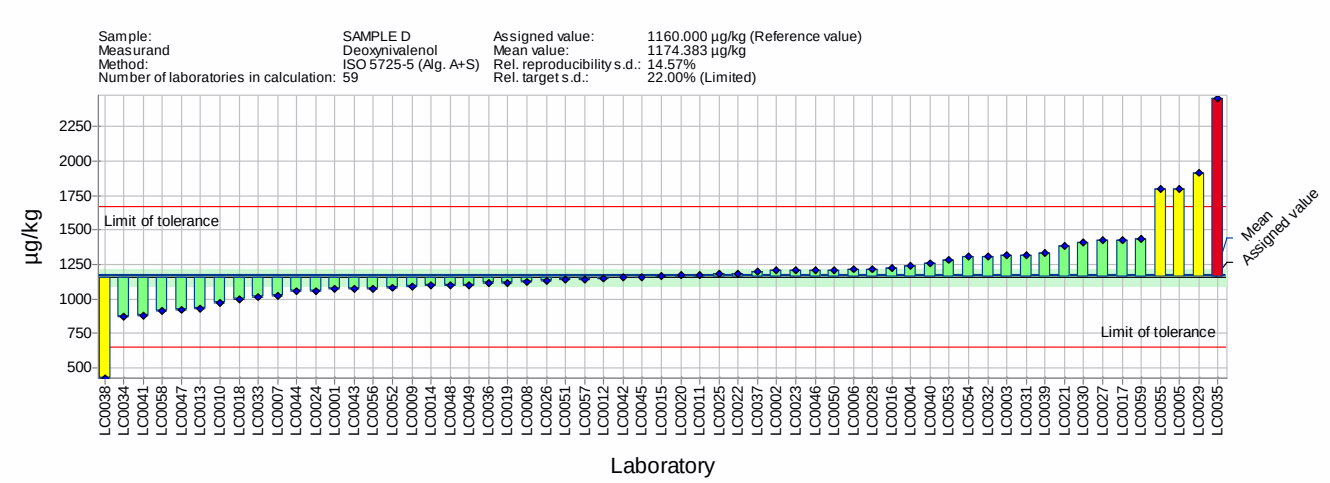


Figure 5. Sigmoidal plot of individual results reported for test Material D

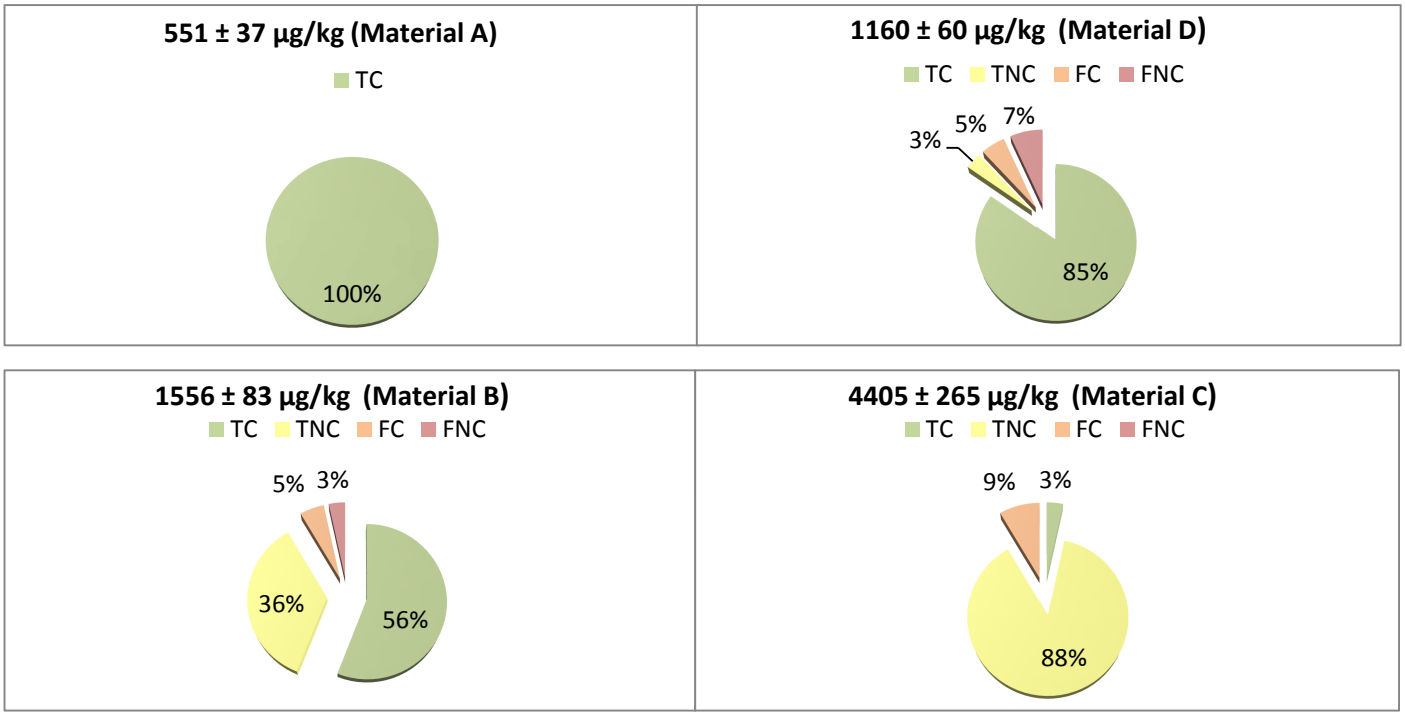


7.4 Compliance assessment

Participants were requested to assess the compliance of the test items assuming they fell into category 2.4.1 of the Annex in Commission Regulation (EC) No 1881/2006: maximum level for DON of 1250 µg/kg in unprocessed cereals other than durum wheat, oats and maize [1].

The answers received (Annex 8) are summarised in Figure 6. For material A (assigned value of 551 ± 37 µg/kg), 100 % of the laboratories correctly assessed the test item received to be compliant. For material D (assigned value of 1160 ± 60 µg/kg), 85 % of the laboratories classified correctly the test item to be compliant, whereas 3 % assumed the material to be non-compliant based on their high measurement results. Other 7 laboratories (12 %) gave an inconsistent assessment contradicting their reported results (false compliances and false non-compliances). Only 36 % of the laboratories assessed correctly Material B (1556 ± 83 µg/kg) as non-compliant, and 56 % of the participants classified appropriately (providing proper justification) the test item as compliant taking into account their measurement result and reported uncertainty. A total of 5 laboratories (8 %) gave inconsistent statements: 5 % stated the material to be compliant (false compliances), and 3 % stated the material to be non-compliant (false non-compliances). For material C (4405 ±265 µg/kg), most of the laboratories (88 %) assessed the test item correctly to be non-compliant. Two laboratories (3 %) assumed the material to be compliant based on their measurement result, while 9 % of the laboratories provided an inconsistent assessment (false-compliances).

Figure 6. Overview of the laboratory statements in the compliance assessment.
The statements are categorised as TC (true compliant), TNC (true non-compliant), FC (false compliant) and FNC (false non-compliant).



7.5 Evaluation of questionnaire

All 59 laboratories answered the questionnaire. The summary of the answers is presented in Annex 10.

Recovery rates varied from 19 to 159 % and the main techniques used for the determination of DON were LC-MS (46 % of laboratories) followed HPLC-UV (42 % of laboratories). One of the laboratories used HPLC-DAD, 1 GC-MS and five laboratories used screening methods: 4 ELISA and 1 lateral flow strips (Annex 9).

