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# Report on the 2016 Proficiency Test of the European Union Reference Laboratory for Mycotoxins

*Determination of regulated mycotoxins and enniatins and beauvericin in cereals*

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European Union Reference Laboratory
Mycotoxins

# EURL MYCO PT2016
# Proficiency test report

## *Determination of regulated mycotoxins and enniatins and beauvericin in cereals*

C. Oliveira Gonçalves, E. Cubero-Leon, V. Tamosiunas, C. Mischke, S. Bratinova, J. Stroka

268-PT Accredited by the
Belgian Accreditation Body (BELAC)

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## Executive summary

The number of known mycotoxins, their precursors and metabolites has been steadily increasing over the past years. The European Commission puts special emphasis on the need to monitor the co-occurrence of mycotoxins of various families at levels that allow for a sound risk assessment, taking into account possible additive or synergistic effects. Prior any regulatory action is taken for mycotoxins for which a health concern has been expressed (e.g. enniatins and beauvericin) valid data on their prevalence in food is required.

LC-MS-based multi-mycotoxin methods have the potential of streamlining and widening the monitoring work carried out by the official control laboratories. Although many practical advantages have been recognised, such methodologies have not been adopted by all routine laboratories and only a handful of such methods are just on the verge to become standardised. It is of great interest to assess how well laboratories using diverse sample preparation methodologies and determination techniques perform.

Therefore, a proficiency test was organised by the European Union Reference Laboratory (EURL) for Mycotoxins for this purpose. The focus was the assessment of the measurement performance of EU Member States laboratories regarding the determination of aflatoxin B1, deoxynivalenol, zearalenone, fumonisins B1 & B2,T-2 & HT-2 toxins, enniatins B, B1, A, A1 and beauvericin in two test materials (corn and oat) using single- or multi-mycotoxin methodologies.

Fifty-three laboratories, among them thirty-six National Reference Laboratories for mycotoxins in food and feed from the 28 EU Member States and 17 Official Control Laboratories, participated in the PT. For the regulated mycotoxins, 83.7 % of the results were rated with satisfactory z-scores. The performance of the laboratories was best for AFB1 (94 %), followed by DON (91 %), ZON (89 %), FB1 (87 %), FB2 (78 %), T-2 (75 %) and HT-2 (64 %). Additionally, 11 laboratories submitted results for all enniatins and beauvericin. LC-MS/MS is gaining much preference as it allowed for the determination of all the proposed analytes (12) in the test materials. Nevertheless, the results provided by multi-mycotoxin methodologies did not differ statistically from those produced by single-analyte procedures. Many participants uphold the will to implement a methodology to analyse enniatins and beauvericin in the near future, while other laboratories' methods require improvements in the extraction efficiency and sensitivity.

## Acknowledgements

The organisers of the study would like to thank the colleagues involved in the project for their support. The laboratories that participated in this exercise, listed in **Table 1**, are also immensely acknowledged.

**Table 1.** Participating laboratories

| Department | Country |
|---|---|
| LVA GmbH | Austria |
| AGES GmbH | Austria |
| CODA-CERVA | Belgium |
| Bulgarian Food Safety Agency | Bulgaria |
| Andrija Stampar Teaching Institute of Public Health | Croatia |
| State General Laboratory | Cyprus |
| Czech Agriculture and Food Inspection Authority (CAFIA) | Czech Republic |
| UKZUZ (Central Institute for Supervising and Testing in Agriculture) | Czech Republic |
| Danish Veterinary and Food Administration | Denmark |
| DTU Food | Denmark |
| Agricultural Research Centre | Estonia |
| Finnish Food Safety Authority Evira | Finland |
| Laboratoire SCL de Rennes | France |
| Laboratoire des Pyrenees et des Landes | France |
| CVUA Rheinland | Germany |
| Federal Institute for Risk Assessment | Germany |
| Lower Saxony State Office for Consumer Protection and Food Safety | Germany |
| CVUA Sigmaringen | Germany |
| Feedstuffs Control Laboratory of Athens, Ministry of Rural Development & Food | Greece |
| General Chemical State Laboratory | Greece |
| Chemical State Laboratory, Division of Piraeus and the 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 National Reference Laboratory | Hungary |
| Public Analyst's Laboratory Dublin | Ireland |
| Ireland State Laboratory, Contaminants Department | Ireland |
| Azienda USL Toscana centro | Italy |
| Istituto Zooprofilattico Sperimentale del Mezzogiorno | Italy |
| IZSLER | Italy |
| ARPAL | Italy |
| ATS Val Padana | Italy |
| IZS Sicilia | Italy |
| Istituto Superiore di Sanità – National Reference Laboratory for Mycotoxins | Italy |
| Institute of Food Safety, Animal Health and Environment "BIOR" | Latvia |
| National Food And Veterinary Risk Assessment Institute | Lithuania |
| Laboratoire national de santé | Luxembourg |
| Public Health Laboratory | Malta |
| RIKILT - Wageningen University and Research | Netherlands |
| National Veterinary Research Institute | Poland |
| ASAE | Portugal |
| Institute for Hygiene and Veterinary Public Health | Romania |
| Regional Public Health Authority in Poprad | Slovakia |
| State veterinary and food institute Dolný Kubín, Veterinary and food institute in Košice" | Slovakia |
| National laboratory for health, environment and food | Slovenia |
| National Centre for Food (Spanish Consuming Affairs, Food Safety and Nutrition Agency | Spain |
| Consejeria de Desarrollo Rural y Rec. Naturales – Laboratorio de Sanidad Animal | Spain |
| GV. Conselleria de Sanidad Universal y Salud Pública. Centro de Salud Pública | Spain |
| CNTA | Spain |
| Laboratorio de Salud Pública de Albacete. Junta de Comunidades de Castilla-La Mancha | Spain |
| National Food Agency | Sweden |

| | |
|---|---|
| National Veterinary Institute (SVA) | Sweden |
| Fera Science Ltd | UK |
| West Yorkshire Analytical Services | UK |
| Public Analyst Scientific Services Limited | UK |

## List of abbreviations and definitions

| | |
|---|---|
| AFB1 | Aflatoxin B1 |
| BEA | Beauvericin |
| CEN | European Committee for Standardization |
| DON | Deoxynivalenol |
| ELISA | Enzyme-linked immunosorbent assay |
| EN | Enniatin |
| EMD-IDMS | Exact-Matching Double Isotope Dilution Mass Spectrometry |
| EURL | European Union Reference Laboratory |
| FAO | Food and Agriculture Organization |
| FB1&2 | Fumonisins B1 and B2 |
| HPLC-FLD | High performance liquid chromatography-fluorescence detection |
| HPLC-UV(DAD) | High performance liquid chromatography-ultraviolet/diode array detection |
| IAC | Immunoaffinity extraction column/clean-up |
| ISO | International Organization for Standardization |
| JRC | Joint Research Centre |
| LC-HiResMS | Liquid chromatography-high resolution mass spectrometry |
| LC-MS/MS | Liquid chromatography-tandem mass spectrometry |
| GC-MS(/MS) | Gas chromatography-mass spectrometry or tandem mass spectrometry |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| MS | Member States |
| NRL | National Reference Laboratory |
| OCL | Official Control Laboratory |
| PT | Proficiency Test |
| QuEChERS | Quick, Easy, Cheap, Effective, Rugged and Safe |
| ZON | Zearalenone |

# 1 Introduction

Mycotoxins are products of fungal secondary metabolism produced by filamentous fungi that can infect agricultural commodities both in the field and during storage [1]. Over 400 mycotoxins are known nowadays, but just about 30 occur in food and feed [2]. Aflatoxins, trichothecenes, fumonisins, ochratoxin A, zearalenone, patulin and *Alternaria* toxins are considered to be of the greatest importance to human and animal health, and to have the biggest detrimental economic impact in food trade [2-4].

They can display a range of severe toxic effects in humans and animals. Aflatoxin B1 (AFB1) is the most potent natural carcinogen in experimental animals (rats), ochratoxin A (OTA) is nephrotoxic, fumonisins B1 and B2 (FB1 and FB2) exhibit neuro- or hepatotoxicity and carcinogenicity depending on the target species affected, deoxynivalenol (DON) shows immunotoxic effects, zearalenone (ZON) is an endocrine disruptor, binding to the oestrogen receptors; and T-2 and HT-2 toxins inhibit protein synthesis and are highly haematotoxic [1,2,5,6].

Nowadays, their co-occurrence and combined toxicity is gaining increased interest. The same fungus might produce different mycotoxins, and various fungi can affect the same crop. Exposure to several classes of mycotoxins often results in an additive effect, not excluding a possible synergistic interaction [6-8]. Maize is an example where several mycotoxins have been reported to occur simultaneously.

The Food and Agriculture Organization (FAO) has estimated that 25% of the world's food crops are contaminated with mycotoxins, but the actual figures might well be much higher [9]. Kovalsky *et al.* [6] reported contamination rates varying between 7 and 79 % for B trichothecenes and 88 % for ZON, while enniatins (ENs) were ubiquitous.

Presently, AFs are regulated with maximum levels in 18 food categories, OTA in 13, DON in 9, ZON in 10 and fumonisins in 6 [10]. Indicative levels were established for the sum of T-2 and HT-2 toxins in unprocessed cereals and cereal products [11]. Food safety concerns have been extended recently to the so-called ''emerging'' mycotoxins such as ENs and beauvericin (BEA). ENs exhibit biological activity acting as enzyme inhibitors, are antifungal and antibacterial agents, and immunomodulatory substances [12]. BEA is cytotoxic and can induce apoptosis and DNA fragmentation. ENs and BEA act as ionophores, disturbing the pH and physiological ionic balance [13]. Enniatin B, the most prevalent EN, was found by Juan et *al.* in 70 % of the baby food samples at levels of up to 1100 $\mu g\ kg^{-1}$ and in 44 % of the pasta samples at levels of up to 106 $\mu g\ kg^{-1,}$ while other authors reported contamination rates of between 50-90 % of the wheat, maize and barley samples with total concentrations of EN and BEA of up to 500 mg $kg^{-1}$ [12].

The trends in food analysis go in the direction of developing multi-analyte methods, combining a generic sample preparation protocol with a highly selective instrumental analysis, such as liquid chromatography-mass spectrometry (LC-MS) [14,15]. This may allow monitoring for larger numbers of potential contaminants and revealing heretofore unknown potential hazards [3]. LC-MS-based multi-mycotoxin methods offer improved selectivity and sensitivity, a substantial reduction of the sample preparation, and simultaneous quantification and confirmation of the identity [1,2]. Despite the fact that the performance of the laboratories has enhanced over the recent years, improvements in accuracy, efficiency and the management of matrix effects are still needed [1,2]. A previous proficiency test (PT) highlighted that matrix-matched calibrations or calibrations using $^{13}C$-labelled mycotoxins as internal standards were essential for accurate mycotoxin quantification [1]. Nevertheless, neither all national reference laboratories (NRLs) use LC-MS methods for mycotoxin determinations nor they resort to the above tools for matrix compensation. Therefore, the continued evaluation of their proficiency is required. Besides, efforts should be undertaken to foster their analytical capability on the determination of emerging mycotoxins (e.g. ENs and BEA).

# 2 Scope

As stated in Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council [16], one of the core duties of the EURL is to organise PTs for the benefit of the NRLs.

Given the fact that single mycotoxin PTs are only of relevance where a particular mycotoxin is regulated the conduction of multi-mycotoxin PTs is an elegant way of recognising the fact that for some food matrices a number of mycotoxins are potential contaminants. Therefore, a proficiency test including naturally contaminated test materials was organised covering all regulated mycotoxins in cereals, except ochratoxin A. Mycotoxins of emerging concern, such as ENs and BEA were also included to gauge the analytical capability and proficiency among the participants. The determination of enniatins A, A1, B, B1 and beauvericin was not mandatory but highly encouraged.

The proficiency test was addressed to all NRLs for mycotoxins and to appointed Official Control Laboratories (OCLs). Participation was mandatory and free of charge for the NRLs. Fifty-six laboratories from 28 Member States registered for the PT.

The EURL Mycotoxins performed the planning, execution and assessment of the measurement results based on the requirements laid down in ISO/IEC 17043:2010 [17]. Participants' results were evaluated using the ProLab software package (Quodata, Dresden, DE). The team that organised this PT is an ISO/IEC 17043:2010 accredited PT provider [18].

# 3 Confidentiality

The procedures used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 [17] and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the NRLs appointed in line with the Regulation (EC) No 882/2004 will be disclosed to DG SANTE upon request for (long-term) performance assessment.

# 4 Time frame

The PT was announced to the National Reference Laboratories by email and through the EURL Mycotoxins web page [18] on 15th July 2016. Registration for this PT was open until 02nd September 2016 (**Annex 1**). The participants were given six weeks after the dispatch of the samples (13th and 14th September 2016) for analysing them and reporting back the results together with the duly filled questionnaire. The deadline for reporting the results was 28th October 2016.

# 5 Materials

## 5.1 Preparation

The oat test material was produced by combining two contaminated oat batches with one blank oat. The corn material was produced by combining two low contaminated materials with a small amount of a highly contaminated corn supplied by Trilogy (Washington, USA). The materials were thoroughly homogenised, bottled and stored in the freezer until dispatch. Batches of approximately 5 kg of oat and 10 kg of corn were prepared and approximately 55 g and 100 g portions, respectively, were packed in amber plastic bottles. The materials were envisaged to contain as many regulated mycotoxins as possible, as well as enniatins and beauvericin. The contamination levels were in the range as commonly found in cereal samples.

## 5.2 Homogeneity

For checking the homogeneity of the test materials, 10 units per material (oat and corn) were randomly selected from the production lot. Two independent determinations were performed per bottle using a liquid chromatography-isotope dilution tandem mass spectrometry (LC-ID-MS/MS) method that was collaboratively validated to be published as CEN standard. The determination of beauvericin was carried out separately, consisting of an extraction with a mixture of water:ethyl acetate 1:2 followed by salting-out with sodium sulfate and clean-up with a silica solid-phase extraction column. Both methodologies are described in the working instruction D-00797 of the JRC Geel. The order of measurements was randomised. Homogeneity was evaluated according to ISO 13528:2015 [19]. The materials proved to be adequately homogeneous (**Annex 2**).

## 5.3 Stability study

The stability study was conducted following an isochronous experimental design [20]:
-70 °C was chosen as the reference temperature for sample storage. Stability was assessed at the following test temperatures: room temperature (≈20 °C), 4 °C and -18 °C. The time periods considered in this study were: 14, 28 and 55 days. The stability was evaluated according to the requirements of ISO 13528:2015 [19]. 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 (**Annex 3**). The materials proved to be adequately stable at room temperature, 4 °C and -18 °C for the period between dispatch (t=0) and the submission date of the last results (t=55 days). Based on a similar PT (mycotoxins in cereals) of 2013, the regulated mycotoxins should be stable in the present PT matrix during 1-2 days shipment without cooling.

## 5.4 Distribution

The test materials were dispatched in polystyrene boxes at ambient temperature on 13$^{th}$ and 14$^{th}$ September 2016. The samples were mostly received within 24 hours after dispatch. Storage was required to be at -18 °C until analysis.

Each participant received:
a) two test materials for analysis, packed in amber plastic bottles
- Sample O-1## – oat (approx. 55 g)
- Sample C-2## – corn (approx. 100 g)
b) an accompanying letter with instructions on sample handling and reporting (**Annex 4**)
d) a sample receipt form (**Annex 5**) and
e) laboratory specific files for reporting with a lab code (by email).

# 6 Instructions to the participants

The scope of the PT and the instructions for sample handling and reporting of the results was communicated to the participants via an accompanying letter (**Annex 4**). The laboratories were required to report the mass fractions of the regulated mycotoxins and enniatins and beauvericin in µg kg$^{-1}$ (mass as received) following their routine practices, accompanied by the measurement uncertainty (µg kg$^{-1}$) for at least the regulated mycotoxins (k=2). Then, in the Questionnaire (**Annex 6**), participants were asked to mention whether the results were corrected for recoveries or not and to provide the recoveries figures (in %).

The results were reported by the participants using the RingDat software, which is part of the ProLab software [21]. Laboratory specific files generated by ProLab were sent to each laboratory by email. A detailed questionnaire was also included. The questionnaire was

intended to provide additional information on method-related aspects and laboratory capabilities to allow insights on potential individual and general results' trends as well as to improve the planning of future PTs.

Method-related details such as the type of extraction and clean-up protocols, chromatographic and detection conditions, calibration strategy and quality control; and performance parameters such as LODs and LOQs were requested.

Participants were informed about the shipment of the materials at ambient temperature and that upon arrival they should be transferred to -18 °C. Participants were also encouraged to perform the analysis as soon as possible to allow enough time for data treatment, get acquainted with the software for reporting and resolve any unexpected instrumental issue.

# 7 Reference values and their uncertainties

The assigned values of the regulated mycotoxins in the test materials and their uncertainties were established by Exact-Matching Double Isotope Dilution Mass Spectrometry (EMD-IDMS) at JRC-Geel (Table 2). This methodology is considered to provide the highest degree of accuracy of the assigned values [22].

The reference values for the enniatins were obtained by standard addition, due to the lack of the corresponding isotope-labelled standards required for performing EMD-IDMS. These values should be regarded as indicative.

**Table 2.** Assigned values of the analytes and their associated expanded uncertainties in oat and corn test items

| Matrix | Analyte | Technique | Assigned value ($\mu g\ kg^{-1}$) | U (k=2) ($\mu g\ kg^{-1}$) |
|---|---|---|---|---|
| Corn | Deoxynivalenol | EMD-IDMS | 611 | 32 |
| | Aflatoxin B1 | EMD-IDMS | 10.61 | 0.65 |
| | Zearalenone | EMD-IDMS | 161.6 | 8.8 |
| | Fumonisin B1 | EMD-IDMS | 768 | 50 |
| | Fumonisin B2 | EMD-IDMS | 224 | 16 |
| Oat | T-2 toxin | EMD-IDMS | 70.3 | 2.1 |
| | HT-2 toxin | EMD-IDMS | 150.3 | 9.5 |
| | Enniatin B | Stand. Add. | 36.4 | 2.8 [1] |
| | Enniatin B1 | Stand. Add. | 26.3 | 2.2 [1] |
| | Enniatin A1 | Stand. Add. | 7.95 | 0.84 [1] |
| | Enniatin A | Stand. Add. | <LOQ | – |

U - expanded uncertainty of the assigned value

[1] A conservative approach was adopted in the absence of information about the uncertainty of the purity of the calibrants

# 8 Evaluation of the results

## 8.1 General observations

Fifty-six participants from 28 countries registered for the exercise and 53 datasets were reported back. Thirty-six laboratories were NRLs for mycotoxins and 17 were OCLs. Both NRLs for food and feed from Czech Republic, Denmark, Greece, Ireland, Hungary, Slovakia and Sweden have participated in this PT.

It was intended that the test materials distributed would contain the widest possible range of regulated mycotoxins and additionally, enniatins and beauvericin. The

concentrations of ochratoxin A and enniatin A were, however, too low to be reliably quantified and most probably fall below the LOQs of routine analytical methods. The mycotoxins that were requested to be analysed were split between the two materials to limit the analytical work of the laboratories, although such materials may contain other mycotoxins.

The laboratories were free to use their method of choice reflecting their routine procedures. More than half of the laboratories (33) used LC-MS/MS-based multi-mycotoxin methods while many laboratories still used HPLC-UV(DAD) for the determination of DON, and HPLC-FLD was the preferred technique for the determination of AFB1.

## 8.2 Scores and evaluation criteria

The individual laboratory performance was assessed in terms of z- and zeta- (ζ) scores following ISO 13528:2015 [19]. The following formulas were used:

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

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

where:

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

$\sigma_p$ was calculated as 22 % of the assigned value. The coefficient derived from the Horwitz equation for a mass fraction of 120 µg kg$^{-1}$ ($\sigma_p = 0.22\,C$) was applied regardless of the magnitude of the mass fraction of each given analyte. Data collected in previous PTs indicated that this coefficient often closely resembles the reproducibility standard deviation of the participants' data.

The z-score compares the participants' deviation from the reference value with the target standard deviation accepted for the proficiency test, $\sigma_p$. 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 participants' estimate of the uncertainty is consistent with the observed deviation from the assigned value. The ζ-score is the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value, its uncertainty as well as the uncertainty of the assigned value.

The interpretation of the ζ-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 |ζ|-score ≥3 might be due to an underestimation of the uncertainty, a large deviation from the reference value or to a combination of the two factors.

## 8.3 Laboratory results and scoring

The statistical evaluation of the results was performed using the ProLab software [21]. Z- and ζ-scoring was based on the reference values (and respective uncertainties) assigned by EMD-IDMS instead of the consensus values (robust mean). The robust mean and the reproducibility standard deviation were computed according to the Algorithm A of ISO 13528:2015, and are given in Table 3 just for information purposes [19].

No performance scoring was attempted for the enniatins and beauvericin, as the reduced number of participants (max. 15) limited the robustness of the consensus estimation of the actual mass fraction while the calibration standards used in the organiser's estimations were not accompanied by the uncertainty of the purity. The two estimations above for enniatin B and A1 deviated 6.6 and 7.6 % from each other, respectively, which is in the range of their uncertainties, while the estimations for enniatin B1 deviated by 43 %. The mass fraction of enniatin A is below the method's LOQ, while, in general, the calibrations for beauvericin didn't meet the requirement of $r^2>0.99$ due to the dispersion of the results. The values supplied should be seen as indicative, and any technical judgement on the analytical performance is reserved to each of the participants.

83.7 % of the results reported by the participants were rated with satisfactory z-scores ($|z| \leq 2$), taking into consideration all regulated mycotoxins requested in the two matrices (DON, AFB1, ZON, FB1, FB2, HT-2 and T-2) (see figure 1).

6.1 % of the results fell into the unsatisfactory range with $|z| \geq 3$

Figure 2 shows that the performance of the laboratories analysing DON, AFB1, ZON, FB1 and FB2 present in the corn material was better than analysing HT-2 and T-2 (for which just a Commission Recommendation exists) in oat. Additionally, the number of laboratories that reported results for HT-2 (39) and T-2 (40) was lower than for AFB1 (52), ZON (49) and DON (48), which are strictly mandatory determinations in a variety of food matrices.

The determination of HT-2 and T-2 toxins was almost exclusively done by LC-MS/MS, which requires an appropriate compensation of the matrix effects by using the corresponding isotopologues, opposite to other mycotoxins which can be analysed by robust techniques such as HPLC-FLD after immunoaffinity clean-up (IAC).

Figures 3 and 4 present 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 numerical values of the calculated z-scores and ζ-scores are compiled in Tables 4 and 5. All z- and ζ-scores in the satisfactory performance range are shown with a green background; those in the questionable range are displayed with a yellow background and scores indicating unsatisfactory performance are presented with a light-red background.

**Figure 1.** Overall distribution of the z-scores obtained by the participants for the regulated mycotoxins in the corn and oat materials

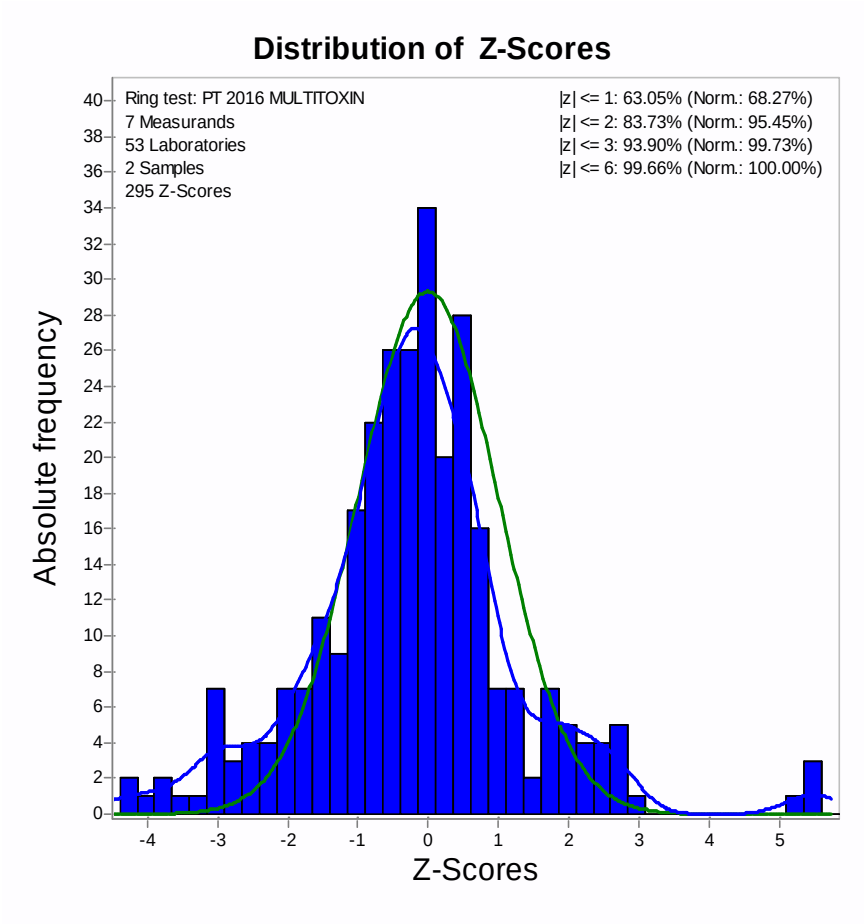
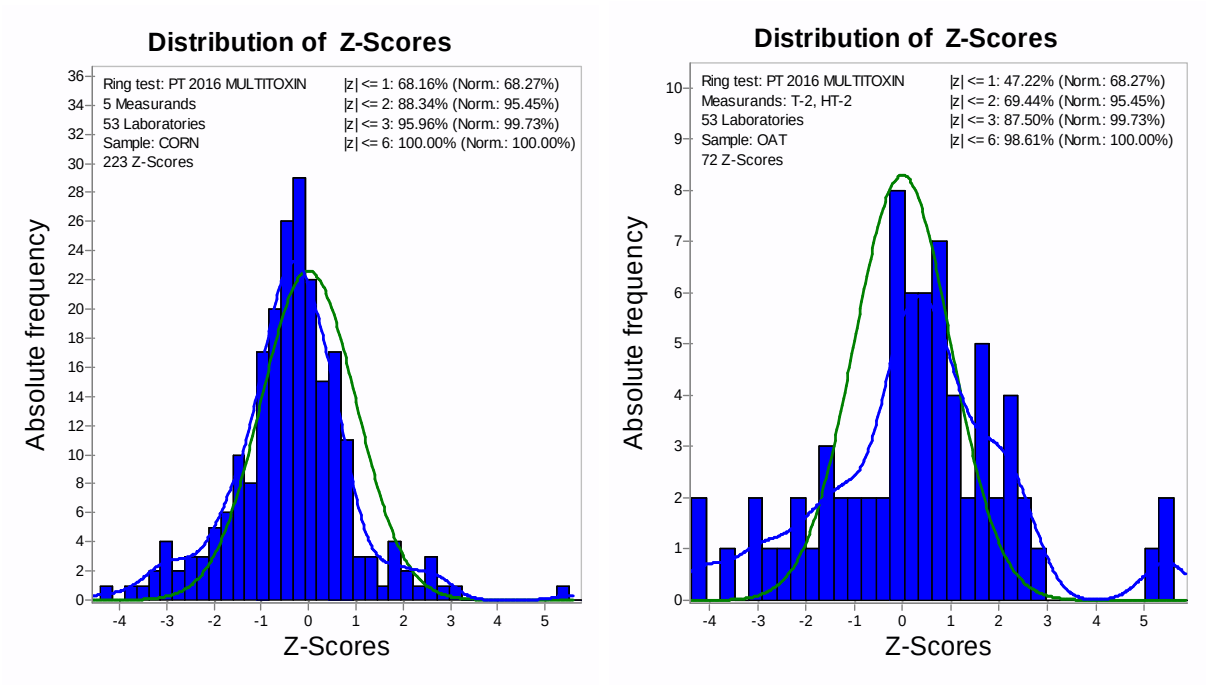



**Figure 2.** Distribution of the z-scores for the regulated mycotoxins present in the corn (left) and oat (right) materials



The graphical representations of the sigmoidal distribution of the results (µg $kg^{-1}$) for each combination of analyte/sample are given in Figure 5. Reported values are shown as bars. The green line corresponds to Xref; the green shadow covers the boundary of the reference interval (Xref ± $u_{ref}$), and the red lines mark the boundary of the target interval (Xref ± 2σ). Green bars represent results with |z-score| ≤2, yellow bars represent results with 2<|z-score|<3, while the red bars represent results with |z-score|≥3.

