Proficiency test for deoxynivalenol (DON), acetyl-DONs and DON-3G in cereals

EURL-PT-MP01 (2018)

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Distribution list:
- Participating laboratories
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Contents

Summary

A proficiency test (PT) for quantitative of deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON), and deoxynivalenol-3-glucoside (DON-3G) in wheat and maize was organised by the European Union Reference Laboratory for mycotoxins & plant toxins between March-June 2018. DON is a regulated mycotoxin in the EU. Acetyl-DONs and DON-3G were included in this PT because data collection and monitoring is recommended by EFSA, and insight in analytical performance is needed also for these substances. The primary goal was to assess the proficiency of National Reference Laboratories (NRLs).

In total 50 participants from 29 countries registered (Annex 1). This included NRLs from all EU member states, and a number of official laboratories.

Two food/feed materials, wheat (A) and maize (B), were prepared containing DON, 3-Ac-DON, 15-Ac-DON, and DON-3G. The starting materials were naturally contaminated with low levels of DON, and in case of maize also with 15-acetyl-DON and DON-3G. Levels were artificially increased by spiking with DON, 3-Ac-DON and 15-Ac-DON, and wheat also with DON-3G. Both materials were sufficiently homogeneous and stable during the course of the PT. Each participant received one test sample per material.

The assigned values were derived from the consensus of the results submitted by the participants and ranged from 35 to 750 µg/kg for the different mycotoxins. The proficiency of the participants was assessed through z-scores, calculated using the assigned value and a relative target standard deviation of 25%.

All participants submitted results for DON and satisfactory z-scores were obtained by all participants exept 2. Acetyl-DONs and DON-3G were covered by less than half and less than one third of the laboratories, respectively. The laboratories that did have these mycotoxins in their scope had adequate performance in most cases (≥79%). In this PT, four false positives and two false negatives were reported, all related to 15-acetyl-DON. In some cases, the limits of quantification (LOQ) were high in relation to typical occurrence data.

Approximately two third of the laboratories used methods based on LC-MS/MS. The others mainly used methods based on LC-UV involving an IAC clean-up. The interlaboratory reproducibility (RSD_R) ranged from 14% to 28% without clear dependency regarding the mycotoxin or concentration.

Characteristics of the PT materials and the outcome of this PT are summarised in Table 1.

Table 1 *Summary of proficiency test parameters and participants' performance.*

Mycotoxin	Matrix	Assigned value (µg/kg)	Uncert. (µg/kg)	Robust RSD$_R$[1] %	Included in scope of labs		No of labs reporting:		
					No	%	quant value	<LOQ	FN
DON	A	572	15.5	15%	50	100%	50	0	0
	B	753	21.5	16%			50	0	0
3-Ac-DON	A	34.5	2.16	21%	22	44%	19	3	0
	B	93.4	4.53	18%			22	0	0
15-Ac-DON	A	<20	-	-	22	44%	9[2]	13	0
	B	154	11.6	26%			20	2	2
DON-3G	A	209	19.0	28%	16	32%	16	0	0
	B	35.1	1.91	14%			11	5	0

Mycotoxin	Matrix	Assigned value (µg/kg)	z-scores[3]			Labs out of 50 with acceptable z-score	
			satisfactory	questionable	unsatisfactory	No	
DON	A	572	96%	0%	4%	48	96%
	B	753	98%	0%	2%	49	98%
3-Ac-DON	A	34.5	79%	0%	21%	15	30%
	B	93.4	95%	0%	5%	21	42%
15-Ac-DON	A	<20	-	(1xFP)	(3xFP)	-	-
	B	154	86%	0%	14%	19	38%
DON-3G	A	209	88%	6%	6%	14	28%
	B	35.1	91%	0%	9%	10	20%

Matrix: A= Wheat, B= Maize

[1] robust relative standard deviation (interlaboratory RSD based on participants' results)

[2] of which four results were false positives

[3] calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted here as unsatisfactory z-score.

[4] the number and percentage here means: mycotoxin determined, at sufficiently low LOQ to be quantified, and obtaining a satisfactory z-score.

1 Introduction

Deoxynivalenol (DON) is a secondary fungal metabolite produced by *Fusarium* species growing on the cereals in the field, especially at temperate climates. It is one of the most frequently occurring mycotoxins in food and feed. Mainly cereals and cereal-based products like pasta, bread and beer are affected. Chemically, DON is classified as type-B trichothecene. In addition to DON, the structurally related acetylated DON and modified forms of DON (e.g. plant-conjugates) have been found in the same type of matrices, of which 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), and DON-3-glucoside (DON-3G) are the most relevant ones. In a scientific opinion by EFSA [1], the relative concentrations of 3-Ac-DON, 15-Ac-DON and DON-3G to DON were estimated as 10%, 15% and 20%, respectively. In the EFSA opinion, a group-TDI of 1 µg/kg bw per day for the sum of the four DON forms has been set, and a group-ARfD of 8 µg/kg bw per eating occasion. In current EU legislation maximum levels have been set for DON in food [2] ranging from 200 to 1750 µg/kg. In feed guidance values have been set at 0.9 to 12 mg/kg [3]. Although the acetyl-DONs and DON-3G are not yet included in legislation, their monitoring is recommended [1,4] and therefore the DON-derivatives were included in this proficiency test.

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [5] and is demanded by ISO/IEC 17025:2017 [6]. Organisation of proficiency tests (PT) is one of the tasks of European Union Reference Laboratories (EURLs) [7]. Here the primary goal is to assess the proficiency of the National Reference Laboratories (NRLs). To facilitate NRLs in their task, official laboratories (OLs) can also participate, in consultation with their NRL.

2 PT Material

2.1 Scope of the PT

This proficiency test focused on the mycotoxins DON, 3-Ac-DON, 15-Ac-DON and DON-3G in food and feed, using wheat and maize as representative matrices. The target concentrations aimed for (see Table 2) took regulatory limits and commonly found concentrations into account. Levels for the acetyl-DONs and DON-3G included enhanced levels because this was the first time these derivatives were included in an EURL-PT for mycotoxins. The proficiency test was carried out according to ISO/IEC 17043:2010 [8]. At the time of conduct not all of these analyte/matrix combinations were yet part of the accreditation scope, this was achieved in July 2018.

Table 2   *Target concentrations µg/kg of mycotoxins in the PT materials.*

Material	Target concentrations (µg/kg)			
	DON	3-Ac-DON	15-Ac-DON	DON-3G
A	400	100	100	200
B	750	100	150	

2.2 Material preparation

For preparation of the two PT materials A and B, wheat flour and maize flour were used. The starting materials were naturally contaminated with low levels of DON, and in case of maize also 15-acetyl-DON and DON-3G. Levels were artificially increased by spiking with DON, 3-Ac-DON and 15-Ac-DON, and wheat also with DON-3G. For each material, four kilograms were first fortified by adding a solution of a mycotoxin mix in acetonitrile, aiming at the levels as presented in Table 2. The materials were mixed with approximately six litres of water, homogenized using an industrial mixer according to an in-house standard operating procedure [9]. The fortified slurries were freeze-dried, homogenized in a Stephan cutter, and stored in the freezer until use.

2.3 Sample identification

After homogenization, materials A and B were divided into sub-portions of approximately 35 grams and stored in polypropylene, airtight closed containers of 125 ml. After preparation the containers were stored in the freezer until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was EURLPT-MP 01/xxx, in which the three-digit number of the code was automatically generated by the web application. One sample set was prepared for each laboratory consisting of one randomly selected sample of each material A and B. The codes of the samples for each sample set are presented in Annex 2. For homogeneity and stability testing, randomly selected containers of materials A and B were used.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, ten containers of materials A and B were analysed in duplicate for DON, 3-Ac-DON, 15-Ac-DON and DON-3G. The method of analysis is described in detail

in [10]. In brief, DON and related mycotoxins were extracted from the homogenised sample material after addition of water, by shaking with acidified acetonitrile. After a salt-induced phase partitioning step and centrifugation, an aliquot of the acetonitrile phase was dried with magnesium sulfate. After addition of isotopically labelled internal standards for each of the four mycotoxins, an aliquot of extract was taken, evaporated to dryness, and reconstituted in methanol/water. Analysis was then done by high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The homogeneity of the materials was assessed according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [11] and ISO 13528:2015 [12]. The results of the homogeneity study, grand mean with the corresponding RSD_r, are presented in Table 3, and the statistical evaluation of materials A and B is presented in Annex 3. Both materials proved to be sufficiently homogeneous for this PT.

Table 3 *Concentrations of mycotoxins in material A and B obtained during homogeneity testing.*

Material code	DON		3-Ac-DON		15-Ac-DON		DON-3G	
	Conc. (µg/kg	RSD_r (%)	Conc. (µg/kg)	RSD_r (%)	Conc. (µg/kg)	RSD_r (%)	Conc. (µg/kg)	RSD_r (%)
A	536	5.2	31.8	2.9	<20*	(27*)	261	6.8
B	730	5.7	98.8	4.0	144	8.6	24.9	8.6

* below lowest validated level, indicative concentration 6 µg/kg with RSD_r of 27%.