Forty-eight laboratories used a method for analysing DON in wheat for which they had been accredited. The standard analytical methods, EN 15791:2009 and EN 15891:2010 (or modified) were the most used (15 and 11 laboratories, respectively) [9,10].

No correlation between the performance and the use of an accredited method or laboratory experience (evaluated as the number of analysis per year) could be identified.

8 Conclusions

Fifty-nine laboratories (41 NRLs and 18 OCLs) participated in this study and the performance of most of the participants was satisfactory (≥ 93 %). Only 1 NRL reported a result with an unsatisfactory z-score. This confirms the analytical capabilities of the NRLs to enforce the Commission's Regulation (EC) No 1881/2006 setting the maximum levels for certain contaminants in foodstuffs.

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

Regarding compliance assessment, the majority of the participants (≥ 85 %) stated correctly that the test items were compliant (materials A and D) and non-compliant (material C). For material B, 36 % of the laboratories reported correctly that the test item was non-compliant whereas 56 % of the laboratories concluded that the test item was compliant. This is mainly due to the larger uncertainty estimations of the laboratories. The remaining laboratories (8-12 %) wrongly interpreted their analytical results. This clearly indicates that compliance assessment remains to be improved.

References

[1] EC, Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, of 19 December 2006, and successive amendments, Official Journal of the European Union, L 364/5-24 (2006).

[2] A. Astoreca, L. Ortega, C. Fígoli, M. Cardós, L. Cavaglieri, A. Bosch and T. Alconada, Analytical techniques for deoxynivalenol detection and quantification in wheat destined for the manufacture of commercial products, World Mycotoxin Journal. 10 (2017) 111-120. doi: 10.3920/WMJ2016.2121.

[3] Commission Regulation (EC) No 882/2004 of the European Parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. L191/1-141 (2004).

[4] Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis, Official Journal of the European Union, L 147/29-43 (2014).

[5] ISO/IEC 17043:2010. Conformity assessment - General requirements for proficiency testing.

[6] ISO 13528:2015. Statistical methods for use in proficiency testing by interlaboratory comparisons.

[7] A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fresenius Journal of Analytical Chemistry, 360 (1998) 359-361. doi: 10.1007/s002160050711.

[8] PROLab Plus - Software for PT programs and collaborative studies, Quodata, Dresden, Germany; http://quodata.de/en/software/for-interlaboratory-tests.html.

[9] EN 15791:2009. Determination of deoxynivalenol in animal feed. HPLC method with immunoaffinity column clean-up. European Committee for Standardization.

[10] EN 15891:2010. Foodstuffs. Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children. HPLC method with immunoaffinity column cleanup and UV detection. European Committee for Standardization.

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Annexes

Annex 1. Announcement- opening of registration

Proficiency test for the determination of deoxynivalenol (DON) in wheat

Fields marked with * are mandatory.

On behalf of the European Union Reference Laboratory for Mycotoxins (EURL Mycotoxins), I have the pleasure to announce the opening for registration of the proficiency test (PT) for the determination of deoxynivalenol (DON) in unprocessed wheat (Commission Regulation (EC) No 1881/2006).

The deadline for registration is 12th April 2017.

According to Regulation (EC) No 882/2004 it is obligatory for EU National Reference Laboratories (NRLs) to participate. The PT materials consist of 4 naturally contaminated wheat products. The dispatch of the samples is expected by the end of April 2017. Participants will have 6 weeks from the dispatch date to report back the results. Laboratories are encouraged to use screening methods according to Commission Regulation (EU) No 519/2014. Classification of the materials as "compliant" or "non-compliant" is mandatory assuming the material falls into category 2.4.1 of the Annex in Commission Regulation (EC) No 1881/2006 (1250 µg/kg DON). In addition, we will ask for quantitative results.

Further information will be sent after the registration period.

For NRLs the participation is free of charge. The participation fee for official control laboratories is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. Official control laboratories will be contacted for payment details upon registration.

Confidentiality of results is guaranteed.

Thank you in advance for your consideration.

Best regards

The Operating Manager of the EURL for Mycotoxins

* Contact person

* Second contact person

* Organisation

Department

* Address

* Postcode

* City

* Country

* Telephone number

Fax

* Email address

Additional comments

Annex 2. Sample accompanying letter

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

Geel, 25 April 2017

EURL Proficiency Test 2017 on the determination of deoxynivalenol in wheat

Dear Participant,

Please read the following information carefully before starting any analysis. This PT on deoxynivalenol (DON) in wheat aims to assess the content of DON in four naturally contaminated test samples ("Sample A", "Sample B", "Sample C" and "Sample D").

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

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

The use of screening methods according to Commission Regulation (EU) No 519/2014 is encouraged. Therefore each material **has to be classified as 'compliant' or 'non-compliant'** assuming the material falls into category 2.4.1 of the Annex in Commission Regulation (EC) No 1881/2006 (1250 µg/kg DON).

If a laboratory uses a screening method we ask you to mention the established cut-off of your method and the actual reading indicating the dimension of the signal (µg/kg, AU, etc.) in the questionnaire. If a laboratory uses a quantitative confirmatory method, we request that the laboratory reports the quantitative result corrected for recovery in (µg/kg), including the recovery rate (%) and measurement uncertainty (MU) in µg/kg for a coverage factor of 2 (k=2) in the results table.

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

The deadline for reporting the PT results is the 12th June 2017.

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

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

Elena Cubero Leon
PT Coordinator
E-mail: JRC-EURL-MYCOTOX@ec.europa.eu
Tel: +32-14-571274

Cc: Frans Verstraete, Hendrik Emons, Joerg Stroka

Annex 1: Instructions for reporting the results using RingDat.

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

User: *ringdat*

Password: *prolabdata*

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

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

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

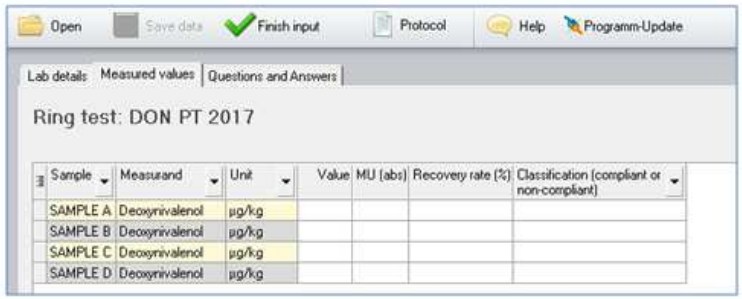
- The **"*.LA2"** file contains information about the participant – laboratory name and laboratory code.
- The **"*.LAB"** file is unique to each laboratory and contains information about the samples and measurands that have to be analysed and reported.