**Figure 3.** Individual laboratory z-scores for the regulated mycotoxins in corn.

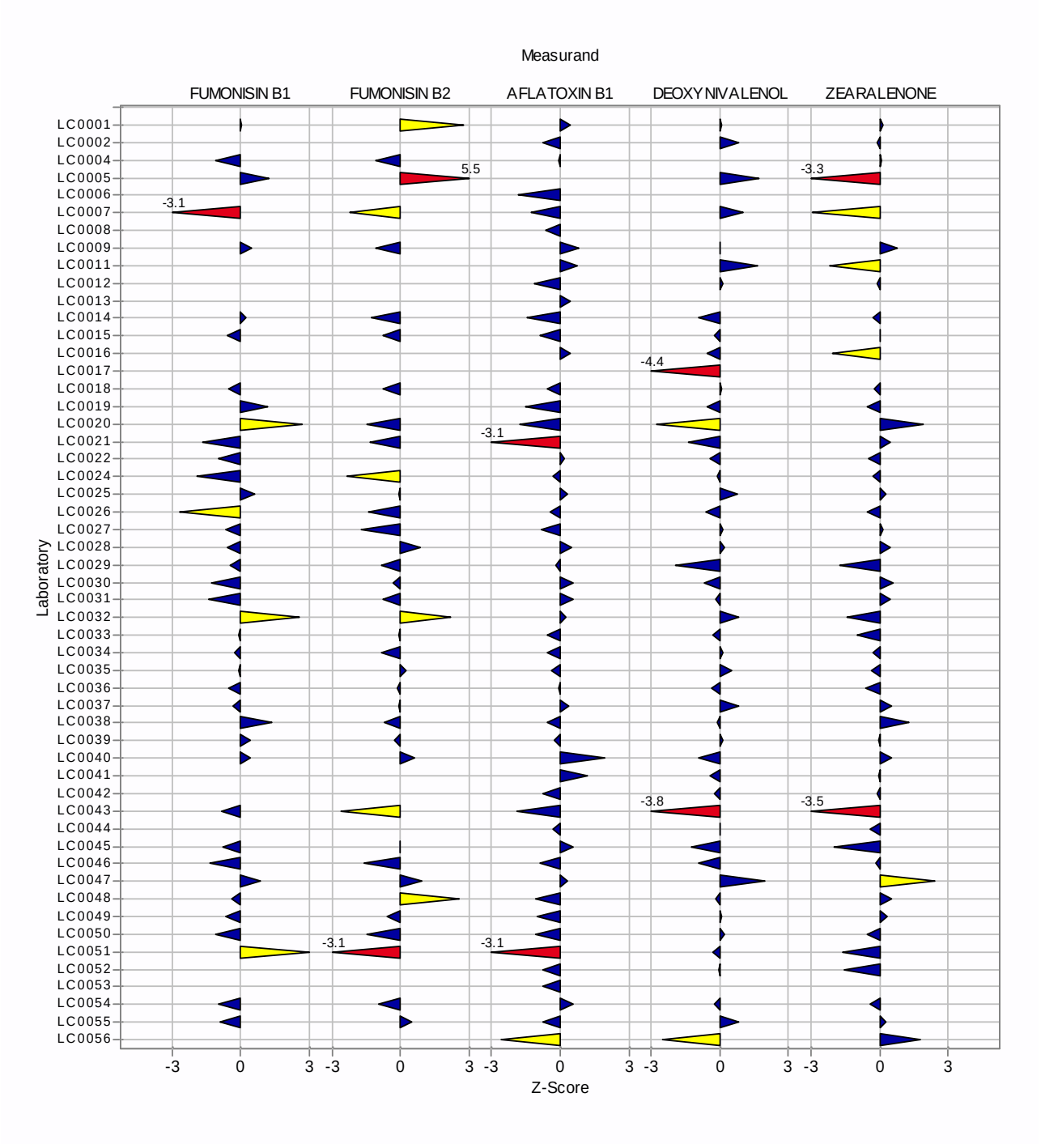
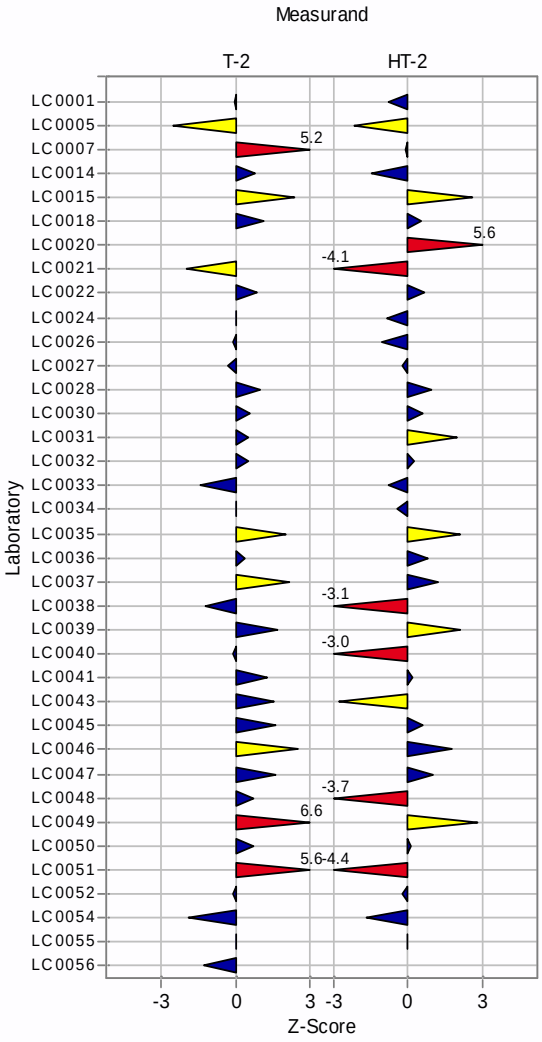


**Figure 4.** Individual laboratory z-scores for the regulated mycotoxins in oat.




A summary of the statistical evaluation of the results of the regulated mycotoxins, enniatins and beauvericin is presented in Table 3. The robust standard deviations of the reported results for AFB1, DON and ZON were close or below the target standard deviation (22 %) while they were somewhat higher for FB1 and FB2. On the other hand, the robust standard deviations for HT-2 and T-2 toxins are clearly above the target standard deviation (45 and 33 %, respectively). This finding reflects the maturity of the laboratories analysing these mycotoxins. Not all NRLs submitted results for the HT-2 and T-2 toxins and efforts are still needed to improve the analytical performance.

The limited number of laboratories reporting results for enniatins and the fact that these mycotoxins are not part of a routine monitoring may explain the higher robust standard deviation that was calculated (from 41 to 60 %). At the highest extreme is beauvericin, which suffers from considerable signal variability from run to run when analysed by LC-MS/MS. Presently, there are no commercially available isotope-labelled internal standards that can effectively compensate these analytical inconsistencies.

**Annex 7** shows the individual kernel density plots for the mycotoxins covered by the PT. The confidence intervals of the robust means calculated from the participants' results overlap with the confidence intervals of the assigned values for all the analytes, except for the T-2 toxin.

**Table 3.** Summary statistics of the results submitted for the regulated mycotoxins, enniatins and beauvericin in corn and oat

| | | CORN | | | | | OAT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Units** | **DON** | **AFB1** | **ZON** | **FB1** | **FB2** | **HT-2** | **T-2** | **ENB** | **ENB1** | **ENA1** | **ENA** | **BEA** |
| No. of participants | | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 |
| No. of laboratories that submitted results | | 48 | 52 | 49 | 41 | 39 | 39 | 40 | 15 | 13 | 12 | 13 | 15 |
| Assigned value | µg $kg^{-1}$ | 611 | 10.61 | 161.6 | 768 | 224 | 150.3 | 70.3 | 36.4 | 26.3 | 7.95 | <LOQ | – [1] |
| Uncertainty of the assigned value (k=2) | µg $kg^{-1}$ | 32 | 0.65 | 8.8 | 50 | 16 | 9.5 | 2.1 | 2.8 [2] | 2.2 [2] | 0.84 [2] | – | – |
| Mean (robust) | µg $kg^{-1}$ | 587 | 9.6 | 151 | 715 | 196 | 145 | 80 | 34.1 | 17.0 | 7.4 | 1.7 | 15.8 |
| Reproducibility s.d. | µg $kg^{-1}$ | 113 | 2.2 | 37 | 188 | 60 | 68 | 23 | 14.2 | 6.9 | 4.4 | 1.6 | 13.9 |
| Target s.d. | µg $kg^{-1}$ | 134 | 2.3 | 36 | 169 | 49 | 33 | 16 | – | – | – | – | – |
| Rel. reproducibility s.d. | % | 18 | 21 | 23 | 24 | 27 | 45 | 33 | 42 | 41 | 60 | 94 | 88 |
| $\sigma_p$ | % | 22 | 22 | 22 | 22 | 22 | 22 | 22 | – | – | – | – | – |

[1] No reliable value could be established, see chapter 8.3 on page 10 for details.
[2] A conservative approach was adopted in the absence of information about the uncertainty of the purity of the calibrants

**Table 4.** Reported results and respective z-scores and ζ-scores in the corn test material

| | Deoxynivalenol | | | | | Aflatoxin B1 | | | | | Zearalenone | | | | | Fumonisin B1 | | | | | Fumonisin B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lab code | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C* | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$)) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C |
| LC0001 | 619 | 87.0 | 0.1 | 0.2 | a | 11.6 | 2.30 | 0.4 | 0.8 | a | 166 | 50.0 | 0.1 | 0.2 | a | 778 | 110.0 | 0.1 | 0.2 | a | 361 | 43.00 | 2.8 | 6.0 | a |
| LC0002 | 723 | 115.0 | 0.8 | 1.9 | a | 8.91 | 1.20 | -0.7 | -2.5 | a | 158.5 | 36.1 | -0.1 | -0.2 | a | | | | | | | | | | |
| LC0004 | | | | | | 10.5 | 2.70 | 0.0 | -0.1 | a | 165 | 35.0 | 0.1 | 0.2 | a | 581 | 107.0 | -1.1 | -3.2 | a | 171 | 39.0 | -1.1 | -2.5 | a |
| LC0005 | 842.2 | 151.8 | 1.7 | 2.98 | a | - | | | | | 43.8 | 8.8 | -3.3 | -18.9 | a | 975.5 | 117.1 | 1.2 | 3.3 | a | 495.2 | 87.5 | 5.5 | 6.1 | a |
| LC0006 | | | | | | 6.4 | 1.60 | -1.8 | -4.9 | a | | | | | | | | | | | | | | | |
| LC0007 | 747 | 329.0 | 1.0 | 0.8 | c | 7.63 | 3.36 | -1.3 | -1.7 | a | 56 | 25.0 | -2.97 | -8.0 | a | 248 | 109.0 | -3.1 | -8.7 | a | 116 | 51.0 | -2.2 | -4.0 | a |
| LC0008 | | | | | | 9.12 | 1.37 | -0.6 | -2.0 | a | | | | | | | | | | | | | | | |
| LC0009 | 608 | 122.0 | 0.0 | 0.0 | a | 12.5 | 2.50 | 0.8 | 1.5 | a | 190 | 38.0 | 0.8 | 1.5 | a | 846 | 169.0 | 0.5 | 0.9 | a | 170 | 51.0 | -1.1 | -2.003 | a |
| LC0011 | 835 | | 1.7 | | | 12.34 | 4.94 | 0.7 | 0.7 | b | 84.2 | | -2.2 | | | <1000 | | | | | <1000 | | | | |
| LC0012 | 625.75 | 188.0 | 0.1 | 0.2 | a | 7.97 | 1.40 | -1.1 | -3.4 | a | 156.8 | 34.0 | -0.1 | -0.3 | a | | | | | | | | | | |
| LC0013 | | | | | | 11.7 | 2.30 | 0.5 | 0.9 | a | | | | | | | | | | | | | | | |
| LC0014 | 482 | 33.0 | -1.0 | -5.6 | a | 7.2 | 0.70 | -1.5 | -7.1 | a | 151 | 15.0 | -0.3 | -1.2 | a | 808 | 36.0 | 0.2 | 1.3 | b | 161 | 8.0 | -1.3 | -6.9 | b |
| LC0015 | 576 | 140.0 | -0.3 | -0.5 | a | 8.6 | 3.90 | -0.9 | -1.0 | a | 163 | 48.0 | 0.0 | 0.1 | a | 671 | 160.0 | -0.6 | -1.2 | a | 186 | 54.0 | -0.8 | -1.3 | a |
| LC0016 | 533 | 187.0 | -0.6 | -0.8 | a | 11.6 | 5.10 | 0.4 | 0.4 | c | 89 | 39.0 | -2.04 | -3.6 | a | | | | | | | | | | |
| LC0017 | 20 | 8.0 | -4.4 | -35.8 | b | <0.1 | | | | | <10 | | | | | <20 | | | | | <20 | | | | |
| LC0018 | 617.5 | 64.9 | 0.0 | 0.2 | a | 9.3 | 1.00 | -0.6 | -2.2 | a | 152.9 | 7.5 | -0.2 | -1.5 | b | 683.8 | 47.6 | -0.5 | -2.4 | b | 185.1 | 20.5 | -0.8 | -2.9 | a |
| LC0019 | 536 | 85.9 | -0.6 | -1.6 | a | 7.01 | 1.06 | -1.5 | -5.8 | a | 142.5 | 28.5 | -0.5 | -1.3 | a | 967 | 193.0 | -0.11 | -0.24 | a | | | | | |
| LC0020 | 239.5 | 45.51 | -2.8 | -13.3 | a | 6.43 | 0.96 | -1.8 | -7.2 | a | 230 | 36.8 | 1.9 | 3.6 | a | 1220 | 244.0 | 2.7 | 3.6 | a | 153 | 27.54 | -1.4 | -4.4 | a |
| LC0021 | 422.28 | | -1.4 | | | 3.39 | | -3.1 | | | 176.65 | | 0.4 | | | 485.78 | | -1.7 | | | 158.24 | | -1.3 | | |
| LC0022 | 550 | 165.0 | -0.5 | -0.7 | a | 11 | 5.50 | 0.2 | 0.1 | c | 144 | 43.2 | -0.5 | -0.8 | a | 602 | 301.0 | -1.0 | -1.1 | a | | | | | |
| LC0024 | 597 | 191.0 | -0.1 | -0.1 | a | 9.87 | 5.43 | -0.3 | -0.3 | c | 150 | 37.0 | -0.3 | -0.6 | a | 447 | 214.0 | -1.9 | -2.9 | a | 109 | 31.0 | -2.3 | -6.5 | a |
| LC0025 | 716.1 | 94.5 | 0.8 | 2.1 | a | 11.34 | 7.60 | 0.3 | 0.2 | c | 171.2 | 33.6 | 0.3 | 0.6 | a | 873 | 222.0 | 0.6 | 0.9 | a | 220 | 70.0 | -0.1 | -0.1 | a |
| LC0026 | 527.75 | 211.1 | -0.6 | -0.8 | a | 9.51 | 3.80 | -0.5 | -0.6 | a | 141.67 | 56.67 | -0.6 | -0.7 | a | 319.48 | 127.79 | -2.7 | -6.5 | a | 154.04 | 61.62 | -1.4 | -2.2 | a |
| LC0027 | 630 | 221.0 | 0.1 | 0.2 | a | 8.75 | 4.40 | -0.8 | -0.8 | a | 167 | 84.0 | 0.2 | 0.1 | c | 657 | 263.0 | -0.7 | -0.8 | a | 138 | 55.0 | -1.7 | -2.98 | a |
| LC0028 | 634 | 253.0 | 0.2 | 0.2 | a | 11.75 | 4.70 | 0.5 | 0.5 | c | 178 | 71.0 | 0.5 | 0.5 | a | 668 | 267.0 | -0.6 | -0.7 | a | 266 | 106.0 | 0.9 | 0.8 | c |
| LC0029 | 347 | 104.1 | -2.0 | -4.8 | a | 10.1 | 3.03 | -0.2 | -0.3 | a | 98.4 | 29.5 | -1.8 | -4.1 | a | 686.1 | 205.8 | -0.5 | -0.8 | a | 182.8 | 54.8 | -0.8 | -1.4 | a |
| LC0030 | 521 | 182.0 | -0.7 | -1.0 | a | 12 | 3.60 | 0.6 | 0.8 | a | 182 | 62.0 | 0.6 | 0.7 | a | 557 | | -1.2 | | | 208 | | -0.3 | | a |
| LC0031 | 589 | 177.0 | -0.2 | -0.2 | a | 11.9 | 3.60 | 0.6 | 0.7 | a | 177 | 53.1 | 0.4 | 0.6 | a | 536 | 214.0 | -1.4 | -2.1 | a | 185 | 55.5 | -0.8 | -1.3 | a |

| | Deoxynivalenol | | | | | Aflatoxin B1 | | | | | Zearalenone | | | | | Fumonisin B1 | | | | | Fumonisin B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lab code | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C* | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$)) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C |
| LC0032 | 724.9 | 13.5 | 0.8 | 6.5 | b | 11.16 | 1.85 | 0.2 | 0.6 | a | 109.5 | 8.0 | -1.5 | -8.8 | b | 1201 | 260.0 | 2.6 | 3.3 | a | 332 | 70.0 | 2.2 | 3.0 | a |
| LC0033 | 572.2 | 199.1 | -0.3 | -0.4 | a | 9.3 | 4.10 | -0.6 | -0.6 | a | 126.9 | 55.4 | -1.0 | -1.2 | a | 756.0 | 252.3 | -0.1 | -0.1 | a | 220.3 | 88.5 | -0.1 | -0.1 | a |
| LC0034 | 630 | 37.0 | 0.1 | 0.8 | a | 9.34 | 4.43 | -0.5 | -0.6 | a | 150 | 35.0 | -0.3 | -0.6 | a | 727 | 74.0 | -0.2 | -0.9 | a | 182 | 9.0 | -0.8 | -4.5 | b |
| LC0035 | 679.0 | 119.5 | 0.5 | 1.1 | a | 9.7 | 2.80 | -0.4 | -0.6 | a | 148.1 | 44.2 | -0.4 | -0.6 | a | 751.0 | 223.7 | -0.1 | -0.1 | a | 234.6 | 63.1 | 0.2 | 0.3 | a |
| LC0036 | 556.74 | 16.73 | -0.4 | -3.0 | b | 10.4 | 2.90 | -0.1 | -0.1 | a | 139.14 | 1.69 | -0.6 | -5.0 | b | 679 | 230.9 | -0.5 | -0.8 | a | 217 | 73.4 | -0.1 | -0.2 | a |
| LC0037 | 719 | 144.0 | 0.8 | 1.5 | a | 11.5 | 2.30 | 0.4 | 0.7 | a | 180 | 36.0 | 0.5 | 1.0 | a | 708 | 156.0 | -0.4 | -0.7 | a | 220 | 44.0 | -0.1 | -0.2 | a |
| LC0038 | 592 | 118.0 | -0.1 | -0.3 | a | 9.3 | 1.90 | -0.6 | -1.3 | a | 207 | 41.0 | 1.3 | 2.2 | a | 999 | 200.0 | 1.4 | 2.2 | a | 190 | 38.0 | -0.7 | -1.6 | a |
| LC0039 | 630 | 110.0 | 0.1 | 0.3 | a | 10 | 2.10 | -0.3 | -0.6 | a | 160 | 60.0 | 0.0 | -0.1 | a | 840 | 180.0 | 0.4 | 0.8 | a | 210 | 27.0 | -0.3 | -0.9 | a |
| LC0040 | 482 | 226.0 | -1.0 | -1.1 | a | 15.2 | 8.60 | 2.0 | 1.1 | c | 179 | 100.0 | 0.5 | 0.3 | c | 843 | 489.0 | 0.4 | 0.3 | c | 253 | 111.0 | 0.6 | 0.5 | c |
| LC0041 | 552 | 276.0 | -0.4 | -0.4 | c | 13.4 | 6.50 | 1.2 | 0.9 | c | 160 | 80.0 | 0.0 | 0.0 | c | | | | | | | | | | |
| LC0042 | 581.4 | 173.4 | -0.2 | -0.3 | a | 8.9 | 3.70 | -0.7 | -0.9 | a | 158.3 | 52.9 | -0.1 | -0.1 | a | | | | | | | | | | |
| LC0043 | 99.9 | 10.8 | -3.8 | -30.2 | b | 6.18 | 1.05 | -1.9 | -7.2 | a | 35.4 | 8.37 | -3.5 | -20.8 | b | 630 | 85.4 | -0.8 | -2.8 | a | 96.3 | 15.8 | -2.6 | -11.2 | b |
| LC0044 | 609.8 | 208.6 | 0.0 | 0.0 | a | 9.8 | 4.30 | -0.3 | -0.4 | a | 146.9 | 62.3 | -0.4 | -0.5 | a | | | | | | | | | | |
| LC0045 | 443 | 133.0 | -1.2 | -2.5 | a | 11.9 | 4.80 | 0.6 | 0.5 | c | 91 | 27.0 | -2.0 | -5.0 | a | 638 | 223.0 | -0.8 | -1.1 | a | 222 | 78.0 | 0.0 | 0.0 | a |
| LC0046 | 485.9 | 114.4 | -0.9 | -2.1 | a | 8.48 | 3.70 | -0.9 | -1.1 | a | 154.8 | 43.3 | -0.2 | -0.3 | a | 540.8 | 125.3 | -1.3 | -3.4 | a | 145.2 | 41.0 | -1.6 | -3.6 | a |
| LC0047 | 874 | 350.0 | 2.0 | 1.5 | c | 11.4 | 4.60 | 0.3 | 0.3 | a | 247 | 99.0 | 2.4 | 1.7 | c | 911 | 364.0 | 0.8 | 0.8 | c | 270 | 108.0 | 0.9 | 0.8 | c |
| LC0048 | 583 | 60.0 | -0.2 | -0.8 | a | 8.1 | 0.90 | -1.1 | -4.5 | a | 180 | 27.0 | 0.5 | 1.3 | a | 707 | 127.0 | -0.4 | -0.9 | a | 352 | 57.0 | 2.6 | 4.3 | a |
| LC0049 | 621 | 257.0 | 0.1 | 0.1 | a | 8.3 | 1.80 | -1.0 | -2.4 | a | 173.1 | 64.6 | 0.3 | 0.4 | a | 657 | 335.0 | -0.7 | -0.7 | a | 196 | 100.0 | -0.6 | -0.5 | c |
| LC0050 | 637 | 192.0 | 0.2 | 0.3 | a | 8.1 | 2.50 | -1.1 | -1.9 | a | 141 | 43.0 | -0.6 | -0.9 | a | 586 | 293.0 | -1.1 | -1.2 | a | 153 | 77.0 | -1.4 | -1.8 | a |
| LC0051 | 569.0 | | -0.3 | | | 3.3 | | -3.1 | | | 103.0 | | -1.6 | | | 1274.2 | | 2.998 | | | 71.0 | | -3.1 | | |
| LC0052 | 601 | 30.0 | -0.1 | -0.5 | b | 8.8 | 1.90 | -0.8 | -1.8 | a | 107 | 4.6 | -1.5 | -11.0 | b | | | | | | | | | | |
| LC0053 | | | | | | 8.9 | 3.90 | -0.7 | -0.9 | a | | | | | | | | | | | | | | | |
| LC0054 | 580 | 180.0 | -0.2 | -0.3 | a | 11.9 | 3.90 | 0.6 | 0.7 | a | 146 | 44.0 | -0.4 | -0.7 | a | 603 | 190.0 | -1.0 | -1.7 | a | 176 | 53.0 | -1.0 | -1.7 | a |
| LC0055 | 719 | 259.0 | 0.8 | 0.8 | a | 8.9 | 2.30 | -0.7 | -1.4 | a | 170 | 70.0 | 0.2 | 0.2 | a | 621 | 217.0 | -0.9 | -1.3 | a | 247 | 79.0 | 0.5 | 0.6 | a |
| LC0056 | 271.66 | 45.29 | -2.5 | -12.2 | a | 4.52 | 0.75 | -2.6 | -12.3 | a | 225.74 | 37.63 | 1.8 | 3.3 | a | | | | | | | | | | |

* Classification of the uncertainty reported by the participant

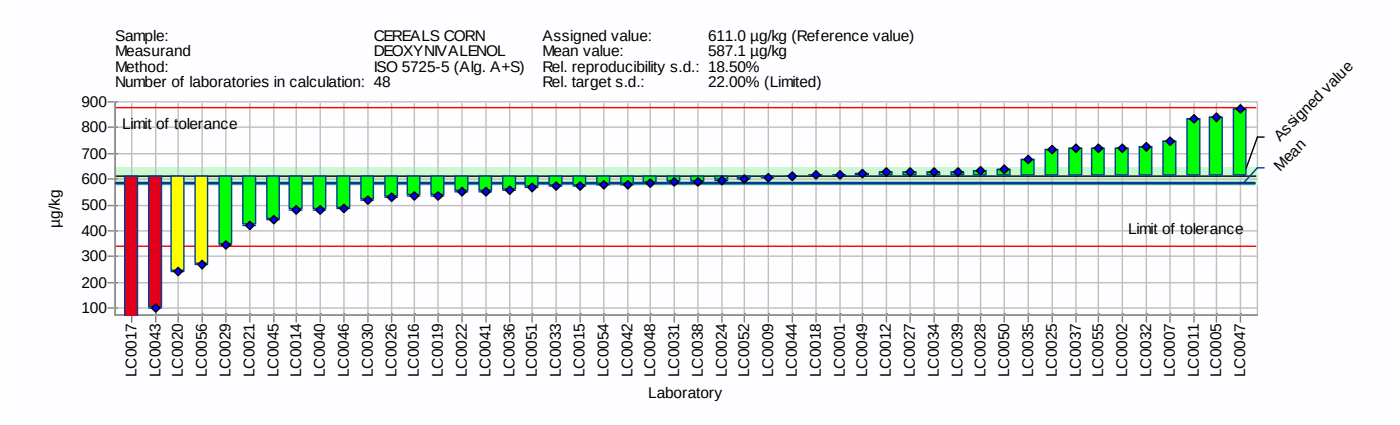
**Table 5.** Reported results and respective z-scores and ζ-scores in the oat test material

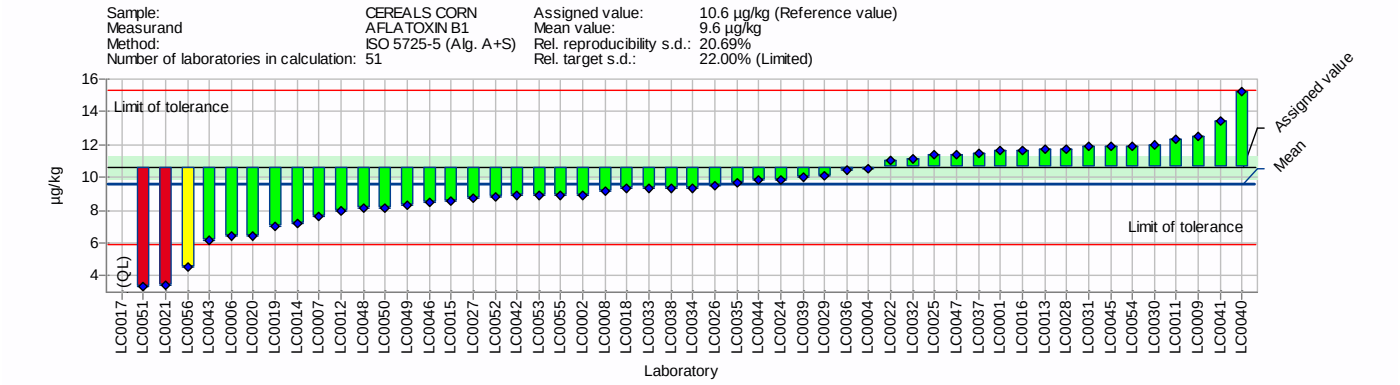
| | HT-2 toxin | | | | | T-2 toxin | | | | | SUM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lab code | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C * | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | Z-Score |
| LC0001 | 124 | 22.00 | -0.8 | -2.2 | a | 69.5 | 7.00 | 0.0 | -0.2 | a | 193.5 | -0.6 |
| LC0005 | 78.0 | 15.70 | -2.2 | -7.9 | a | 31.4 | 6.80 | -2.5 | -10.9 | a | 109.4 | -2.4 |
| LC0007 | 147 | 65.00 | -0.1 | -0.1 | a | 150 | 66.00 | 5.2 | 2.4 | c | 297 | 1.6 |
| LC0009 | <50 | | | | | <50 | | | | | | |
| LC0011 | <250 | | | | | <250 | | | | | | |
| LC0014 | 101 | 7.00 | -1.5 | -8.4 | b | 82 | 5.00 | 0.8 | 4.3 | a | 183 | -0.8 |
| LC0015 | 237 | 66.00 | 2.6 | 2.6 | a | 107 | 34.00 | 2.4 | 2.2 | c | 344 | 2.5 |
| LC0017 | <10 | | | | | <10 | | | | | | |
| LC0018 | 168.0 | 34.00 | 0.5 | 1.0 | a | 88,3 | 3.80 | 1.2 | 8.3 | a | 256.3 | 0.7 |
| LC0020 | 334 | 50.10 | 5.6 | 7.2 | a | <1.6 | | | | | 334 | 5.6 |
| LC0021 | 15.09 | | -4.1 | | | 39.09 | | -2.02 | | | 54.18 | -3.4 |
| LC0022 | 171.8 | 51.50 | 0.6 | 0.8 | a | 83.5 | 25.10 | 0.9 | 1.1 | a | 255.3 | 0.7 |
| LC0024 | 122 | 37.00 | -0.9 | -1.5 | a | 71 | 22.00 | 0.0 | 0.1 | a | 193 | -0.6 |
| LC0026 | 113.93 | 45.57 | -1.1 | -1.6 | a | 68.51 | 27.40 | -0.1 | -0.1 | a | 182.44 | -0.8 |
| LC0027 | 143 | 46.00 | -0.2 | -0.3 | a | 65 | 22.00 | -0.3 | -0.5 | a | 208 | -0.3 |
| LC0028 | 182 | 73.00 | 1.0 | 0.9 | c | 86 | 34.00 | 1.0 | 0.9 | c | 268 | 1.0 |
| LC0030 | 169 | 49.00 | 0.6 | 0.7 | a | 79 | 26.00 | 0.6 | 0.7 | a | 248 | 0.6 |
| LC0031 | 217 | 109.00 | 2.02 | 1.2 | c | 77.9 | 23.40 | 0.5 | 0.6 | a | 294.9 | 1.5 |
| LC0032 | 158.0 | 35.00 | 0.2 | 0.4 | a | 78.3 | 21.00 | 0.5 | 0.8 | a | 236.3 | 0.3 |
| LC0033 | 123.0 | 53.90 | -0.8 | -1.0 | a | 48.0 | 21.10 | -1.4 | -2.1 | a | 171 | -1.0 |
| LC0034 | 136 | 17.00 | -0.4 | -1.5 | a | 70.9 | 2.90 | 0.0 | 0.4 | a | 206.9 | -0.3 |
| LC0035 | 221.0 | 61.20 | 2.1 | 2.3 | a | 102.3 | 27.30 | 2.1 | 2.3 | a | 323.3 | 2.1 |
| LC0036 | 176.1 | 74.00 | 0.8 | 0.7 | c | 76 | 33.40 | 0.4 | 0.3 | c | 252.1 | 0.6 |
| LC0037 | 191 | 38.20 | 1.2 | 2.1 | a | 104 | 20.80 | 2.2 | 3.2 | a | 295 | 1.5 |
| LC0038 | 48 | 9.60 | -3.1 | -15.1 | a | 51 | 10.00 | -1.2 | -3.8 | a | 99 | -2.5 |