In material A (wheat), the concentrations of the acetyl-DONs were much lower than the anticipated target concentrations. During preparation of this material, the slurry mixing with water at ambient temperature took relatively long and it was hypothesized that acetyl-DON might be (enzymatically) de-acetylated. A follow up experiment in which the wheat and maize starting materials were spiked individually with the acetylated DONs, slurried with water, and left for 4 and 24 hours confirmed conversion of the acetylated DONs into DON in the wheat flour. In maize flour, no 15-Ac-DON and only very minor 3-Ac-DON conversion occurred.

2.5 Stability of the materials

The stability of the mycotoxins in the PT materials was assessed according to [11,12]. At the day of distribution of the PT samples, six randomly selected containers of each material A and B were stored at <-70°C. Under these conditions it is assumed that the mycotoxins are stable in the materials. Another twelve containers remained stored in the freezer. In addition, to mimic a possible thaw situation during transport, six containers were stored at room temperature for one day and then stored again in the freezer.

On June 5th, 2017, 43 days after distribution of the samples, for each of the storage conditions (<-70°C, freezer, one-day room temperature) six samples of materials A and B were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes occurred [11,12] in the materials stored in the freezer or stored at room temperature for one day. A consequential instability is observed when the average value of an analyte in the samples stored in the freezer or stored at room temperature for one day is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-70°C. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 4. In none of the mycotoxin/storage condition combinations, a consequential difference was observed. The mycotoxins in the materials were therefore considered stable for the duration of the PT.

3　Organisational details

3.1　Participants

This proficiency test focused on the mycotoxins DON, 3-Ac-DON, 15-Ac-DON and DON-3G in food and feed, using wheat. Invitations to the NRL network were sent out on 7th of March 2018 (Annex 5). Fifty laboratories registered for the PT (Annex 1). This included 39 NRLs (38 from EU countries and one from Serbia), ten OLs, and one external laboratory. Each participant was asked *a priori* to indicate which compounds were included in the scope of their method. The participants were asked to report the results through an existing web application designed for proficiency tests organised by RIKILT.

3.2　Material distribution and instructions

Each of the participants received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding number, consisting of two coded samples (Annex 2) were sent to the participants on April 23th 2018. The sample sets were packed in an insulation box containing dry ice and were dispatched to the participants immediately by courier. The samples were accompanied by a letter describing the requested analysis (Annex 6) and an acknowledgement of receipt form. By e-mail the participants received instructions on how to use the web application to report the results.

The participants were asked to store the samples in the freezer and to analyse the samples according to their routine method. A single analysis result for the mycotoxins in each sample was requested. The deadline for submitting the quantitative results was June 4th, 2018, allowing the participants six weeks for the analysis.

All samples were received in good order by the participants. Results were submitted within the deadline with two exceptions. Participants PT052 and PT065 were unable to report results in time (a.o. due to instrument problems).

Participants were asked to provide information on their analysis method (extraction solvent/procedure, clean-up procedure, internal standards used, detection technique, limit of detection, limit of quantification).

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [11], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [12] in combination with the insights published by the Analytical Methods Committee [13,14] regarding robust statistics.

The evaluation is based on assigned values and the standard deviation for proficiency assessment (σP). From this, z-scores are calculated to classify the participants' performance. Details on the methods used for the statistical evaluation can be found in the background document 'EURL-MP PT performance assessment' on the EURL-MP website.

4.1 Calculation of the assigned value

The consensus value based on the participants' results (NRLs and OLs) was used as the assigned value. The robust mean was used as consensus value in this PT. The values and their uncertainties are summarised in Table 1 in the summary section. Consensus values could be established for all analytes in both materials, except for 15-Ac-DON in material A (wheat) which was below the LOQ as used by the EURL-MP and below the LOQ of the majority of the participants.

4.2 Standard deviation for proficiency assessment (σP)

A fixed relative target standard deviation for proficiency assessment of 25% was used, irrespective the mycotoxin, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and best practises for mycotoxin and plant toxin determination in food and feed. The rationale behind this is provided in the background document 'EURL-MP PT performance assessment' on the EURL-MP website.

4.3 Quantitative performance (z-scores)

For evaluation of numerical results submitted by the participant, z-scores are calculated based on the assigned value, its uncertainty, and the standard deviation for proficiency assessment (σ_P). When the uncertainty of the assigned value is negligible and no instability of the analytes in the PT material is observed, z-scores are calculated by:

$$Z = \frac{x - C}{\sigma_p}$$

Equation 1

where:

z   =   z-score;
x   =   the result of the laboratory;
C   =   assigned value, here the consensus value;
σ_P =   standard deviation for proficiency assessment.

The z-score compares the participants' deviation from the assigned value, taking the target standard deviation accepted for the proficiency test into account, and is interpreted as indicated in Table 4.

Table 4   *Classification of z-scores.*

$\|z\| \leq 2$	Satisfactory
$2 < \|z\| < 3$	Questionable
$\|z\| \geq 3$	Unsatisfactory

If not negligible, the uncertainty of the assigned value and, if applicable, instability of analytes in the PT material, are taken into account in the determination of the z-scores. If applicable, this is indicated by assigning a z'-, z_i-, or z_i'-score. For details see the background document 'EURL-MP PT performance assessment' on the EURL-MP website.

In this PT, the uncertainty of the assigned value for DON-3G in material A and 15-Ac-DON in material B were not negligible and taken into account in the assignment of the z-score (z'). In all other cases, the uncertainty of the assigned value was negligible. No instability of the analytes in the PT material was observed.

4.4   Evaluation of non-quantified results

In case the participant reported '<[value]', i.e. below their limit of quantification (LOQ), 'proxy-z-scores' were calculated as a way to assess possible false negatives and to benchmark the LOQ relative to the assigned value and the LOQ of the other participants.

A proxy-z-score was calculated by Equation 1, using the LOQ value as result. Proxy-z-scores are for information only and indicated as a value between brackets. Values below -2 are considered as false negatives (see 4.5). Values above 2 indicate that the LOQ is high in relation to the assigned value and high in comparison to other participants.

Other types of results, e.g. 'detected', or 'not detected' without specification of an LOQ, were excluded from the evaluation. In these cases the participant was considered not to have a quantitative method available for the applicable mycotoxin/matrix.

4.5   False positives and false negatives

A false positive is a quantitative result reported by the participant while the toxin is:
i) not detected in the PT material by the organiser, and/or
ii) not detected by the majority of the other participants.
A threshold may apply, below which results are not considered false positives, e.g. when the analyte concentration is below the LOQ of the organiser and/or the majority of the participants. This is decided on a case-to-case basis. False positives are indicated as 'FP'. False positives are to be interpreted as unsatisfactory performance.

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', and this value is well below the assigned value, then the result can be classified as a false negative. This is the case when the proxy-z-score (see 4.4) is <-2. False negatives are indicated as 'FN'. False negatives are to be interpreted as unsatisfactory performance.

5 Assessment of participants' performance

5.1 Scope and LOQ

This PT was dedicated to DON, 3-Ac-DON, 15-Ac-DON and DON-3G. In Annex 7 the quantitative scope for each participant is provided, with indication of the LOQ provided. It was noted that three participants did not report results for the acetyl-DONs or DON-3G, despite the fact that these compounds were indicated to be in their scope during the *a priori* survey at the time of registration for the PT. While all laboratories have methods for determination of DON, only 22 out of 50 reported quantitative results for the acetyl-DONs, and only 16 out of 50 for DON-3G. Fourteen laboratories determined all four mycotoxins requested. The LOQs as provided by the participants varied widely, from low µg/kg up to 500 µg/kg. The median LOQs were 50 µg/kg for DON and DON-3G and 25 µg/kg for the acetyl-DONs.

There can be several causes for the gap in the scope observed for many laboratories. A first reason is that only DON is currently regulated, i.e. for analysis in the frame of enforcement inclusion of acetyl-DONs and DON-3G is not yet required. This could be a reason to not (yet) including the other DON forms in the method. Another reason might be that a number of laboratories are using methods involving an immuno-affinity-based clean-up (see 5.2) which may not be suited for simultaneous determination of all four toxins due to poor cross-reactivity [15]. Insight in the reasons for not covering the full scope will be obtained through a follow up questionnaire from the EURL-MP.

The quite extreme differences in LOQs may have several causes. The first is due to differences in analysis methods, i.e. different degrees of concentration factors of the final extract, and differences in sensitivity of (MS) instruments. Another cause may lie in the different ways that LOQs are defined and calculated. Finally, it can also not be excluded that in some cases the LOQ actually is a reporting limit, i.e. a cut-off value below which no results are reported, and is a rather arbitrary value below the regulatory limit but above the actual method LOQ.

Since NRLs are expected to have analytical capabilities not only in the frame of compliance testing of regulatory limits but also in the frame of data generation for risk assessment, efforts should be made toward inclusion of acetyl-DONs and DON-3G, and laboratories are recommended to aim for LOQs in the range of ≤50-100 µg/kg for DON, and ≤10-20 µg/kg for the three DON derivatives.

5.2 Analysis methods

Details on the analytical methods used by the participants are included in Annex 8. The methods used can roughly be categorised in methods based on LC-MS/MS (two thirds), often without clean-up, and methods based on LC-UV (one third) with immunoaffinity column (IAC) clean-up. GC-MS was used by one laboratory.