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

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

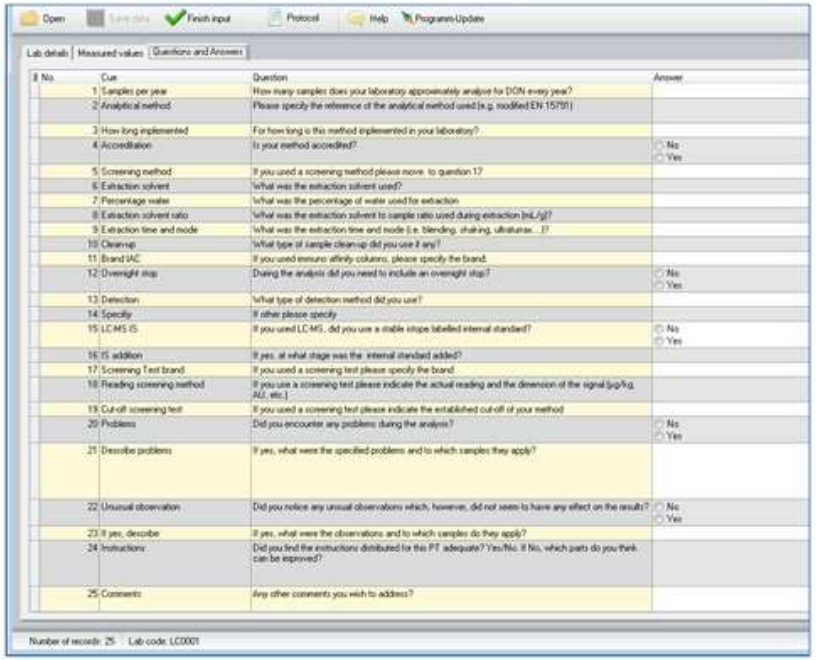
4. Fill in the results table ("Measured values") with your data. Please find below some captures of the RingDat pages that have been configured for this PT.

Figure 1 – Capture of the "Measured Values" page



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

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



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

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

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

Annex 3. Material receipt form

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

Geel, 20 September 2017

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) 4 test materials (wheat) for analysis:

- Sample A
- Sample B
- Sample C
- Sample D

b) Material receipt form

Your Signature / Stamp here:

Please e-mail the completed form to:

Elena Cubero Leon

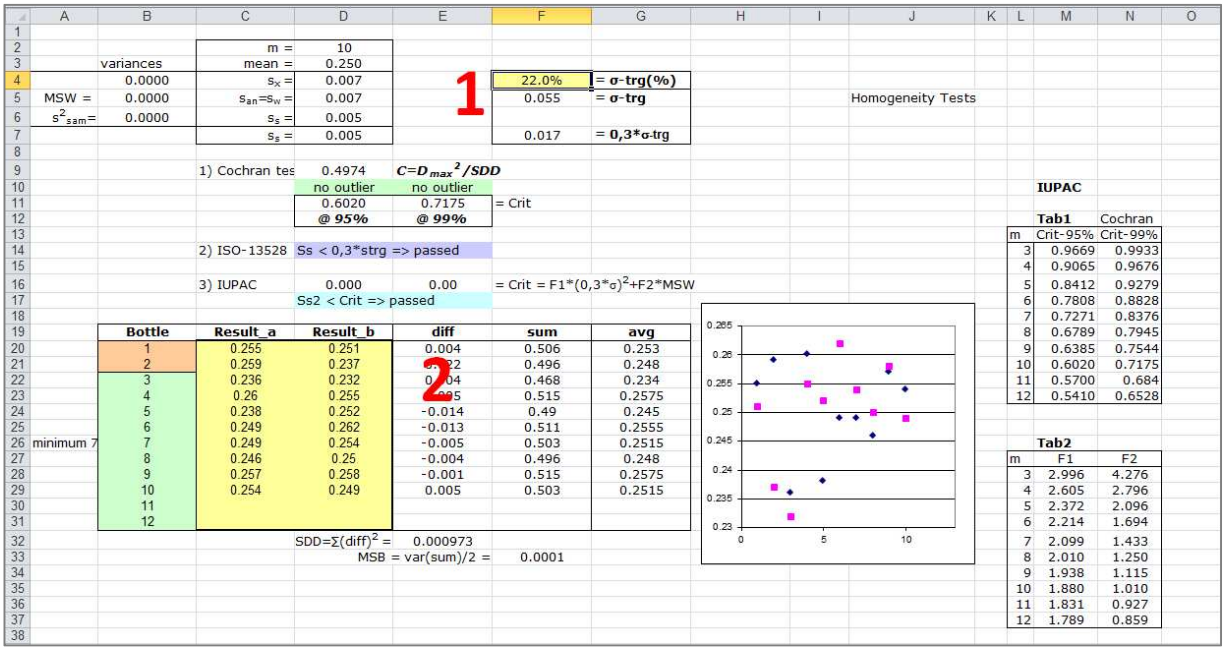
JRC-EURL-MYCOTOX@ec.europa.eu

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

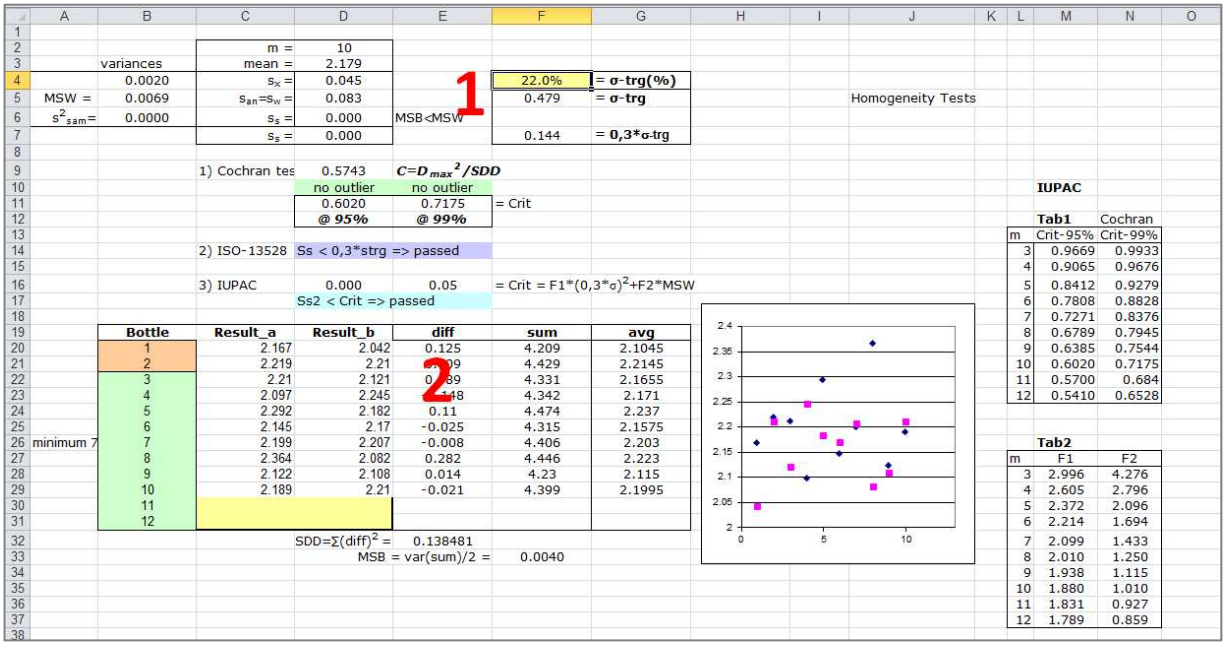
Annex 4. Homogeneity study

Homogeneity according to ISO 13528:2015	Material A	Material B	Material C	Material D
Mean (area)	0.250	2.179	1.547	1.468
$\hat{\sigma}$	0.055 (22 %)	0.479 (22 %)	0.340 (22 %)	0.323 (22 %)
0.3 $\hat{\sigma}$ (critical value)	0.017	0.144	0.102	0.097
S_X (standard deviation of sample averages)	0.007	0.045	0.063	0.045
S_W (within-sample standard deviation)	0.007	0.083	0.062	0.045
S_S (between-sample standard deviation)	0.005	0.000	0.045	0.032
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**