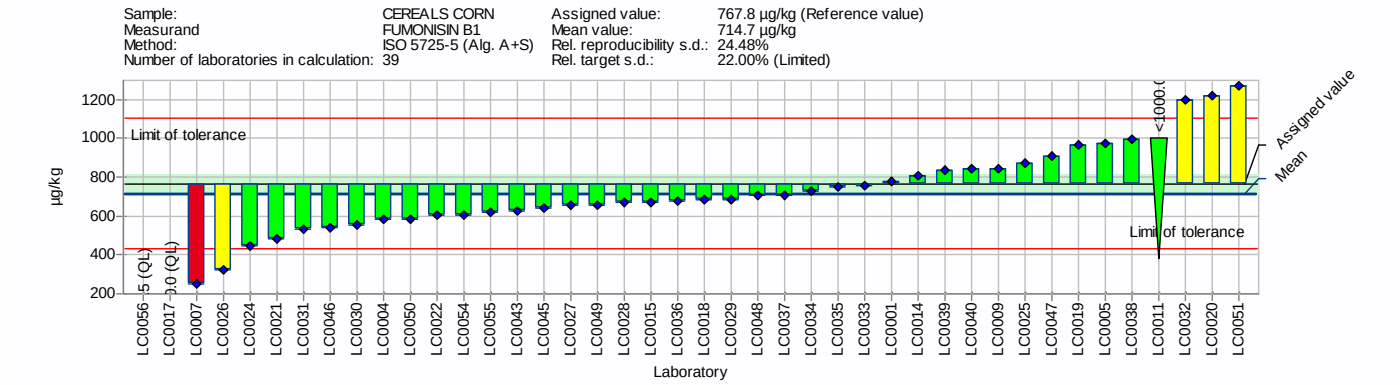
| | HT-2 toxin | | | | | T-2 toxin | | | | | SUM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lab code | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | U lab (µg kg$^{-1}$) | Z-Score | Zeta score | C | Result (µg kg$^{-1}$) | Z-Score |
| LC0039 | 220 | 72.00 | 2.1 | 1.9 | c | 97 | 24.00 | 1.7 | 2.2 | a | 317 | 1.99 |
| LC0040 | 49.5 | 18.80 | -3.0 | -9.6 | a | 68.6 | 26.80 | -0.1 | -0.1 | a | 118.1 | -2.1 |
| LC0041 | 157 | 79.00 | 0.2 | 0.2 | c | 90.5 | 45.00 | 1.3 | 0.9 | c | 247.5 | 0.6 |
| LC0043 | 58.4 | 10.50 | -2.8 | -13.0 | a | 94.5 | 12.50 | 1.6 | 3.8 | a | 152.9 | -1.4 |
| LC0045 | 171.2 | 51.40 | 0.6 | 0.8 | a | 95.1 | 28.50 | 1.6 | 1.7 | a | 266.3 | 0.9 |
| LC0046 | 208.1 | 55.70 | 1.7 | 2.04 | a | 109.0 | 32.10 | 2.5 | 2.4 | c | 317.1 | 1.99 |
| LC0047 | 183 | 73.00 | 1.0 | 0.9 | c | 95.2 | 38.00 | 1.6 | 1.3 | c | 278.2 | 1.2 |
| LC0048 | 29.5 | 8.40 | -3.7 | -19.0 | b | 81.6 | 16.90 | 0.7 | 1.3 | a | 111.1 | -2.3 |
| LC0049 | 243 | 97.00 | 2.8 | 1.9 | c | 172 | 69.00 | 6.6 | 2.9 | c | 415 | 4.0 |
| LC0050 | 154 | 47.00 | 0.1 | 0.2 | a | 81 | 25.00 | 0.7 | 0.9 | a | 235 | 0.3 |
| LC0051 | 6.3 | | -4.4 | | | 156.9 | | 5.6 | | | 163.2 | -1.2 |
| LC0052 | 142 | 14.00 | -0.3 | -1.0 | a | 69 | 7.00 | -0.1 | -0.3 | a | 211 | -0.2 |
| LC0054 | 94.1 | 29.00 | -1.7 | -3.7 | a | 40.2 | 13.00 | -1.9 | -4.6 | a | 134.3 | -1.8 |
| LC0055 | 150 | 29.00 | 0.0 | 0.0 | a | 71 | 14.00 | 0.0 | 0.1 | a | 221 | 0.0 |
| LC0056 | | | | | | 50.79 | 8.50 | -1.3 | -4.4 | a | 50.79 | -1.3 |

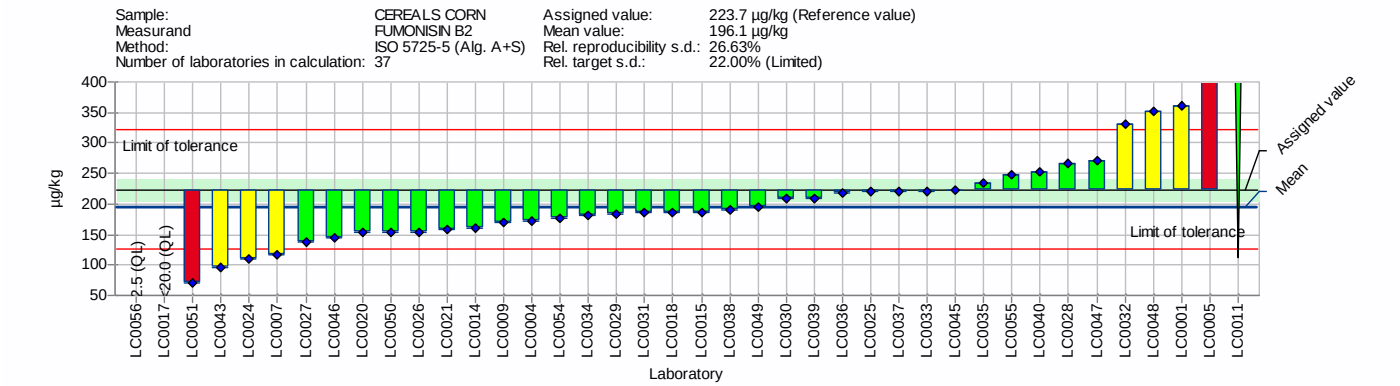
* Classification of the uncertainty reported by the participant

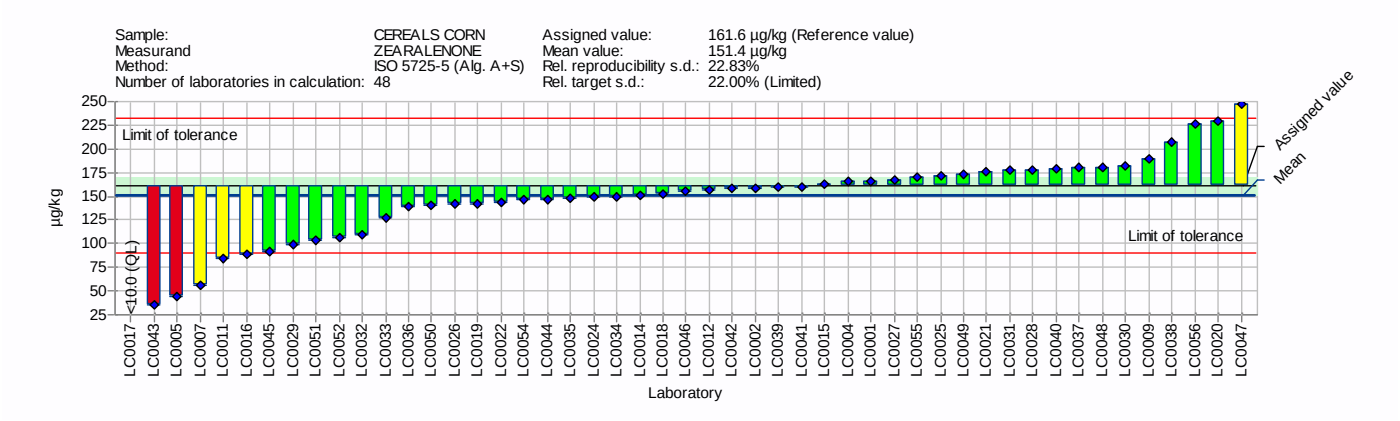
**Figure 5.** Sigmoidal plots of individual laboratory results reported for the regulated mycotoxins, enniatins and beauvericin in corn and oat.

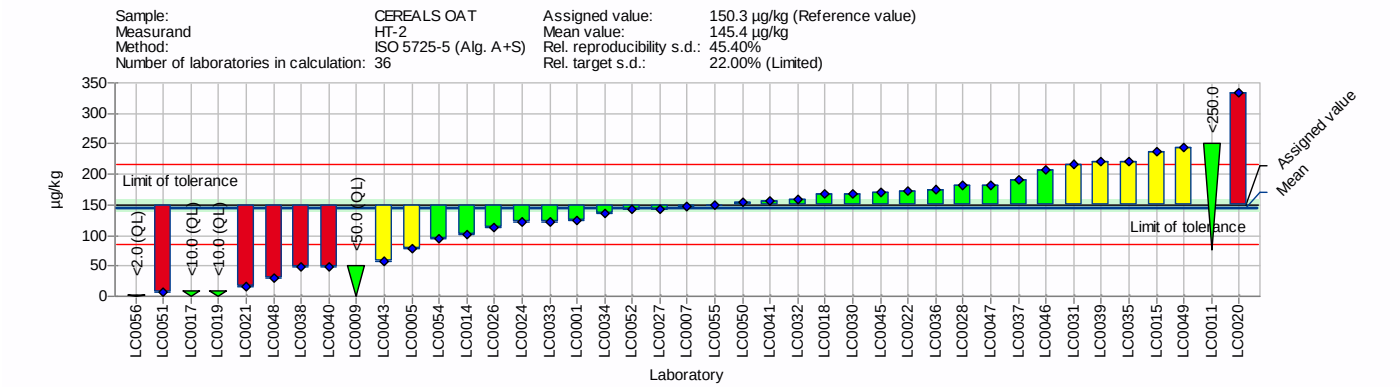


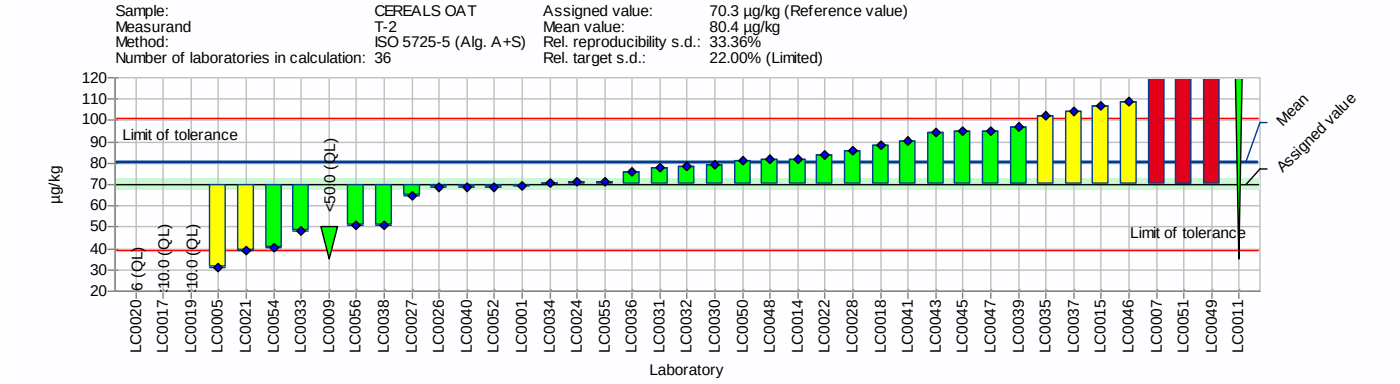



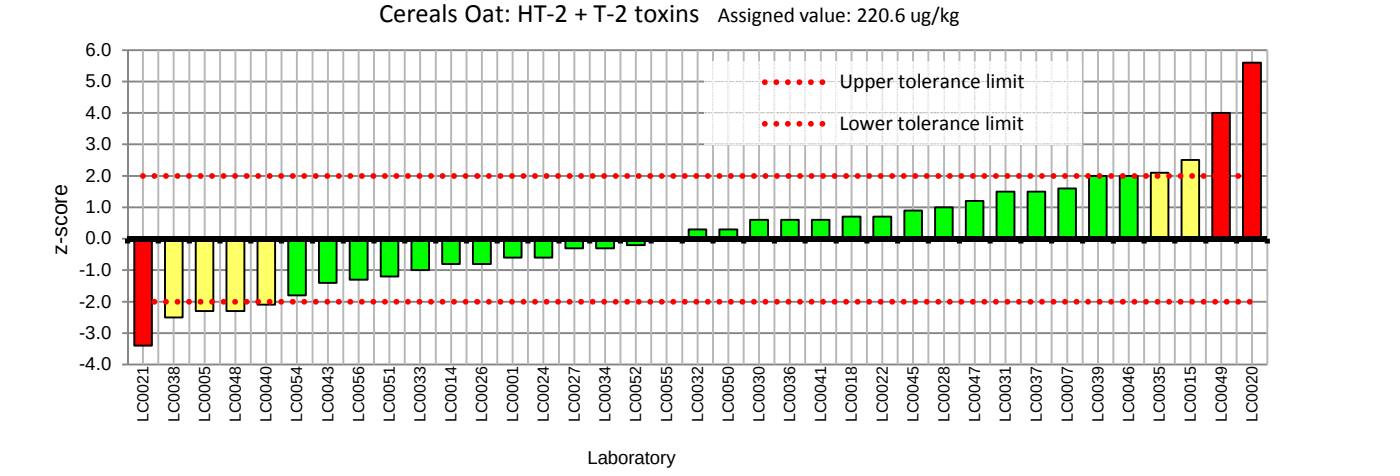



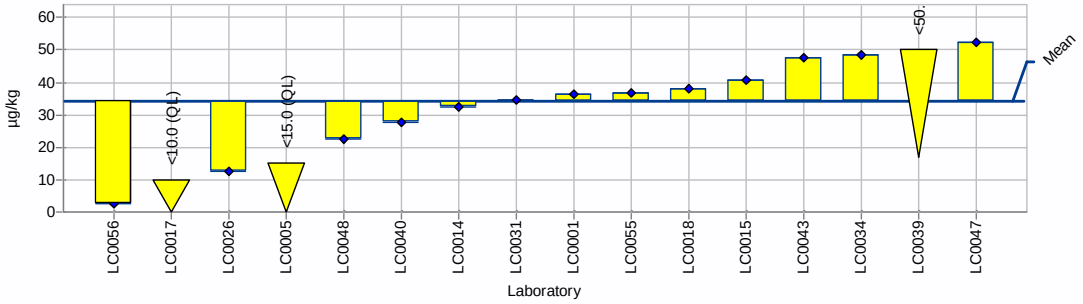

Sample: CEREALS CORN
Measurand ZEARALENONE
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 48
Assigned value: 161.6 µg/kg (Reference value)
Mean value: 151.4 µg/kg
Rel. reproducibility s.d.: 22.83%
Rel. target s.d.: 22.00% (Limited)
Limit of tolerance
Assigned value
Mean
µg/kg
<10.0 (QL)
Laboratory


Sample: CEREALS OAT
Measurand HT-2
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 36
Assigned value: 150.3 µg/kg (Reference value)
Mean value: 145.4 µg/kg
Rel. reproducibility s.d.: 45.40%
Rel. target s.d.: 22.00% (Limited)
Limit of tolerance
Assigned value
Mean
µg/kg
<2.0 (QL)
<10.0 (QL)
<10.0 (QL)
<50.0 (QL)
<250.0
Laboratory

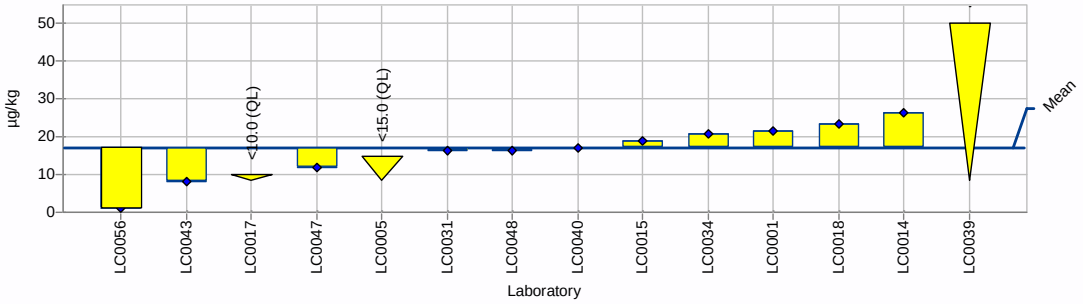

Sample: CEREALS OAT
Measurand T-2
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 36
Assigned value: 70.3 µg/kg (Reference value)
Mean value: 80.4 µg/kg
Rel. reproducibility s.d.: 33.36%
Rel. target s.d.: 22.00% (Limited)
Limit of tolerance
Mean
Assigned value
µg/kg
<6 (QL)
<10.0 (QL)
<10.0 (QL)
<50.0 (QL)
Laboratory


Cereals Oat: HT-2 + T-2 toxins Assigned value: 220.6 ug/kg
Upper tolerance limit
Lower tolerance limit
z-score
Laboratory

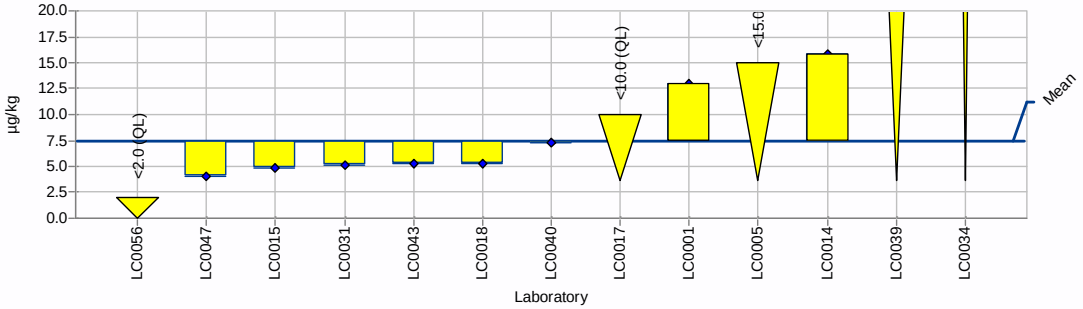
Sample: CEREALS OAT
Measurand ENNIATIN B
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 13
Mean value: 34.1 µg/kg
Rel. reproducibility s.d.: 41.72%



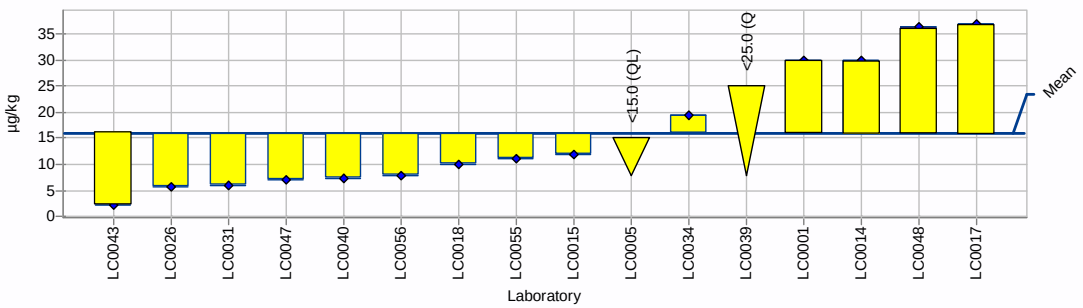

Sample: CEREALS OAT
Measurand ENNIATIN B1
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 11
Mean value: 17.0 µg/kg
Rel. reproducibility s.d.: 40.85%




Sample: CEREALS OAT
Measurand ENNIATIN A1
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 8
Mean value: 7.4 µg/kg
Rel. reproducibility s.d.: 60.01%




Sample: CEREALS OAT
Measurand BEAUVERICIN
Method: ISO 5725-5 (Alg. A+S)
Number of laboratories in calculation: 14
Mean value: 15.8 µg/kg
Rel. reproducibility s.d.: 87.68%



The plausibility of the uncertainty statements of the laboratories was assessed by classifying every reported uncertainty into one of the three groups (see column C in Tables 4 and 5) according to the following rules.

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 ($u(x_{pt})$). 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 ($\sigma_{pt}$). Consequently, case "a" becomes: $u(x_{pt}) \leq u(x_i) \leq \sigma_{pt}$.

If $u(x_i)$ is smaller than $u(x_{pt})$ (case "b": $u(x_i) < u(x_{pt})$) the laboratory might have underestimated its measurement uncertainty.

If $u(x_i)$ is larger than $\sigma_{pt}$ (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 amendment.

The rate of the satisfactory ζ-scores is lower than the one for z-scores. 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 8** presents the sigmoidal distribution of the results associated with the respective uncertainties (k=2).

# 9 Evaluation of the questionnaire

The questionnaire distributed to the participants has provided very useful information concerning the approaches and capabilities of the laboratories in the determination of regulated mycotoxins, enniatins and beauvericin in cereals (**Annex 6**).

The questionnaire will be discussed in two parts:

1) the first part will address the answers regarding the previous experience of the participants and general organisational matters: questions Q.1-3 and Q.19-29.

2) the second part will deal with the outcome of the answers concerning analytical features (questions Q.4-16 and Q.18) and will present the validation data of the methods used by the PT participants.

## 9.1 Experience and organisational aspects

The participants were asked to classify their yearly work load on the analysis of mycotoxins in 3 categories (Q.1). The results are summarised in Figure 6. The most frequently analysed mycotoxins were: aflatoxins, deoxynivalenol and zearalenone, while the least were the enniatins and beauvericin, both in terms of the number of samples (mainly <50) and the number of laboratories that conducted it (18). The type of matrices was diverse: figs, nuts, spices, cereals/flour and cereal products, feed products, peanuts, baby-food, etc. (Q.2) (**Annex 9**). Of the 46 laboratories that answered the question on accreditation (Q. 3), 87 % hold an accreditation for measuring aflatoxin B1, 83 % for deoxynivalenol and 80 % for zearalenone. 35 % of the accredited methods were multi-mycotoxin procedures (Table 6).

Presently, 79 % of the participants don't analyse enniatins and beauvericin, and out of these, 35 % don't plan to implement this determination in the near future (Q. 19 and 20). Still, another 38 % foresee implementing a suitable analytical procedure in the mid-term future (within 1-2 years).

**Figure 6.** Number of mycotoxin determinations performed by the laboratories on a yearly basis.

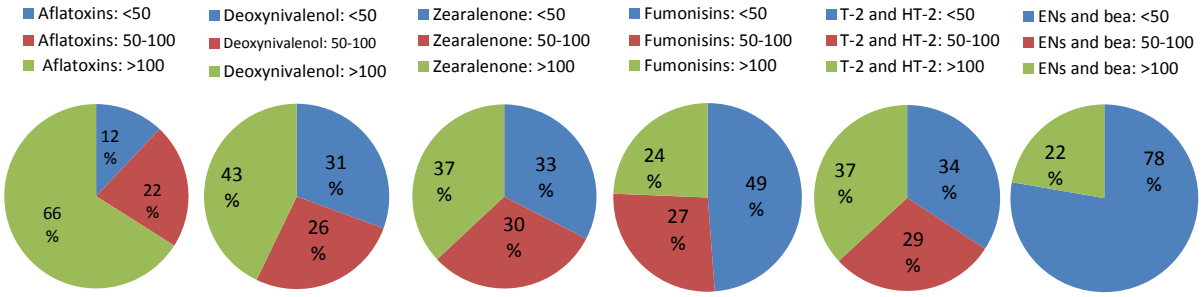



**Table 6.** Number of laboratories accredited for the determination of mycotoxins in food.

| | AfB1 | DON | ZON | FB1 | FB2 | HT-2 | T-2 | ENs | BEA | Multitoxin method |
|---|---|---|---|---|---|---|---|---|---|---|
| N. labs | 40 | 38 | 37 | 23 | 23 | 30 | 29 | 4 | 3 | 16 |
| **%** | **87** | **83** | **80** | **50** | **50** | **65** | **63** | **9** | **7** | **35** |

The majority of the participants did not experience any difficulties during this PT (Q.21, Table 7). Those who mentioned issues related them mainly to the sensitivity of their analytical instruments and the complexity of the matrices (Q.22, Table 8). For 92 % of the participants the time allowed for reporting the results was adequate (Q.24), while 84 % also found the amount of sample provided as sufficient for the analysis (Q.25). The vast majority of the participants didn't face any difficulties with the software for reporting the results (Q. 27), and they were all happy with the instructions to carry-out the PT (Q.28) (**Annex 4**). About 65 % of the participants completed the PT analyses in one week or less (Q.26). Despite the general satisfaction with the layout of the PT and the information provided, some participants took the opportunity to raise some remarks in the Comments section (Q.29). A compilation can be found in Table 9.

The most effective route to spread information on upcoming PTs still seems to be by direct contact, via email or during the annual workshops (Q.23, Table 10).

**Table 7.** Answers related to the experience of the participants during this PT and the evaluation of organisational aspects.

| Response | | Q.21 | Q.24 | Q.25 | Q.27 | Q.28 |
|---|---|---|---|---|---|---|
| **NO** | Nr. | 40 | 4 | 8 | 43 | 0 |
| | ***%*** | ***77*** | ***8*** | ***16*** | ***91*** | ***0*** |
| **YES** | Nr. | 12 | 48 | 43 | 4 | 48 |
| | ***%*** | ***23*** | ***92*** | ***84*** | ***9*** | ***100*** |

**Table 8.** Analytical difficulties experienced running the PT

| |
|---|
| Sensitivity loss of 80 % for the multimethod |
| Recovery rates for enniatins and BEA were very low. We never had rates lower than about 90 % for the last 3 years in equal matrices! Probably due to the samples provided? |
| No, for the routine samples, but yes for new ones - sensitivity of the instrument, insufficient clean-up |
| not the right matrix for calibration |
| We have no LC MSMS, and for example we had problems with derivatisation of the T2/HT2 toxins (both the standard solutions and the samples) |
| complex matrix, therefore insufficient clean-up |
| Probably depending to the thinness of the sample particle it was impossible to obtain a clear test solution especially for DON-ZEA-T-2-HT-2 analysis. |
| Carry-over between subsequent LC-MS/MS runs was observed for fumonisin B1&B2 and beauvericin, which required thorough rinsing |
| Sensitivity of the instrument, pump leak, difficulties with recovery estimation ... |
| matrix effects for oat sample sensitivity problems on our MS/MS for enniatins and beauvericin |
| Some matrix effects due to insufficient clean-up of the PT samples |
| problem of filtration for Afla B1 in corn sample only |
| Sensitivity of the instrument |

**Table 9.** Comments submitted by the participants

| |
|---|
| Besides Aflatoxin B1 in corn, there was Aflatoxin B2. The sum of aflatoxins is 9.83 μg/Kg. |
| The results for all enniatins and beauvericin are <LOD, where LOD was estimated as 5 ug/kg |
| Enniatin-amount is quite low in comparison to our routine samples. |
| For Fumonisins, the result is the sum of Fum B1+B2 together, but there is no possibility to type together, so I put it in the column for Fum B1. For T2 and HT2, I didn't obtain adequate recoveries, so I am not sending the results. Time and sample amount were enough for routine analyses, for new - I would appreciate more time and more samples to work on methods. We tried to analyse enniatins and beauvericin too, but the technique was not sensitive enough. |
| These samples were analysed with the newly validated multitoxin method. Will be accredited in few weeks. |
| The high uncertainty values obtained in some cases (AFB1 and FB1) do not exclude a lack of sufficient homogeneity in the sample. |
| Thank you for the opportunity to participate |
| No data reported for beauvericin, enniatins and fumonisins. This is due to the absence of recovery data. The chosen spiking level was not sufficient to significantly add to the residue already present, and therefore recovery values could not be calculated. |
| From the measurands requested, the only 3 mycotoxins which are reported are those which are analysed in the laboratory; AFLA, DON and ZON. Our laboratory does not have an LC-MS/MS and future analysis of the mycotoxins not reported here will depend on whether such instrumentation is procured. |
| We were not able to provide results for enniatins and beauvericin since with our current MS/MS detector (Waters TQD), it was not possible to implement a multimycotoxin method up to now. We hope to do this after installation of our new Xevo-TQ-S next month. |
| We don't use the terminology of "LOD/LOQ", for our routine analyses we report the "reporting limit" to the customer. This (usually) coincides with the LOQ. Hence we didn't report an LOD. |
| Unfortunately, we have no experience for enniatin and beauvericin. |
| The method used is screening |
| The recoveries indicated are "apparent recoveries", i.e to account for both extraction recovery and matrix effects |
| Beauvericin and Enniatins integrated relative to the internal standard associated with T-2. |

**Table 10.** Information source about the PT on multi-mycotoxins

| **Invitation/announcement of the PT** | **%** |
|---|---|
| Invitation by email | 73 |
| Through the EURL Mycotoxins website | 0 |
| During the EURL workshop for the NRLs on mycotoxins | 13 |
| By the NRL in your country | 23 |

## 9.2 Overview of the analytical methodologies

A considerable number of laboratories resorted to LC-MS/MS-based multi-mycotoxin methods for analysing the distributed PT samples (see Table 11). This was the only methodology that allowed analysing all the requested analytes, including regulated and emerging mycotoxins (enniatins and beauvericin). LC-MS/MS analysis was mostly preceded by a sample preparation of the "dilute and shoot" type (14 cases), but QuEChERS, IACs and SPE also found application in 4 to 5 cases each. Pressurised liquid extraction (PLE) was employed just by a single laboratory. HPLC-UV(DAD) was used by 11 participants to analyse DON while HPLC-FLD was used chiefly to analyse AFB1 but also ZON, fumonisins, HT-2 and T-2. These two techniques were almost invariably preceded by an IAC clean-up. LC coupled with high-resolution MS (HiResMS) was used as a screening method. One laboratory reported the sum of FB1 and FB2 as their ELISA did not allow individuating the analytes. Another laboratory also using ELISA reported results <LOQ for FB1, FB2, HT-2 and T-2.

Of the laboratories that employed LC-MS/MS methods, 62 % used $^{13}$C-labelled internal standards (Q.10) which were added in the majority of cases (88 %) after the extraction (Q.11). The analysis of calibration standards prepared in the pure solvent was the dominant strategy (78 %, Q.12) in good correlation with the use of either individual mycotoxin methods or multi-methods employing $^{13}$C internal standards.