LC-UV–based methods always involved a clean-up using IAC, and an extraction with water. In most cases, only DON was determined, although one laboratory also reported on all four analytes. The inclusion of other DON derivatives besides DON itself with methods involving IAC clean-up might be difficult as IAC columns often have no or limited cross-reactivity for the DON derivatives [15].

In LC-MS/MS based methods extraction was mostly done using acetonitrile/water (23x), with or without acidification (acetic acid or formic acid). In six cases a salt-induced phase partitioning was done (QuEChERS type of extraction/clean-up). Methanol/water was used by three laboratories. In many cases no clean-up was performed, apart from a phase partitioning in case of QuEChERS-based approaches, or a dilution of the extract. When a clean-up was included, this was by solid phase

extraction (SPE, 8x) or by IAC (4x). Fourteen laboratories used isotopically labelled internal standards, in most cased only for DON. Despite the good possibilities to cover all forms of DON in LC-MS/MS-based methods, ten laboratories reported only DON.

Based on the results and method details provided by the laboratories, no obvious effects of extraction, clean-up or measurement methods on the results were observed.

5.3 Performance

The quantitative performance was assessed through z-scores. For each participant, the individual z-scores for the mycotoxins in material A (wheat) and B (maize) are provided in Annex 9 and 10, respectively. These annexes also show graphical representations of the z-scores.

For DON satisfactory z-scores were obtained by almost all participants in both materials. There were only two exceptions (one NRL and one OL). Combining the results for the two materials, 97% of the z-scores were satisfactory.

As indicated in 5.1, 22 out of the 50 laboratories determined the acetyl-DONs. For 3-Ac-DON in total five unacceptable z-scores were observed, mostly for wheat that contained the lower concentration. 15-Ac-DON was not present in material A (<RL [20 µ/kg] used by the EURL, indicative level 6 µg/kg). 15-Ac-DON was quantified in this material above 20 µg/kg by four laboratories. Those results were classified as false positives. In material B, 15-Ac-DON was present at 154 µg/kg but reported as below LOQ by two laboratories. As the assigned value was well above their LOQs, these results were classified as false negatives. Besides the false negatives, unsatisfactory performance was observed for one other laboratory (z-score >3). Combining the results for both acetyl-DONs in both materials, 81% of the z-scores were satisfactory (here the false positives were considered as unsatisfactory). The poorer performance for the acetyl-DONs may be due to difficulties in the chromatographic analysis. 3-Ac-DON and 15-Ac-DON are often co-eluting under generic chromatographic conditions which makes their determination less straightforward. However, it is possible to separate them chromatographically, and to a certain extend also mass spectrometrically (details see [10]).

DON-3G was included in the analysis by 16 out of the 50 laboratories. In general satisfactory z-scores were obtained in both materials, although due to the relatively low level in material B (maize, 35 µg/kg) only eleven laboratories could quantify this DON conjugate.

A summary of the characteristics and performance of the participants in this PT for each mycotoxins in each material is provided in Table 1 in the Summary.

In Annex 11 an overview is given of the overall performance for each participant in this PT. For the two materials combined, a maximum of seven satisfactory z-scores could be obtained, and '7 out of 7' reflects optimal performance in terms of scope and capability for quantitative determination. The number of laboratories that analysed the materials for all four mycotoxins was fourteen. Of these, seven achieved optimal performance. For the other seven, either the LOQ was too high, false positives or false negatives were reported, or a non-satisfactory z-score was obtained.

5.4 Robust relative standard deviation

For informative purposes the robust standard deviation (RSD_R) was calculated according to ISO13528:2015 [12]. This provides a good estimation of the interlaboratory variability. The individual RSD_R values for each toxin in both materials are included in Annex 9 and 10, and also in Table 1. They ranged from 14% for DON-3G in material B (35 µg/kg) to 28% for DON-3G in material A (209 µg/kg).

6   Conclusions

Fifty laboratories, including NRLs from all member states, participated in EURL-PT-MP01 on the quantitative determination of DON, 3-Ac-DON, 15-Ac-DON and DON-3G in cereals (wheat and maize). All laboratories determined DON, but only 44% included the acetyl-DONs, and only 32% DON-3G. Fourteen laboratories analysed the materials for all four target toxins. LOQs varied widely from low µg/kg to 500 µg/kg (medians in the range 25-50 µg/kg). LOQs were generally adequate for compliance testing for DON, but not always for monitoring in the frame of risk assessment.

Two-thirds of the laboratories used methods based on LC-MS/MS, either with or without clean-up. One third used methods based on LC-UV with IAC as clean-up step.

For DON satisfactory results were obtained in almost all cases. For the other three mycotoxins satisfactory performance rates were lower, 81% for the acetyl-DONs and 89% for DON-3G. Only seven out of 50 laboratories obtained satisfactory performance for all four toxins.

The quantitative performance of the participants was generally good, but extension of the scope is needed in many cases (and lower LOQs in some) to align with EFSA monitoring recommendations. In a relatively limited number of cases, a follow up is needed regarding questionable or unsatisfactory z-scores and false positive/false negative results.

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Annex 1   List of participants

Country	Organisation
AUSTRIA*	AGES Austrian Agency for Health and Food Safety
AUSTRIA	University of Natural Resources and Life Sciences Vienna (BOKU)
BELGIUM*	Sciensano (pka Veterinary and Agrochemical Research Centre (CODA-CERVA))
BULGARIA*	Bulgarian Food Safety Agency
CROATIA*	Andrija Stampar Teaching Institute of Public Health
CYPRUS*	Feeding Stuffs Quality Control Laboratory
CYPRUS*	State General Laboratory
CZECH REPUBLIC*	Central Institute for Supervising and Testing in Agriculture (UKZUZ)
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
DENMARK*	Danish Veterinary and Food Administration
DENMARK*	National Food Institute
ESTONIA*	Agricultural Research Centre
FINLAND*	Finnish Customs Laboratory
FINLAND*	Finnish Food Safety Authority Evira
FINLAND	Natural Resources Institute Finland
FRANCE	LABOCEA
FRANCE	Laboratoires des Pyrenees et des Landes
FRANCE*	Service Commun des Laboratoires DGCCRF-DGDDI (SCL-L35) Laboratoire de RENNES
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GREECE*	General Chemical State Laboratory (GCSL)
HUNGARY*	National Food Chain Safety Office, Analytical NRL
HUNGARY*	National Food Chain Safety Office, Toxicological NRL
IRELAND*	Public Analyst's Laboratory (HSE)
IRELAND*	The State Laboratory
ITALY	ARPA FVG
ITALY	ARPAM
ITALY	ATS MILANO
ITALY*	Istituto Superiore di Sanita (ISS)
LATVIA*	Institute of Food Safety, Animal Health and Environment (BIOR)
LITHUANIA*	National Food and Veterinary Risk Assessment Institute
LUXEMBOURG*	Laboratoire National de Santé surveillance alimentaire
MALTA*	Public Health Laboratory
NETHERLANDS	Nederlandse Voedsel en Waren Autoriteit
POLAND*	National Institute of Public Health - National Institute of Hygiene
POLAND*	National Veterinary Research Institute
PORTUGAL*	Autoridade Seguranca Alimentar e Economica
ROMANIA*	Directia Sanitara Veterinara si pentru Siguranta Alimentelor (DSVSA) Bucuresti
ROMANIA*	Hygiene and Veterinary Public Health Institute
SERBIA	SP Laboratorija A.D.
SLOVAKIA*	Regional Public Health Authority in Poprad (RUVZ)
SLOVAKIA*	State veterinary and food institute
SLOVENIA*	National laboratory of health, environment and food
SLOVENIA*	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
SPAIN	AINIA
SPAIN	Laboratori Agroalimentari
SPAIN*	National Center for Food (AESAN)
SWEDEN*	National Food Agency (SLV)
SWEDEN*	National Veterinary Institute (SVA)
UNITED KINGDOM*	Fera Science Ltd
UNITED KINGDOM	Public Analyst Scientific Services Limited

* National Reference Laboratory of EU Member State

Annex 2　Codification of the samples

Participants code	Material A*	Material B*	Participants code	Material A*	Material B*
PT031	837	137	PT056	274	922
PT032	561	169	PT057	247	847
PT033	703	916	PT058	228	583
PT034	426	431	PT059	366	593
PT035	269	224	PT060	104	972
PT036	344	321	PT061	181	463
PT037	724	205	PT062	663	377
PT038	885	616	PT063	795	386
PT039	596	352	PT064	311	612
PT040	782	808	PT065	642	166
PT041	409	539	PT066	361	995
PT042	552	405	PT067	400	290
PT043	154	468	PT068	354	500
PT044	737	615	PT069	842	245
PT045	515	991	PT070	110	200
PT046	220	665	PT071	326	342
PT047	850	647	PT072	139	327
PT048	419	542	PT073	393	280
PT049	824	691	PT074	576	293
PT050	499	252	PT075	757	168
PT051	261	216	PT076	626	720
PT052	902	731	PT077	516	793
PT053	867	861	PT9958	775	889
PT054	268	451	PT9959	491	638
PT055	575	978	PT9960	316	246

* All sample codes start with EURLPT-MP 01/.