Material A



Material B



Material C

			m =	10											
	variances		mean =	1.547											
	0.0040		s_x =	0.063	1	22.0%	= σ-trg(%)								
MSW =	0.0038		$s_{an}=s_w$ =	0.062		0.340	= σ-trg			Homogeneity Tests					
s^2_{sam}=	0.0021		s_s =	0.045											
			$s_{\bar{s}}$ =	0.045		0.102	= 0,3*σ-trg								
		1) Cochran tes		0.5256	$C=D_{max}^2/SDD$										
				no outlier	no outlier										
				0.6020	0.7175	= Crit									
				@ 95%	@ 99%										
		2) ISO-13528		Ss < 0,3*strg => passed											
		3) IUPAC		0.002	0.02	= Crit = F1*(0,3*σ)^2+F2*MSW									
				Ss2 < Crit => passed											

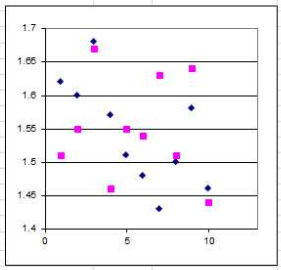
Bottle	Result_a	Result_b	diff	sum	avg
1	1.62	1.51	0.11	3.13	1.565
2	1.6	1.55	0.05	3.15	1.575
3	1.68	1.67	0.01	3.35	1.675
4	1.57	1.46	0.11	3.03	1.515
5	1.51	1.55	-0.04	3.06	1.53
6	1.48	1.54	-0.06	3.02	1.51
7	1.43	1.63	-0.2	3.06	1.53
8	1.5	1.51	-0.01	3.01	1.505
9	1.58	1.64	-0.06	3.22	1.61
10	1.46	1.44	0.02	2.9	1.45
11					
12					

minimum 7

2

SDD=Σ(diff)^2 = 0.0761

MSB = var(sum)/2 = 0.0079



IUPAC

Tab1	Cochran	
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2		
m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

Material D

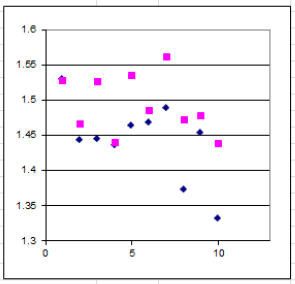
			m =	10											
variances			mean =	1.468											
0.0020			s_x =	0.045	1	22.0%	= σ-trg(%)								
0.0020			$s_{an}=s_w$ =	0.045		0.323	= σ-trg			Homogeneity Tests					
0.0010			s_s =	0.032											
			$s_{\bar{s}}$ =	0.032		0.097	= 0,3*σ-trg								
		1) Cochran tes		0.2908	$C=D_{max}^2/SDD$										
				no outlier	no outlier										
				0.6020	0.7175	= Crit									
				@ 95%	@ 99%										
		2) ISO-13528		Ss < 0,3*strg => passed											
		3) IUPAC		0.001	0.02	= Crit = F1*(0,3*σ)^2+F2*MSW									
				Ss2 < Crit => passed											

Bottle	Result_a	Result_b	diff	sum	avg
1	1.53	1.528	0.002	3.058	1.529
2	1.443	1.466	-0.023	2.909	1.4545
3	1.445	1.526	-0.081	2.971	1.4855
4	1.435	1.44	-0.005	2.875	1.4375
5	1.464	1.536	-0.072	3	1.5
6	1.468	1.485	-0.017	2.953	1.4765
7	1.488	1.562	-0.074	3.05	1.525
8	1.373	1.472	-0.099	2.845	1.4225
9	1.454	1.478	-0.024	2.932	1.466
10	1.331	1.439	-0.108	2.77	1.385
11					
12					

2

SDD=Σ(diff)^2 = 0.040109

MSB = var(sum)/2 = 0.0041



IUPAC

Tab1	Cochran	
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2		
m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

Annex 5. Stability study

Material A				
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope
4	0.00037	-0.00227	0.00199	**YES**
20	-0.00084	-0.00152	0.00031	**YES**

Material B				
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope
4	0.00060	-0.00032	0.00151	**YES**
20	-0.00044	-0.00121	0.00033	**YES**

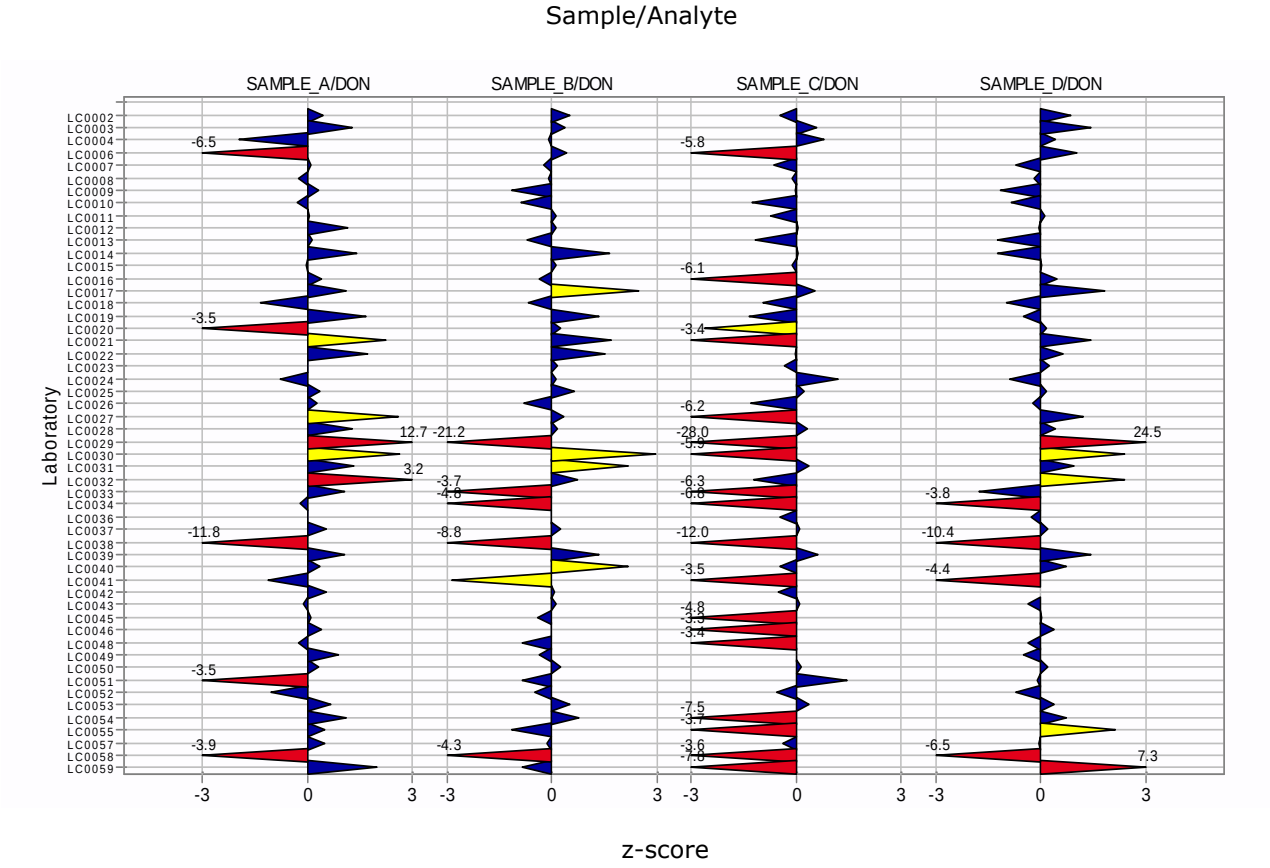
Material C				
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope
4	0.00016	-0.00101	0.00132	**YES**
20	0.00054	-0.00058	0.00166	**YES**

