*Proficiency Tests*

**DLA**

food
cosmetics
consumer goods
**www.dla-lvu.de**

# Evaluation Report

proficiency test

**DLA 22/2018**

# Mycotoxins: DON and ZEA
# Deoxynivalenol and Zearalenone in Cereals

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## Allgemeine Informationen zur Eignungsprüfung (EP)
## General Information on the proficiency test (PT)

| | |
|---|---|
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| *EP-Nummer*<br>*PT-Number* | DLA 22/2018 |
| *EP-Koordinator*<br>*PT-Coordinator* | Dr. Gerhard Wichmann |
| *Status des EP-Bericht*<br>*Status of PT-Report* | Abschlussbericht / Final report : 22 January 2019 |
| *EP-Bericht Freigabe*<br>*PT-Report Authorization* | Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager)<br>- *gezeichnet / signed M. Besler-Scharf*<br>Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager)<br>- *gezeichnet / signed G. Wichmann*<br>Datum / Date: 22 January 2019 |
| *Unteraufträge*<br>*Subcontractors* | Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben.<br>The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA. |
| *Vertraulichkeit*<br>*Confidentiality* | Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben.<br>Participant result are named anonymously with evalutation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant. |

# Inhalt / Content

# 1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

# 2. Realisation

## 2.1 Test material

The test material is a mixture of different batches of corn grits (naturally contaminated with DON and ZEA) and a microtracer premix (wheat flour, microtracer iron particles (FSS red lake) for homogeneity verification.

The raw materials were ground, sieved, combined, homogenized and then sieved again.

Approximately 3 kg of the material was packaged in about 50 grams in metallized PET film bags. The portions were numbered chronologically.

***Note:*** *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

### 2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15]. The microtracer analysis of the present PT sample showed probability of 41%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat value of 1,1. The results of microtracer analysis are given in the documentation.

The calculation of the **variation coefficient** of the repeatability standard deviation ($CV_r$) was used as an indicator of homogeneity. It is 3,8% for deoxynivalenol. The coefficient of variation $CV_r$ is thus comparable to the precision data of the official method ASU §64 LFGB L 15.00-9 or DIN EN 15891/2010, see 3.6.2 (see Tab. 1). The repeatability standard deviation of the participants is given at the characteristics (4.1).

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked and optionally the evaluation of the results of the participants will be done using the z´-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3].

### *2.1.2 Stability*

A water activity ($a_W$) of < 0,6 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the $a_W$ value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity ($a_W$ value <0,6).
The $a_W$ value of the PT samples was approx. 0,55 (21,0°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

## 2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 47th week of 2018. The testing method was optional. The tests should be finished at January 4th 2019 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

Further information see 5.3.

## 2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).

The finally calculated concentrations as average of duplicate determinations of both numbered samples was used for the statistical evaluation. For the calculation of the Repeatability- and Reproducibility standard deviation the single values of the double determination were used.

Queried and documented were single results, recovery and the used testing method, information on the limit of quantification, the date of the analysis and general points to the method.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

Of the 11 participants, 10 submitted at least one result in time.

# 3. Evaluation

## 3.1 Consensus values from participants (Assigned value)

The robust mean of the submitted results was used as assigned value (X) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 $\sigma_{pt}$) [3].

The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values ($X_{pti}$) are made whenever possible.

The statistical evaluation is carried out for all the parameters for a minimum of 7 values are present, in justified cases an evaluation may also be carried out from 5 results onwards.

The actual measurement results will be drafted. Individual results, which are outside the specified measurement range of the participating laboratory (for example with the result > 25 mg/kg or < 2,5 mg/kg) or the indicating "0" will not be considered for the statistic evaluation [3].

## 3.2 Robust standard deviation

For comparison to the target standard deviation $\sigma_{pt}$ (standard deviation for proficiency assessment) a robust standard deviation ($S^x$) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

## 3.3 Repeatability standard deviation

The repeatability standard deviation $S_r$ is based on the laboratory´s standard deviation of (outlier free) individual participant results, each under repeatability conditions, that means analyses was performed on the same sample by the same operator using the same equipment in the same laboratory within a short time. It characterizes the mean deviation of the results within the laboratories [3] and is used by DLA as an indication of the homogeneity of the sample material.

In case single results from participants are available the calculation of the repeatability standard deviation $S_r$, also known as standard deviation within laboratories $S_w$, is performed by: [3, 4].