Most of the laboratories (88 %) have estimated the methods´recoveries based on spiking experiments while the remaining used certified reference materials or an alternative strategy (Q.14). Nevertheless, in only 71 % of the cases, the results were corrected for recoveries (Q.15). The use of certified reference materials for quality control is not a common practice for the laboratories, but about 30 % of them mentioned the use of reference/quality control materials from FAPAS or Trilogy (Q.16).

The preferred approach for estimating the measurement uncertainty was using "initial method validation data" accounting for 53 % of the participants (Q.13).

**Annex 9** presents a compilation of the main analytical conditions of the methods used by the participants such as the type of method, extraction conditions, clean-up, LC-MS acquisition settings, quantification strategy, amongst others. **Annex 10** compiles some important analytical figures of merit (recoveries, LODs and LOQs). The median recoveries for all analytes varied from 91 to 98 %. In general, the sensitivity of the methods was sufficient to analyse the mycotoxin levels present in the PT materials.

**Table 11.** Analytical methods and number of laboratories that have adopted them for participating in the PT

| | DON | AFB1 | ZON | FB1 | FB2 | HT-2 | T-2 | EN A | EN A1 | EN B | EN B1 | BEA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LC-MS/MS | 33 | 24 | 30 | 28 | 27 | 31 | 32 | 13 | 12 | 15 | 13 | 15 |
| HPLC-UV(DAD) | 11 | - | - | - | - | - | - | - | - | - | - | - |
| HPLC-FLD | - | 26 | 15 | 9 | 9 | 3 | 3 | - | - | - | - | - |
| ELISA | 2 | 1 | 3 | 3 | 3 | 3 | 3 | - | - | - | - | - |
| LC-HiRes | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - | - | - | - |
| GC-MS(/MS) | 1 | - | - | - | - | 1 | 1 | - | - | - | - | - |

# 10 Conclusions

A PT was organised by the EURL mycotoxins covering 12 mycotoxins spread over two test materials (oat and maize) to allow the participants to test their multi-mycotoxin procedures. Laboratories were also encouraged to report results for enniatins and beauvericin which, although not mandatory, was an important focus of this PT and of special interest for DG SANTE.

A total of 53 laboratories submitted results providing from 39 to 52 datasets for the

regulated mycotoxins and a maximum of 15 datasets for the enniatins and beauvericin. Overall, 83.7 % of the results reported by the participants for the regulated mycotoxins were classified as satisfactory. The rate of satisfactory z-scores for the individual mycotoxins was ranked as follows: AFB1 - 94 %, DON – 91 %, ZON - 89 %, FB1 – 87 %, FB2 – 78 %, T-2 – 75 % and HT-2 – 64 %. Twelve laboratories had satisfactory performance in all the 7 regulated mycotoxins while 8 additional laboratories had one questionable result.

Eleven laboratories reported results for all enniatins and beauvericin. The consensus values for enniatin B and A1 were very close to the EURL's estimate although no z-scoring was attempted as the reference values didn't meet the required level of accuracy to make sound statements.

Up to 33 laboratories used LC-MS/MS for analysing a combination of mycotoxins while 11 laboratories could analyse all the requested mycotoxins (12). HPLC-UV(DAD) was used by 11 laboratories to analyse DON exclusively. HPLC-FLD was the technique of choice for analysing AFB1 (26 laboratories) although it was also selected for analysing ZON (15 laboratories) and fumonisins (9 laboratories). ELISA was employed by 3 laboratories while GC-MS/MS and LC-HiResMS were used by a single laboratory each. The determination of mycotoxins by LC-MS/MS often included a straightforward sample preparation based on "extract, dilute & shoot", whereas HPLC-UV(DAD), HPLC-FLD and LC-HiResMS were preceded by an IAC clean-up.

No bias was observed when comparing LC-MS/MS-based multi-mycotoxin procedures and analyte-specific protocols based on the t-student test. The reproducibility standard deviation of the multi-mycotoxin and analyte-specific procedures was equivalent.

The aim of this PT on providing insight on the performance of multi-mycotoxin methods was successfully achieved as 24 laboratories determined the whole range of regulated mycotoxins. Some laboratories could also analyse enniatins and beauvericin although technical difficulties such as inappropriate recoveries and sensitivity issues hampered others to contribute as well.

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# Annexes

## Annex 1. Opening of the registration

Proficiency test for the determination of regulated mycotoxins and enniatins and beauvericin in cereal products

Fields marked with * are mandatory.


On behalf of the European Union Reference Laboratory for Mycotoxins (EU-RL Mycotoxins), I have the pleasure to announce the opening for registration of the inter-laboratory comparison/proficiency test (PT) for the determination of regulated mycotoxins (Commission Regulation (EC) No 1881/2006) and additionally enniatins and beauvericin in cereal products.

**The deadline for registration is 2nd September 2016.**

The PT materials consist of 2 naturally contaminated cereal products (oat and maize) with selected combinations of mycotoxins. The dispatch of the samples is expected by mid-September 2016. Participants will have 6 weeks from the dispatch date to report back the results.

The aim of this study is to evaluate the proficiency of the European National Reference Laboratories (NRLs) and Official Control Laboratories (OCLs) on the determination of the regulated mycotoxins, as well as to assess their capability for analysing mycotoxins that might be subject to future regulation. The determination of enniatins and beauvericin is not mandatory but highly encouraged.

For NRLs the participation is free of charge. The participation fee for OCLs is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. Enrolled 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

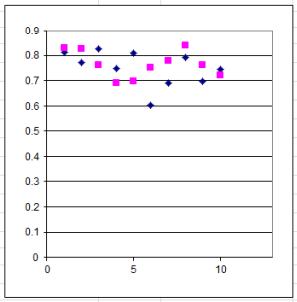
* Email address

## Annex 2. Homogeneity test

| Homogeneity according to ISO 13528:2015 | Corn: samples C-1## | | | | |
|---|---|---|---|---|---|
| | **Aflatoxin B1** | **DON** | **ZON** | **FB1** | **FB2** |
| $\hat{\sigma}$ | 0.167 (22 %) | 0.173 (22 %) | 0.061 (22 %) | 0.489 (22 %) | 0.297 (22 %) |
| 0.3 $\hat{\sigma}$ (critical value) | 0.050 | 0.053 | 0.018 | 0.147 | 0.082 |
| $S_X$ (standard deviation of sample averages) | 0.047 | 0.029 | 0.011 | 0.130 | 0.112 |
| $S_W$ (within-sample standard deviation) | 0.055 | 0.038 | 0.025 | 0.177 | 0.020 |
| $S_S$ (between-sample standard deviation) | 0.027 | 0.011 | 0.000 | 0.037 | 0.020 |
| **$S_S$ < 0.3 $\hat{\sigma}$** | **Passed** | **Passed** | **Passed** | **Passed** | **Passed** |

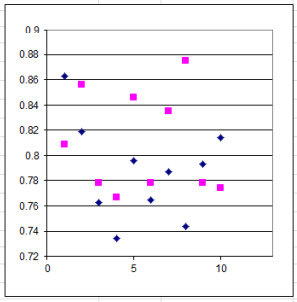
*Aflatoxin B1*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 0.758 | | | | | | | | | | |
| 0.0022 | | $s_x$ = | 0.047 | | 22.0% | = σ-trg(%) | | | | | | | | |
| MSW = | 0.0030 | $s_{an}=s_w$ = | 0.055 | 1 | 0.167 | = σ-trg | | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0008 | $s_s$ = | 0.027 | | | | | | | | | | | |
| | | $s_s$ = | 0.027 | | 0.050 | = 0,3*σ-trg | | | | | | | | |
| | | 1) Cochran test | 0.3678 | $C=D_{max}{}^2/SDD$ | | | | | | | | | | |
| | | | no outlier | no outlier | | | | | | | | | IUPAC | |
| | | | 0.6020 | 0.7175 | = Crit | | | | | | | | Tab1 | Cochran |
| | | | @ 95% | @ 99% | | | | | | | | | m | Crit-95% Crit-99% |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | 3 | 0.9669 0.9933 |
| | | 3) IUPAC | 0.001 | 0.01 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | 4 | 0.9065 0.9676 |
| | | | Ss2 < Crit => passed | | | | | | | | | | 5 | 0.8412 0.9279 |
| | | | | | | | | | | | | | 6 | 0.7808 0.8828 |
| | Bottle | Result_a | Result_b | diff | sum | avg | | | | | | | 7 | 0.7271 0.8376 |
| | 1 | 0.811 | 0.83 | -0.019 | 1.641 | 0.8205 | | | | | | | 8 | 0.6789 0.7945 |
| | 2 | 0.771 | 0.827 | -0.056 | 1.598 | 0.799 | | | | | | | 9 | 0.6385 0.7544 |
| | 3 | 0.825 | 0.762 | 0.063 | 1.587 | 0.7935 | 2 | | | | | | 10 | 0.6020 0.7175 |
| | 4 | 0.747 | 0.691 | 0.056 | 1.438 | 0.719 | | | | | | | 11 | 0.5700 0.684 |
| | 5 | 0.81 | 0.699 | 0.111 | 1.509 | 0.7545 | | | | | | | 12 | 0.5410 0.6528 |
| | 6 | 0.604 | 0.752 | -0.148 | 1.356 | 0.678 | | | | | | | | |
| minimum 7 | 7 | 0.692 | 0.78 | -0.088 | 1.472 | 0.736 | | | | | | | Tab2 | |
| | 8 | 0.793 | 0.841 | -0.048 | 1.634 | 0.817 | | | | | | | m | F1 F2 |
| | 9 | 0.698 | 0.762 | -0.064 | 1.46 | 0.73 | | | | | | | 3 | 2.996 4.276 |
| | 10 | 0.745 | 0.721 | 0.024 | 1.466 | 0.733 | | | | | | | 4 | 2.605 2.796 |
| | 11 | | | | | | | | | | | | 5 | 2.372 2.096 |
| | 12 | | | | | | | | | | | | 6 | 2.214 1.694 |
| | | | SDD=Σ(diff)² = | 0.059547 | | | | | | | | | 7 | 2.099 1.433 |
| | | | MSB = var(sum)/2 = | 0.0045 | | | | | | | | | 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 |




*DON*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 0.799 | | | | | | | | | | |
| 0.0009 | | $s_x$ = | 0.029 | | 22.0% | = σ-trg(%) | | | | | | | | |
| MSW = | 0.0015 | $s_{an}=s_w$ = | 0.038 | 1 | 0.176 | = σ-trg | | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0001 | $s_s$ = | 0.011 | | | | | | | | | | | |
| | | $s_s$ = | 0.011 | | 0.053 | = 0,3*σ-trg | | | | | | | | |
| | | 1) Cochran test | 0.5806 | $C=D_{max}{}^2/SDD$ | | | | | | | | | | |
| | | | no outlier | no outlier | | | | | | | | | IUPAC | |
| | | | 0.6020 | 0.7175 | = Crit | | | | | | | | Tab1 | Cochran |
| | | | @ 95% | @ 99% | | | | | | | | | m | Crit-95% Crit-99% |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | 3 | 0.9669 0.9933 |
| | | 3) IUPAC | 0.000 | 0.01 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | 4 | 0.9065 0.9676 |
| | | | Ss2 < Crit => passed | | | | | | | | | | 5 | 0.8412 0.9279 |
| | | | | | | | | | | | | | 6 | 0.7808 0.8828 |
| | Bottle | Result_a | Result_b | diff | sum | avg | | | | | | | 7 | 0.7271 0.8376 |
| | 1 | 0.863 | 0.809 | 0.054 | 1.672 | 0.836 | | | | | | | 8 | 0.6789 0.7945 |
| | 2 | 0.819 | 0.856 | -0.037 | 1.675 | 0.8375 | | | | | | | 9 | 0.6385 0.7544 |
| | 3 | 0.763 | 0.778 | -0.015 | 1.541 | 0.7705 | 2 | | | | | | 10 | 0.6020 0.7175 |
| | 4 | 0.734 | 0.767 | -0.033 | 1.501 | 0.7505 | | | | | | | 11 | 0.5700 0.684 |
| | 5 | 0.796 | 0.846 | -0.05 | 1.642 | 0.821 | | | | | | | 12 | 0.5410 0.6528 |
| | 6 | 0.765 | 0.778 | -0.013 | 1.543 | 0.7715 | | | | | | | | |
| minimum 7 | 7 | 0.787 | 0.835 | -0.048 | 1.622 | 0.811 | | | | | | | Tab2 | |
| | 8 | 0.744 | 0.875 | -0.131 | 1.619 | 0.8095 | | | | | | | m | F1 F2 |
| | 9 | 0.793 | 0.778 | 0.015 | 1.571 | 0.7855 | | | | | | | 3 | 2.996 4.276 |
| | 10 | 0.814 | 0.774 | 0.04 | 1.588 | 0.794 | | | | | | | 4 | 2.605 2.796 |
| | 11 | | | | | | | | | | | | 5 | 2.372 2.096 |
| | 12 | | | | | | | | | | | | 6 | 2.214 1.694 |
| | | | SDD=Σ(diff)² = | 0.029558 | | | | | | | | | 7 | 2.099 1.433 |
| | | | MSB = var(sum)/2 = | 0.0017 | | | | | | | | | 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 |



*ZON*

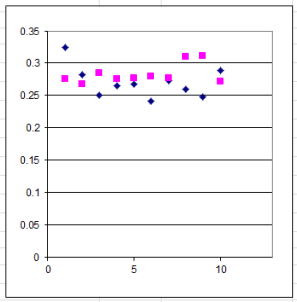
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 0.276 | | | | | | | | | | |
| | 0.0001 | | $s_x$ = | 0.011 | | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 0.0006 | | $s_{an}=s_w$ = | 0.025 | 1 | 0.061 | = σ-trg | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0000 | | $s_s$ = | 0.000 | MSB<MSW | | | | | | | | | |
| | | | $s_s$ = | 0.000 | | 0.018 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.3272 | $C=D_{max}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | | Ss < 0,3*strg => passed | | | | | | | | | | |
| | | 3) IUPAC | | 0.000 | 0.00 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | | Ss2 < Crit => passed | | | | | | | | | | |

| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 0.324 | 0.275 | 0.049 | 0.599 | 0.2995 |
| 2 | 0.282 | 0.267 | 0.015 | 0.549 | 0.2745 |
| 3 | 0.25 | 0.284 | -0.034 | 0.534 | 0.267 |
| 4 | 0.264 | 0.275 | -0.011 | 0.539 | 0.2695 |
| 5 | 0.267 | 0.277 | -0.01 | 0.544 | 0.272 |
| 6 | 0.241 | 0.279 | -0.038 | 0.52 | 0.26 |
| minimum 7 | 0.272 | 0.277 | -0.005 | 0.549 | 0.2745 |
| 8 | 0.26 | 0.309 | -0.049 | 0.569 | 0.2845 |
| 9 | 0.248 | 0.311 | -0.063 | 0.559 | 0.2795 |
| 10 | 0.288 | 0.271 | 0.017 | 0.559 | 0.2795 |
| 11 | | | | | |
| 12 | | | | | |

2

SDD=∑(diff)² = 0.012131

MSB = var(sum)/2 = 0.0002




**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 |

*FB1*

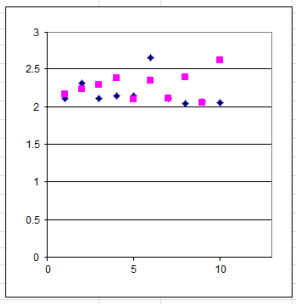
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 2.222 | | | | | | | | | | |
| | 0.0170 | | $s_x$ = | 0.130 | | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 0.0312 | | $s_{an}=s_w$ = | 0.177 | 1 | 0.489 | = σ-trg | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0014 | | $s_s$ = | 0.037 | | | | | | | | | | |
| | | | $s_s$ = | 0.037 | | 0.147 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.4973 | $C=D_{max}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | | Ss < 0,3*strg => passed | | | | | | | | | | |
| | | 3) IUPAC | | 0.001 | 0.07 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | | Ss2 < Crit => passed | | | | | | | | | | |

| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 2.113 | 2.168 | -0.055 | 4.281 | 2.1405 |
| 2 | 2.312 | 2.232 | 0.08 | 4.544 | 2.272 |
| 3 | 2.108 | 2.286 | -0.178 | 4.394 | 2.197 |
| 4 | 2.141 | 2.378 | -0.237 | 4.519 | 2.2595 |
| 5 | 2.144 | 2.102 | 0.042 | 4.246 | 2.123 |
| 6 | 2.655 | 2.352 | 0.303 | 5.007 | 2.5035 |
| minimum 7 | 2.114 | 2.109 | 0.005 | 4.223 | 2.1115 |
| 8 | 2.047 | 2.397 | -0.35 | 4.444 | 2.222 |
| 9 | 2.065 | 2.049 | 0.016 | 4.114 | 2.057 |
| 10 | 2.058 | 2.615 | -0.557 | 4.673 | 2.3365 |
| 11 | | | | | |
| 12 | | | | | |

2

SDD=∑(diff)² = 0.623881

MSB = var(sum)/2 = 0.0339




**IUPAC**

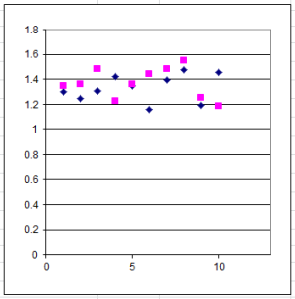
| | Tab1 | Cochran |
|---|---|---|
| m | Crit-95% | Crit-99% |
| 3 | 0.9669 | 0.9933 |
| 4 | 0.9065 | 0.9676 |
| 5 | 0.8412 | 0.9279 |
| 6 | 0.7808 | 0.8828 |
| 7 | 0.7271 | 0.8376 |
| 8 | 0.6789 | 0.7945 |
| 9 | 0.6385 | 0.7544 |
| 10 | 0.6020 | 0.7175 |
| 11 | 0.5700 | 0.684 |
| 12 | 0.5410 | 0.6528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

**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 |

*FB2*

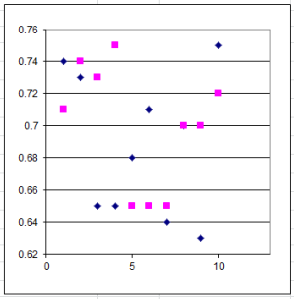
| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | | |
| | variances | | mean = | 1.349 | | | | | | | | | | | |
| | 0.0067 | | $s_x$ = | 0.082 | | 22.0% | = σ-trg(%) | | | | | | | | |
| MSW = | 0.0126 | | $s_{an}$=$s_w$ = | 0.112 | 1 | 0.297 | = σ-trg | | | Homogeneity Tests | | | | | |
| $s^2_{sam}$= | 0.0004 | | $s_s$ = | 0.020 | | | | | | | | | | | |
| | | | $s_s$ = | 0.020 | | 0.089 | = 0,3*σ-trg | | | | | | | | |
| | | 1) Cochran test | | 0.3074 | $C=D_{max}^2/SDD$ | | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | IUPAC | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | Tab1 | Cochran |
| | | | | @ 95% | @ 99% | | | | | | | | m | Crit-95% | Crit-99% |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | 3 | 0.9669 | 0.9933 |
| | | | | | | | | | | | | | 4 | 0.9065 | 0.9676 |
| | | 3) IUPAC | | 0.000 | 0.03 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | 5 | 0.8412 | 0.9279 |
| | | | Ss2 < Crit => passed | | | | | | | | | | 6 | 0.7808 | 0.8828 |
| | | | | | | | | | | | | | 7 | 0.7271 | 0.8376 |
| | Bottle | Result_a | Result_b | diff | sum | avg | | | | | | | 8 | 0.6789 | 0.7945 |
| | 1 | 1.298 | 1.349 | -0.051 | 2.647 | 1.3235 | | | | | | | 9 | 0.6385 | 0.7544 |
| | 2 | 1.249 | 1.359 | -0.11 | 2.608 | 1.304 | | | | | | | 10 | 0.6020 | 0.7175 |
| | 3 | 1.304 | 1.48 | -0.176 | 2.784 | 1.392 | | | | | | | 11 | 0.5700 | 0.684 |
| | 4 | 1.424 | 1.228 | 0.196 | 2.652 | 1.326 | | | | | | | 12 | 0.5410 | 0.6528 |
| | 5 | 1.349 | 1.359 | -0.01 | 2.708 | 1.354 | | | | | | | | | |
| | 6 | 1.161 | 1.439 | -0.278 | 2.6 | 1.3 | | | | | | | | | |
| minimum 7 | 7 | 1.397 | 1.483 | -0.086 | 2.88 | 1.44 | | | | | | | | Tab2 | |
| | 8 | 1.475 | 1.552 | -0.077 | 3.027 | 1.5135 | | | | | | | m | F1 | F2 |
| | 9 | 1.189 | 1.25 | -0.061 | 2.439 | 1.2195 | | | | | | | 3 | 2.996 | 4.276 |
| | 10 | 1.457 | 1.187 | 0.27 | 2.644 | 1.322 | | | | | | | 4 | 2.605 | 2.796 |
| | 11 | | | | | | | | | | | | 5 | 2.372 | 2.096 |
| | 12 | | | | | | | | | | | | 6 | 2.214 | 1.694 |
| | | | SDD=Σ(diff)² = | 0.251423 | | | | | | | | | 7 | 2.099 | 1.433 |
| | | | MSB = var(sum)/2 = | 0.0134 | | | | | | | | | 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 |



| Homogeneity according to ISO 13528:2015 | Oat: samples O-2## | | | | | |
|---|---|---|---|---|---|---|
| | **HT-2 toxin** | **T2 toxin** | **Enniatin B** | **Enniatin B1** | **Enniatin A1** | **Beauvericin** |
| $\hat{\sigma}$ | 0.153 (22 %) | 0.167 (22 %) | 1280210 (22 %) | 192729 (22 %) | 66968 (22 %) | 891.5 (22 %) |
| 0.3 $\hat{\sigma}$ (critical value) | 0.046 | 0.050 | 384063 | 57819 | 20090 | 267.4 |
| $S_X$ (standard deviation of sample averages) | 0.031 | 0.024 | 148345 | 46432 | 22710 | 151.6 |
| $S_W$ (within-sample standard deviation) | 0.037 | 0.054 | 146096 | 53406 | 19735 | 222.7 |
| $S_S$ (between-sample standard deviation) | 0.016 | 0.000 | 106463 | 27016 | 17917 | 0.000 |
| **$S_S$ < 0.3 $\hat{\sigma}$** | **Passed** | **Passed** | **Passed** | **Passed** | **Passed** | **Passed** |

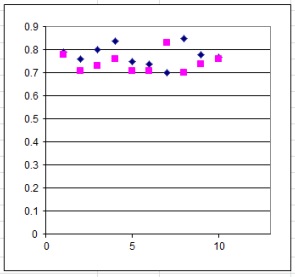
*HT-2 toxin*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | | |
| | variances | | mean = | 0.694 | | | | | | | | | | | |
| | 0.0010 | | $s_x$ = | 0.031 | | 22.0% | = σ-trg(%) | | | | | | | | |
| MSW = | 0.0014 | | $s_{an}=s_w$ = | 0.037 | 1 | 0.153 | = σ-trg | | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0003 | | $s_s$ = | 0.016 | | | | | | | | | | | |
| | | | $s_s$ = | 0.016 | | 0.046 | = 0,3*σ-trg | | | | | | | | |
| | | 1) Cochran test | | 0.3597 | $C=D_{max}{}^2/SDD$ | | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | IUPAC | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | Tab1 | Cochran |
| | | | | @ 95% | @ 99% | | | | | | | | | m | Crit-95% Crit-99% |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | | 3 | 0.9669 0.9933 |
| | | | | | | | | | | | | | | 4 | 0.9065 0.9676 |
| | | 3) IUPAC | | 0.000 | 0.01 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | 5 | 0.8412 0.9279 |
| | | | Ss2 < Crit => passed | | | | | | | | | | | 6 | 0.7808 0.8828 |
| | | | | | | | | | | | | | | 7 | 0.7271 0.8376 |
| | Bottle | Result_a | Result_b | diff | sum | avg | | | | | | | | 8 | 0.6789 0.7945 |
| | 1 | 0.74 | 0.71 | 0.03 | 1.45 | 0.725 | | | | | | | | 9 | 0.6385 0.7544 |
| | 2 | 0.73 | 0.74 | -0.01 | 1.47 | 0.735 | | | | | | | | 10 | 0.6020 0.7175 |
| | 3 | 0.65 | 0.73 | -0.08 | 1.38 | 0.69 | | | | | | | | 11 | 0.5700 0.684 |
| | 4 | 0.65 | 0.75 | -0.1 | 1.4 | 0.7 | | | | | | | | 12 | 0.5410 0.6528 |
| | 5 | 0.68 | 0.65 | 0.03 | 1.33 | 0.665 | | | | | | | | | |
| | 6 | 0.71 | 0.65 | 0.06 | 1.36 | 0.68 | | | | | | | | | |
| minimum 7 | 7 | 0.64 | 0.65 | -0.01 | 1.29 | 0.645 | | | | | | | | Tab2 | |
| | 8 | 0.7 | 0.7 | 0 | 1.4 | 0.7 | | | | | | | | m | F1 F2 |
| | 9 | 0.63 | 0.7 | -0.07 | 1.33 | 0.665 | | | | | | | | 3 | 2.996 4.276 |
| | 10 | 0.75 | 0.72 | 0.03 | 1.47 | 0.735 | | | | | | | | 4 | 2.605 2.796 |
| | 11 | | | | | | | | | | | | | 5 | 2.372 2.096 |
| | 12 | | | | | | | | | | | | | 6 | 2.214 1.694 |
| | | | SDD=Σ(diff)² = | | 0.0278 | | | | | | | | | 7 | 2.099 1.433 |
| | | | MSB = var(sum)/2 = | | 0.0019 | | | | | | | | | 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 |




*T2 toxin*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | | |
| | variances | | mean = | 0.761 | | | | | | | | | | | |
| | 0.0006 | | $s_x$ = | 0.024 | | 22.0% | = σ-trg(%) | | | | | | | | |
| MSW = | 0.0029 | | $s_{an}=s_w$ = | 0.054 | 1 | 0.167 | = σ-trg | | | Homogeneity Tests | | | | | |
| $s^2_{sam}$ = | 0.0000 | | $s_s$ = | 0.000 | MSB<MSW | | | | | | | | | | |
| | | | $s_s$ = | 0.000 | | 0.050 | = 0,3*σ-trg | | | | | | | | |
| | | 1) Cochran test | | 0.3913 | $C=D_{max}{}^2/SDD$ | | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | IUPAC | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | Tab1 | Cochran |
| | | | | @ 95% | @ 99% | | | | | | | | | m | Crit-95% Crit-99% |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | | 3 | 0.9669 0.9933 |
| | | | | | | | | | | | | | | 4 | 0.9065 0.9676 |
| | | 3) IUPAC | | 0.000 | 0.01 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | 5 | 0.8412 0.9279 |
| | | | Ss2 < Crit => passed | | | | | | | | | | | 6 | 0.7808 0.8828 |
| | | | | | | | | | | | | | | 7 | 0.7271 0.8376 |
| | Bottle | Result_a | Result_b | diff | sum | avg | | | | | | | | 8 | 0.6789 0.7945 |
| | 1 | 0.79 | 0.78 | 0.01 | 1.57 | 0.785 | | | | | | | | 9 | 0.6385 0.7544 |
| | 2 | 0.76 | 0.71 | 0.05 | 1.47 | 0.735 | | | | | | | | 10 | 0.6020 0.7175 |
| | 3 | 0.8 | 0.73 | 0.07 | 1.53 | 0.765 | | | | | | | | 11 | 0.5700 0.684 |
| | 4 | 0.84 | 0.76 | 0.08 | 1.6 | 0.8 | | | | | | | | 12 | 0.5410 0.6528 |
| | 5 | 0.75 | 0.71 | 0.04 | 1.46 | 0.73 | | | | | | | | | |
| | 6 | 0.74 | 0.71 | 0.03 | 1.45 | 0.725 | | | | | | | | | |
| minimum 7 | 7 | 0.7 | 0.83 | -0.13 | 1.53 | 0.765 | | | | | | | | Tab2 | |
| | 8 | 0.85 | 0.7 | 0.15 | 1.55 | 0.775 | | | | | | | | m | F1 F2 |
| | 9 | 0.78 | 0.74 | 0.04 | 1.52 | 0.76 | | | | | | | | 3 | 2.996 4.276 |
| | 10 | 0.77 | 0.76 | 0.01 | 1.53 | 0.765 | | | | | | | | 4 | 2.605 2.796 |
| | 11 | | | | | | | | | | | | | 5 | 2.372 2.096 |
| | 12 | | | | | | | | | | | | | 6 | 2.214 1.694 |
| | | | SDD=Σ(diff)² = | | 0.0575 | | | | | | | | | 7 | 2.099 1.433 |
| | | | MSB = var(sum)/2 = | | 0.0012 | | | | | | | | | 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 |



*Enniatin B*

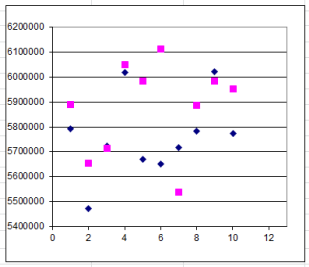
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 5819136.4 | | | | | | | | | | |
| | 22006371660.5 | | $s_x$ = | 148345.4 | 1 | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 21344000381.7 | | $s_{an}=s_w$ = | 146095.9 | | 1280210.0 | = σ-trg | | | Homogeneity Tests | | | | |
| $s^2_{sam}$= | 11334371469.7 | | $s_s$ = | 106463.0 | | | | | | | | | | |
| | | | $s_s$ = | 106463.0 | | 384063.0 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.4921 | $C=D_{max}{}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | | |
| | | 3) IUPAC | | 11334371469.7 | 298853023810 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | Ss2 < Crit => passed | | | | | | | | | | | |

| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 5793488 | 5888783 | -95295 | 11682271 | 5841135.5 |
| 2 | 5473414 | 5653207 | -179793 | 11126621 | 5563310.5 |
| 3 | 5724523 | 5713106 | 11417 | 11437629 | 5718814.5 |
| 4 | 6017863 | 6048325 | -30462 | 12066188 | 6033094 |
| 5 | 5669382 | 5984339 | -314957 | 11653721 | 5826860.5 |
| 6 | 5652763 | 6111088 | -458325 | 11763851 | 5881925.5 |
| minimum 7 | 5715687 | 5539073 | 176614 | 11254760 | 5627380 |
| 8 | 5783550 | 5886895 | -103345 | 11670445 | 5835222.5 |
| 9 | 6021398 | 5982664 | 38734 | 12004062 | 6002031 |
| 10 | 5772452 | 5950728 | -178276 | 11723180 | 5861590 |
| 11 | | | | | |
| 12 | | | | | |