Material D				
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope
4	-0.00024	-0.00180	0.00131	**YES**
20	-0.00034	-0.00179	0.00112	**YES**

Annex 6. Questionnaire

No of answers	Cue	Question	Answer
59	Samples per year	How many samples does your laboratory approximately analyse for DON every year?	Less than 50 Between 50 and 100 More than 100
54	Analytical method	Please specify the reference of the analytical method used (e.g. modified EN 15791)	
54	How long implemented	For how long is this method implemented in your laboratory?	
59	Accreditation	Is your method accredited?	No Yes
8	Screening method	If you used a screening method, please move to question 17	
54	Extraction solvent	What was the extraction solvent used?	
50	Percentage water	What was the percentage of water used for extraction?	
48	Extraction solvent ratio	What was the extraction solvent to sample ratio used during extraction (mL/g)?	
54	Extraction time and mode	What was the extraction time and mode (i.e. blending, shaking, ultraturrax....)?	
49	Clean-up	What type of sample clean-up did you use if any?	
42	Brand IAC	If you used immunoaffinity columns, please specify the brand:	
55	Overnight stop	During the analysis, did you need to include an overnight stop?	No Yes
54	Detection	What type of detection method did you use?	HPLC-UV LC-MS Lateral flow strips (screening test) Other
11	Specify	If other, please specify	
33	LC-MS IS	If you used LC-MS, did you use a stable isotope labelled internal standard?	No Yes
14	IS addition	If yes, at what stage was the internal standard added?	Before extraction After extraction
12	Screening Test brand	If you used a screening test, please specify the brand	
12	Reading screening method	If you use a screening test, please indicate the actual reading and the dimension of the signal (µg/kg, AU, etc.)	
11	Cut-off screening test	If you used a screening test, please indicate the established cut-off of your method	
59	Problems	Did you encounter any problems during the analysis?	No Yes
10	Describe problems	If yes, what were the specified problems and to which samples they apply?	
56	Unusual observation	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	
4	If yes, describe	If yes, what were the observations and to which samples do they apply?	
52	Instructions	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	
19	Comments	Any other comments you wish to address?	

Annex 7. Zeta scores graphs



Annex 8. Compliance statements. The statements are categorised as TC (true compliant: green), TNC (true non-compliant: yellow), FC (false compliant: orange) and FNC (false non-compliant: red).

Laboratory code	Compliance assessment			
	Material A	Material B	Material C	Material D
Assigned value	551 ± 37 µg/kg: C	1556 ± 83 µg/kg: NC	4405 ± 265 µg/kg: NC	1160 ± 60 µg/kg: C
LC0001	TC	FC	FC	TC
LC0002	TC	TNC	TNC	FNC
LC0003	TC	TNC	TNC	TC
LC0004	TC	TC	TNC	TC
LC0005	TC	TC	FC	FC
LC0006	TC	TNC	TNC	TC
LC0007	TC	TC	TNC	TC
LC0008	TC	TC	TNC	TC
LC0009	TC	TNC	TNC	TC
LC0010	TC	TC	TNC	TC
LC0011	TC	TNC	TNC	TC
LC0012	TC	TC	FC	TC
LC0013	TC	TC	TNC	TC
LC0014	TC	TNC	TNC	TC
LC0015	TC	TC	TNC	TC
LC0016	TC	TC	TNC	TC
LC0017	TC	TNC	TNC	TC
LC0018	TC	TC	TNC	TC
LC0019	TC	TNC	TNC	FNC
LC0020	TC	TNC	TNC	TC
LC0021	TC	TNC	TNC	TC
LC0022	TC	TNC	TNC	TC
LC0023	TC	TC	TNC	TC
LC0024	TC	TNC	TNC	TC
LC0025	TC	TNC	TNC	TC
LC0026	TC	TC	TNC	TC
LC0027	TC	TC	TNC	TC
LC0028	TC	TNC	TNC	TC
LC0029	TC	TC	TC	FC
LC0030	TC	TNC	TNC	FNC
LC0031	TC	TNC	TNC	FNC
LC0032	TC	TNC	TNC	TNC
LC0033	TC	TC	TNC	TC
LC0034	TC	TC	TNC	TC
LC0035	TC	FC	FC	FC
LC0036	TC	TC	TNC	TC
LC0037	TC	TC	TNC	TC
LC0038	TC	TC	TC	TC
LC0039	TC	TNC	TNC	TC
LC0040	TC	TNC	TNC	TC
LC0041	TC	TC	TNC	TC
LC0042	TC	TC	TNC	TC
LC0043	TC	TC	TNC	TC

Laboratory code	Compliance assessment			
	Material A	Material B	Material C	Material D
Assigned value	**551 ± 37 µg/kg: C**	**1556 ± 83 µg/kg: NC**	**4405 ± 265 µg/kg: NC**	**1160 ± 60 µg/kg: C**
LC0044	TC	TNC	TNC	TC
LC0045	TC	TC	TNC	TC
LC0046	TC	FNC	TNC	TC
LC0047	TC	FC	TNC	TC
LC0048	TC	TC	TNC	TC
LC0049	TC	TC	FC	TC
LC0050	TC	TC	TNC	TC
LC0051	TC	TC	TNC	TC
LC0052	TC	FNC	TNC	TC
LC0053	TC	TC	TNC	TC
LC0054	TC	TC	TNC	TC
LC0055	TC	TC	TNC	TC
LC0056	TC	TC	TNC	TC
LC0057	TC	TC	TNC	TC
LC0058	TC	TC	TNC	TC
LC0059	TC	TNC	TNC	TNC

Annex 9. Recoveries

Laboratory code	Recovery %				Technique
	A	B	C	D	
LC0001	95	95	95	95	LC-MS
LC0002	96.25	96.25	96.25	96.25	HPLC-UV
LC0003	88.6	91	89.3	86.1	HPLC-UV
LC0004	65	65	65	65	HPLC-UV
LC0005	-	-	-	-	Screening test
LC0006	87.39	87.39	87.39	87.39	LC-MS
LC0007	83	83	83	83	HPLC-UV
LC0008	90	90	90	90	HPLC-UV
LC0009	100	100	100	100	HPLC-UV
LC0010	90.1	95.8	87.9	81.1	LC-MS
LC0011	99	99	99	99	HPLC-UV
LC0012	100	100	100	100	LC-MS
LC0013	100	100	100	100	LC-MS
LC0014	103	99	104	109	LC-MS
LC0015	101.5	101.5	101.5	101.5	HPLC-UV
LC0016	92	92	92	92	HPLC-UV
LC0017	122	148	156	159	LC-MS
LC0018	108	108	108	108	LC-MS
LC0019	103	103	103	103	ELISA
LC0020	99.8	99.8	99.8	99.8	LC-MS
LC0021	83	83	83	83	LC-MS
LC0022	100	100	100	100	HPLC-UV
LC0023	94	94	94	94	HPLC-UV
LC0024	104	101	90	103	HPLC-UV
LC0025	96.2	96.2	96.2	96.2	HPLC-UV
LC0026	94	94	94	94	LC-MS
LC0027	78	76	76	76	LC-MS
LC0028	100	100	100	100	LC-MS
LC0029	19	19	19	19	HPLC-DAD
LC0030	96	96	96	96	HPLC-UV