Annex 3   Statistical evaluation of homogeneity data

Sample No.	DON in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	613	560
Hom/A002	550	536
Hom/A003	550	518
Hom/A004	533	520
Hom/A005	550	525
Hom/A006	580	557
Hom/A007	521	493
Hom/A008	532	516
Hom/A009	506	523
Hom/A010	530	502
Grand mean	536	
Cochran's test		
C	0.377	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	134	
s_x	25.0	
s_w	19.2	
s_s	21.0	
Critical= 0.3 σ_P	40.2	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	3-Ac-DON in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	33.1	31.7
Hom/A002	32.2	32.2
Hom/A003	30.4	32.0
Hom/A004	31.7	32.3
Hom/A005	32.2	33.6
Hom/A006	31.8	32.4
Hom/A007	30.6	31.8
Hom/A008	32.6	31.6
Hom/A009	30.1	30.4
Hom/A010	31.5	32.8
Grand mean	31.8	
Cochran's test		
C	0.220	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.96	
s_x	0.752	
s_w	0.763	
s_s	0.524	
Critical= 0.3 σ_P	2.39	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	DON-3G in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	316	247
Hom/A002	249	262
Hom/A003	261	250
Hom/A004	261	267
Hom/A005	235	266
Hom/A006	292	254
Hom/A007	253	255
Hom/A008	252	260
Hom/A009	277	249
Hom/A010	251	271
Grand mean	261	
Cochran's test		
C	0.546	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	65.3	
s_x	9.58	
s_w	20.9	
s_s	0.00	
Critical= 0.3 σ_P	19.6	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	DON in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	787	806
Hom/B002	762	785
Hom/B003	756	720
Hom/B004	685	745
Hom/B005	741	765
Hom/B006	745	740
Hom/B007	719	703
Hom/B008	693	696
Hom/B009	642	676
Hom/B010	706	733
Grand mean	730	
Cochran's test		
C	0.422	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	183	
s_x	39.5	
s_w	20.8	
s_s	36.6	
Critical= 0.3 σ_P	54.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	3-Ac-DON in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	103	98.9
Hom/B002	102	101
Hom/B003	100	105
Hom/B004	88.4	98.0
Hom/B005	96.0	99.2
Hom/B006	99.4	98.4
Hom/B007	96.2	101
Hom/B008	95.2	92.2
Hom/B009	99.5	102
Hom/B010	96.7	104
Grand mean	98.8	
Cochran's test		
C	0.388	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	24.7	
s_x	3.15	
s_w	3.43	
s_s	2.01	
Critical= 0.3 σ_P	7.41	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation

Sample No.	15-Ac-DON in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	161	158
Hom/B002	153	145
Hom/B003	143	146
Hom/B004	142	139
Hom/B005	147	147
Hom/B006	156	143
Hom/B007	136	134
Hom/B008	127	116
Hom/B009	126	146
Hom/B010	152	167
Grand mean	144	
Cochran's test		
C	0.412	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	36.1	
s_x	11.6	
s_w	7.18	
s_s	10.4	
Critical= 0.3 σ_P	10.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation

Sample No.	DON-3G in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	24.8	24.2
Hom/B002	25.0	25.1
Hom/B003	22.2	26.1
Hom/B004	25.6	24.7
Hom/B005	23.6	25.1
Hom/B006	25.6	29.4
Hom/B007	19.4	23.9
Hom/B008	23.7	28.7
Hom/B009	24.6	25.6
Hom/B010	23.5	27.2
Grand mean	24.9	
Cochran's test		
C	0.272	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.22	
s_x	1.51	
s_w	2.16	
s_s	0.00	
Critical= 0.3 σ_P	1.87	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation

Annex 4   Statistical evaluation of stability data

*Stability evaluation for **DON in material A.***

Storage temperature	<-70°C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	507	491	501
	532	527	517
	497	517	506
	531	518	513
	512	503	507
	508	510	493
Average amount (µg/kg)	514	511	506
n	6	6	6
st. dev (µg/kg)	14.1	12.6	8.66
Difference		3.36	7.91
$0.3 * \sigma_P$		38.6	38.6
Consequential difference? Diff < $0.3 * \sigma_P$		No	No

*Stability evaluation for **3-Ac-DON in material A.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	28.8	35.2	32.6
	31.3	31.3	30.8
	33.1	33.1	30.8
	32.4	29.0	31.2
	28.5	31.9	34.5
	29.8	31.9	31.9
Average amount (µg/kg)	30.6	32.1	31.9
n	6	6	6
st. dev (µg/kg)	1.93	2.03	1.42
Difference		-1.44	-1.32
$0.3 * \sigma_P$		2.30	2.30
Consequential difference? Diff < $0.3 * \sigma_P$		No	No

*Stability evaluation for **DON-3-G in material A.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	249	242	236
	253	256	251
	240	260	260
	253	248	241
	240	231	241
	244	230	235
Average amount (µg/kg)	247	245	244
n	6	6	6
st. dev (µg/kg)	6.18	12.5	9.50
Difference		2.00	2.81
$0.3 * \sigma_P$		18.5	18.5
Consequential difference? Diff < $0.3 * \sigma_P$		No	No

*Stability evaluation for **DON in material B.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	723	699	734
	688	680	722
	705	711	713
	711	683	714
	696	717	692.4
	660	707	712
Average amount (µg/kg)	697	699	715
n	6	6	6
st. dev (µg/kg)	21.8	15.4	13.8
Difference		-2.12	-17.3
$0.3*\sigma_P$		52.3	52.3
Consequential difference? Diff < $0.3*\sigma_P$		No	No

*Statistical evaluation for **3-Ac-DON in material B.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	99	98	90
	100	92	102
	100	95	95
	91	103	101
	95	100	97
	96	99	95
Average amount (µg/kg)	97	98	97
n	6	6	6
st. dev (µg/kg)	3.56	3.87	4.48
Difference		-0.97	0.28
$0.3*\sigma_P$		7.26	7.26
Consequential difference? Diff < $0.3*\sigma_P$		No	No

*Statistical evaluation for **15-Ac-DON in material B.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	169	155	162
	156	166	159
	168	163	156
	172	168	167
	166	152	159
	154	158	153
Average amount (µg/kg)	164	160	159
n	6	6	6
st. dev (µg/kg)	7.41	6.21	4.57
Difference		3.71	4.76
$0.3*\sigma_P$		12.3	12.3
Consequential difference? Diff < $0.3*\sigma_P$		No	No

*Statistical evaluation for **DON-3G in material B.***

Storage temperature	<-70 °C	<-18 °C	1 day RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	45.0	37.8	43.0
	43.9	43.7	45.8
	35.0	35.8	41.5
	48.4	38.6	42.5
	34.2	46.1	45.6
	36.8	37.6	40.9
Average amount (µg/kg)	40.6	39.9	43.2
N	6	6	6
st. dev (µg/kg)	5.97	4.05	2.06
Difference		0.62	-2.66
$0.3*\sigma_P$		3.04	3.04
Consequential difference? Diff < $0.3*\sigma_P$		No	No

Annex 5   Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European
Union
Reference
Laboratory

RIKILT

DATE
March 7, 2018

SUBJECT
Invitation first EURL
mycotoxins & plant toxins
proficiency test deoxynivalenol
and related compounds in food
and feed matrices

YOUR REFERENCE
NR

OUR REFERENCE
RIKILT/EURLPT-MP01/2018

POSTAL ADDRESS
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

VISITORS' ADDRESS
Wageningen Campus
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COC NUMBER
09098104

HANDLED BY
Diana Pereboom

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EMAIL
eurl.mycotoxins-
planttoxins@wur.nl

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam/Sir,

The European Union Reference Laboratory for mycotoxins and plant toxins announces the first proficiency test on deoxynivalenol and related compounds in food and feed matrices, EURLPT-MP01.

Aim of the PT is to provide laboratories with an assessment of their analytical performance and the reliability of their data - in comparison to other laboratories.

Obliged and eligible laboratories

According to Regulation (EU) 2017/625 it is obligatory for EU National Reference Laboratories (NRLs) mycotoxins in food and/or feed to participate.

For NRLs the participation is free of charge. If an extra batch of test materials is needed after the first shipping, the courier costs will be charged.

Official laboratories (OLs) can also participate as long as sufficient test material is available, at a first come first serve basis. The participation fee for OLs is 270 EURO per participant. OLs will be contacted for payment details upon registration.

Deadline for registration is 1 April 2018

Test materials

The test materials will be wheat flour and corn flour. The participants will receive approximately 35 gram of each test material.

100years
1918 — 2018

Target analytes

This PT will focus on the quantification of deoxynivalenol (DON) as included in Commission Regulation (EC) No 1881/2006 (food) and Commission Recommendation 2006/576/EC (feed).

It furthermore will include three related compounds of deoxynivalenol: 3-acetyl deoxynivalenol (3-Ac-DON), 15-acetyl deoxynivalenol (15-Ac-DON) and deoxynivalenol-3-glucoside (DON-3-G). EFSA recommends monitoring of these DON related compounds[1].

Participation

You can participate by completing the accompanying "EURLPT-MP01 Participation form" and return it before April 1, 2018 to: **eurl.mycotoxins-planttoxins@wur.nl**.

Shipment of test materials and deadline for submission

The shipment of test materials is scheduled in April week 16-17, 2018. The distribution of the test materials will be announced by e-mail. If any laboratories have holidays during the shipment period, please inform us.

Results must be submitted via the electronic submission form for which each participant must register, as explained in the "EURLPT-MP01 Participation form".