The relative repeatability standard deviation as a percentage of the mean

value is indicated as coefficient of variation $CV_r$ in the table of statistical characteristics in the results section in case single results from participants are available.

## 3.4 Reproducibility standard deviation

The reproducibility standard deviation $S_R$ represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation $S_r$ and the within-laboratory standard deviation $S_S$. Reproducibility standard deviations of PT´s may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.

In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

In case single results from participants are available the calculation of the reproducibility standard deviation $S_R$ is performed by: [3, 4].

The relative reproducibility standard deviation as a percentage of the mean value is indicated as coefficient of variation $CV_R$ in the table of statistical characteristics in the results section in case single results from participants are available. Its meaning is explained in more detail in 3.9.

## 3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. Even if a result clearly deviates from the robust mean (e.g. factor >10) and has an influence on the robust statistics, a result can be excluded from statistical evaluation [3].

All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

## 3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value $\sigma_{pt}$ (= standard deviation for proficiency assessment) can be determined according to the following methods.

If an acceptable quotient $S^*/\sigma_{pt}$ is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for evaluation of interlaboratory studies, where different methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information, the z-scores of both models are given in the evaluation, if available.

**For the valuation of deoxynivalenol the target standard deviation from the general model of Horwitz (s. 3.6.1) was applied. The general model of Horwitz/ Thompson was used for the evaluation of zearalenone (s. 3.6.1). For information the target standard deviation for both parameters was calculated from evaluation of a precision experiment (see 3.6.2) was given additionally.**

### 3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation $\sigma_R$ [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation $\sigma_R$ can be applied as the relative target standard deviation $\sigma_{pt}$ in % of the assigned values and calculated according to the following equations [3]. For this the assigned value $X_{pt}$ is used for the concentration c.

| **Equations** | **Range of concentrations** | **corresponds to** |
|---|---|---|
| $\sigma_R = 0{,}22c$ | $c < 1{,}2 \times 10^{-7}$ | < 120 µg/kg |
| $\sigma_R = 0{,}02c^{0{,}8495}$ | $1{,}2 \times 10^{-7} \leq c \leq 0{,}138$ | ≥ 120 µg/kg |
| $\sigma_R = 0{,}01c^{0{,}5}$ | $c > 0{,}138$ | > 13,8 g/100g |

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = $10^{-6}$ kg/kg)

### 3.6.2 Precision experiment

Using the reproducibility standard deviation $\sigma_R$ and the repeatability standard deviation $\sigma_r$ of a precision experiment (collaborative trial or proficiency test) the target standard deviation $\sigma_{pt}$ can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2\left(m - 1/m\right)}$$

The relative repeatability standard deviations ($RSD_r$) and relative reproducibility standard deviation ($RSD_R$) given in Table 1 were determined in ring tests using the indicated methods.
The resulting target standard deviations $\sigma_{pt}$, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 1: relative repeatability standard deviations ($RSD_r$) and relative reproducibility standard deviation ($RSD_R$) according to selected evaluations of tests for precision and the resulting target standard deviation $\sigma_{pt}$ [18, 19, 22]

| **Parameter** | **Matrix** | **Mean** | **$RSD_r$** | **$RSD_R$** | **$\sigma_{pt}$** | **Method / Literature** |
|---|---|---|---|---|---|---|
| DON | Rice | 458 µg/kg | 6,5% | 11,5% | 11,5% | HPLC / 18 |
| DON | Wheat | 678 µg/kg | 6,0% | 16,3% | 15,7% | HPLC / 18 |
| DON | Wheat | 165 µg/kg | 21% | 39% | 36,1% | HPLC / 18 |
| DON | Corn | 501 µg/kg | 10%* | 23%* | 21,9% | HPLC / 18 |
| ZEA | Corn | 87,2 µg/kg | 14,2% | 20,6% | 10,5% | HPLC / 22 |
| ZEA | Corn | 66,5 µg/kg | 8,9%* | 16,4%* | 15,1% | HPLC / 22 |
| ZEA | Rye | 26,3 µg/kg | 8,9% | 19,7% | 18,7% | HPLC / 19 |
| ZEA | Rye | 58,4 µg/kg | 3,8% | 23,0% | 22,9% | HPLC / 19 |

The values marked with "*" are used to calculated the target standard deviation **$\sigma_{pt}$** from tests for precision, which are specified for information in the evaluation (see under 4.1 and 4.2).