Laboratory code	Recovery %				Technique
	A	B	C	D	
LC0031	98	98	98	103	HPLC-UV
LC0032	106	106	106	106	ELISA
LC0033	95	95	95	93	ELISA
LC0034	92	92	92	92	HPLC-UV
LC0035	97	97	97	97	LC-MS
LC0036	86	86	86	86	HPLC-UV
LC0037	97	97	97	97	HPLC-UV
LC0038	100	100	100	100	LC-MS
LC0039	99	98	98	101	LC-MS
LC0040	97	97	97	97	LC-MS
LC0041	98.1	98.1	98.1	98.1	LC-MS
LC0042	93	93	93	93	HPLC-UV
LC0043	97	97	97	97	LC-MS
LC0044	85	85	85	85	ELISA
LC0045	93	93	93	93	HPLC-UV
LC0046	92	91	104	94	HPLC-UV
LC0047	84	84	84	84	LC-MS
LC0048	71	97	94	96	LC-MS
LC0049	86.7	86.7	86.7	86.7	LC-MS
LC0050	96	96	96	96	HPLC-UV
LC0051	82	82	82	82	LC-MS
LC0052	95	95	95	95	LC-MS
LC0053	85.1	85.1	85.1	85.1	LC-MS
LC0054	85	85	85	85	HPLC-UV
LC0055	91	91	91	91	GC-MS
LC0056	100	100	100	100	HPLC-UV
LC0057	88	88	88	88	HPLC-UV
LC0058	89.2	89.2	89.2	89.2	LC-MS
LC0059	93	93	93	93	HPLC-UV

Annex 10. Experimental details as reported by the participants in the questionnaire

Lab Code	How many samples does your laboratory approximately analyse for DON every year?	Please specify the reference of the analytical method used (e.g. modified EN 15791)	For how long is this method implemented in your laboratory?	Is your method accredited?	If you used a screening method please move to question 17	What was the extraction solvent used?
LC0001	More than 100	None, validated LC-HRMS based multianalyte method	1.5 years	No		
LC0002	Between 50 and 100	R-Biopharm Rhone Application Note	8 years	Yes		Water
LC0003	Less than 50	Rhone-Biopharm DONPREP immunoaffinity column P50/P50B application note	Less than a year	No		Water
LC0004	Less than 50	In-house method based on AOAC Vol 88, No 4 , 2005	3 years	No		Water
LC0005	Less than 50	Quantitative immunoreceptor test	three years	Yes		Distilled water
LC0006	Between 50 and 100	In house		Yes		Water
LC0007	More than 100	In house method	since 2005	Yes		water
LC0008	More than 100	EN15791	10 years	Yes		ACN/water (84/16; v/v)
LC0009	Less than 50	EN 15891	5 years	Yes		water with polyethylene glycol according to EN 15891
LC0010	More than 100		>5 years	Yes		80% ACN 20% Acetic Acid (2%)
LC0011	Between 50 and 100	modified EN 15791	Since 2010	Yes		Water
LC0012	More than 100	In-house method, CON-PV 01126, LC-MS/MS	12 years	Yes	-	Acetonitrile/Water (80/20, v/v)
LC0013	Between 50 and 100	none	2013	Yes		water/acetone/isopropanol/acetic acid
LC0014	More than 100	in-house-method	approx. 10 years	Yes		Acetonitrile, methanole, water
LC0015	More than 100	modified EN 15791	12 years	No		Yes
LC0016	Less than 50	ROMER immunoaffinity column method	7 years	No		H2O
LC0017	More than 100		1 year	Yes		acetonitrile
LC0018	Between 50 and 100	Modified EURL Mycotoxins instruction of "Determination of deoxynivalenol, aflatoxin B1, fumonisin B1&B2, T-2, & HT-2 -toxins, zearalenon and ochratoxin A in unprocesseed cereals and cereal-based compound feed by LC-MSMS"	about one year	Yes		Acetonitrile/water/formic acid (79/20/1; v:v:v)
LC0019	Between 50 and 100	ELISA	5 years	Yes		
LC0020	More than 100	Food Additives & Contaminants. Part A. 2008, 25(4), 472-489.	4 years	Yes		ACN:H2O
LC0021	Less than 50	in-house method	5 years	Yes	Not used	methanol + water 6+4 vol/vol
LC0022	More than 100	15891 L 15.00-09	2014	Yes		Water
LC0023	More than 100	EN 15891	since 2002	Yes	no	5 g PEG + 200 mL H2O
LC0024	Less than 50	EN 15891	5 years	Yes		water
LC0025	Less than 50	internal method	since 2012	Yes		water

Lab Code	How many samples does your laboratory approximately analyse for DON every year?	Please specify the reference of the analytical method used (e.g. modified EN 15791)	For how long is this method implemented in your laboratory?	Is your method accredited?	If you used a screening method please move to question 17	What was the extraction solvent used?
LC0026	More than 100	In house method build from several references		Yes		Acetonitrile 85%, water 15%
LC0027	Less than 50	Journal of Chromatography A, 1400 (2015) 91 - 97	2 years	Yes		methanol :water
LC0028	More than 100		4 years	Yes		80:20 acetonitrile:water
LC0029	Less than 50	STN EN 15791 STN EN 15891	5 years	Yes		water
LC0030	More than 100	In house method	4 years	Yes		Water
LC0031	Less than 50	modified EN 15791	7 years	No		water
LC0032	Between 50 and 100	Protocol Elisa R- Biopharm	9 years	Yes		
LC0033	More than 100	ELISA	10 years	No		
LC0034	Between 50 and 100	SR EN 15791 DON PREP Immunoaffinity column	2011	Yes	-	water
LC0035	More than 100	LCMSMS	10 YEARS	Yes	NO	ACETONITRILE
LC0036	Less than 50	UNI EN 15891	06 months	No		water
LC0037	Between 50 and 100	modified EN 15791	since 2005	Yes		water
LC0038	More than 100	house method	more then 10 years	Yes		Acetonitril
LC0039	More than 100	internal method	6 years	Yes 2	no	water
LC0040	More than 100	Zachariasova M, et al, Analytica Chimica Acta, 662 (2010), 51-61	5 years	Yes 2		acetonitrile/1%formic acid
LC0041	Between 50 and 100	CEN/TC275/WG5 N672 (modified)	one year	Yes 2		acetonitrile/water
LC0042	Between 50 and 100	modified EN UNI 15791		No		water
LC0043	More than 100	EN 15791	about 8 years	Yes 2		water
LC0044	Less than 50	ELISA	new method	No		
LC0045	More than 100	in house validated (based on CEN 15891	15 years	Yes		H2O
LC0046	More than 100	EN 15791	>3 year	Yes		Ultra pure water
LC0047	More than 100	Internal method	3 years	Yes		methanol
LC0048	More than 100	In-house method		No		Water
LC0049	More than 100		4 Year	Yes		1% acetic acid in acetonitril (quechers method)
LC0050	Between 50 and 100	EN 15891:2010	10 years	Yes		water (+PEG)
LC0051	Between 50 and 100	In-house method	since 2009	Yes		acetonitrile, water, formic acid
LC0052	More than 100	Romerlabs mycosep	about 10 years	Yes	no	ACN:Water
LC0053	Less than 50		5 years	Yes		Acetonitrile:Water:Acetic acid
LC0054	More than 100	Modified UNI EN 15891:2010	two months	Yes		water
LC0055	Between 50 and 100	In-house method	Since 2007	Yes		Acetonitril-water
LC0056	Less than 50	EN 15891		Yes	NO	water
LC0057	More than 100	Susan J. MacDonald, Danny Chan, and Paul Brereton, Roger Wood, 2005, Determination of Deoxynivalenol in Cereals and Cereal Products by Immunoaffinity Column Cleanup with Liquid Chromatography: Interlaboratory Study, J. Assoc. Off. Anal. Chem. Int., 88, (4), 1197-1204. (Equivalent to EN 15891:2010)	>15 years	Yes		Water
LC0058	Between 50 and 100	P50/V13/19.01.15 R-Biopharm	8 years	Yes		water
LC0059	Between 50 and 100	modified 15891	since 2014	Yes		water