See calendar below for complete time schedule EURLPT-MP01.

Reporting

Laboratory proficiency will be determined through z-scores. Confidentiality of results is guaranteed. The results of the proficiency test will be presented anonymously in the report. The report will be published in the public domain of the EURL Mycotoxins & plant toxins website. The results of this PT will be discussed during the EURL workshop.

Kind regards,

D. Pereboom

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins
RIKILT Wageningen University & Research
the Netherlands

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[1] http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4718/epdf

Invitation letter (continued)

Calendar EURLPT-MP01
(last update March 7 2018)

Activity	Date
Announcement; Calendar; Target mycotoxins; Registration form	March 7, 2018
Deadline for registration	April 1, 2018
Distribution of test materials	April week 16-17, 2018
Deadline for receipt and acceptance of test materials	within 24 hr on receipt
Deadline for result submission	6 weeks after shipment
Preliminary report (only compilation of results) published	August 2018
Discussion on results	October 9-10, 2018
Final Report published	November 2018

Annex 6   Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

RIKILT

DATE
April 23, 2018

SUBJECT
Instruction proficiency test deoxynivalenol and related compounds in food and feed matrices

OUR REFERENCE
1810940/RIK
(RIKILT/EURLPT-MP01/2018)

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CoC NUMBER
09098104

HANDLED BY
D.P.K.H. Pereboom - de Fauw

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Dear Madam, Sir,

Thank you very much for your interest in the proficiency test for the analysis of deoxynivalenol and related compounds in food and feed matrices. Hereby I send you a parcel containing two randomly coded samples. Each sample consists of approximately 35 grams of test material.

Please fill out the accompanying acknowledgement of receipt form and return it immediately upon receipt of the samples, preferably by e-mail (pt.rikilt@wur.nl)

Instructions:
- After arrival store the samples in the freezer.
- Before analysis, homogenize them according to your laboratory's procedure.
- Treat the test material as if it was a sample for routine analysis.
  Report one result and not an average of multiple measurements.
- Report all results in µg/kg relative to a feed with a moisture content of 12% (assuming 0% moisture in the sample).
- Please use the web application for entering your results (https://crlwebshop.wur.nl/apex/f?p=107:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- The deadline for submitting test-results for this test is **June 4th 2018.**

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

- Please inform us about your applied method and detection technique (via the web application).

Please contact me if you have any questions or need any assistance.
With kind regards,

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins
RIKILT Wageningen University & Research, the Netherlands

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1918 — 2018

Annex 7   Scope and LOQ

Participant code	DON	3-Ac-DON	15-Ac-DON	DON-3G
		LOQ in µg/kg		
PT031	50			50
PT032	30	40	40	
PT033	50			
PT034	26			
PT035	10	10	20	10
PT036	50	500	500	500
PT037	75	20	20	20
PT038	25	25	25	
PT039	50			
PT040	256			
PT041	50	50	50	
PT042	157			
PT043	120			
PT044	200			
PT045	100	100	100	
PT046	20	10	40	
PT047	0.195			
PT048	50			
PT049	250			
PT050	100			
PT051	?			
PT052	200	90	150	200
PT053	13	16	20	2.5
PT054	20	20	20	
PT055	50	25	25	50
PT056	40			
PT057	50	50	50	50
PT058	10	10	10	10
PT059	60	40	40	
PT060	20			
PT061	10			150
PT062	20			
PT063	30	30	30	30
PT064	200	200	200	200
PT065	10	10	10	50
PT066	203			
PT067	144			
PT068	2.5	2.1	1.8	1.2
PT069	150			
PT070	100			
PT071	100			
PT072	200			
PT073	80	80	80	200
PT074	100			
PT075	4	4	4	
PT076	13.2	5.32	25	1.3
PT077	180			
PT9958	50			
PT9959	40			
PT9960	120			

Annex 8   Method details

Participant code	Sample weight (g)	Extraction solvent	Extr. solvent volume ml	Extraction conditions	Clean-up	ISTD	Measurement
PT031	25	water (5 g PEG800)	200	shake 30 min	IAC		LC-UV
PT032		ACN/water (84/16)			SPE (mycosep 225)	yes	LC-MS/MS
PT033		MeOH/water			IAC	none	LC-MS/MS
PT034		ACN/water (70/30)		shake 60 min	dilution	none	LC-MS/MS
PT035	5	ACN/water (84/16)	20	shake 120 min	SPE (OASIS prime HLB)		LC-MS/MS
PT036		QuEChERS			salt-out phase partitioning	none	LC-MS/MS
PT037							
PT038	20	ACN/water (84/16)			SPE (mycosep 227)	19-nortestosterone	GC-MS (SIM) after silylation
PT039		Water		ultraturrax	IAC		LC-UV
PT040		Water			IAC		LC-UV
PT041		ACN/water/HAc (79/20/1)			none	13C15-DON, not for Ac-DONs	LC-MS/MS
PT042					IAC	none	LC-UV
PT043	5	Water	200	blend 3 min	IAC	none	LC-UV
PT044	12.5	MeOH/water (70/30)	70	blend 2min	IAC (DZT)	none	LC-MS/MS
PT045							
PT046		ACN/water (84/16)			SPE (mycosep 225)	13C15-DON	LC-MS/MS
PT047							
PT048		Water			IAC	none	LC-UV
PT049		Water			IAC		LC-UV
PT050	10	ACN/water/HAc (80/20/1)		stir 60 min		13C15-DON	LC-MS/MS
PT051							
PT052		ACN/water			dilution	13C label for each toxin	LC-MS/MS
PT053		ACN/water/HAc (79/20/1)		90 min	dilution	none	LC-MS/MS
PT054		ACN/water			SPE		LC-MS/MS
PT055		QuEChERS			salt-out phase partitioning	none	LC-MS/MS
PT056		Water		shaker 60 min	IAC (DONprep)		LC-UV
PT057		QuEChERS (ACN/water)			salt-out phase partitioning		LC-MS/MS

Participant code	Sample weight (g)	Extraction solvent	Extr. solvent volume ml	Extraction conditions	Clean-up	ISTD	Measurement
PT058							
PT059	5	ACN/water/FA (79/20/1)	25	60 min	solvent switch	13C-labeled	LC-MS/MS
PT060		water with PEG			IAC	none	LC-UV
PT061	2	QuEChERS (ACN-1%FA/water/ (1:1))	20	shake 30 min	salt-out phase partitioning	13C15-DON	LC-MS/MS
PT062		MeOH/water		shaker	IAC (DZT)	none	LC-MS/MS
PT063		Water			IAC	none	LC-UV
PT064					IAC	none	LC-MS/MS
PT065		EtOAC/water-1% HAc (2:1)				13C15-DON	LC-MS/MS
PT066		ACN/water			SPE (Oasis HLB)	13C15-DON	LC-MS/MS
PT067		Water			IAC	none	LC-UV
PT068		ACN/water/FA (79/20/1)				none	LC-MS/MS
PT069		Water			IAC		LC-UV
PT070		Water			IAC		LC-UV
PT071	10	ACN/water/FA (74/52/1)	50		dilution	none	LC-MS/MS
PT072					SPE	13C	LC-MS/MS
PT073	25	ACN/water/HAc (79/20/1)	100	stir 120 min	dilution	13C label for each toxin	LC-MS/MS
PT074		Water			IAC	none	LC-UV
PT075	5	ACN/water; ACN	20; 20	shake 30 min	SPE (mycosep afla/zon)	13C15-DON	LC-MS/MS
PT076	1	ACN/water (84/16)	8		SPE (mycosep Trich 225)	13C15DON and 13C21DON3-G	LC-MS/MS
PT077		QuEChERS (modified)			salt-out phase partitioning	13C label for each toxin	LC-MS/MS
PT9958	5	QuEChERS (ACN-1% FA/water)	10;10	shake 1 min	salt-out phase partitioning	13C15-DON	LC-MS/MS
PT9959							
PT9960		Water			IAC (DONprep)		LC-UV

ACN = acetonitrile; EtOAc = ethyl acetate; FA = formic acid; HAc = acetic acid; MeOH = methanol; PEG = polyethylene glycol (PEG)

SPE = solid phase extraction; IAC = immunoaffinity column

Annex 9   Results material A (wheat)

Part. code	Material A DON C: 572 µg/kg u: 15.5 µg/kg σ$_p$: 143 µg/kg (25%) robust σ: 86.5 µg/kg (15%)		Material A 3-Ac-DON C: 34.5 µg/kg u: 2.16 µg/kg σ$_p$: 8.62 µg/kg (25%) robust σ: 7.32 µg/kg (21%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT031	556.03	-0.1		
PT032	770	1.4	133	**11**
PT033	113	**-3.2**		
PT034	606	0.2		
PT035	452	-0.8	33.1	-0.2
PT036	572.3	0.0	<500	(54)
PT037	792	1.5	33	-0.2
PT038	542.9	-0.2	27.6	-0.8
PT039	574.6	0.0		
PT040	745	1.2		
PT041	672.5	0.7	42.2	0.9
PT042	835	1.8		
PT043	667	0.7		
PT044	508	-0.5		
PT045	643	0.5	66.9	**3.8**
PT046	497	-0.5	34.3	-0.0
PT047	685.464	0.8		
PT048	600.3	0.2		
PT049	528	-0.3		
PT050	760	1.3		
PT051	670	0.7		
PT052	555	-0.1	< 90	(6)
PT053	527	-0.3	42.2	0.9
PT054	440	-0.9	21	-1.6
PT055	488.1	-0.6	31.5	-0.3