2

SDD=Σ(diff)² = 426880007634

MSB = var(sum)/2 = 44012743321




**IUPAC**

| m | Tab1 Crit-95% | Cochran 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 |
| # | 0.6020 | 0.7175 |
| # | 0.5700 | 0.684 |
| # | 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 |
| # | 1.880 | 1.010 |
| # | 1.831 | 0.927 |
| # | 1.789 | 0.859 |

*Enniatin B1*

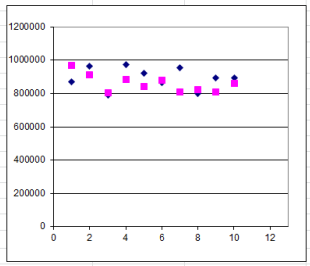
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 876041.1 | | | | | | | | | | |
| | 2155971005.3 | | $s_x$ = | 46432.4 | 1 | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 2852172504.8 | | $s_{an}=s_w$ = | 53405.7 | | 192729.0 | = σ-trg | | | Homogeneity Tests | | | | |
| $s^2_{sam}$= | 729884752.9 | | $s_s$ = | 27016.4 | | | | | | | | | | |
| | | | $s_s$ = | 27016.4 | | 57818.7 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.3761 | $C=D_{max}{}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | Ss < 0,3*strg => passed | | | | | | | | | | | |
| | | 3) IUPAC | | 729884752.9 | 9165707273 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | Ss2 < Crit => passed | | | | | | | | | | | |

| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 870541 | 967178 | -96637.86366 | 1837718.867 | 918859.43 |
| 2 | 966396 | 910868 | 55527.32817 | 1877263.466 | 938631.73 |
| 3 | 792627 | 806502 | -13874.86324 | 1599129.688 | 799564.84 |
| 4 | 973519 | 882521 | 90997.47421 | 1856040.217 | 928020.11 |
| 5 | 921806 | 844577 | 77228.91461 | 1766382.592 | 883191.3 |
| 6 | 864170 | 880271 | -16101.33644 | 1744440.596 | 872220.3 |
| minimum 7 | 953821 | 807344 | 146477.3203 | 1761165.386 | 880582.69 |
| 8 | 797949 | 821678 | -23729.79553 | 1619626.889 | 809813.44 |
| 9 | 892571 | 809552 | 83019.23185 | 1702123.156 | 851061.58 |
| 10 | 894383 | 862548 | 31835.26852 | 1756931.222 | 878465.61 |
| 11 | | | | | |
| 12 | | | | | |

2

SDD=Σ(diff)² = 57043450096

MSB = var(sum)/2 = 4311942011




**IUPAC**

| m | Tab1 Crit-95% | Cochran 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 |
| # | 0.6020 | 0.7175 |
| # | 0.5700 | 0.684 |
| # | 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 |
| # | 1.880 | 1.010 |
| # | 1.831 | 0.927 |
| # | 1.789 | 0.859 |

*Enniatin A1*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 304400.0 | | | | | | | | | | |
| | 515763844.5 | | $s_x$ = | 22710.4 | | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 389456858.3 | | $s_{an}=s_w$ = | 19734.7 | 1 | 66968.0 | = σ-trg | | | Homogeneity Tests | | | | |
| $s^2_{sam}$= | 321035415.4 | | $s_s$ = | 17917.5 | | | | | | | | | | |
| | | | $s_s$ = | 17917.5 | | 20090.4 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.4033 | $C=D_{max}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | | Ss < 0,3*strg => passed | | | | | | | | | | |
| | | 3) IUPAC | | 321035415.4 | 1152193681 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | | Ss2 < Crit => passed | | | | | | | | | | |

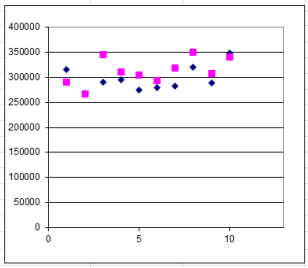
| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 315028 | 289606 | 25421.96858 | 604633.6127 | 302316.81 |
| 2 | 268711 | 266270 | 2441.89314 | 534980.8975 | 267490.45 |
| 3 | 289547 | 345595 | -56047.95732 | 635142.4281 | 317571.21 |
| 4 | 294609 | 310750 | -16141.33426 | 605359.1139 | 302679.56 |
| 5 | 274125 | 304632 | -30507.04948 | 578757.8232 | 289378.91 |
| 6 | 279526 | 293411 | -13885.59854 | 572936.6668 | 286468.33 |
| 7 | 282595 | 318836 | -36240.98276 | 601430.8626 | 300715.43 |
| 8 | 320139 | 349760 | -29621.05974 | 669898.7053 | 334949.35 |
| 9 | 289138 | 307701 | -18563.21456 | 596839.1263 | 298419.56 |
| 10 | 348372 | 339649 | 8722.545738 | 688021.1271 | 344010.56 |
| 11 | | | | | |
| 12 | | | | | |

2

minimum 7

SDD=Σ(diff)² = 7789137167

MSB = var(sum)/2 = 1031527689




**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 |
| # | 0.6020 | 0.7175 |
| # | 0.5700 | 0.684 |
| # | 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 |
| # | 1.880 | 1.010 |
| # | 1.831 | 0.927 |
| # | 1.789 | 0.859 |

*Beauvericin*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m = | 10 | | | | | | | | | | |
| | variances | | mean = | 4052.197 | | | | | | | | | | |
| | 22990.7328 | | $s_x$ = | 151.627 | | 22.0% | = σ-trg(%) | | | | | | | |
| MSW = | 49618.3960 | | $s_{an}=s_w$ = | 222.752 | 1 | 891.483 | = σ-trg | | | Homogeneity Tests | | | | |
| $s^2_{sam}$= | 0.0000 | | $s_s$ = | 0.000 | MSB<MSW | | | | | | | | | |
| | | | $s_s$ = | 0.000 | | 267.445 | = 0,3*σ-trg | | | | | | | |
| | | 1) Cochran test | | 0.2817 | $C=D_{max}^2/SDD$ | | | | | | | | | |
| | | | | no outlier | no outlier | | | | | | | | | |
| | | | | 0.6020 | 0.7175 | = Crit | | | | | | | | |
| | | | | @ 95% | @ 99% | | | | | | | | | |
| | | 2) ISO-13528 | | Ss < 0,3*strg => passed | | | | | | | | | | |
| | | 3) IUPAC | | 0.000 | 184586.42 | = Crit = F1*(0,3*σ)²+F2*MSW | | | | | | | | |
| | | | | Ss2 < Crit => passed | | | | | | | | | | |

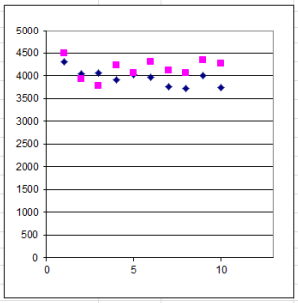
| Bottle | Result_a | Result_b | diff | sum | avg |
|---|---|---|---|---|---|
| 1 | 4301 | 4500 | -198.762119 | 8801.0325 | 4400.51625 |
| 2 | 4045 | 3919 | 125.926454 | 7963.85647 | 3981.92824 |
| 3 | 4060 | 3780 | 280.11807 | 7840.71826 | 3920.35913 |
| 4 | 3901 | 4226 | -325.003529 | 8127.10614 | 4063.55307 |
| 5 | 4011 | 4062 | -50.543675 | 8073.17694 | 4036.58847 |
| 6 | 3966 | 4296 | -330.230899 | 8262.43839 | 4131.2192 |
| 7 | 3754 | 4118 | -364.119111 | 7871.7595 | 3935.87975 |
| 8 | 3716 | 4055 | -339.088218 | 7770.87231 | 3885.43615 |
| 9 | 3996 | 4334 | -338.080472 | 8330.12226 | 4165.06113 |
| 10 | 3737 | 4266 | -528.693716 | 8002.86401 | 4001.43201 |
| 11 | | | | | |
| 12 | | | | | |

2

minimum 7

SDD=Σ(diff)² = 992367.92

MSB = var(sum)/2 = 45981.4655




**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 3. Stability study**

Oat material: O-1##

| | HT-2 toxin | | | | T2 toxin | | | |
|---|---|---|---|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % * | Upper 95 % * | Null slope | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | -0.00040 | -0.00378 | 0.00298 | **YES** | -0.00170 | -0.00464 | 0.00124 | **YES** |
| 4 | -0.00019 | -0.00274 | 0.00236 | **YES** | -0.00192 | -0.00465 | 0.00081 | **YES** |
| 20 | 0.00057 | -0.00151 | 0.00266 | **YES** | 0.00039 | -0.00233 | 0.00310 | **YES** |

* Upper and lower intervals of the regression slope at 95 % confidence level.

| | Enniatin B | | | | Enniatin B1 | | | |
|---|---|---|---|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % | Upper 95 % | Null slope | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | -2157.8 | -12242.8 | 7927.3 | **YES** | 808.0 | -2648.6 | 4264.5 | **YES** |
| 4 | -1945.3 | -5116.6 | 1226.0 | **YES** | -303.2 | -2814.9 | 2208.5 | **YES** |
| 20 | -1397.9 | -11833.8 | 9038.0 | **YES** | -982.1 | -3446.3 | 1482.0 | **YES** |

| | Enniatin A1 | | | | Beauvericin | | | |
|---|---|---|---|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % | Upper 95 % | Null slope | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | 225.78 | -167.27 | 618.83 | **YES** | -228.94 | -555.15 | 97.27 | **YES** |
| 4 | 53.10 | -344.74 | 450.94 | **YES** | -74.32 | -582.69 | 434.05 | **YES** |
| 20 | -232.40 | -893.36 | 428.57 | **YES** | -311.08 | -898.89 | 276.72 | **YES** |

Corn material: C-2##

| | Aflatoxin B1 | | | | DON | | | |
|---|---|---|---|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % | Upper 95 % | Null slope | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | -0.00029 | -0.00585 | 0.00528 | **YES** | -0.00064 | -0.00274 | 0.00146 | **YES** |
| 4 | -0.00064 | -0.00459 | 0.00330 | **YES** | 0.00143 | -0.00032 | 0.00318 | **YES** |
| 20 | -0.00018 | -0.00660 | 0.00623 | **YES** | -0.00107 | -0.00520 | 0.00307 | **YES** |

| | FB1 | | | | FB2 | | | |
|---|---|---|---|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % | Upper 95 % | Null slope | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | 0.00682 | -0.00072 | 0.01436 | **YES** | 0.00131 | -0.00255 | 0.00517 | **YES** |
| 4 | 0.00653 | -0.00549 | 0.01856 | **YES** | 0.00105 | -0.00175 | 0.00386 | **YES** |
| 20 | -0.00011 | -0.01169 | 0.01148 | **YES** | -0.00044 | -0.00129 | 0.00042 | **YES** |

| | ZON | | | |
|---|---|---|---|---|
| T (ºC) | Slope | Lower 95 % | Upper 95 % | Null slope |
| -18 | -0.00075 | -0.00215 | 0.00065 | **YES** |
| 4 | 0.00021 | -0.00123 | 0.00165 | **YES** |
| 20 | 0.00024 | -0.00101 | 0.00150 | **YES** |

## Annex 4. Accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Food and Feed Compliance (F.5)
**European Union Reference Laboratory for Mycotoxins**

Ref. Ares(2016)5432622 - 19/09/2016

Geel, 12th of September 2016

**2016 PROFICIENCY TESTING TO THE NATIONAL REFERENCE LABORATORIES (NRLs) AND APPOINTED OFFICIAL CONTROL LABORATORIES (OCLs) REGARDING THE DETERMINATION OF REGULATED MYCOTOXINS AND ENNIATINS AND BEAUVERICIN IN CEREAL PRODUCTS**

Dear Participant,

**Please read the following information carefully before starting any analysis. If doubts remain, do not hesitate to contact us either by phone or e-mail (see details at end of this doc.)**.

Please confirm the receipt of the parcel by e-mail upon arrival, by using the "**Materials Receipt Form**" that was provided. If some test material is damaged, please request new material immediately.

**The materials are shipped at ambient temperature. After receipt transfer the samples immediately to -18°C until the analysis is performed. Begin the analysis as soon as possible.**

The 2016 EURL PT on regulated mycotoxins and enniatins and beauvericin aims to assess the content of two naturally contaminated cereal products (Oat and Corn) on a combination of mycotoxins. You will be asked to analyse each mycotoxin just once, as follows:
Oat – HT-2 toxin, T-2 toxin, enniatins (A, A1, B and B1) and beauvericin
Corn – deoxynivalenol, aflatoxin B1, fumonisins (B1 and B2) and zearalenone

Please report their **concentration in µg kg$^{-1}$**, as you do in routine analysis, accompanied by the **measurement uncertainty (µg kg$^{-1}$)** for (at least) the regulated mycotoxins with a coverage factor of 2 (k=2). In the Questionnaire please mention whether the results **WERE CORRECTED for recoveries OR NOT a**nd provide the recoveries in the "Measured values" table (in %).

Additional information will be asked to enable us to interpret methodological trends and therefore allow the deepest insight in laboratory independent method-related aspects.

Please homogenise the test materials with a spatula before analysis, as segregation might have occurred during transport.

**Reporting the results and Questionnaire**

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 at the end of this document.

**The deadline for reporting the PT results is the 28th October 2016.**

If some incident happens during the analysis, please let us know as soon as possible, as an extension of the deadline is not foreseen.

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.

Success with the analysis!

With kind regards,

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

Tel: +32-14-571823 / Fax: +32-14-571 783
E-mail: JRC-EURL-MYCOTOX@ec.europa.eu

Cc: Frans Verstraete, Hendrick Emons, Joerg Stroka

**Annex: 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.

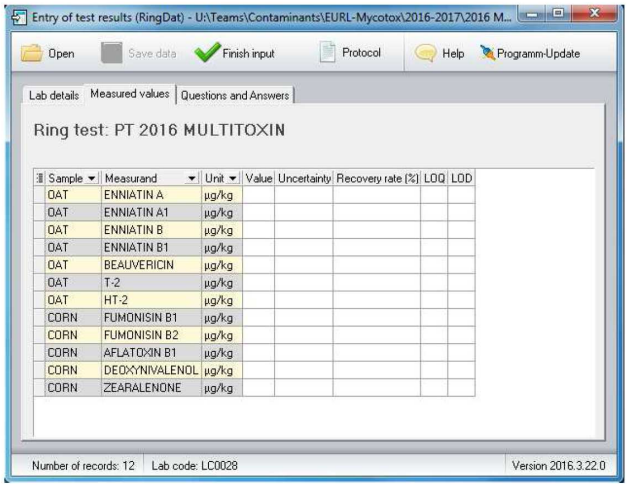
2. Save the two lab specific files with the extension **"*.Lab"** and **"*.LA2",** generated by the ProLab software and provided to each individual laboratory (personal files attached to this email) to the same folder as RingData.exe.
The name of each laboratory and the samples are codified by the software, so that each participant will receive samples with unique codified numbers (i.e., C-229).

- 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 for every measurand/sample combination (Measured values)
- The third tab contains a general questionnaire (Questions and Answers).

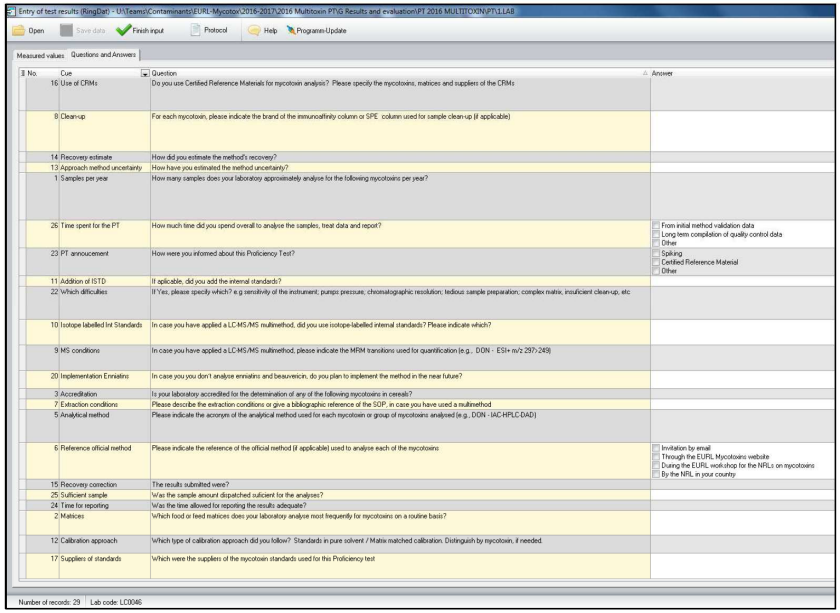
4. Fill in the results table (Measured values) with your data. Please find below some captures of the RingDat pages that have been configured for this PT.

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 menu of the window. 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 **"*.LA"** 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 5. Materials receipt form**

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Geel Site

**European Union Reference Laboratory for Mycotoxins**

Geel, 12th of September 2016

# PROFICIENCY TESTING MATERIALS RECEIPT FORM

| |
|---|
| **Name:** |
| **Institute:** |
| **Address:** |
| **Member State:** |

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

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

| | |
|---|---|
| Date of receipt | |
| Samples' numbers<br>(e.g. O-138 and C-223) | |
| All items have been received undamaged | YES / NO |
| *If NO, please list damaged items:* | |

**Contents of the parcel:**

a) **Two** test materials for analysis packed in ambar bottles:
- 1 Corn sample and 1 Oat sample

b) A bag containing the following documents:
- This **materials receipt form**
- The pro-forma invoice

**Your Signature / Stamp here:**

Please sign this completed form and e-mail it to:

Carlos GONÇALVES

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

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

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

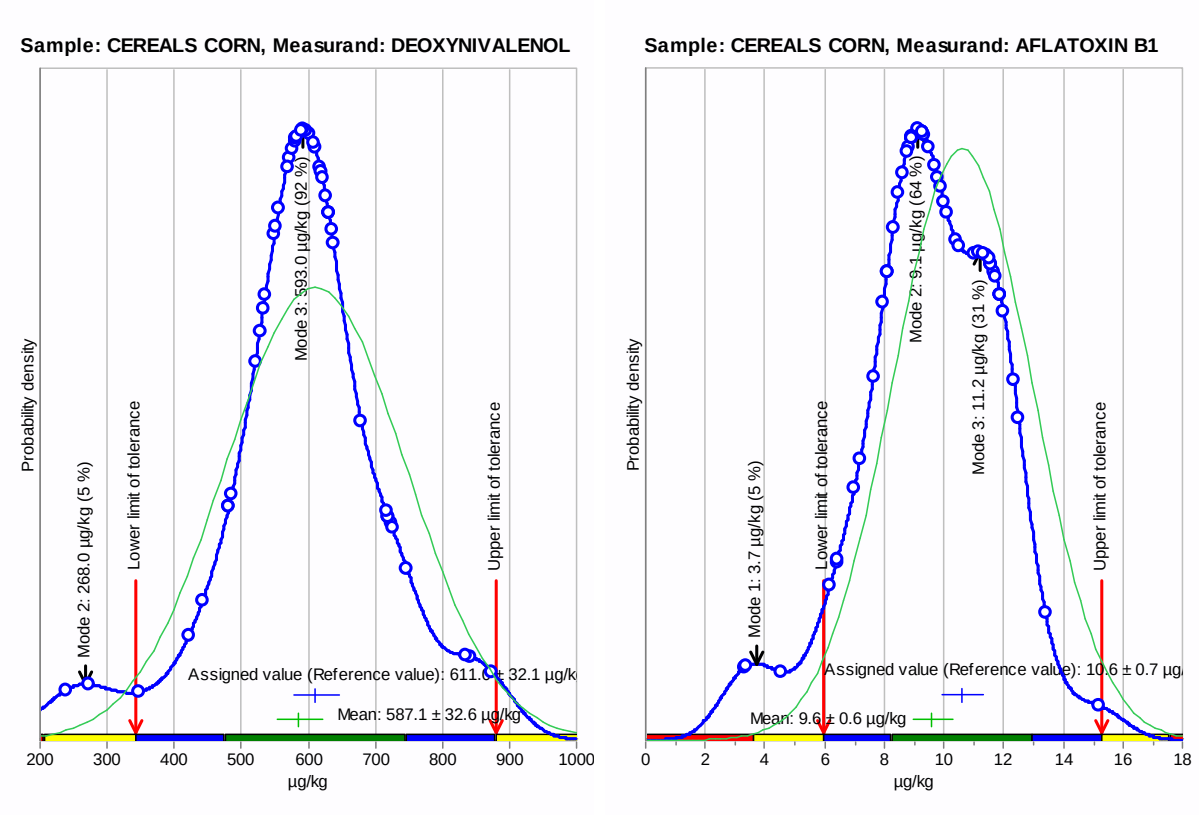
## Annex 6. Questionnaire

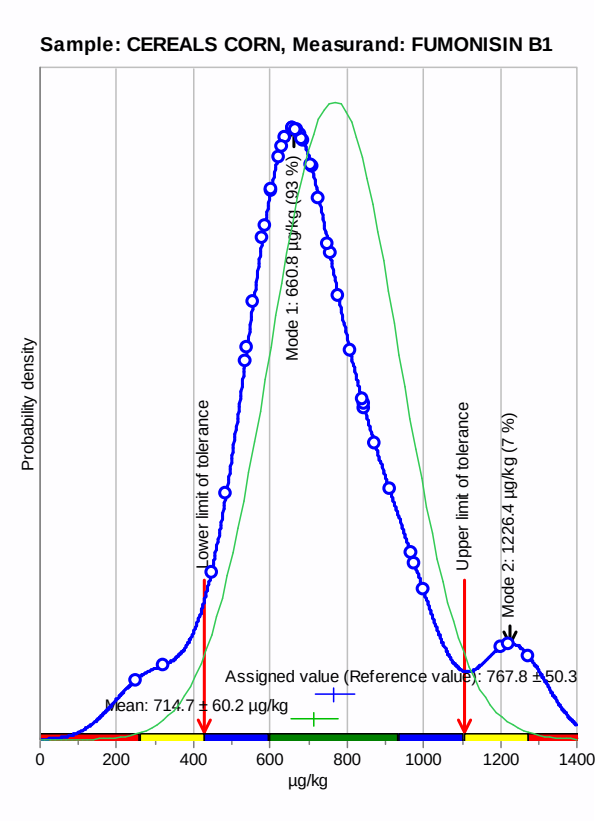
| Ring test : PT 2016 MULTITOXIN (29 questions, 1277 answers) | | | |
|---|---|---|---|
| **Nr.** | **Cue** | **Question** | **Answers** |
| 1 | Samples per year | How many samples does your laboratory approximately analyse for the following mycotoxins per year? Aflatoxins, enniatins and beauvericin, T-2 and HT-2, deoxynivalenol, zearalenone, fumonisins | 51 Answers |
| 2 | Matrices | Which food or feed matrices does your laboratory analyse most frequently for mycotoxins on a routine basis? | 50 Answers |
| 3 | Accreditation | Is your laboratory accredited for the determination of any of the following mycotoxins in cereals? Aflatoxin B1, fumonisin B1, fumonisin B2, deoxynivalenol, zearalenone, T-2, HT-2, enniatins, beauvericin, multitoxin method | 46 Answers |
| 4 | Multitoxin / individual method | Did you use a multitoxin method or individual methods? | 53 Answers |
| 5 | Analytical method | Please indicate the acronym of the analytical method used for each mycotoxin or group of mycotoxins analysed (e.g., DON - IAC-HPLC-DAD) | 53 Answers |
| 6 | Reference official method | Please indicate the reference of the official method (if applicable) used to analyse each of the mycotoxins | 37 Answers |
| 7 | Extraction conditions | Please describe the extraction conditions or give a bibliographic reference of the SOP, in case you have used a multimethod | 43 Answers |
| 8 | Clean-up | For each mycotoxin, please indicate the brand of the immunoaffinity column or SPE column used for sample clean-up (if applicable) | 45 Answers |
| 9 | MS conditions | In case you have applied a LC-MS/MS multimethod, please indicate the MRM transitions used for quantification (e.g., DON - ESI+ m/z 297>249) | 36 Answers |
| 10 | Isotope labelled Int Standards | In case you have applied a LC-MS/MS multimethod, did you use isotope-labelled internal standards? Please indicate which? | 36 Answers |
| 11 | Addition of ISTD | If applicable, did you add the internal standards? | 21 Answers |
| 12 | Calibration approach | Which type of calibration approach did you follow? Standards in pure solvent / Matrix matched calibration. Distinguish by mycotoxin, if needed. | 49 Answers |
| 13 | Approach method uncertainty | How have you estimated the method uncertainty? | 51 Answers |
| 14 | Recovery estimate | How did you estimate the method's recovery? | 49 Answers |
| 15 | Recovery correction | The results submitted were? | 47 Answers |
| 16 | Use of CRMs | Do you use Certified Reference Materials for mycotoxin analysis? Please specify the mycotoxins, matrices and suppliers of the CRMs | 46 Answers |
| 17 | Suppliers of standards | Which were the suppliers of the mycotoxin standards used for this Proficiency test | 47 Answers |
| 18 | Special precautions | Do you take special precautions to avoid the loss of analytes (e.g., acid washing of the glassware, amber glassware and protection from daylight, etc.)? Please indicate for which mycotoxins | 48 Answers |
| 19 | Analysis of enniatins and beau | Did you analyse before enniatins and beauvericin in cereal samples? In case YES, for how long? | 41 Answers |
| 20 | Implementation Enniatins | In case you you don't analyse enniatins and beauvericin, do you plan to implement the method in the near future? | 32 Answers |
| 21 | Difficulties | Did you have major difficulties analysing the distributed samples? | 52 Answers |
| 22 | Which difficulties | If Yes, please specify which? e.g. sensitivity of the instrument; pumps pressure; chromatographic resolution; tedious sample preparation; complex matrix, insufficient clean-up, etc. | 17 Answers |
| 23 | PT announcement | How were you informed about this Proficiency Test? | 52 Answers |
| 24 | Time for reporting | Was the time allowed for reporting the results adequate? | 52 Answers |
| 25 | Sufficient sample | Was the sample amount dispatched sufficient for the analyses? | 51 Answers |
| 26 | Time spent for the PT | How much time did you spend overall to analyse the samples, treat data and report? | 51 Answers |
| 27 | Problems with Prolab/RingDat | Did you have any problems using the ProLab/RingDat platform for results reporting? If Yes, describe which? | 47 Answers |
| 28 | Instructions clear | Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved? | 48 Answers |
| 29 | Comments | Any other comments you wish to address? | 26 Answers |

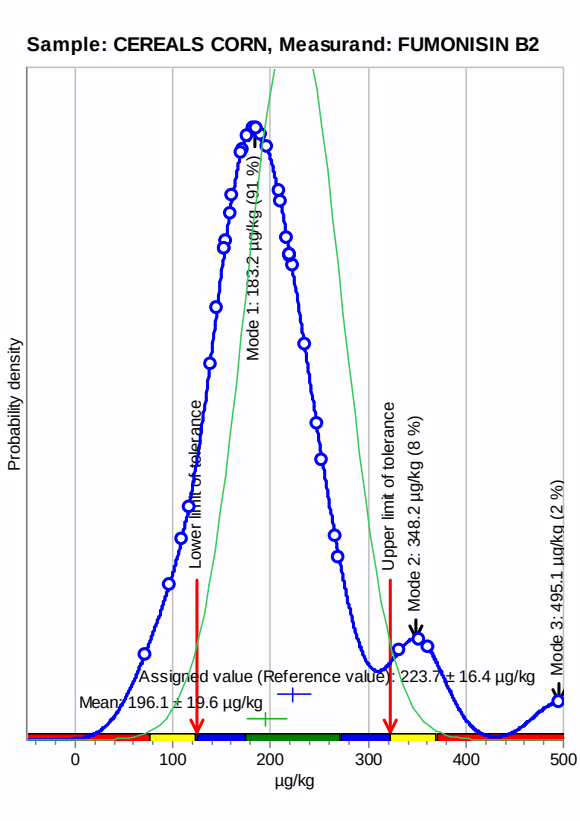
## Annex 7. Kernel density plots

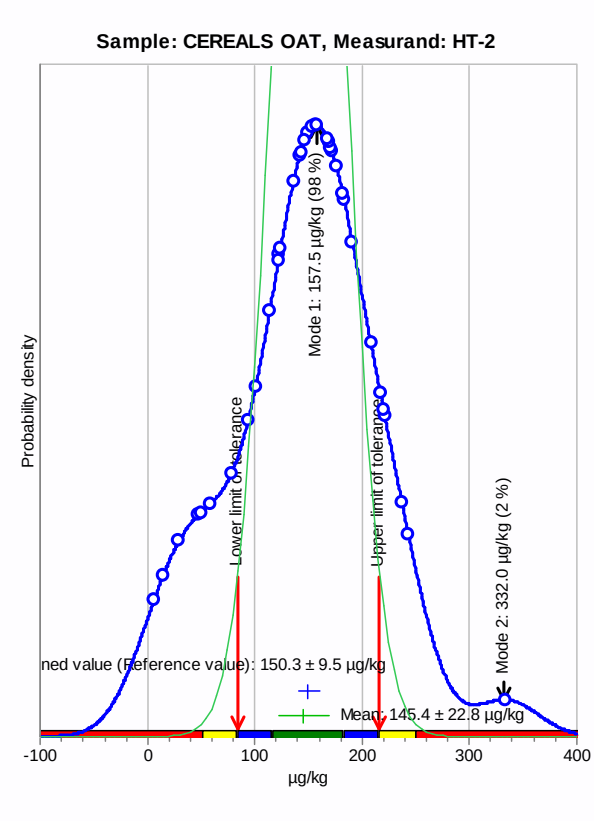
The assigned (reference) values for DON and HT-2 toxin cluster very closely with the respective major modes and the robust means calculated from the results of the participants. The pairs of kernel density plots FB1/FB2 and HT-2/T-2 toxins show a similar and minor deviation from a Gaussian distribution. There is seemingly a significant number of laboratories which underestimated HT-2 and T-2 mass fractions, that despite using LC-MS/MS, didn't use $^{13}$C-labelled internal standards and the calibration standards were prepared in pure solvent. Although other participants following similar calibration strategy reached a satisfactory performance, this approach renders the procedure more vulnerable to systematic errors.