Lab Code	What was the percentage of water used for extraction	What was the extraction solvent to sample ratio used during extraction (mL/g)?	What was the extraction time and mode (i.e. blending, shaking, ultraturrax....)?	What type of sample clean-up did you use if any?	If you used immunoaffinity columns, please specify the brand:	During the analysis did you need to include an overnight stop?
LC0001						
LC0002	100	200 ml/25 g	Ultra Turrax for 2 minutes	Centrifuge and filter	R-Biopharm Rhone DONPrep	No
LC0003	100%	8	60 minutes. Shaking	IAC	Tecna IClean C+ DON	No
LC0004	80%	4ml/g	3 minutes ultr-turrax; 15 minutes centrifugation	Immunoaffinty	r-Biopharm	No
LC0005	50 mL	10 g sample and 50 mL distilled water	shaking			No
LC0006	20	5	20, shaking	NA	NA	No
LC0007	100%	20 mL/g	half an hour in ultrasonic bath	immuno affinity columns	R-Biopharm	No
LC0008	16 %	4	120 min; stiring	IAC	r-Biopharm	No
LC0009	100	8	30 min stirring with magnetic stirrer	immunoaffinity column	r-biopharm DONPREP	No
LC0010	18%	8	shaking for 2 x 45 min	Filtration and degreasing using 5 mL n-hexane		No
LC0011	100%		blanding and shaking	IAC	Vicam	No
LC0012	20%	8 mL/g	30 minutes shaking	None	-	No
LC0013	40	17.4/4	60 min / overhead shaking	Modified QuEChERS		No
LC0014	50	different, e. g. 50/5	2 hours, stirring	sedimentation, freezing, centrifugation	none	Yes
LC0015	100	40 ml/5g	shaking 2 hours	Immuno affinity columns	R-BIOPHARM RHONE LTD DONPREP	Yes
LC0016	100%	60/6	30 min, shaker	immunoaffinity columns	ROMER	No
LC0017	50	20/5	10 min, shaking	d-SPE		No
LC0018	20	5 mL/g	60 min shaking	none		No
LC0019						
LC0020	30%	4/1	1h shaking			No
LC0021	40%	10 mL/g	60 min, rotary shaker	IAC	VICAM Myco6+1	No
LC0022	100	100 ml water / 10 g Probe	1 h	IAC	r-biopharm	No
LC0023	100%	8 (200 mL/25g)	Waring blendor 3 min	IAC Don Prep	RBiopharm	No
LC0024	100%	40ml/g	3min blending	immuno affinity column	R-Biopharm	No
LC0025	100%	8	Blending	immuno affinity columns	R-Biopharm Rhône LTD	No
LC0026	15%	10	60 min, shaking	SPE Mycosep 227		No
LC0027	30%	50 ml extraction solvent for 12,5 grams of sample	ultraturrax for 3 minutes	immunoaffinity columns	DZT MS-PREP, R-BiopharmRhone Ltd (Glasqow, UK);	No
LC0028	20	4/1	shaking for 20 mins	filter		No
LC0029	100 %	12 500 mg/L	shaking	IAC	Jemo	Yes

Lab Code	What was the percentage of water used for extraction	What was the extraction solvent to sample ratio used during extraction (mL/g)?	What was the extraction time and mode (i.e. blending, shaking, ultraturrax....)?	What type of sample clean-up did you use if any?	If you used immunoaffinity columns, please specify the brand:	During the analysis did you need to include an overnight stop?
LC0030	25 g sample/200 ml solvent		30 minutes shaking	IAC	R-biopharm	No
LC0031	100	50	1 hour	IAC	DONPREP	No
LC0032						
LC0033						No
LC0034	100%	-	1 h	Immunoaffinity column	R-Biopharm Rhone	No
LC0035	85:15 (ACETONITRILE:WATER)		Ultraturrax		PHENOMENEX	No
LC0036	100%	200/25	30 min, shaking	filtration	R_BIOPHARM_RHONE LTD	No
LC0037	100%	10	shaking 60 min	IAC	R-BIOPHARM RHONE LTD	No
LC0038	16	2 ml/g	15min extraction time and 2 min shaking with collomix	Romer Labs MycoSep 227 Trich		No
LC0039	100%	8	shaking 30 min and centrifugate	immuno affinity columns	R-Biopharm	No
LC0040	50	5mL/1g	30 min, shaking			No
LC0041	16%	5:1	one hour shaking	Oasis HLB 3cc (60 mg)	Waters	No
LC0042			Shaking	Immuno Affinity column	Bio-Pharm	No
LC0043		200/25	1 hour by shaking + 10 min centrifuge	IAC	R-Biopharm	No
LC0044						
LC0045	100%	8	blending, 3 min	IAC	R-Biopharm	No
LC0046			2h, shaking	IAC	Protealummun	No
LC0047			ASE - 20 min			No
LC0048	100%	0,125	Shaking	IAC	Romer	No
LC0049	50%	4 ml/g	30 min. shaking	-	-	No
LC0050	100%	200 mL/25g	horixontal shaking, 1 hour	immunoaffinity column	R-Biopharm Rhone	No
LC0051	25	5	shaking 60 minutes	Filter	-	No
LC0052	16	2/100, 5/100, 10/100	2 hours shaking	SPE	no	No
LC0053	20	6 sample A, 4 Sample B-D	Shaking 30 min	Non		No
LC0054	100	8	ultraturrax for 2 minutes	IAC	r-Biopharm	No
LC0055	16	100/10	Shaking, 1 hour	MycoSep 227 Trich+ columns (Romer Labs)	N.A.	No
LC0056	100	8	blending	immunoaffinity	Biopharm-Rhone	No
LC0057	100%	160 mL water / 20g sample	Blending 3 minutes high speed Ultra Turrax	Immunoaffinity column	R-Biopharm Rhone DONPREP	No
LC0058	100%	8	3 minutes	immuno-affinity	R-Biopharm	Yes
LC0059	100	200 ml water to 25 g sample	shaking	immuno affinity column	Romer	No