C = consensus value (robust mean)

u = uncertainty of consensus value

σ$_p$ = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

Part. code	Material A DON C: 572 µg/kg u: 15.5 µg/kg σₚ: 143 µg/kg (25%) robust σ: 86.5 µg/kg (15%)		Material A 3-Ac-DON C: 34.5 µg/kg u: 2.16 µg/kg σₚ: 8.62 µg/kg (25%) robust σ: 7.32 µg/kg (21%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT056	543.6	-0.2		
PT057	552	-0.1	96	**7.1**
PT058	610	0.3	35	0.1
PT059	560	-0.1	< 40	(0.6)
PT060	600	0.2		
PT061	556.3	-0.1		
PT062	510	-0.4		
PT063	533	-0.3	88	**6.2**
PT064	842	1.9	28.8	-0.7
PT065	609	0.3	34	-0.1
PT066	446.3	-0.9		
PT067	569	-0.0		
PT068	621	0.3	37.6	0.4
PT069	503	-0.5		
PT070	529	-0.3		
PT071	560	-0.1		
PT072	564	-0.1		
PT073	466	-0.7	31	-0.4
PT074	582.5	0.1		
PT075	491.9	-0.6	20.7	-1.6
PT076	502	-0.5	32	-0.3
PT077	563	-0.1		
PT9958	600	0.2		
PT9959	452	-0.8		
PT9960	1004	**3.0**		

C = consensus value (robust mean)

u = uncertainty of consensus value

σₚ = target standard deviation for proficiency

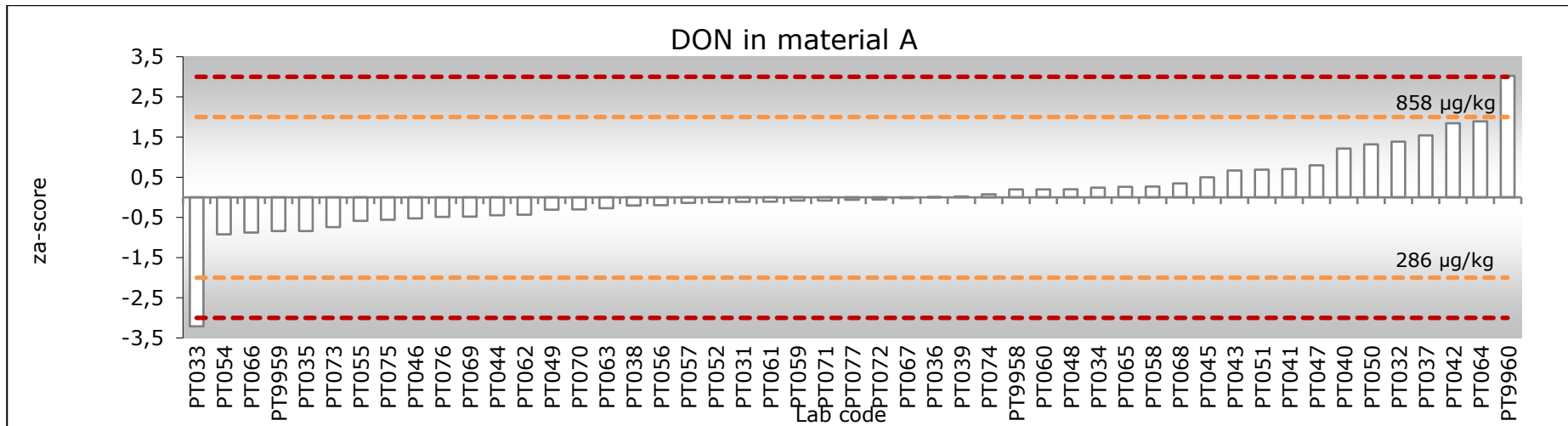robust σ = robust (relative) standard deviation based on participants' results

Part. code	Material A 15-Ac-DON C: <20 µg/kg (~6 µg/kg)			Material A DON-3G C: 209 µg/kg u: 19.0 µg/kg σ_p: 52.2 µg/kg (25%) robust σ: 59.0 µg/kg (28%)	
	Result (µg/kg)			Result (µg/kg)	z'-score
PT031				210.84	0.0
PT032	169		**FP**		
PT033					
PT034					
PT035	< 20			195	-0.3
PT036	< 500			334.2	**2.3**
PT037	< 20			205	-0.1
PT038	< 25				
PT039					
PT040					
PT041	49.4		**FP**		
PT042					
PT043					
PT044					
PT045	< 100				
PT046	< 40				
PT047					
PT048					
PT049					
PT050					
PT051					
PT052	< 150			222	0.2
PT053	<20			289	1.5
PT054	18				
PT055	< 25			170.7	-0.7

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

| Part. code	Material A 15-Ac-DON C: <20 µg/kg (~6 µg/kg)			Material A DON-3G C: 209 µg/kg u: 19.03 µg/kg σ_p: 52.2 µg/kg (25%) robust σ: 59.0 µg/kg (28%)	
	Result (µg/kg)			Result (µg/kg)	z'-score
PT056					
PT057	< 50			320	**2.0**
PT058	< 10			160	-0.9
PT059	< 40				
PT060					
PT061				292.9	1.5
PT062					
PT063	290		**FP**	128	-1.5
PT064	28.6		**(FP?)**	436	**4.1**
PT065	11			103	-1.9
PT066					
PT067					
PT068	7.1			181	-0.5
PT069					
PT070					
PT071					
PT072					
PT073	11			193	-0.3
PT074					
PT075	6.9				
PT076	< 25			215	0.11
PT077					
PT9958					
PT9959					
PT9960					

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

Figure a   *Graphical representation of the z-scores for DON in material A (wheat). Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.*
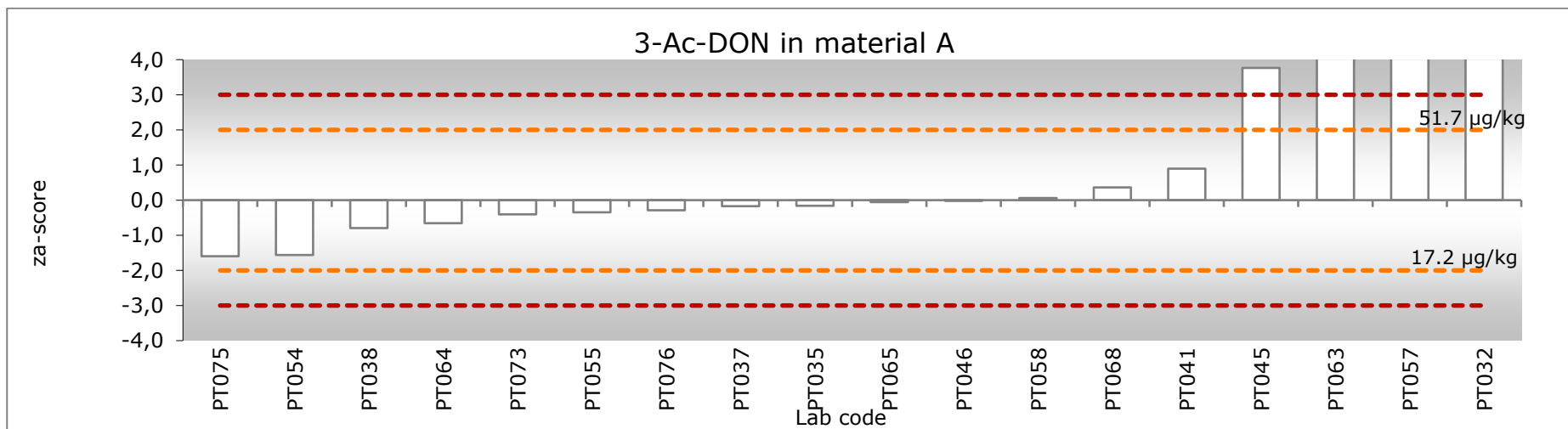
Figure b   *Graphical representation of the z-scores for 3-Ac-DON in material A. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.*
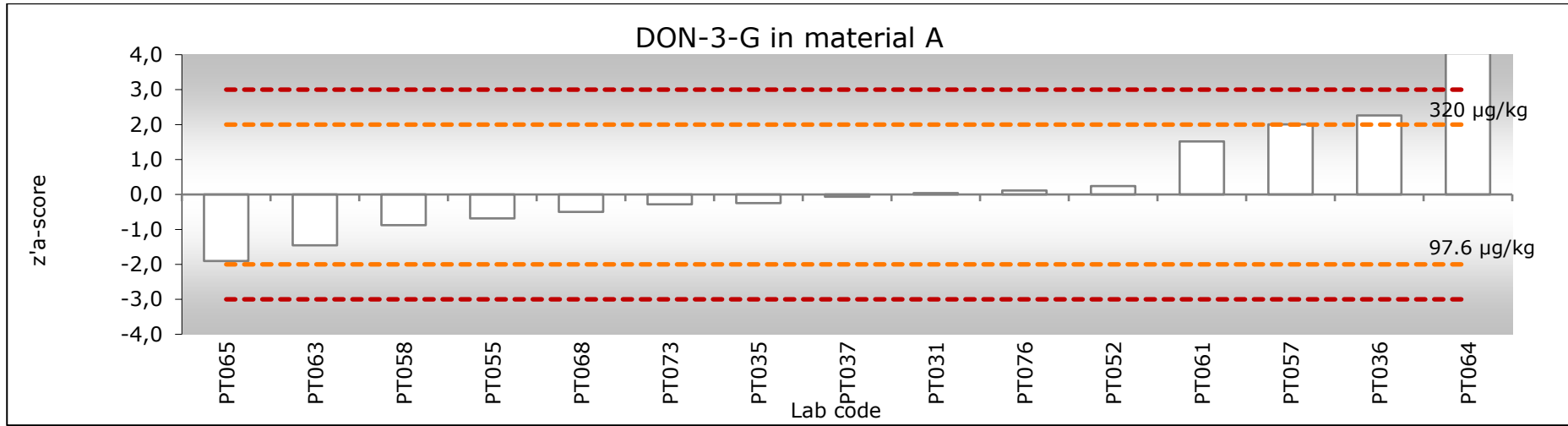
Figure c Graphical representation of the z'-scores for DON-3G in material B. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.