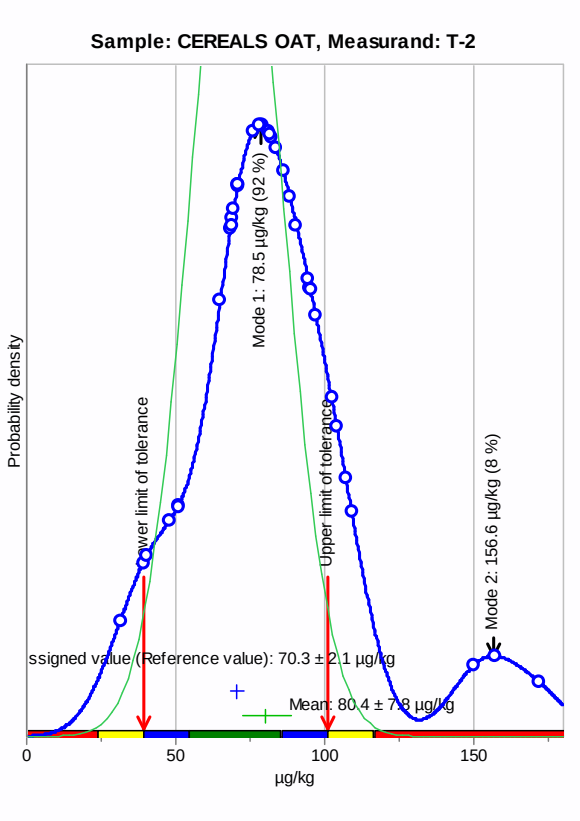
The deviation from normality in the AFB1 kernel density plot was investigated. Neither the different sample preparation techniques (IAC, dilute&shoot and QuEChERS) nor the analytical methods (LC-MS/MS and HPLC-FLD) used by or the participants could be unequivocally implicated in the apparent bimodality. Likewise, the origin of the calibration standards does not seem to have played any role in that regard.



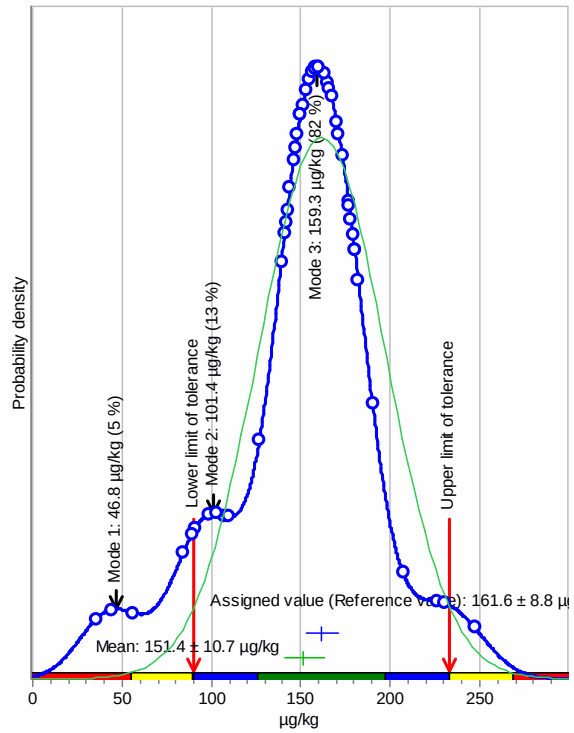

Sample: CEREALS CORN, Measurand: FUMONISIN B1
Probability density
Mode 1: 660.8 µg/kg (93 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 1226.4 µg/kg (7 %)
Assigned value (Reference value): 767.8 ± 50.3
Mean: 714.7 ± 60.2 µg/kg
0
200
400
600
800
1000
1200
1400
µg/kg


Sample: CEREALS CORN, Measurand: FUMONISIN B2
Probability density
Mode 1: 183.2 µg/kg (91 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 348.2 µg/kg (8 %)
Mode 3: 495.1 µg/kg (2 %)
Assigned value (Reference value): 223.7 ± 16.4 µg/kg
Mean: 196.1 ± 19.6 µg/kg
0
100
200
300
400
500
µg/kg

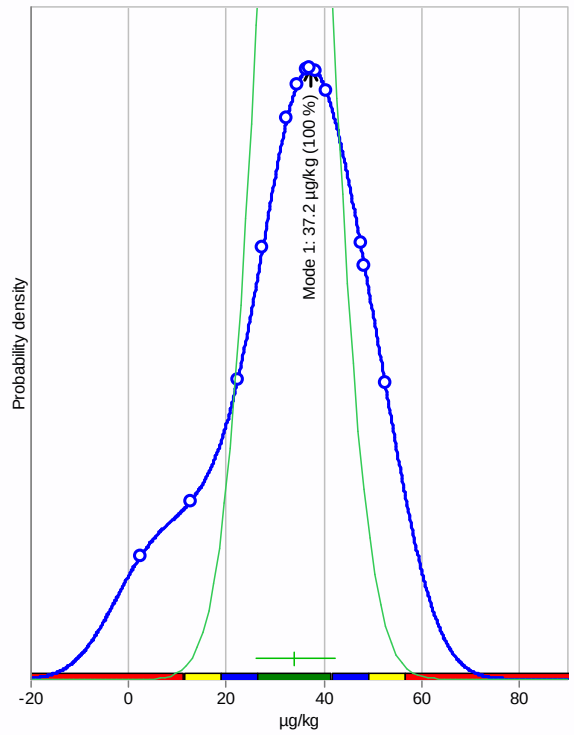

Sample: CEREALS OAT, Measurand: HT-2
Probability density
Mode 1: 157.5 µg/kg (98 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 332.0 µg/kg (2 %)
ned value (Reference value): 150.3 ± 9.5 µg/kg
Mean: 145.4 ± 22.8 µg/kg
-100
0
100
200
300
400
µg/kg


Sample: CEREALS OAT, Measurand: T-2
Probability density
Mode 1: 78.5 µg/kg (92 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 156.6 µg/kg (8 %)
ssigned value (Reference value): 70.3 ± 2.1 µg/kg
Mean: 80.4 ± 7.8 µg/kg
0
50
100
150
µg/kg

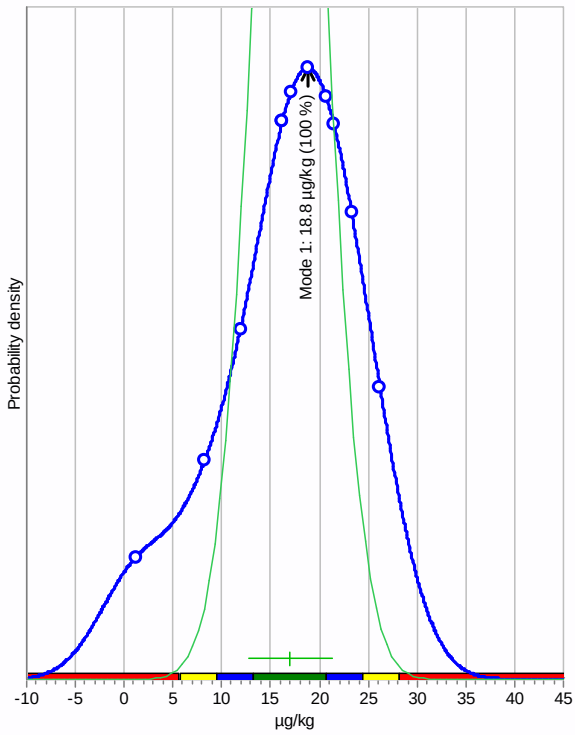
**Sample: CEREALS CORN, Measurand: ZEARALENONE**

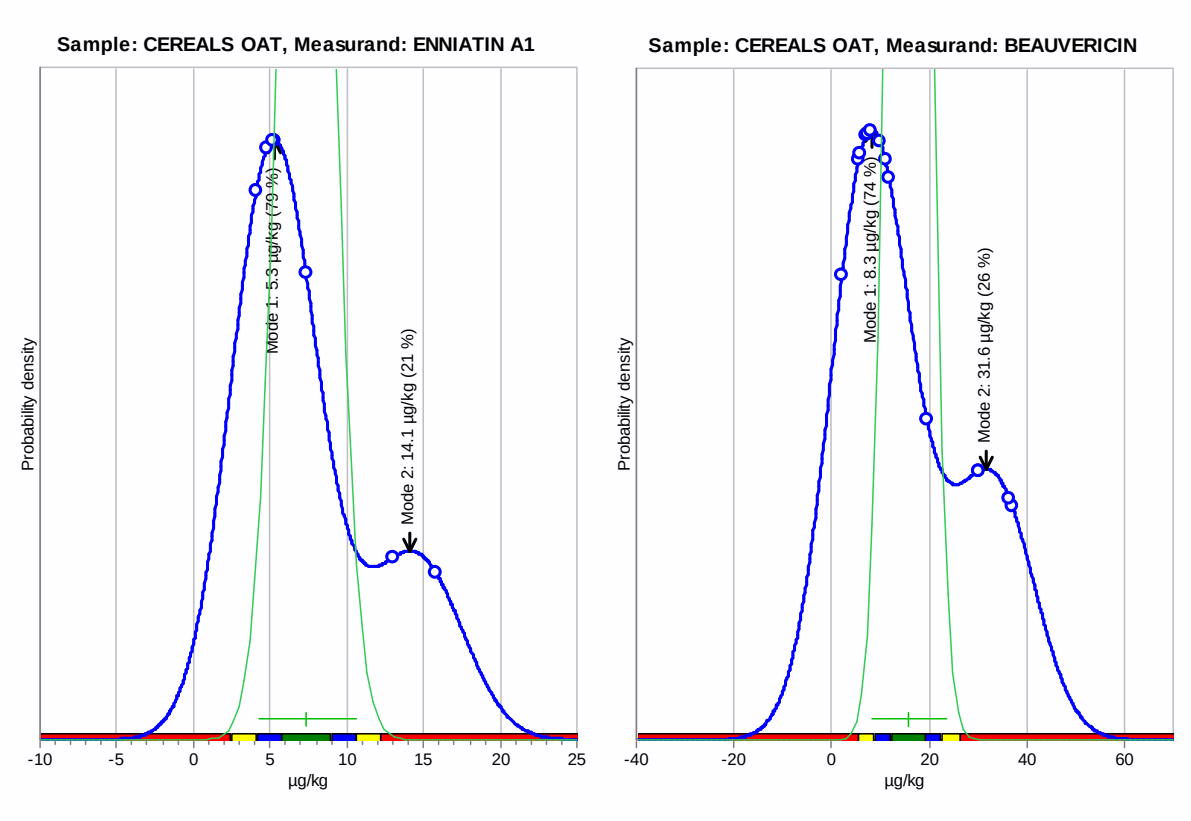



**Sample: CEREALS OAT, Measurand: ENNIATIN B**

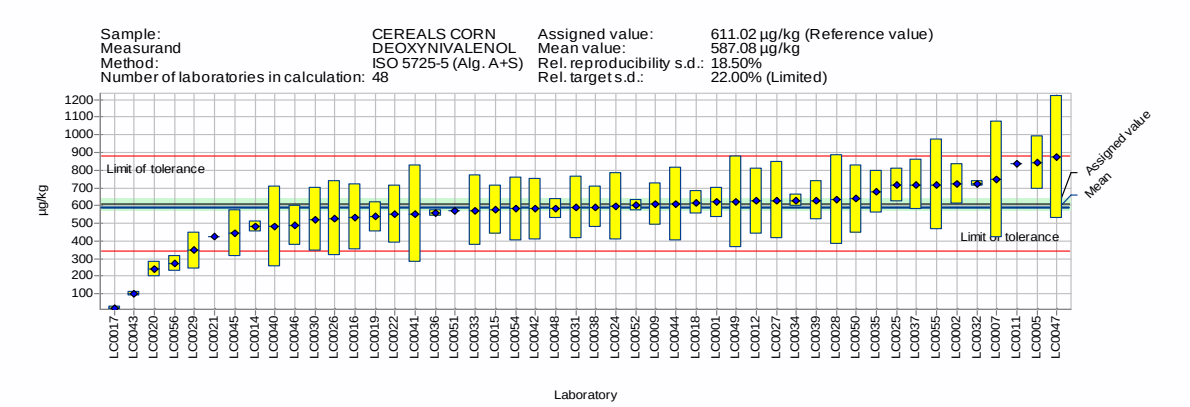


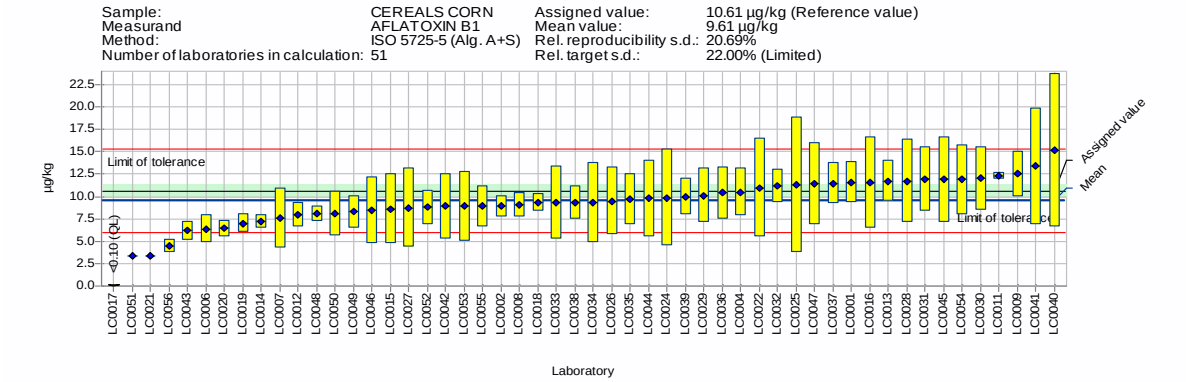

**Sample: CEREALS OAT, Measurand: ENNIATIN B1**

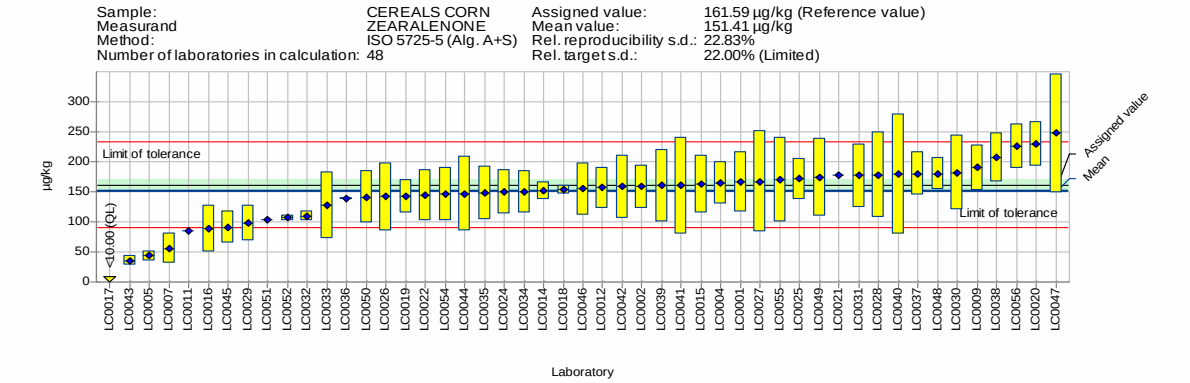


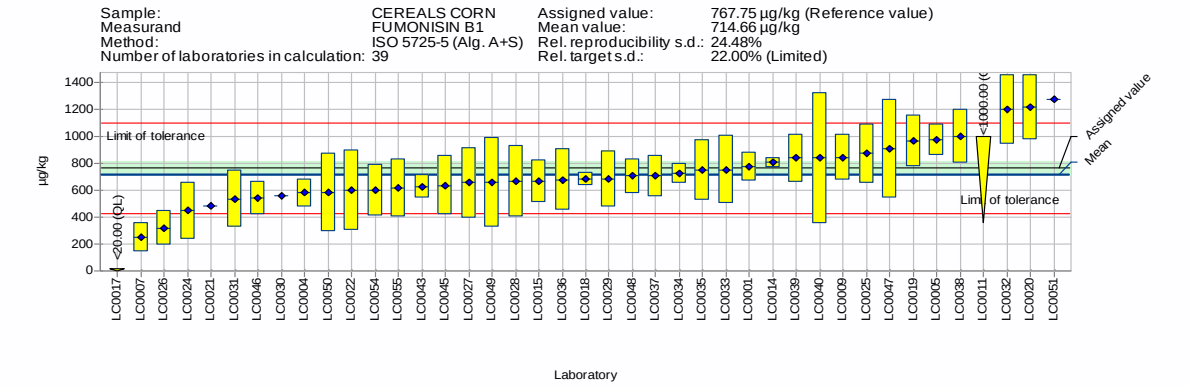

Sample: CEREALS OAT, Measurand: ENNIATIN A1
Probability density
Mode 1: 5.3 µg/kg (79 %)
Mode 2: 14.1 µg/kg (21 %)
-10
-5
0
5
10
15
20
25
µg/kg
Sample: CEREALS OAT, Measurand: BEAUVERICIN
Probability density
Mode 1: 8.3 µg/kg (74 %)
Mode 2: 31.6 µg/kg (26 %)
-40
-20
0
20
40
60
µg/kg

# Annex 8. Distribution of individual results and respective uncertainties (k=2)

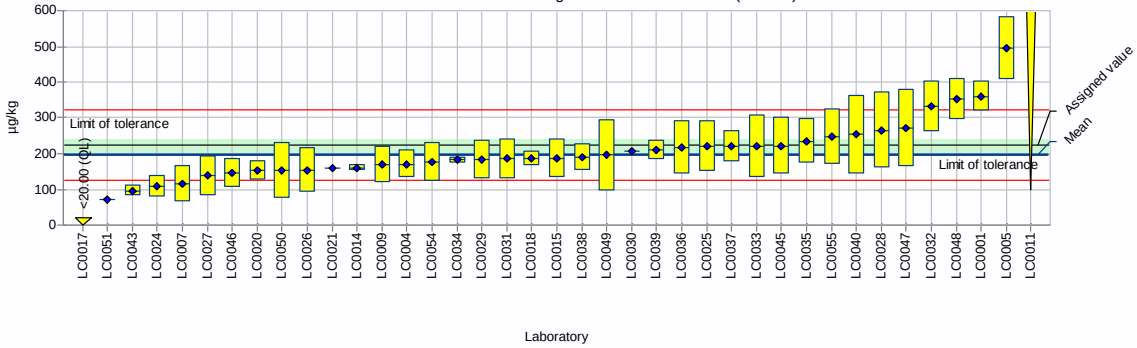


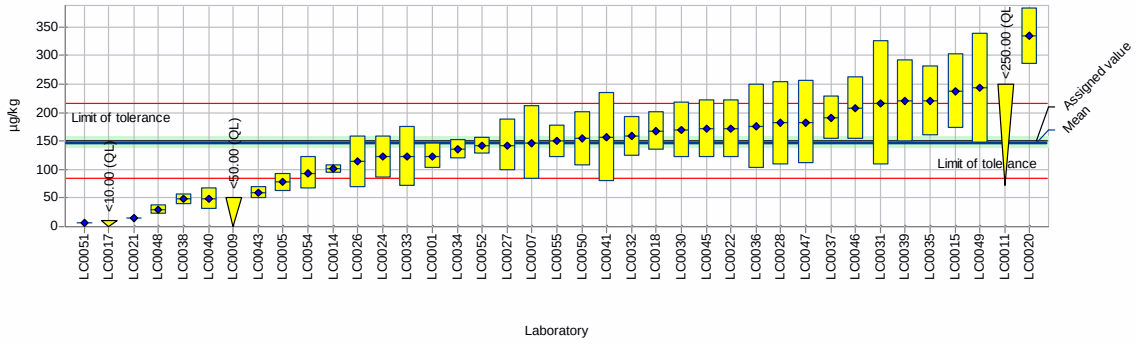



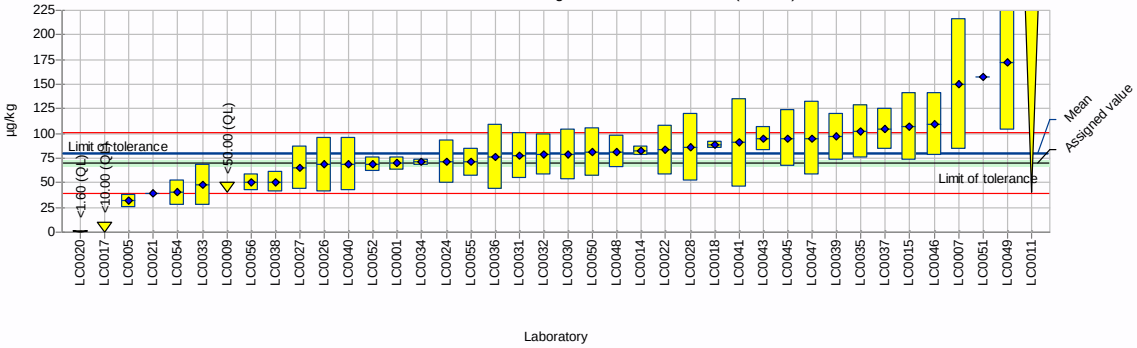
| | | | |
|---|---|---|---|
| Sample: | CEREALS CORN | Assigned value: | 223.66 µg/kg (Reference value) |
| Measurand | FUMONISIN B2 | Mean value: | 196.06 µg/kg |
| Method: | ISO 5725-5 (Alg. A+S) | Rel. reproducibility s.d.: | 26.63% |
| Number of laboratories in calculation: | 37 | Rel. target s.d.: | 22.00% (Limited) |




| | | | |
|---|---|---|---|
| Sample: | CEREALS OAT | Assigned value: | 150.32 µg/kg (Reference value) |
| Measurand | HT-2 | Mean value: | 145.39 µg/kg |
| Method: | ISO 5725-5 (Alg. A+S) | Rel. reproducibility s.d.: | 45.40% |
| Number of laboratories in calculation: | 36 | Rel. target s.d.: | 22.00% (Limited) |




| | | | |
|---|---|---|---|
| Sample: | CEREALS OAT | Assigned value: | 70.27 µg/kg (Reference value) |
| Measurand | T-2 | Mean value: | 80.38 µg/kg |
| Method: | ISO 5725-5 (Alg. A+S) | Rel. reproducibility s.d.: | 33.36% |
| Number of laboratories in calculation: | 36 | Rel. target s.d.: | 22.00% (Limited) |



## Annex 9. Method conditions, quantification approaches and quality control

| Lab code | Q.2 Matrices | Q.4 Multitox/ind. method | Q.5 Analytical method | Q.6 Reference official method | Q.7 Extraction conditions |
|---|---|---|---|---|---|
| LC0001 | Figs, nuts, spices, milk, fish, cereals/flour (feed and food), many feed matrices | Mutitoxin method/ Individual methods | Aflatoxin B1 - IAC-UPLC-FD<br>Fumonisin B1/B2 - IAC-HPLC-FD<br>DON - UPLC-MS/MS<br>ZON - UPLC-MS/MS<br>HT-2 - UPLC-MS/MS<br>T-2 - UPLC-MS/MS<br>ENN A,A1,B,B1 - UPLC-MS/MS<br>BEAU - UPLC-MS/MS | Aflatoxin B1 - ISO 17375/2006<br>Fumonisin B1/B2 - EN 14352 and other<br>DON, ZON, HT-2, T-2, ENN, BEAU several non-official | 10 g and 100 ml acetonitrile/water - 84/16 shaking in 60 min, filtration, dissolving with acetonitrile/water - 84/16 and adding internal standard. Evaporation to dryness. Resolving in 1 ml 40% methanol. |
| LC0002 | Feed | Individual methods | B1 IAC -HPLC -FLD<br>ZON - IAC -HPLC -FLD<br>DON -IAC-HPLC -UV/VIS | B1- SR EN 16050/2011<br>ZON - ISO 17372/2008; SR EN 15792/2010<br>DON- SR EN 15791/2010 | IAC specification |
| LC0004 | Cereals and all products derived from cereals, dry fruits, dried fruits and feed | Individual methods | Aflatoxin - HPLC-FLD<br>Fumonisin B1- HPLC-FLD<br>Fumonisin B2 - HPLC-FLD<br>Zearalenone - HPLC-FLD | | |
| LC0005 | Cereals, cereal products, baby foods | Mutitoxin method | Multi-toxin method "dilute and shoot", LC-MS/MS | Compilation of Waters LC/MS/MS application method and EURL method for multi mycotoxin determination in cereal based feed | weigh 5g sample, dilute with 25 mL of extraction solution (ACN/water/FA 79/20/1), vortex, shaking 1h, centrifuge, evaporation and redilution to MeOH/water 50/50 |
| LC0006 | | Individual methods | HPLC-FLD | | Sample 10 g<br>Methanol/Water 20 ml (extraction) |
| LC0007 | Corn and corn based products, dried fruits (pistachios and peanuts) | Mutitoxin method | LC-MS/MS for all the tested mycotoxins | No official method used | For T-2 and HT-2 the following method was applied: JRC 66507 EN - JRC - IRMM "Validation of an analytical method for the simultaneous determination of deoxynivalenol, zearalenone, T-2 and HT-2 toxins in unprocessed cereals - Validation report. Andreas Breidbach. 2011.<br>The extraction of DON, AFB1, FB1, FB2 and ZON was performed by shaking for 60 minutes 2g of grounded sample with 8ml of AcCN:H2O:HCOOH 79:20:1 |
| LC0008 | Feed Materials | Individual methods | AflaB1-IAC-HPLC-FLD | In House Method based on ISO 17375:2006 | 50g of sample, 250ml extraction solvent acetone/H2O (85/15), 30' shaking |
| LC0009 | Corn, wheat | Mutitoxin method | extraction ASE, LC-MS/MS | | extraction with ASE (ACN, MeOH, water) |
| LC0011 | Cereals, nuts, seeds, spices | Individual methods | Aflatoxin: IAC-HPLC-FLD<br>DON, ZON, FUM, T2/HT2: ELISA | | |
| LC0012 | Frutos secos, cereals | Individual methods | DON-IAC-HPLC-DAD, ZEA-IAC-HPLC-FLD, AFLATOXIN-IAC-HPLC-KOBRACELL-FLD | ZEA: UNE-EN 15850:2010<br>AFLATOXI: UNE-EN 14123:2008, UNE-EN 16050:2011<br>DON: UNE-EN 15891:2010 | |
| LC0013 | Cereals, Dried fruit, Nuts, Baby foods. | Individual methods | B1: IAC-HPLC-PCD-FLD | B1: AOAC Official method 991.31 | B1: MeOH-H2O 62,5%, NaCl |

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| LC0014 | Maize, wheat, barley rye, oats | Individual methods | Aflatoxin, ZEA, FB1, FB2, DON, HT2, T2 and Enniatins analysed by LC-MS/MS | | Extraction is carried out with water-acetonitrile by shaking. |
| LC0015 | nuts, dried fruit, spices, cereals, corn, baby food, coffee | Mutitoxin method | QuEChERS for all compounds | In-house method | 2-gram sample, extraction with acetonitrile/formic acid, addition of magnesium sulphate and sodium chloride, shaking, centrifugation, filtration through syringe filter, LC-MS/MS |
| LC0016 | cereals, spices, wine, coffee, dried fruit | Individual methods | AFLA-IAC-HPLC-FLD<br>DON-IAC-HPLC-DAD<br>ZEA-IAC-HPLC-FLD | AFLA: UNI EN 14123:2007<br>DON: UNI EN 15891:2010<br>ZEA: 15850:2010 | |
| LC0017 | Peanuts, dried figs, chilli powder | Individual methods | T2 + HT2, DON, ZON, Beauvericin + Enniatins - LC-MS/MS<br>Aflatoxins - IAC-LC-FLD | T2 + HT2 - LC/GC Europe (17)11a, 2004, 25-30<br>DON + ZON - Internal method<br>Beauvericin + Enniatins - Internal method<br>Aflatoxins - EN 14123 | T2 + HT2, DON, ZON, Beauvericin + Enniatins - extraction with ACN/H20, SPE Clean-up<br>Aflatoxins - IAC |
| LC0018 | wheat, rye, barley, oat, rice, sunflower seeds, ... | Mutitoxin method | all mycotoxins - LC-MS/MS | | samples were stirred in a water/acetonitrile/methanol-mixture |
| LC0019 | baby food | Individual methods | B1 - IAC-HPLC-FD<br>DON - IAC - HPLC - UV<br>ZON - Elisa<br>FUM - ELisa<br>T2, HT2 - IAC - HPLC - FD<br>EN, BEA - HPLC-UV, DAD | | |
| LC0020 | | Individual methods | AFLA - HPLC - FLD<br>T2, HT2 - HPLC - FLD<br>ZEA - HPLC - FLD<br>DON - HPLC - DAD<br>FUMO B1,B2 HPLC FLD | | |
| LC0021 | cereal and cereal products | Mutitoxin method | dilute and shoot, LC-MS/MS | no reference | extract with ACN/water/acetic acid<br>dilute 1:1 with ACN/water/acetic acid |
| LC0022 | mixed feed | Mutitoxin method | LC-QQQ (dilute & shoot) | | ACN/water/acetic acid (89/20/1); 120 min stirring; 25g; 100 ml |
| LC0024 | cereals (barley, oat, wheat and rye)<br>feed (cereal based) | Mutitoxin method | DON, T-2. TH-2, zearalenone, aflatoxin B1, fumonisins B1 and B2 - UHPLC-MS/MS<br>enniatins and beauvericin - not analysed | | EURL draft "Determination of deoxynivalenol, aflatoxin B1, fumonisins B1&B2, T-2 & HT-2 toxins, zearalenone and ochratoxin A in unprocessed cereals and cereal based compound feeds by liquid chromatography - tandem mass spectrometry. |
| LC0025 | nuts, raisins, dried figs | Individual methods | AFB1-IAC-HPLC-FLD<br>FB1FB2-IAC-HPLC-FLD<br>ZON-IAC-HPLC-FLD<br>DON-SPE-UPLC-MSMS | | |
| LC0026 | cereals, pastries, dried fruits, edible nuts | Mutitoxin method | Multitoxin - HPLC-MS/MS | | Sulyok M et al. 2006. Rapid Communication in Mass Spectrometry 20, 2649-2659. |
| LC0027 | cereals and cereal products, dried fruit, spices | Individual methods | DON-IAC-HPLC/DAD<br>ZON-IAC-HPLC/FLD<br>FUMONIZIN-IAC-LC-MS/MS<br>AFLATOKSIN-IAC-LC-MS/MS | internal methods | |