### *3.6.3 Value by perception*

In the present LVU DLA 22-2018 corn flour was to be tested for the parameters deoxynivalenol (DON) and zearalenone (ZEA). According to EU Regulation 1881/2006 [23] the following maximum levels are set for DON and ZEA:

| | Foodstuffs (1) | Maximum levels (µg/kg) |
|---|---|---|
| 2.4 | **Deoxynivalenol** (17) | |
| 2.4.1 | Unprocessed cereals (18) (19) other than durum wheat, oats and maize | 1 250 |
| 2.4.2 | Unprocessed durum wheat and oats (18) (19) | 1 750 |
| 2.4.3 | Unprocessed maize (18) | 1 750 (20) |
| 2.4.4 | Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits (21)), bran as end product marketed for direct human consumption and germ, with the exception of foodstuffs listed in 2.4.7 | 750 |
| 2.4.5 | Pasta (dry) (22) | 750 |
| 2.4.6 | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals | 500 |
| 2.4.7 | Processed cereal-based foods and baby foods for infants and young children (3) (7) | 200 |

| | | |
|---|---|---|
| 2.5 | **Zearalenone** (17) | |
| 2.5.1 | Unprocessed cereals (18) (19) other than maize | 100 |
| 2.5.2 | Unprocessed maize (18) with the exception of unprocessed maize intended to be processed by wet milling (37) | 350 (20) |
| 2.5.3 | Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.5.6, 2.5.7, 2.5.8, 2.5.9 and 2.5.10 | 75 |
| 2.5.4 | Refined maize oil | 400 (20) |
| 2.5.5 | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals | 50 |
| 2.5.6 | Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals | 100 (20) |
| 2.5.7 | Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children (3) (7) | 20 |
| 2.5.8 | Processed maize-based foods for infants and young children (3) (7) | 20 (20) |
| 2.5.9 | Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 200 (20) |
| 2.5.10 | Milling fractions of maize with particle size ≤ 500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤ 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 300 (20) |

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

For the present evaluation the target standard deviation according to 3.6.1 were regarded suitable.

Table 2 shows selected characteristics of participants results of the present PT in comparison to the previous year.

Table 2: Characteristics of the present PT (on blue-grey) in comparison to previous PTs since 2015 (SD = standard deviation, CV = coefficient of variation)

| Parameter | rob. Mean (µg/kg) | rob. SD (S*) (µg/kg) | rel. SD ($VK_r$) [%] | rel. SD ($VK_R$) [%] | Target-SD ($\sigma_{pt}$) (µg/kg) | Quotient $S^*/\sigma_{pt}$ | DLA-report |
|---|---|---|---|---|---|---|---|
| DON | 773 | 147 | 3,76 | 21,9 | 129 | 1,1 | 22-2018 |
| ZEA | 44,4 | 17,4 | 14,1 | 26,5 | 9,78 | 1,8 | 22-2018 |
| DON | 444 | 152 | 6,8 | 38 | 98,6 | 1,5 | 22-2017 |
| ZEA | 38,1 | 13,2 | 7,7 | 30 | 8,37 | 1,6 | 22-2017 |
| DON | 368 | 163 | 15,2 | 48,1 | 87,3 | 1,9 | 20-2016 |
| ZEA | 16,7 | 9,53 | 26,5 | 61,9 | 3,68 | 2,6 | 20-2016 |
| DON | 225 | 53,0 | 5,05 | - | 45,1 | 1,2 | 15-2015 |
| ZEA | 14,4 | 3,4 | - | - | 3,2 | 1,1 | 15-2015 |

## 3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ($\sigma_{pt}$) the result ($x_i$) of the participant is deviating from the assigned value ($X_{pt}$) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

The z-score valid for the PT evaluation is designated z-score ($\sigma_{pt}$), while the value of z-score (Info) is for information only. The two z-scores are calculated using the different target standard deviations according to 3.6.

### 3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and if necessary appropriate corrective measures should be applied [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

## 3.8 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ( $\hat{\sigma}$ ) and the standard uncertainty ($Ux_{pt}$) [3].

The calculation is performed by:

$$z_i' = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z 'score, we have defined below the expression in the denominator as a target standard deviation $\sigma_{pt}$'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning- and action-signals see 3.7.1.