Annex 10   Results material B

Part. code	Material B DON C: 753 µg/kg u: 21.5 µg/kg σ_p: 188 µg/kg (25%) robust σ: 120 µg/kg (16%) Result (µg/kg)	z-score	Material B 3-Ac-DON C: 93.4 µg/kg u: 4.53 µg/kg σ_p: 23.3 µg/kg (25%) robust σ: 16.6 µg/kg (18%) Result (µg/kg)	z-score
PT031	759.19	0.0		
PT032	549	-1.1	74	-0.8
PT033	144	**-3.2**		
PT034	742	-0.1		
PT035	636	-0.6	105	0.5
PT036	937.4	1.0	117.2	1.0
PT037	770	0.1	129	1.5
PT038	809.1	0.3	68.7	-1.1
PT039	719.8	-0.2		
PT040	1018	1.4		
PT041	826.5	0.4	106.8	0.6
PT042	1100	1.8		
PT043	766	0.1		
PT044	554	-1.1		
PT045	561	-1.0	88.3	-0.2
PT046	683	-0.4	83.6	-0.4
PT047	926.431	0.9		
PT048	811.2	0.3		
PT049	685	-0.4		
PT050	1060	1.6		
PT051	777	0.1		
PT052	753	0.0	95	0.1
PT053	688	-0.3	90.4	-0.1
PT054	616	-0.7	55	-1.6
PT055	616.1	-0.7	94.7	0.1

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

Lab code	Material B DON C: 753 µg/kg u: 21.5 µg/kg σ_p: 188 µg/kg (25%) robust σ: 120 µg/kg (16%)		Material B 3-Ac-DON C: 93.4 µg/kg u: 4.53 µg/kg σ_p: 23.3 µg/kg (25%) robust σ: 16.6 µg/kg (18%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT056	930.9	0.9		
PT057	745	-0.0	219	**5.4**
PT058	770	0.1	85	-0.4
PT059	700	-0.3	89	-0.2
PT060	715	-0.2		
PT061	939.1	1.0		
PT062	670	-0.4		
PT063	605	-0.8	70	-1.0
PT064	838	0.5	93.9	0.0
PT065	1012	1.4	108	0.6
PT066	637.3	-0.6		
PT067	800	0.3		
PT068	746	-0.0	95.5	0.1
PT069	680	-0.4		
PT070	744	-0.1		
PT071	850	0.5		
PT072	713	-0.2		
PT073	682	-0.4	100	0.3
PT074	776.3	0.1		
PT075	741	-0.1	88.4	-0.2
PT076	729	-0.1	90.2	-0.1
PT077	879	0.7		
PT9958	820	0.4		
PT9959	568	-1.0		
PT9960	1064	1.7		

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

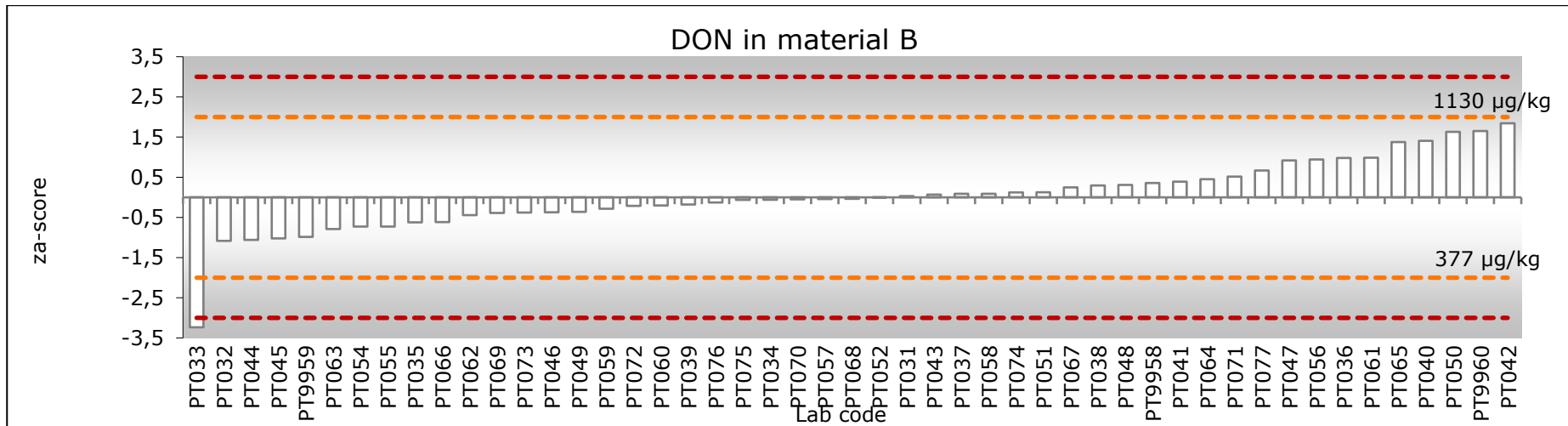robust σ = robust (relative) standard deviation based on participants' results

Part. code	Material B 15-Ac-DON C: 154 µg/kg u: 11.6 µg/kg σ$_p$: 38.6 µg/kg (25%) robust σ: 40.4 µg/kg (26%)		Material B DON-3G C: 35.1 µg/kg u: 1.91 µg/kg σ$_p$: 8.77 µg/kg (25%) robust σ: 4.83 µg/kg (14%)	
	Result (µg/kg)	z'-score	Result (µg/kg)	z-score
PT031			34.63	-0.1
PT032	< 40	(-3.0) **FN**		
PT033				
PT034				
PT035	153	-0.0	33.1	-0.2
PT036	147.3	-0.2	< 500	(53)
PT037	208	1.3	34	-0.1
PT038	137.2	-0.4		
PT039				
PT040				
PT041	128	-0.7		
PT042				
PT043				
PT044				
PT045	91.5	-1.6		
PT046	106	-1.2		
PT047				
PT048				
PT049				
PT050				
PT051				
PT052	167	0.3	< 200	(19)
PT053	113	-1.1	34.7	-0.0
PT054	229	1.9		
PT055	155	0.0	< 50	(1.7)

C = consensus value (robust mean)

u = uncertainty of consensus value

σ$_p$ = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

Part. code	Material B 15-Ac-DON C: 154 µg/kg u: 11.6 µg/kg σ_p: 38.6 µg/kg (25%) robust σ: 40.4 µg/kg (26%)			Material B DON-3G C: 35.1 µg/kg u: 1.91 µg/kg σ_p: 8.77 µg/kg (25%) robust σ: 4.83 µg/kg (14%)	
	Result (µg/kg)	z'-score		Result (µg/kg)	z-score
PT056					
PT057	287	**3.3**		30	-0.6
PT058	130	-0.6		25	-1.2
PT059	115	-1.0			
PT060					
PT061				< 150	(13)
PT062					
PT063	< 30	(-3.2) **FN**		37	0.2
PT064	95.1	-1.5		76.6	**4.7**
PT065	170	0.4		< 50	(1.7)
PT066					
PT067					
PT068	183	0.7		34.4	-0.1
PT069					
PT070					
PT071					
PT072					
PT073	173	0.5		38	0.3
PT074					
PT075	179.2	0.6			
PT076	159	0.1		47.5	1.4
PT077					
PT9958					
PT9959					
PT9960					