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| LC0028 | Feed material and compound feed | Mutitoxin method | LC/MSMS for all analytes analysed | 1. The Detection of Mycotoxins Using a Simple Sample Extraction and LC-MS/MS with Fast Polarity Switching and the Scheduled MRM Algorithm. AB Sciex publication, Jianru Stahl-Zeng, Stephen Lock, Stefanie Krepperhofer, Kristen von Czapiewski<br>2. LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food additives and Contaminants, April 2008;25(4): 472-489, Martien C. Spanjer, Peter M. Rensen and Jos M. Scholten | 80:20 acetonitrile:water |
| LC0029 | peanuts, hazelnuts, pistachios, dried figs, red pepper, cereal crops such as wheat, corn, barley and wheat flour, corn oil. | Individual methods | DON-IAC-HPLC-DAD<br>ZON-IAC-HPLC-FLD<br>AFLB1-IAC-HPLC-FLD<br>FUM B1-IAC-HPLC-FLD<br>FUM B2-IAC-HPLC-FLD<br>T2-IAC-HPLC-FLD<br>HT2-IAC-HPLC-FLD<br>AFL-OTA-FUM-IAC-HPLC_FLD<br>DON-ZON_T2/HT2-IAC-HPLC_FLD_DAD | AFL- EN ISO 16050<br>OTA EN 14132<br>DON EN 15891<br>ZEA - EN 15850<br>FUM -EN 14352 | multitoxin IACs with one sample preparation, but different chromatographic methods and detectors wavelengths.<br>AFL-OTA-FUM-IAC-HPLC_FLD- 10g sample with 2 g NaCl was extracted with 40 ml mixture of methanol-water=60/40. after filtration 10 ml were diluted with 15 ml PBS solution. After filtration with glass microfiber filter, 5 ml are passed through the IAC. The IAC column was washed with 20 ml water. eluting with 1 ml methanol with backflushing and 1 ml of water.<br>DON-ZON_T2/HT2-IAC-HPLC_FLD_DAD -10g sample with 2 g NaCl was extracted with 40 ml mixture |
| LC0030 | Barley and wheat. | Mutitoxin method | QUECHERS-HPLC-MS/MS | internal document: SOP 10575.1 | |
| LC0031 | cereals (wheat, triticale, barley,maize,rye), silage, hay, complete feed, supplementary compound feed | Mutitoxin method | all mycotoxins - QuEChERS-LC-MS | | QuEChERS |
| LC0032 | cereal based food, baby food, infant formula | Individual methods | T2, HT-2 - LC MS/MS<br>Fumonisin - LC MS/MS<br>Aflatoxin B1 - HPLC-FLD<br>DON - HPLC-UV<br>F2 - HPLC-FLD | AFLA UNI EN 14123:2008<br>FUMO UNI EN 14352:2005<br>DON-ZEA-T-2-HT-2 Internal Method | T2, HT2 - ACN:H2O:CH3COOH - 79:20:1<br>Fumonisin - ACN:MEOH:H2O - 25:25:50<br>Aflatoxin B1 - MEOH:H2O - 70:30<br>DON - H2O<br>F2 - ACN:H2O 50:50 |
| LC0033 | Dry fruits, cereals and derived | Mutitoxin method/Individual methods | AFLA IAC-HPLC-FLD<br>FUMO IAC-HPLC-FLD<br>DON-ZEA-T-2-HT-2 SPE-LC/MS/MS | In-house validated method based on draft for ongoing CEN mandate "Multimethod for the screening of ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisin B1 and B2 in foodstuffs by LC-MS/MS" | DON-ZEA-T-2-HT-2 Extraction with Acetonitrile / water 84:16 |
| LC0034 | none (no routine analysis) | Mutitoxin method | All analytes: HPLC-MS/MS | internal methods | Samples were extracted with a mixture of 10 ml water and 10 ml acetonitrile containing 0.1% formic acid. After 30 min shaking and centrifugation, 1 ml of the supernatant was mixed with 100 µl isotope-labelled standard |

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| | | | | | solution and 250 mg MgSO4. After phase separation, 300 µl of the organic phase was mixed with 300 µl water and analysed using LC-MS/MS |
| LC0035 | cereals, baby foods, dryed fruits | Individual methods | IAC-LC-MS/MS for all mycotoxins | Aflatoxins - EN 14123<br>T-2/HT-2 - Method of CRL 2006<br>Fumonisins - EN 14352<br>DON - EN 15891<br>ZON - EN 15850 | Meoh/water or ACN/water |
| LC0036 | Nuts, cereals, cereals products, spices, milk, milk products, premixes<br>pig ration, poultry ration<br>dairy ration | Individual methods | Aflatoxins - IAC - HPLC-FD<br>Fumonisins - IAC - HPLC-FD<br>DON - IAC - LC-MS<br>ZON - IAC - LC-MS<br>T-2 and HT-2 - IAC - GC-MSD | AflaB1 - MSZ EN ISO 17375:2006<br>Zearalenon - MSZ EN 15792:2010 | |
| LC0037 | corn,cereals, pet foods, mixed feed | Mutitoxin method/Individual methods | AflaB1-IAC-HPLC-FLD<br>Zearalenon-IAC-HPLC-FLD<br>Fumonizins-SPE-HPLC-MS<br>DON, T2, HT2 SPE-HPLC-MS/MS multitoxin | | Acn/water 84/16, 2 hours extraction time, cleanup AflaZon 226 SPE |
| LC0038 | | Individual methods | UPLC-ToF | | |
| LC0039 | Feed | Mutitoxin method | multi mycotoxin - LC-MSMS | | Quechers extraction |
| LC0040 | cereals, cereal products, nuts, coffee, dried fruits | Mutitoxin method | LC-MS/MS for all toxins | | Acetonitril:water:acetic acid (79:20:1). Shaking for 30 min. 12,5 g sample+50 ml extraction solvent |
| LC0041 | | Mutitoxin method | LC-MSMS | | Shake with 50:50 water : 1 % HAc in MeCN |
| LC0042 | Nuts<br>Dried Fruits<br>Cereals | Individual methods | AFLA: IAC-HPLC-FLD<br>DON: IAC-HPLC-UV<br>ZON: IAC-HPLC-FLD | AFLA: EN 15851:2010<br>DON: in-house method<br>ZON: in-house method | Individual methods used |
| LC0043 | feed<br>animal tissues<br>milk | Mutitoxin method | LC-MS/MS | Determination of Deoxynivalenol, Aflatoxin B1, Fumonisin B1&B2, T-2 &<br>HT-2 toxins, Zearalenone and Ochratoxin A in unprocessed cereals and<br>cereal-based compound feeds by Liquid Chromatography - Tandem<br>Mass Spectrometry- DRAFT- SOP EURL GEEL | Determination of Deoxynivalenol, Aflatoxin B1, Fumonisin B1&B2, T-2 & HT-2 toxins, Zearalenone and Ochratoxin A in unprocessed cereals and cereal-based compound feeds by Liquid Chromatography – Tandem Mass Spectrometry- DRAFT- SOP EURL GEEL |
| LC0044 | feed, raw materials and cereals for humans consumption | Individual methods | AFLATOXINE B1: UPLC-FD<br>DON: HPLC-UV<br>ZEARALENONE:UPLC-FD | RE(UE)519/2014 AMENDING REGULATION (EC) Nº401/2006 | AFLATOXINE: METHANOL-WATER (80/20) 100mL<br>DON: WATER 200mL<br>ZEARALENONE: ACETONITRILE-WATER (60/40) 200 mL |
| LC0045 | Nuts, dried fruits,cereals and cereal based products. | Individual methods | AFLA B1-LC-MS/MS<br>FUM B1-LC-MS/MS<br>FUM B2-LC-MS/MS<br>ZON-LC-MS/MS | | Extraction with mixture of solvent, acetonitrile:water=70:30 (AFLA, ZON)<br>Extraction with mixture of solvent, methanol:water=70:30 (T2, HT2) |

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| | | | DON-LC-MS/MS<br>T2-LC-MS/MS<br>HT2-LC-MS/MS | | Extraction with mixture of solvent, methanol:acetonitrile:water=1:1:2 (FUM B1, FUM B2)<br>Extraction with water, DON |
| LC0046 | cereal flours, biscuits, pistachios, peanut, baby food, feed ingredients | Individual methods | Afla: IAC-HPLC-FLD<br>Zea: IAC-HPLC-FLD<br>DON: IAC-UPLC-MS/MS<br>FUM: IAC-UPLC-MS/MS<br>T-2/HT-2: IAC-UPLC-MS/MS | in-house methods or derived from publications | not applicable |
| LC0047 | Cereals | Mutitoxin method | All toxins via LC-MS/MS<br>(different method for enniatins & beauvericin) | NA | Modified QuEChERS |
| LC0048 | Cereals, cereal products, nuts and products, dried fruits | Mutitoxin method | Multimycotoxin - LC-MS-MS | | Extraction solvent ACN:H2O (70:30), Shake for 2h, Centrifuge, Filter |
| LC0049 | Feed, cereals, maize, nuts | Individual methods | Aflatoxin B1 - IAC-HPLC-FLD, Kobra cell<br>Fumonisins - IAC-HPLC-FLD,<br>DON - IAC-HPLC-DAD,<br>ZON - IAC-HPLC-FLD,<br>T-2, HT-2 - IAC-HPLC-FLD | Aflatoxin B1 - EN ISO 17375<br>Fumonisins - EN 13585, CEN/TS 16187<br>DON - EN 15791<br>ZON - EN 15792<br>T-2, HT-2 - R-BIOPHARM RHONE LTD IAK Description | |
| LC0050 | food: dried fruits, cerelas and cereals products, nuts,spices,baby food<br>feed: raw materials, compounds feed,pet food | Mutitoxin method/Individual methods | T2;HT2,DON,Zea: LCMS<br>Fumos B1 and B2: IAC-HPLC- fluo after derivatization (OPA)<br>AfB1: IAC-HPLC-fluo after derivatization with Cobra cell | Afla B1: NF EN 14123<br>Fumos B1 and B2 : NF EN 16006<br>T2 HT DON ZEA: in-house method lcms | for Don,THT2,DON and Zea:<br>- Extraction : Weigh 10g of sample ( 1mg precision)in an erlen, add 100ml of extraction solution : CH3CN/H2O/CH3COOH - 80/20/1<br>Agitate magnétically during 1h<br>Filtration with paper<br>- Preparation of extract injection :<br>Transfer 1ml (=0,1g) of extract in a vial deactivated of 4ml. Add 25 µl of Mix IS in all vials at the same time (standards solutions and samples) with multipet and evaporate dry with nitrogen. Solubilize the residue in 500µl of mobile phase containing 1mM ammonium acetate + 0.1% acetic acid as follow: - Add 100µl of mobile phase B (M" |
| LC0051 | Cereals | Mutitoxin method | MULTI IAC - UHPLC - HRMS/MS | | Double extraction: PBS and methanol |
| LC0052 | nuts, dried fruits, wine, coffee, spices, grains | Individual methods | Don, T2, HT2 - GC/MS<br>Zearalenon - IAC - HPLC - FLD<br>Aflatoxin B1 - IAC - HPLC - FLD | | DON, T2; HT2 - ACN/H20 84/16<br>Zearalenone - MeOH/H20 3/2<br>AB1 - MeOH/H20 4/1 |
| LC0053 | cereals, coffee, dried fruit, spices, wine, apple based products, beer | Individual methods | Aflatoxins B1- HPLC-FL | UNI EN ISO 16050 2011 | methanol/water 80/20 extraction |
| LC0054 | Cereals | Mutitoxin method | Aflatoxin B1 - IAC-HPLC-FL<br>The other mycotoxins - UHPLC-MS/MS | In-house methods | AcN - acetonitrile/water 80/20 (v/v)<br>The other mycotoxins - acetonitrile/water/formic acid 74/25/1 (v/v/v) |
| LC0055 | Cereals, nuts, baby food, milk, apple juice, wine. | Mutitoxin method | UPLC-MS/MS Multitoxin method | House Method based in the article Determination of mycotoxins in different food commodities by UPLC-MS/MS. Rapid Commun. Mass Spectrum. 2009; 23; 1801-1809. | 10 g + 40 ml Acetonitrile 80% + 0.1% formic acid. Shaking 90 min. Centrifuge.<br>Dilute 1 ml extract + 1 ml H2O. Filter 0.2 um<br>DON : 1 ml extract, evaporate (dryness). Add 1ml H2O. Filter 0.2 um. |

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| LC0056 | cereals | Mutitoxin method | UPLC-MS/MS | Multi-Method Screening Mycotoxins CEN/TC 275/WG 5 N 720 | 5g of sample shaken with 20 mls of 92:8 ACN:Water |

| Lab code | Q.8 Clean-up | Q.9 MS conditions | Q.10 Isotope-labelled ISTD | Q.11 Addition of ISTD | Q.12 Calibration approach |
|---|---|---|---|---|---|
| LC0001 | Aflatoxin B1 - Aflaprep R-Biopharm Rhone<br>Fumonisin B1/B2 - Fumoniprep R-Biopharm Rhone | DON - ESI - m/z 295>265<br>ZON - ESI- m/z 317>175<br>HT-2 - ESI+ m/z 447>345<br>T-2 - ESI+ m/z 489>387<br>ENN A - ESI+ m/z 704>350<br>ENN A1 - ESI+ m/z 690>350<br>ENN B - ESI+ m/z 662>336<br>ENN B1 - ESI+ m/z 676>336<br>BEAU - ESI+ m/z 806>384 | 13C DON, 13C T-2, 13C HT-2, 13C ZON | After extraction | Standard in pure solvent |
| LC0002 | R-BIOPHARM RHONE | - | - | | Standards in pure solvent (B1;DON; ZON) |
| LC0004 | Zearalenone - Vicam<br>Aflatoxin - R-Biopharm<br>Fumonisin - Vicam | - | - | | Standards in solvent |
| LC0005 | - | DON-ESI+- m/z 297.1->231.2<br>ZON-ESI+-m/z 319->281.2<br>FB1-ESI+-m/z 722.4->352.1<br>FB2-ESI+-m/z 706.4->318.1<br>T2-ESI+-m/z 484.2->215<br>HT2-ESI+-m/z 442.2-> 323<br>ENA-ESI+-m/z 682.6->210.3<br>ENA1-ESI+-m/z 668.5->210<br>ENB-ESI+-m/z 640.5->195.9<br>ENB1-ESI+-m/z 654.5->195.9<br>BVR-ESI+-m/z 784.5->244.0 | No | | Standards in pure solvent (MeOH/water 50/50) |
| LC0006 | Vicam Aflatest | T-2 489.6>327.1; HT-2 489.6>345.1; DON 297.3>231; AFB1 313>285; FB1 723.3>335.1; FB2 706.9>336.5; ZON 319>187. ESI+ for all the texted mycotoxins | | | Standard in pure solvent |
| LC0007 | No IAC or SPE used for clean-up the samples. | | isotope-labelled standard was used for the determination of T-2, HT-2; DON, AFB1, FBs | | For T-2, HT-2, DON, AFB1, FB1, FB2 standards in pure solvent with the addition of the isotope-labelled standard was used. For ZON matrix, matched calibration was used. |
| LC0008 | LC-Tech IAC | | | | Standards in pure solvent |
| LC0009 | | FB1: ES+ 722.4>352.4 / FB2: ES+ 706.5>336.4/ AF B1: ES+ 313.1>241.1/ ZEA: ES+ 319.2>283.2/ DON: ES+ 297.2> 249.1 | Deoxynivalenol C13, zearalenone C13, T2 C13, aflatoxin B1 C13, fumonisin B1 C13 | Before extraction | T2/HT2: matrix matched calibration<br>other mycotoxins: pure solvent |
| LC0011 | R-Biopharm for Aflatoxins | | | | |
| LC0012 | | | | | Standards in pure solvent |
| LC0013 | B1: VICAM Aflaprep WB | | | | |
| LC0014 | Fumonisins : IAC,<br>Aflatoxin and ZEA : mycosep 226<br>DON, HT2, T2: mycosep 225<br>Enniatins, beauvericin: just | Enn A: 699 > 210, Enn A1: 685 > 210, Enn B: 657 > 196, Enn B1: 671 > 196,<br>Beau: 801 > 244,<br>HT2: 442>263, T2: 484>365 | | After extraction | Aflatoxins and Zea: matrix match calibration<br>For the others, component used standard in pure solvent |

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| | extract without clean-up | DON: 355>295<br>ZEA: 317>175<br>Afal B1: 313>213 | | | |
| LC0015 | No clean-up | ENN A: ESI+, 699/210<br>ENN A1: ESI+, 685/210<br>ENN B: ESI+, 657/196<br>ENN B1: ESI+, 671/196<br>BEA: ESI+, 801/244<br>T2: ESI+, 484/215<br>HT2: ESI+, 442/215<br>FB1: ESI+, 722/352<br>FB2: ESI+, 706/336<br>AFB1: ESI+, 313/285<br>DON: ESI-, 355/138<br>ZON: ESI-, 317/175 | No | | matrix-matched calibration |
| LC0016 | BIOPHARM | | | | STANDARDS IN PURE SOLVENTS |
| LC0018 | no columns were used (neither SPE nor IAC) | DON_q 297,1 / 248,9<br>Zenon_q 317,1 / 130,8<br>FB1_q 720,4 / 156,8<br>FB2_q 704,4 / 156,9<br>AflaB1_q 313.1 / 241.1<br>BEA+NH4_q 801.41 / 244<br>EnnA+NH4_q 699.5 / 210.1<br>EnnA1+NH4_q 685.5 / 210.1<br>EnnB+NH4_q 657.4 / 196.1<br>EnnB1+NH4_q 671.4 / 196.1<br>HT2+NH4_q 442.2 / 262.9<br>T2+NH4_q 484.2 / 305 | only for some toxins: T2-C13; HT2-C13; DON-C13; FB1-C13; FB2-C13; AflaB1-C13; ZEA-C13 | After extraction | standards in pure solvent for all mycotoxins |
| LC0019 | B1 - Aflaprep, Romer Lab<br>DON - Donprep, Romer Lab<br>T2, HT2 - Easi extract, Romer Lab | | | | Standards in pure solvents |
| LC0020 | Jemo trading all myko except for OCHRA, OCHRA- NEO CHEM | | | | |
| LC0021 | No clean-up | AFB1 313>241<br>FB1 722>334<br>FB2 706.5>336<br>DON 355>295<br>ZEA 317>131<br>T2 484>185<br>HT2 447.5>345 | no | | matrix matched calibration |
| LC0022 | | Analyte1=Target Name Q1 / Q3<br>Analyte2=Qualifier Name Q1 / Q3<br><br>DON 1 355.0 / 265.0 (-)<br>DON 2 355.0 / 295.2 (-)<br>AflaB1 1 313.1 / 285.1<br>AflaB1 2 313.1 / 128.1<br>HT2 1 442.1 / 263.1<br>HT2 2 442.1 / 105.0 | C13 for all analytes | After extraction | Standards in pure solvent |

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| | | T2 1 484.1 / 214.9<br>T2 2 484.1 / 184.9<br>FumoB1 1 722.3 / 352.3<br>FumoB1 2 722.3 / 334.4<br>ZON 1 317.1 / 131.1 (-)<br>ZON 2 317.1 / 175.0 (-) | | | |
| LC0024 | not used | DON ESI+ m/z 297>231<br>T-2 ESI+ m/z 489>387<br>HT-2 ESI+ m/z 447>345<br>AfB1 ESI+ m/z 313>241<br>FB1 ESI+ m/z 722>352<br>FB2 ESI+ m/z 706>336<br>ZON ESI- m/z 317>175<br>OTA ESI+ m/z 404>239 | 13C15-DON, 13C24-T-2, 13C22-HT-2, 13C17-AfB1, 13C34FB1, 13C34-FB2, 13C18-ZON, 13C20-OTA | After extraction | Standards in pure solvent |
| LC0025 | AFB1,ZON,FB1FB2 - IAC VICAM<br><br>DON - SPE OASIS WATERS | Only for DON - ESI+ m/z 297.1 > 249.1 | DON: 13C-DON | Before extraction | Standards in pure solvent |
| LC0026 | | AFB1 - [M+H]+ 313.1>285.0/241.0<br>BEA - [M+NH4]+ 801.4>784.4/262.1<br>DON - [M+H]+ 297.1>249.0/203.0<br>ENB - [M+NH4]+ 657.4>640.3/196.0<br>FUMB1 - [M+H]+ 722.4>352.4/334.4<br>FUMB2 - [M+H]+ 706.4>336.4/318.3<br>HT2 - [M+NH4]+ 442.2>263.0/215.0<br>T2 - [M+NH4]+ 484.3>305.0/215.1<br>ZON - [M-H]- 317.1>272.9/130.9 | 13C15-DON, 13C17-AFB1, 13C34-FUMB1, 13C34-FUMB2, 13C22-HT2, 13C24-T2, 13C18-ZON | After extraction | Standards in pure Methanol |
| LC0027 | Rhone Biopharm | | | | standards in pure solvent |
| LC0028 | N/A | Analyte, Internal standard, Polarity, Precursor, Product<br>ZON 13C18-ZON Negative 317.1 131.1 175.0<br>DON 13C15-DON Negative 355.018 59.00 295.2<br>AFB1 13C17-AFB1 Positive 313.0 285.2 128.1<br>T-2 13C24-T-2 Positive 484.076 214.9 184.9<br>HT-2 13C22-HT-2 Positive 442.097 263.1 105.0<br>OTA 13C20-OTA Positive 404.0 239.0 102.0<br>FB1 13C34-FB1 Positive 722.267 334.4 352.3<br>FB2 13C34-FB2 Positive 706.354 336.3 318.5<br>3C18-ZON Negative 335.1 140.1<br>13C15- DON | yes, C13 | After extraction | solvent only calibration with C13 Internal stds |
| LC0029 | multi_IAC AFL-OTA-FUM- R-Biopharm<br>multi_IAC- DON-ZON_T2/HT2-R-Biopharm | | | | standards in pure solvent |
| LC0030 | | AFB1 ESI + 313>241<br>DON ESI + 297>249<br>ZEA ESI - 317>131<br>FB1 ESI + 722>334<br>FB2 ESI + 706>336<br>T2 ESI + 484>215<br>HT2 ESI + 442>263 | NO | | Standard addition |
| LC0031 | not applicated | DON: 297 / 231, AFB1: 313 /241, HT-2: 442 / 263, FB1: 722 / 352, T-2: 484 / 215, ZON: 319 / 187, FB2: 706 / | NO | Before extraction | matrix matched calibration |

| | | | | | |
|---|---|---|---|---|---|
| | | 336, ENB: 640 / 196, BEA: 784 / 244, ENB1: 654 / 196, ENA1: 669 / 210, ENA: 683 / 210 | | | |
| LC0032 | F2 - Romer<br>DON - Romer<br>Aflatoxin B1 - R-Biopharm | - | - | | Standards in pure solvent<br>Matrix matched calibration - Fumonisin |
| LC0033 | AFLA R biopharm Easy Extract Aflatoxin<br>FUMO R biopharm Fumoniprep<br>DON-ZEA-T-2-HT-2 Waters Oasis HLB | DON ESI- m/z 355>59 - 355>295<br>HT-2 ESI+ m/z 442>215 - 442>263<br>T-2 ESI+ m/z 484>185 - 484>245<br>ZEA ESI- m/z 317>131 - 317>175 | Yes: 13C15DON, 13C22HT-2, 13C24T-2, 13C18ZEA | After extraction | Standards in pure solvent |
| LC0034 | - | Aflatoxin B1: ESI+ m/z 313.0 / 285.1<br>Beauvericin: ESI+ m/z 784.4 / 244.2<br>Enniatin A: ESI+ m/z 699.4 / 210.1<br>Enniatin A1: ESI+ m/z 685.4 / 210.1<br>Enniatin B: ESI+ m/z 657.5 / 196.1<br>Enniatin B1: ESI+ m/z 671.4 / 196.1<br>Deoxynivalenol: ESI+ m/z 297.1 / 249.0<br>Fumonisin B1: ESI+ m/z 722.4 / 334.3<br>Fumonisin B2: ESI+ m/z 706.4 / 336.3<br>HT2-Toxin: ESI+ m/z 442.1 / 263.0<br>T2-Toxin: ESI+ m/z 484.2 / 305.1<br>Zearalenon: ESI- m/z 317.1 / 131.0 | 13C17-Aflatoxin B1; 13C15-Deoxynivalenol, 15N3-Enniatin A; 15N3-Enniatin B; 13C34-Fumonisin B1; 13C34-Fumonisin B2; 13C24-T2-Toxin; 13C22-HT2-Toxin; 13C18-Zearalenon | After extraction | Standards in pure solvent were used. |
| LC0035 | IAC | | | Before extraction | Standard in pure solvent |
| LC0036 | IAC - R-Biopharm | | no | | in pure solvent |
| LC0037 | AflaB1- Aflastar IAC<br>Zearalenone- Zearastar IAC<br>Fumonisins-SPE- Multisep 211<br>DON, T2, HT2 SPE Aflazon226 | DON - ESI+ m/z 297,1>249,1<br>T2- ESI+ m/z 484,2>305<br>HT2- ESI+ m/z 442,3>263 | DON, T2, HT2 C-13 labeled | After extraction | Standard in pure solvent |
| LC0038 | DON, ZEN, HT2, T2, Alfatoxin B1 - Romer Labs, MycoSep | | | Before extraction | Matrix matched calibration |
| LC0039 | - | Aflatoxin B1 - ESI + m/z 313>285.2<br>Beauvericin - ESI + m/z 784.4>244.2<br>Deoxynivalenol - ESI + m/z 297>249<br>Enniatin A - ESI + m/z 699.4>210.1<br>Enniatin A1 - ESI + m/z 685.4>210.1<br>Enniatin B - ESI + m/z 657.5>196.3<br>Enniatin B1 - ESI + m/z 671.4>196<br>Fumonisin B1 - ESI + m/z 722.5>334.4<br>Fumonisin B2 - ESI + m/z 706.4>336.3<br>HT2 toxin - ESI + m/z 441.9>263.1<br>T-2 Toxin - ESI + m/z 484.3>215.2<br>Zearalenone - ESI - m/z 317.1>175 | No | | All mycotoxins standard addition |
| LC0040 | | DON - ESI+ m/z 297>249<br>Afla B1 - ESI+ m/z 313>269<br>ZEA - ESI+ m/z 319>187<br>HT2 - ESI+ m/z 447>345<br>T2 - ESI+ m/z 484>215<br>FumoB1 - ESI+ m/z 723>352<br>FumoB2 - ESI+ m/z 706>318<br>Enn A - ESI+ m/z 700>210 | yes, for all toxins except enniatins and beauvericin | After extraction | Standards in pure solvent |

| | | | | | |
|---|---|---|---|---|---|
| | | Enn A1 - ESI+ m/z 686>210<br>Enn B - ESI+ m/z 658>196<br>Enn B1 - ESI+ m/z 672>196<br>Beau - ESI+ m/z 802>244 | | | |
| LC0041 | NA | AFB1 - ESI+ m/z 313>285 | NA | | Standards in pure solvent. |
| LC0042 | AFLA, DON, ZON: r-Biopharm | | | | Solvents in pure solvent |
| LC0043 | n.a. | DON - ESI NEG 355>265.1<br>ZEN - ESI NEG 317>131<br>AFLB1 ESI POS 313>241<br>FB1 ESI POS 722.2>352.2<br>FB2 ESI POS 706.5>336.6<br>BEA ESI POS 801>134<br>ENN A ESI POS 699.3>210.2<br>ENN A1 ESI POS 685>210<br>ENN B ESI POS 657>196<br>ENN B1 ESI POS 671>196<br>T-2 ESI POS489>245<br>HT-2 ESI POS 442>215 | DON C13, ZEN C15, AFLB1 C15, FB1 C15, T-2 C15 | After extraction | One point matrix matched calibration |
| LC0044 | AFLATOXINE: VICAN<br>ZEARALENONE: R-BIOPHARM<br>DON: R-BIOPHARM | Doesn't apply | Doesn't apply | | Standards in pure solvent |
| LC0045 | IAC T2, HT2-Neogen<br>IAC AFLA - VICAM<br>IAC DON - ROMER<br>IAC ZON - ROMER<br>IAC FUM B1, FUM B2 - ROMER | AFLA B1 - ESI+ m/z 313 >285<br>DON - ESI+ m/z 297 >249<br>ZON - ESI- m/z 319 >283<br>FUM B1 - ESI+ m/z 722.4 >704.4<br>FUM B2 - ESI+ m/z 706.4 > 336.4<br>T2 - ESI+ m/z 484.2 > 305.0<br>HT2 - ESI+ m/z 442.2 > 263.1 | No | | Standards in pure solvent. |
| LC0046 | Afla, ZON, ZEA, FUM: R-Biopharm<br>all the others: Varian Bond elute | DON: 297.3-249.0<br>FUM B1: 722.2-334.3<br>FUM B2: 706.2-336.3<br>HT-2: 425,3-24; 425,3-263,3<br>T-2: 467,4-305,1; 467,4-365,2<br>Enniatine A : MRM1 : 682.6-99.9 ; MRM2 : 682.6-210.3<br>Enniatine A1 : MRM1 : 668.5-99.9 ; MRM2 : 668.5-210.0<br>Enniatine B 1: MRM1 : 654.5-85.9 ; MRM2 : 654.5-195.9<br>Beauvericine: 784.5-133.9; 784.5-244<br>Enniatine B : MRM1 : 640.5-85.9 ; MRM2 : 640.5-195.9 | for fumonsins, use of a 13-C-fumonisin B2-internal standard | | Standards in pure solvent |
| LC0047 | none | DON 297>249 ESI+<br>AFLA B1 313>241 ESI+<br>HT2 442>263 ESI+<br>T2 484 >305 ESI+<br>ZEN 319 >283 ESI+<br>FB1 722 >334 ESI+<br>FB2 706> 336 ESI+<br>EnnA 683>100 ESI+<br>EnnA1 669>100 ESI+<br>EnnB 641>86 ESI+<br>EnnB1 655>86 ESI+<br>BEA 785>134 ESI+ | Isotope labelled standards for DON,ZEN, HT2,T2 and AfB1 | After extraction | Standards in pure solvent |