Lab Code	What type of detection method did you use?	If other please specify	If you used LC-MS, did you use a stable istope labelled internal standard?	If yes, at what stage was the internal standard added?	If you used a screening test please specify the brand	If you use a screening test please indicate the actual reading and the dimension of the signal (µg/kg, AU, etc.)	If you used a screening test please indicate the established cut-off of your method
LC0001					Brand of what? We're using a Thermo Scientific Q-Exactive	ion count, internal standard calibration using 13C-labelled DON, added just prior to analysis	66 µg/kg
LC0002	HPC-UV						
LC0003	HPC-UV	LC-MS-MS	No				
LC0004	HPC-UV						
LC0005	Lateral flow strips (screening test)		No		Charm	microgram/kilogram	
LC0006	LC-MS	UHPLC-HRMS2	No				
LC0007	HPC-UV						
LC0008	HPC-UV						
LC0009	HPC-UV						
LC0010	LC-MS		No	After extraction			
LC0011	HPC-UV						
LC0012	LC-MS	-	Yes	After extraction	-	-	-
LC0013	LC-MS		Yes	After extraction			
LC0014	LC-MS		Yes	After extraction			
LC0015	HPC-UV						
LC0016	HPC-UV						
LC0017	LC-MS		No				
LC0018	LC-MS		Yes	After extraction	ELISA Ridascreen DON (R-Bioprarm)	µg/kg	not established, screening method is not used for official control samples.
LC0019			No		R-biopharm	ug/kg	1050
LC0020	LC-MS		No				
LC0021	LC-MS		Yes	After extraction	Not used	Not used	Not used
LC0022	HPC-UV						
LC0023	HPC-UV						
LC0024	HPC-UV		No				
LC0025	HPC-UV	-			-	-	-
LC0026	LC-MS		Yes	After extraction			
LC0027	LC-MS	LC-MS/MS	No				
LC0028	LC-MS		Yes	After extraction			
LC0029	Other	HPLC-DAD					
LC0030	HPC-UV						
LC0031	HPC-UV		No				
LC0032					Ridascreen R-Biopharm	ug/kg	1115,81 ug/kg
LC0033					RIDASCREEN R-BIOPHARM	sample A 573 µg/kg, 0,708 AU; sample B 1134 µg/kg, 0,502 AU, sample C 2693 µg/kg,	1002 µg/kg

Lab Code	What type of detection method did you use?	If other please specify	If you used LC-MS, did you use a stable istope labelled internal standard?	If yes, at what stage was the internal standard added?	If you used a screening test please specify the brand	If you use a screening test please indicate the actual reading and the dimension of the signal (µg/kg, AU, etc.)	If you used a screening test please indicate the established cut-off of your method
						0,301 AU; sample D 943 µg/kg, 0,552 AU	
LC0034	HPC-UV	-	No		-	-	-
LC0035	LC-MS						
LC0036	Other	H P L C UV					
LC0037	HPC-UV						
LC0038	LC-MS		Yes	After extraction			
LC0039	LC-MS		Yes	After extraction			
LC0040	LC-MS		Yes	After extraction			
LC0041	LC-MS		Yes	After extraction			
LC0042	HPC-UV		No				
LC0043	LC-MS		No				
LC0044					R-biopharm	microg/kg	not established yet
LC0045	HPC-UV						
LC0046	HPC-UV						
LC0047	LC-MS		Yes	Before extraction			
LC0048	LC-MS		No				
LC0049	LC-MS		No				
LC0050	HPC-UV		No				
LC0051	LC-MS	-	No				
LC0052	LC-MS		No				
LC0053	LC-MS		Yes	After extraction			
LC0054	HPC-UV						
LC0055	Other	GC-MS					
LC0056	HPC-UV		No				
LC0057	HPC-UV						
LC0058	LC-MS		No				
LC0059	HPC-UV	-			-	-	-

Lab Code	Did you encounter any problems during the analysis?	If yes, what were the specified problems and to which samples they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If yes, what were the observations and to which samples do they apply?	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	Any other comments you wish to address?
LC0001	No		No			
LC0002	No		No		Yes	
LC0003	No		No		Yes	
LC0004	Yes	Sample A was very slow passing through IAC	No		Yes	Samples have not been corrected to recovery
LC0005	No		No		Yes	
LC0006	No		No		yes	
LC0007	No		No		Yes	
LC0008	No		No		yes	
LC0009	No		No		Yes.	
LC0010	No		No		From the accompanying letter it was not clear how many values per sample need to be submitted.	Thank you!
LC0011	No				Yes	
LC0012	No	-	No			
LC0013	No		No		Yes	No
LC0014	Yes	- large range of DON-contents in the 4 samples (about 600 to 4500 µg/kg) - huge suppression of DON by the matrix, but only in sample A (about 50 %)	No		Yes	
LC0015	No		No			
LC0016	Yes	slow filtration of sample A				
LC0017	No		No		yes	no
LC0018	No		No		I would have preferred a little more information how to report the result for both screening and confirmation method. Now there is no space for example to report the actual readings for the screening method, since measured values are occupied with confirmation method´s results.	Actual reading for the screening method: Sample A: 547 µg/kg (complient); Sample B: 2570 µg/kg (non-complient); Sample C: 7202 µ/kg (non-complient); Sample D: 1460 µg/kg (non-complient)
LC0019	No		No		Yes	The uncertainty of measurement is 14,63%
LC0020	No		No		Yes	
LC0021	No		No		Yes, it was clear	
LC0022	No		No		yes	
LC0023	No		No		Yes	
LC0024	No		No		Yes	
LC0025	No	-	No	-	Yes	-
LC0026	No		No		Yes	No
LC0027	1				Yes	
LC0028	No		No		yes	

Lab Code	Did you encounter any problems during the analysis?	If yes, what were the specified problems and to which samples they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If yes, what were the observations and to which samples do they apply?	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	Any other comments you wish to address?
LC0029	No		No		YES	no
LC0030	No		No		Yes	
LC0031	No		Yes	more difficult filtration - sample A	yes	
LC0032	No		No		yes	
LC0033	No		No		yes	Notifications The results for samples A, B, C and D are not corrected for recovery. The measurement uncertainty for sample A is 86 µg/kg, for sample B is 170 µg/kg, for sample C is 404 µg/kg and for sample D is 141 µg/kg. I could not open the LA2 file.
LC0034	No	-	No	-	Yes	-
LC0035	No		No		YES	
LC0036	No		No		yes	
LC0037	No		No		Yes	
LC0038	No		No		Yes	
LC0039	No		No		yes	
LC0040	No		No		Yes	
LC0041	No		No			
LC0042	No		No		yes	
LC0043	No		No		yes	
LC0044	No		No		Yes	
LC0045	No		No		Yes	-
LC0046	No		No		Yes	
LC0047	No		No		Yes	
LC0048	Yes	Samples B and C were repeated several times due to loss of results and poor recovery. We have noticed the great impact of the matrix (the samples are quite different from the usual wheat samples we analyze in our laboratory).	No		Yes	No
LC0049	No		No		Yes	
LC0050	No		No		Yes	The concentrations measured in the samples C and D exceeded the calibration curve and therefore, were diluted

Lab Code	Did you encounter any problems during the analysis?	If yes, what were the specified problems and to which samples they apply?	Did you notice any unusual observations which, however, did not seem to have any effect on the results?	If yes, what were the observations and to which samples do they apply?	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	Any other comments you wish to address?
						(the final sample) and re-analyzed the next day.
LC0051	No		No			Sample C was diluted 10 times to be in the range of the calibration curve
LC0052	No		No		yes	
LC0053	No		Yes	Sample A needed more extraction solvent than usually used in the method	Yes	
LC0054	No		No		YES	
LC0055	No	N.A.	No		Yes.	N.A.
LC0056	No		No		Yes	No
LC0057	Yes	Not a problem as such but a modification to the method was used. Sample C exceeded our normal calibration range so further portions of the initial extract were diluted (x5 and x10) and cleaned up to: 1) make sure the capacity of the IAC clean-up column had not been exceeded and 2) to bring the extracts run on HPLC within calibration range.	No		We have no record of receiving the reporting files and had to request them on the reporting date. While we understand we should have checked this earlier, we would normally expect to receive them at the same time as the samples, so we each presumed someone else in the team had them! Not a big problem as they were sent immediately but caused us some confusion.	
LC0058	Yes	fluctuations in recovery	No			/
LC0059	No		No		yes	-

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