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

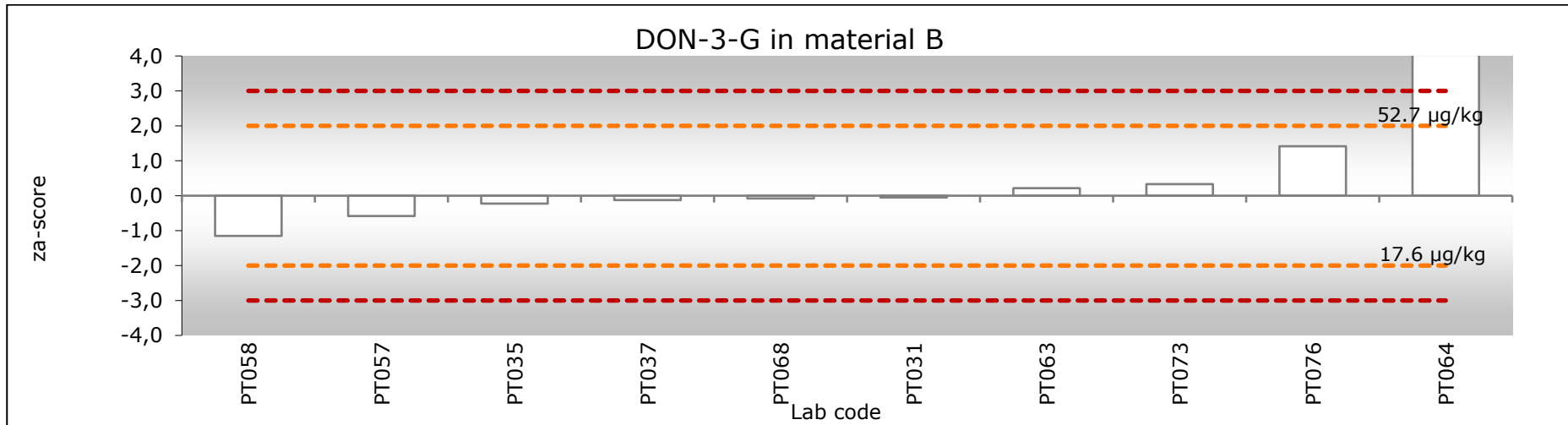robust σ = robust (relative) standard deviation based on participants' results

Figure d   Graphical representation of the z-scores for DON in material B. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.
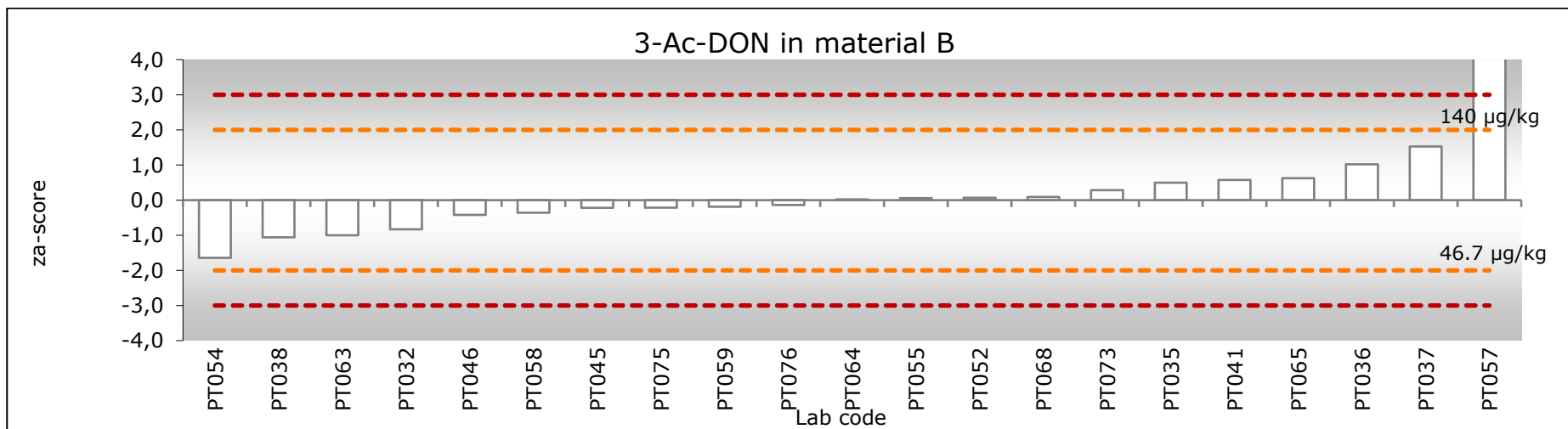
Figure e   Graphical representation of the z-scores for 3-Ac-DON in material B. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.
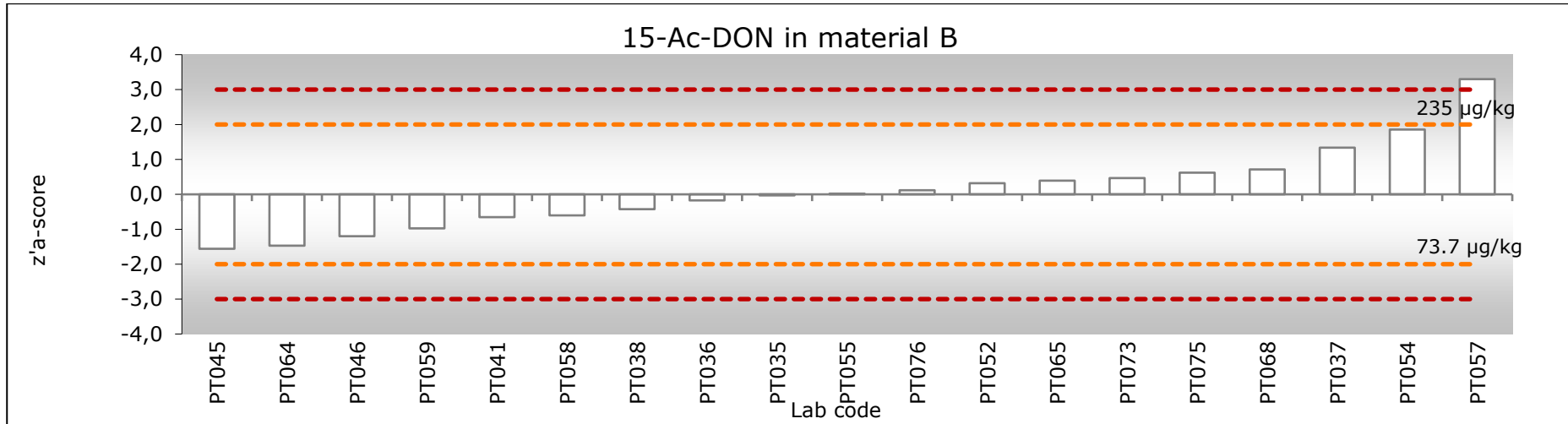
Figure f *Graphical representation of the z'-scores for 15-Ac-DON in material B. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.*

Figure g *Graphical representation of the z-scores for DON-3G in material B. Dotted lines show PT performance boundaries ±2 (also in µg/kg) and ±3.*

Annex 11 Overview performance per laboratory

Participant code	DON Satisfactory performance*	DON, 3-Ac-DON, 15-Ac-DON, DON-3G Satisfactory performance*
PT031	**2 out of 2**	4 out of 7
PT032	**2 out of 2**	3 out of 7 [and 1 FP!]
PT033	0 out of 2	0 out of 7
PT034	**2 out of 2**	2 out of 7
PT035	**2 out of 2**	**7 out of 7**
PT036	**2 out of 2**	4 out of 7
PT037	**2 out of 2**	**7 out of 7**
PT038	**2 out of 2**	5 out of 7
PT039	**2 out of 2**	2 out of 7
PT040	**2 out of 2**	2 out of 7
PT041	**2 out of 2**	5 out of 7 [and 1 FP!]
PT042	**2 out of 2**	2 out of 7
PT043	**2 out of 2**	2 out of 7
PT044	**2 out of 2**	2 out of 7
PT045	**2 out of 2**	4 out of 7
PT046	**2 out of 2**	5 out of 7
PT047	**2 out of 2**	2 out of 7
PT048	**2 out of 2**	2 out of 7
PT049	**2 out of 2**	2 out of 7
PT050	**2 out of 2**	2 out of 7
PT051	**2 out of 2**	2 out of 7
PT052	**2 out of 2****	5 out of 7**
PT053	**2 out of 2**	**7 out of 7**
PT054	**2 out of 2**	5 out of 7
PT055	**2 out of 2**	6 out of 7
PT056	**2 out of 2**	2 out of 7
PT057	**2 out of 2**	4 out of 7
PT058	**2 out of 2**	**7 out of 7**
PT059	**2 out of 2**	4 out of 7
PT060	**2 out of 2**	2 out of 7
PT061	**2 out of 2**	3 out of 7
PT062	**2 out of 2**	2 out of 7
PT063	**2 out of 2**	5 out of 7 [and 1 FP!]
PT064	**2 out of 2**	5 out of 7 [and 1 FP?]
PT065	**2 out of 2****	6 out of 7**
PT066	**2 out of 2**	2 out of 7
PT067	**2 out of 2**	2 out of 7
PT068	**2 out of 2**	**7 out of 7**
PT069	**2 out of 2**	2 out of 7
PT070	**2 out of 2**	2 out of 7
PT071	**2 out of 2**	2 out of 7
PT072	**2 out of 2**	2 out of 7
PT073	**2 out of 2**	**7 out of 7**
PT074	**2 out of 2**	2 out of 7
PT075	**2 out of 2**	5 out of 7
PT076	**2 out of 2**	**7 out of 7**
PT077	**2 out of 2**	2 out of 7
PT9958	**2 out of 2**	2 out of 7
PT9959	**2 out of 2**	2 out of 7
PT9960	1 out of 2	1 out of 7

* satisfactory performance means a satisfactory z-score was obtained for the mycotoxins present in material A and B.

** reported too late

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

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