| | | | | | |
|---|---|---|---|---|---|
| LC0048 | | Enniatins B - ESI+ m/z 640>86, m/z 640>196<br>Enniatin B1 - ESI+ m/z 654>86, m/z 654>196<br>Enniatin A - ESI+ m/z 682>100, m/z 682>210<br>Beauvericin - ESI+ m/z 784>134, m/z 784>244<br>DON - ESI+ m/z 297>231, m/z 297>249<br>AFB1 - ESI+ m/z 313>241, m/z 313>285<br>AFB2 - ESI+ m/z 315>259, m/z 315>287<br>ZON - ESI+ m/z 319>187, m/z 319>283<br>AFG1 - ESI+ m/z 329>200, m/z 329>243<br>AFG2 - ESI+ m/z 331>245, m/z 331>313<br>OTA - ESI+ m/z 404>239, m/z 404>358<br>HT2 - ESI+ m/z 425>245, m/z 425>263<br>T2 - ESI+ m/z 467>245, m/z 467>305<br>FB2 - ESI+ m/z 706>318, m/z 706>336<br>FB1 - ESI+ m/z 722>334, m/z 722>352 | No | | Matrix matched calibration |
| LC0049 | R-BIOPHARM RHONE LTD:<br>Aflatoxin B1 - EASI-EXTRACT RP70N<br>Fumonisins - FUMONIPREP P31<br>DON - DONPREP P50<br>ZON - EASI-EXTRACT RP91<br>T-2, HT-2 - EASI-EXTRACT P43 | | | | Standards in pure solvent |
| LC0050 | IAC for AflaB1: Neogen<br>IAC for fumos B1 and B2 : RBiopharm | Analyte Ions Précurseurs Transition 1 (T1) Transition 2 (T2)<br>DON 295 265 138<br>13C-DON 309 279<br>HT-2 toxine 442 263 215<br>13C-HT-2 464.5 229<br>T-2 toxine 484 215 185<br>13C-T-2 508 322<br>ZON 317 131 175<br>13C-ZON 335 290 | 13C-DON;13C-HT-2;13C-T-2;13C-ZON | After extraction | external calibration |
| LC0051 | Vicam | B1- ESI+m/z 313.07>241.05<br>FB1- ESI+m/z 722.39>352.32<br>FB2- ESI+m/z 706.40>336.32<br>T2- ESI+m/z 484.25>305.14<br>HT2- ESI+m/z 442.24>263.12<br>ZON- ESI-m/z 317.20>131.05<br>DON- ESI-m/z 355.14>265.11 | no | | Matrix matched calibration |
| LC0052 | all Romer Labs | | | After extraction | Standard in pure solvent |
| LC0053 | R-biopharm | n.a. | n.a. | | standards in pure solvent |
| LC0054 | Aflatoxin B1 - Vicam | DON - ESI+ 297>249<br>ZEN - ESI+ 319>283<br>T2 - ESI+ 484>305<br>HT2 - ESI+ 442.2>263<br>FB1 - ESI+ 722.3>352.6<br>FB2 - ESI+706.3>336.4 | No | | Standards in pure solvents |
| LC0055 | Dilute 1 ml extrate + 1 ml H2O<br>Filter 0.2 um | AFB1- ESI+ m/z 313.10 > 241.20 : 313.10 > 285.20<br>DON - ESI+ m/z 297.30 > 249.20 : 314.30 > 249.20<br>FB1 - ESI+ m/z 706.50 > 74.00 : 706.50 > 318.20 | No | | Matrix matched calibration |

| | | | | | |
|---|---|---|---|---|---|
| | | FB2 - ESI+ m/z 722.60 > 334.50 : 722.60 > 352.30<br>ZON - ESI- m/z 317.20 > 131.10 : 317.20 > 175.10<br>HT-2 - ESI+ m/z 442.40 > 215.20 : 442.40 > 263.20<br>T-2 - ESI+ m/z 484.40 > 185.20 : 484.40 > 305.30<br>EN A - ESI+ m/z 682.50 > 100.00 : 682.50 > 209.90<br>EN B- ESI+ m/z 640.00 > 196.10 : 640.00 > 214.10<br>BEA - ESI+ m/z 784.00 > 244.00 : 784.00 > 262.00 | | | |
| LC0056 | Mycosep 226 AflaZON | BEAUVERECIN ES+ m/z 783.9 > 243.9<br>ENNIATIN A ES+ m/z 699.2 > 209.8<br>ENNIATIN A1 ES+ m/z 685.1 > 209.8<br>ENNIATIN B ES+ m/z 657.1 > 196<br>ENNIATIN B1 ES+ m/z 671.2 > 195.9<br>HT2 ES+ m/z 447 > 345<br>T2 ES+ m/z 489.1 > 245<br>AFLA B1 ES+ m/z 312.8 > 284.9<br>DON ES+ m/z 296.9 > 248.9<br>FB1 ES+ m/z 722.3 > 334.4<br>ZON ES+ m/z 318.8 > 186.8 | Yes for DON, ZON, T2 and HT2 | After extraction | Standards in pure solvent |

| Lab code | Q.13 Approach uncertainty | Q.14 Recovery estimate | Q.15 Recovery correction | Q.16 Use of CRMs | Q.17 Supplier of standards | Q.18 Special precautions |
|---|---|---|---|---|---|---|
| LC0001 | From initial method validation data/ Long term compilation of quality control data | Spiking/ Certified Reference Material | Corrected for recoveries | No. We did earlier, but the quality is too bad. We have our own in-house material and are participating in many PT´s | DON - Biopure, ZON - Biopure, HT-2 - Biopure, T-2 - Biopure, ENN A - Sigma/Aldrich,<br>ENN A1 - Sigma/Aldrich,<br>ENN B - Sigma/Aldrich,<br>ENN B1 - Sigma/Aldrich,<br>BEAU - Sigma/Aldrich | Protection from daylight, especially for aflatoxins |
| LC0002 | From initial method validation data | Spiking | Not corrected for recoveries | No | FLUKA | NaOCl -washing of the glassware, amber glassware and protection from daylight |
| LC0004 | Long term compilation of quality control data | Spiking | Corrected for recoveries | Aflatoxin - maize, dry fruit, feed - Fapas<br>Zearalenone - maize, feed - Fapas<br>Fumonisin - maize - Fapas | Aflatoxin - Sigma<br>Zearalenone - Sigma<br>Fumonisin B1, Fumonisin B2 - R-Biopharm | For all mycotoxins tested we use ambar glassware and protection from daylight |
| LC0005 | Other | Spiking | Corrected for recoveries | No | | No |
| LC0006 | From initial method validation data | Spiking | Corrected for recoveries | | Sigma Aldrich Aflatoxin B1+B2+G1+G2 | |
| LC0007 | Other | Spiking | Corrected for recoveries | No | Biopure, for all the tested mycotoxins | No special precautions were taken, but no new glassware was used. Sample extracts were protected from daylight by wrapping the tubes with aluminium foil |
| LC0008 | Long term compilation of quality control data | Spiking | | No | SIGMA | Yes for aflatoxin B1 |
| LC0009 | From initial method | Spiking/ Certified | Not corrected for | PT Bipea (corn and oat) | Romer Labs | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | validation data | Reference Material | recoveries | | | |
| LC0011 | Long term compilation of quality control data | Spiking | | No | R-Biopharm | Protection from daylight |
| LC0012 | From initial method validation data | Spiking | Corrected for recoveries | | R-BIOPHARM (TRILOGY) | AMBAR GLASSWARE |
| LC0013 | Long term compilation of quality control data | Spiking | Corrected for recoveries | No | B1: Biopure, Romer Labs | |
| LC0014 | From initial method validation data | Spiking | | - | | No |
| LC0015 | From initial method validation data | Spiking | Corrected for recoveries | No | DON, ZON, AFB1, FB1, FB2, T2, HT2: Sigma Aldrich<br>BEA, ENN A, ENN A1, ENN B, ENN B1: Enzo Lifesciences | amber vials, acid washing glassware |
| LC0016 | From initial method validation data | Spiking/ Certified Reference Material | Corrected for recoveries | | TRILOGY | NO SPECIAL PRECAUTIONS |
| LC0017 | Long term compilation of quality control data | Spiking | Corrected for recoveries | | | Acid-washed glassware |
| LC0018 | Other | Spiking | Corrected for recoveries | Not yet regularly | several | no glassware, only disposables |
| LC0019 | From initial method validation data | Spiking | Corrected for recoveries | | LGC Standards, | protection from daylight |
| LC0020 | From initial method validation data | Spiking/ Certified Reference Material | Not corrected for recoveries | | | |
| LC0021 | | | | No | sigma aldrich | No |
| LC0022 | From initial method validation data | Spiking | Not corrected for recoveries | No | Romer Labs | No |
| LC0024 | From initial method validation data | Spiking/ Other | Not corrected for recoveries | not yet | Sigma, Biopure | acid washing of the glassware<br>deactivated vials<br>protection from the sunlight |
| LC0025 | Long term compilation of quality control data/ Other | Spiking/ Other | Corrected for recoveries | Just Reference Materials from FAPAS for DON,ZON,FB1FB2 - Maize flour | SIGMA | acid washing of the glassware, use of amber glassware, precaution during the evaporation step (ZON,DON,FB1FB2) and protection from daylight |
| LC0026 | From initial method validation data | | Not corrected for recoveries | No | | all mycotoxins are protected from daylight by using special glassware |
| LC0027 | Long term compilation of quality control data | Spiking | Corrected for recoveries | FAPAS; corn; DON, ZON, AFLA, FUMONIZIN; T2, HT2 | Romer Labs (Biopure) | deactivated glassware; dark vials |
| LC0028 | From initial method validation data | Certified Reference Material | Not corrected for recoveries | FAPAS sample analysed in every batch, maize, Zon, Don, T-2. HT_2, AFB1, OTA, Fum B1, Fum B2 | LGC | We use silanised glassware for fumonisin stds |
| LC0029 | From initial method validation data | Spiking | Not corrected for recoveries | We don't use CRM; we use QC materials of FAPAS in matrices like nutmeg,dried figs, pistachio, red pepper | Biopure | Yes for aflatoxins -acid washing of the glassware and amber glassware |
| LC0030 | From initial method validation data/ Long term | | Corrected for recoveries | No | SIGMA ALDRICH | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| | compilation of quality control data | | | | | |
| LC0031 | From initial method validation data | Spiking/ Certified Reference Material | Corrected for recoveries | Corn naturally contaminated with mycotoxins (aflatoxins, DON, OTA, T-2, HT-2, ZON, FB1 and FB2): Trilogy Analytical laboratory | Sigma Aldrich (aflatoxins, DON, OTA, T-2, HT-2, ZON, FB1 and FB2), Enzo life sciences (Enns + Bea) | Aflatoxins: protection from daylight |
| LC0032 | Other | Spiking | Corrected for recoveries | - | T-2, HT-2 - Romer<br>Fumonisin - Romer<br>DON - Romer<br>Aflatoxin - Supelco<br>F2 - Romer | standards and samples are protected from daylight |
| LC0033 | From initial method validation data | Spiking | Corrected for recoveries | DON-ZEA-T-2-HT-2 Maize Flour by FAPAS | Orsell | No |
| LC0034 | Other | Spiking/ Certified Reference Material | Corrected for recoveries | Aflatoxin B1, Deoxynivalenol, Zearalenone: maize quality-control test material supplied by FAPAS (T04201QC)<br>T2-Toxin, HT2-Toxin: oat flakes certified reference material supplied by BAM (ERM-BC720)<br>Fumonisin B1: maize powder from 2013 EURL-PT multitoxin | Aflatoxin B1: IRMM (ERM AC057) certified reference standard solution<br>Deoxynivalenol, Fumonisin B1+B2; HT2-Toxin, T2-Toxin, Zearalenone: Romer Labs "Biopure" certified standard solutions<br>Enniatins, Beauvericin: Cfm Oskar Tropitzsch, bulk | amber, silanised glassware, including HPLC vials |
| LC0035 | Other | Spiking | Corrected for recoveries | No | Biopure | No |
| LC0036 | Long term compilation of quality control data | Spiking | | | Sigma-Aldrich | amber glassware protection from daylight |
| LC0037 | From initial method validation data | Spiking | Corrected for recoveries | No | Romerlabs | Aflatoxins |
| LC0038 | From initial method validation data | Spiking | Not corrected for recoveries | No | Romer Labs, biopure | No |
| LC0039 | Long term compilation of quality control data | Spiking | Corrected for recoveries | FAPAS animal feed;, DON, T2, HT2 and Zearalenone | Sigma-Aldrich<br>Biopure | No |
| LC0040 | From initial method validation data/ Long term compilation of quality control data | Spiking | Corrected for recoveries | No | Romer Labs | Acid washing of glassware, amber LC-vials, protection from UV (window filters and LED light) |
| LC0041 | Other | Spiking | Corrected for recoveries | NA | | No. |
| LC0042 | Long term compilation of quality control data/ Other | Spiking | Corrected for recoveries | CRMs are sometimes used for Aflatoxin analysis for nuts and dried fruit matrices supplied by FAPAS | AFLA: Sigma<br>DON: Biopure<br>ZON: Biopure | Amber glassware and protection from daylight are applied through analysis |
| LC0043 | Other | Spiking | Corrected for recoveries | Yes, but not for this analysis. | Sigma Aldrich<br>Romer labs | polypropylene tubes, amber vials |
| LC0044 | Long term compilation of quality control data | Spiking | Corrected for recoveries | Yes, we use for quality control, and the supplier is R- Biopharm/Trilogy | SUPELCO/ALDRICH | Acid washing of glassware, amber glassware and protection from daylight |
| LC0045 | From initial method validation data | Spiking | Corrected for recoveries | No | AFLA - Sigma Aldrich<br>LGC for all others | - |
| LC0046 | Long term compilation of quality control data/ Other | Spiking | Not corrected for recoveries | No | Biopure except for Enniatins et beauvericin (Sigma-Aldrich) | acid washing of the glass ware |
| LC0047 | Long term compilation of quality control data | Spiking | Corrected for recoveries | No | Romerlabs | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| LC0048 | From initial method validation data/ Long term compilation of quality control data | Certified Reference Material | Corrected for recoveries | No | Sigma | Daylight protection |
| LC0049 | Long term compilation of quality control data | Spiking | Corrected for recoveries | FAPAS, various matrices | Aflatoxin B1 - SUPELCO<br>Fumonisins - SIGMA-ALDRICH<br>DON - BIOPURE<br>ZON - BIOPURE<br>T-2, HT-2 - BIOPURE | For Aflatoxin B1 - acid washing of the glassware, for all mycotoxins - amber glassware and protection from daylight. |
| LC0050 | Long term compilation of quality control data | Spiking | Corrected for recoveries | No | DON: Libios<br>Zéa:Libios<br>AflaB1: libios<br>T2 and HT2 and fumos B1 and B2: Biopure | acid washing of the glassware and protection from daylight |
| LC0051 | | | Corrected for recoveries | No | Romer | No |
| LC0052 | From initial method validation data | Spiking | Corrected for recoveries | Zearalenone: ERM Wheat<br>DON: ERM Wheat | Biopure, Supelco | |
| LC0053 | From initial method validation data | Spiking | Corrected for recoveries | We use CRMs for aflatoxins. | Romer Labs | Protection from daylight |
| LC0054 | From initial method validation data | Spiking | Corrected for recoveries | Yes. Fapas QC materials | Aflatoxin B1 - Sigma Aldrich<br>The other mycotoxins - Biopure | Acid washing of glass ware (all mycotoxins), daylight protection (aflatoxin B1) |
| LC0055 | From initial method validation data | Spiking | | No | Sigma Aldrich | Ambar glassware and protection from daylight. |
| LC0056 | Other | Spiking | Corrected for recoveries | No | Romer Labs, BioPure | Silanised UPLC Vials |

## Annex 10. Method validation data

| | DON | | | AFB1 | | | ZON | | | FB1 | | | FB2 | | | HT-2 | | | T-2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab code** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** |
| LC0001 | 95 | | 44 | 88 | | 0.1 | 100 | | 15 | 80 | | 25 | 77 | | 25 | 100 | | 24 | 99 | | 13 |
| LC0002 | 94 | 60 | 116 | 98 | 0.1 | 0.2 | 103 | 6 | 20 | | | | | | | | | | | | |
| LC0004 | | | | 91.5 | 0.3 | 0.8 | 97.3 | 3 | 8 | 82 | 41 | 122 | 79 | 19 | 38 | | | | | | |
| LC0005 | 102 | 15 | 45 | | | | 72.1 | 1.6 | 5 | 110 | 10 | 30 | 115 | 10 | 30 | 118 | 5 | 15 | 103 | 5 | 15 |
| LC0006 | | | | 75 | 1 | 1.2 | | | | | | | | | | | | | | | |
| LC0007 | 80 | 13 | 26 | 96 | 0.1 | 0.1 | 92 | 13 | 26 | 136 | 13 | 26 | 97 | 13 | 26 | 67 | 2.5 | 18 | 75 | 1.5 | 10.8 |
| LC0008 | | | | 100 | 0.1 | 0.5 | | | | | | | | | | | | | | | |
| LC0009 | 116 | 17 | 50 | 95 | 0.2 | 0.5 | 105 | 1.7 | 5 | 104 | 17 | 50 | 87 | 17 | 50 | 100 | 17 | 50 | 100 | 17 | 50 |
| LC0011 | | | | 70.6 | 0.2 | 1 | | | 25 | | | 1000 | | | 1000 | | | 250 | | | 250 |
| LC0012 | 99 | | 90 | 100 | | 0.6 | 107 | | 12 | | | | | | | | | | | | |
| LC0013 | | | | 80.9 | | 0.6 | | | | | | | | | | | | | | | |
| LC0014 | 60 | 19 | 57 | 36 | 0.1 | 0.1 | 51 | | | 106 | 40 | 120 | 110 | 25 | 75 | 96 | 5 | 15 | 92 | 8 | 24 |
| LC0015 | 106 | 16 | 80 | 94 | 0.2 | 1 | 119 | 1 | 5 | 70 | 1 | 5 | 71 | 1 | 5 | 88 | 34 | 170 | 91 | 1 | 5 |
| LC0016 | 100 | 6 | 18 | 90 | 0.1 | 0.3 | 95 | 7 | 21 | | | | | | | | | | | | |
| LC0017 | 100 | 5 | 10 | | | 0.1 | | 5 | 10 | | | 20 | | | 20 | | 5 | 10 | | 5 | 10 |
| LC0018 | 105 | 20 | 40 | 98 | 0.2 | 0.4 | 97 | 4 | 8 | 100 | 20 | 40 | 101 | 20 | 40 | 99 | 3 | 6 | 96 | 3 | 6 |
| LC0019 | 89 | 16 | 40 | 91 | 0 | 0.1 | 85 | 1.8 | 1.8 | 90 | 25 | 25 | | | | | 5 | 10 | | 5 | 10 |
| LC0020 | 105 | 1.3 | 4 | 103 | 0.1 | 0.2 | 69 | 2.7 | 8 | 72 | 17 | 51 | 75 | 15 | 45 | 71 | 0.4 | 1.3 | 71 | 0.5 | 1.6 |
| LC0021 | 100 | 250 | 100 | 100 | 1 | 0.5 | 100 | 10 | 5 | 100 | 25 | 10 | 100 | 25 | 10 | 100 | 10 | 5 | 100 | 10 | 5 |
| LC0022 | 102 | 20 | 80 | 87 | 2.5 | 10 | 86 | 5 | 20 | | 300 | 1000 | | | | 102 | 40 | 160 | 98 | 20 | 80 |
| LC0024 | 98 | 60 | 18 | 92 | 3.3 | 1 | 108 | 10 | 3 | 94 | 5 | 2 | 82 | 4 | 1 | 93 | 6 | 2 | 96 | 6 | 2 |
| LC0025 | 76.9 | 1.7 | 202.9 | 89.7 | 0.1 | 0.2 | 95.2 | 0.1 | 5.1 | 72 | 0.3 | 60 | 117.1 | 1.4 | 30 | | | | | | |
| LC0026 | | 13.3 | 40 | | 0.1 | 0.2 | | 6.7 | 20 | | 33.3 | 100 | | 33.3 | 100 | | 3.3 | 10 | | 3.3 | 10 |
| LC0027 | 84 | 20 | 50 | 83 | 0.1 | 0.2 | 79 | 2.5 | 5 | 67 | 25 | 50 | 69 | 25 | 50 | 118 | 3 | 5 | 110 | 3 | 5 |
| LC0028 | 92 | | 200 | 104 | | 2.5 | 101 | | 20 | 92 | | 100 | 92 | | 100 | 86 | | 50 | 96 | | 10 |
| LC0029 | 71.6 | 40 | 120 | 89.5 | 0.2 | 0.6 | 74.3 | 8.3 | 25 | 91.1 | 18.3 | 55 | 94 | 15 | 45 | | | | | | |
| LC0030 | | | 75 | | | 1 | | | 30 | | | 75 | | | 75 | | | 100 | | | 20 |
| LC0031 | 96 | 50 | 100 | 97 | 1 | 2.5 | 96 | 10 | 20 | 98 | 10 | 20 | 95 | 10 | 20 | 90 | 10 | 20 | 80 | 10 | 20 |
| LC0032 | 98 | 106 | 29.9 | 66.4 | 0.2 | 0.8 | 96.5 | 6.8 | 23.8 | 87 | 10 | 33 | 100 | 10 | 33 | 100 | 1.5 | 5 | 100 | 0.5 | 1.7 |
| LC0033 | 107 | | 50 | 96 | | 0.1 | 110 | | 5 | 86 | | 38 | 82 | | 38 | 116 | | 2 | 113 | | 2 |
| LC0034 | | 44 | 140 | | 0.4 | 1.4 | | 2.9 | 9.6 | | 45 | 150 | | 33 | 110 | | 4.7 | 15 | | 4.4 | 14 |
| LC0035 | 100 | 25 | 50 | 70 | 0.3 | 0.5 | 100 | 5 | 10 | 100 | 12.5 | 25 | 100 | 12.5 | 25 | 70 | 2.5 | 5 | 70 | 2.5 | 5 |
| LC0036 | | | 115 | | | 0.2 | | | 15 | | | 80 | | | 24 | | | 8 | | | 7 |
| LC0037 | 89 | 8 | 40 | 96.2 | 0.1 | 0.2 | 97.8 | 4 | 10 | 99.1 | 10 | 25 | 91 | 10 | 25 | 72 | 2 | 10 | 77 | 2 | 10 |
| LC0038 | | | 50 | | | 1 | | | 10 | | | 50 | | | 50 | | | 5 | | | 5 |
| LC0039 | 89 | | 200 | 91 | | 2.5 | 92 | | 50 | 97 | | 100 | 101 | | 40 | 94 | | 20 | 92 | | 20 |
| LC0040 | 107.9 | 4 | 100 | 120.9 | 0.1 | 0.5 | 101.3 | 1 | 2 | 80.4 | 20 | 100 | 93.9 | 20 | 100 | 83.2 | 2 | 5 | 105.4 | 2 | 5 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LC0041 | 69 | | 20 | 58 | | 0.5 | 129 | | 10 | | | | | | 116 | | 10 | 82 | | 10 |
| LC0042 | 76.1 | 25 | 50 | 72.8 | 0.5 | 1 | 108.7 | 5 | 10 | | | | | | | | | | | |
| LC0043 | 81 | 10 | 25 | 75 | 0.5 | 1 | 76 | 1 | 2.5 | 86 | 10 | 25 | 85 | 5 | 20 | 74 | 2.5 | 5 | 84 | 1 | 2.5 |
| LC0044 | 96.8 | | 240 | 102.7 | | 1 | 105.4 | | 50 | | | | | | | | | | | |
| LC0045 | 100 | 50 | 200 | 98 | 0.2 | 0.5 | 90 | 5 | 20 | 80 | 50 | 250 | 80 | 50 | 250 | 65 | 2 | 5 | 78 | 2 | 5 |
| LC0046 | 85.8 | 10 | 5 | 109.7 | 0 | 0 | 97 | 3 | 0.3 | 82.5 | 20 | 60 | 83.5 | 20 | 60 | 55.6 | 30 | 10 | 60.6 | 60 | 20 |
| LC0047 | 85 | | 180 | 100 | | 1 | 85 | | 25 | 100 | | 100 | 100 | | 100 | 85 | | 20 | 85 | | 10 |
| LC0048 | 99 | 8 | 25 | 91 | 0.2 | 0.7 | 101.8 | 3 | 10 | 64.1 | 30 | 99 | 68.8 | 8 | 26 | 108.2 | 3.3 | 11 | 100.2 | 6 | 21 |
| LC0049 | 101.5 | 20 | 100 | 99 | 0.1 | 0.2 | 109.7 | 3 | 10 | 82 | 6 | 19 | 93 | 6 | 21 | 84 | 1.4 | 5 | 94 | 1.4 | 5 |
| LC0050 | 95 | 50 | 150 | 95 | 0.2 | 0.6 | 95 | 3 | 10 | 100 | 20 | 60 | 100 | 30 | 90 | 100 | 5 | 15 | 95 | 3 | 10 |
| LC0051 | 100 | | | 100 | | | 100 | | | 100 | | | 100 | | | 100 | | | 100 | | |
| LC0052 | 100 | 25 | 50 | 100 | 0.2 | 0.4 | 82 | 5 | 10 | | | | | | | 100 | 25 | 50 | 100 | 25 | 50 |
| LC0053 | | | | 114 | 0.3 | 0.5 | | | | | | | | | | | | | | | |
| LC0054 | 68 | | 300 | 97 | | 0.2 | 77 | | 30 | 151 | | 100 | 145 | | 100 | 44 | | 40 | 62 | | 40 |
| LC0055 | 85 | | 200 | 107 | | 0.6 | 98 | | 20 | 81 | | 100 | 89 | | 100 | 106 | | 10 | 103 | | 5 |
| LC0056 | 165.98 | 10 | 2.5 | 84.23 | 0.4 | 0.1 | 56.25 | 10 | 2.5 | | 10 | 2.5 | | 10 | 2.5 | | 0.5 | 2 | 75.87 | 0.5 | 2 |
| **Median** | **97** | **19** | **50** | **95** | **0.2** | **0.5** | **97** | **4** | **10** | **91.1** | **18.3** | **51** | **93.45** | **15** | **40** | **95** | **4** | **10** | **95** | **3.15** | **10** |

| | **EN B** | | | **EN B1** | | | **EN A1** | | | **EN A** | | | **BEA** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab code** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** | **Rec** | **LOD** | **LOQ** |
| LC0001 | 95 | | 10 | 95 | | 10 | 95 | | 10 | 95 | | 10 | 100 | | 15 |
| LC0005 | 62 | 5 | 15 | 118.1 | 5 | 15 | 86 | 5 | 15 | 108.5 | 5 | 15 | 63.4 | 5 | 15 |
| LC0014 | 104 | | | 98 | | | 104 | | | 109 | | | 109 | | |
| LC0015 | 108 | 0.5 | 2 | 101 | 0.5 | 2 | 112 | 0.5 | 2 | 105 | 2 | 10 | 96 | 0.5 | 2 |
| LC0016 | | | | | | | | | | | | | | | |
| LC0017 | | 3 | 10 | | 3 | 10 | | 3 | 10 | | 3 | 10 | 100 | 3 | 10 |
| LC0018 | 63 | 2 | 4 | 57 | 2 | 4 | 67 | 2 | 4 | | 2 | 2 | 45 | 2 | 4 |
| LC0026 | | 1.7 | 5 | | | | | | | | | | | 3.3 | 10 |
| LC0031 | 95 | 5 | 10 | 104 | 5 | 10 | 101 | 5 | 10 | 80 | 5 | 10 | 81 | 5 | 10 |
| LC0034 | | 1.8 | 5.9 | | 2.2 | 7.1 | | 52 | 172 | | 4.3 | 14 | | 1.8 | 6 |
| LC0039 | | | 50 | | | 50 | | | 50 | | | 50 | | | 25 |
| LC0040 | 69.9 | 0.1 | 2 | 44.5 | 0.1 | 2 | 43.9 | 0.1 | 2 | 58.9 | 0.1 | 2 | 39.2 | 0.1 | 2 |
| LC0043 | 80 | 0.1 | 0.1 | 80 | 0.1 | 0.1 | 85 | 0.1 | 0.1 | 85 | 0.1 | 0.1 | 85 | 0.1 | 0.1 |
| LC0047 | 100 | | 1 | 100 | | 1 | 100 | | 1 | 100 | | 1 | 100 | | 1 |
| LC0048 | 97.3 | 0.2 | 0.6 | 94.1 | 0.2 | 0.6 | | | | 93.1 | 0.2 | 0.6 | 81.8 | 0.2 | 0.6 |
| LC0055 | 100 | | 1 | | | | | | | | | 1 | 100 | | 5 |
| LC0056 | 113.02 | 0.5 | 2 | 100.55 | 0.5 | 2 | 112.15 | 0.5 | 2 | 110.49 | 0.5 | 2 | 92.49 | 0.5 | 2 |
| **Median** | **96** | **1.7** | **4** | **98** | **1.25** | **4** | **98** | **2** | **7** | **98** | **2** | **6** | **92** | **1.8** | **5** |

Rec – recovery (%), LOD – limit of detection (μg $kg^{-1}$), LOQ - limit of quantification (μg $kg^{-1}$)

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