## 3.9 Reproducibility coefficient of variation (CV)

The variation coefficient (CV) of the reproducibility (= *relative reproducibility standard deviation)* is calculated from the standard deviation and the mean as follows [4, 13]:

$$CV_R = \frac{S_R * 100}{X}$$

In contrast to the standard deviation as a measure of the absolute variability the CV gives the relative variability within a data region. While a low CV, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV of more than 50% indicates a "strong inhomogeneity of statistical mass", so that the suitability for certain applications such as the assessment of exceeded maximum levels or the performance evaluation of the participating laboratories possibly can not be done [3].

## 3.10 Quotient $S^*/\sigma_{pt}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation $\sigma_{pt}$ does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

## 3.11 Standard uncertainty

The consensus value has a standard uncertainty $U(X_{pt})$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories (P) and perhaps on other factors. The standard uncertainty of the assigned value ($U_{(Xpt)}$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the consensus value needs not to be included in the interpretation of the results of the PT [3]. A clear exceeded the value of 0.3 is an indication that the target standard deviation was possibly set too low for the standard uncertainty of the assigned value.

The quotient $U(X_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

## 4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

In the first table the characteristics are listed:

| **Statistic Data** |
|---|
| *Number of results* |
| *Number of outliers* |
| Mean |
| Median |
| Robust mean($X_{pt}$) |
| Robust standard deviation ($S^*$) |
| *Number with 2 replicates* |
| *repeatability standard deviation ($S_r$)* |
| Repeatability ($Cv_r$) in % |
| *reproducibility standard deviation ($S_R$)* |
| Reproducibility ($CV_R$) in % |
| *Target range:* |
| Target standard deviation $\sigma_{pt}$ or $\sigma_{pt}$' |
| Target standard deviation (for information) |
| lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') * |
| upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$') * |
| *Quotient S*/$\sigma_{pt}$ or S*/$\sigma_{pt}$'* |
| *Standard uncertainty $U(X_{pt})$* |
| *Quotient $U(X_{pt})/\sigma_{pt}$ or $U(X_{pt})/\sigma_{pt}$'* |
| *Results in the target range* |
| *Percent in the target range* |

* Target range is calculated with z-score or z'-score

In the table below, the results of the participating laboratories are formatted in 3 valid digits**:

| Auswerte-nummer<br>Evaluation number | Parameter [Einheit/ Unit] | Abweichung<br>Deviation | Z´-Score $\sigma_{pt}$´ | z-Score (Info) | Hinweis<br>Remark |
|---|---|---|---|---|---|

** *In the documentation part, the results are given as they were transmitted by the participants.*

## 4.1 Deoxynivalenol in µg/kg

**Vergleichsuntersuchung / Proficiency Test**

| **Statistic Data** | |
|---|---|
| *Number of results* | 10 |
| *Number of outliers* | 0 |
| Mean | 757 |
| Median | 745 |
| **Robust Mean (X)** | **773** |
| **Robust standard deviation (S*)** | **147** |
| *Number with 2 replicates* | 10 |
| Repeatability SD ($S_r$) | 28,5 |
| Repeatability ($CV_r$) | 3,76% |
| Reproducibility SD ($S_R$) | 166 |
| Reproducibility ($CV_R$) | 21,9% |
| *Target range:* | |
| **Target standard deviation $\sigma_{pt}$** | **129** |
| Target standard deviation (for Information) | 169 |
| **lower limit of target range** | **516** |
| **upper limit of target range** | **1030** |
| *Quotient S*/$\sigma_{pt}$* | *1,1* |
| *Standard uncertainty $U_{(Xpt)}$* | *58,0* |
| *Quotient $U_{(Xpt)}/\sigma_{pt}$* | *0,45* |
| *Results in the target range* | *9* |
| *Percent in the target range* | *90,0%* |

*Comments:*

The standard target deviation was evaluated using the model of Horwitz. The distribution of results showed an increased variability. The target standard deviation "for information" was calculated from values by perception (ASU §64 L 15.00-9)[18], see 3.6.2.

The distribution of the results showed an acceptable variability. The quotient $S^*/\sigma_{pt}$ was 1,1. The robust standard deviation is comparable to those of prior PTs (see 3.6.3). The comparability of results is given.

Repeatability- and reproducibility standard deviation are in the range of established values for the methods used (see 3.6.2).

The quotient $U(X_{pt})/\sigma_p$ (0,45) is increased, but is acceptable on the basis of the other characteristics and the use of different methods.

90,0% of the results were in the target area.

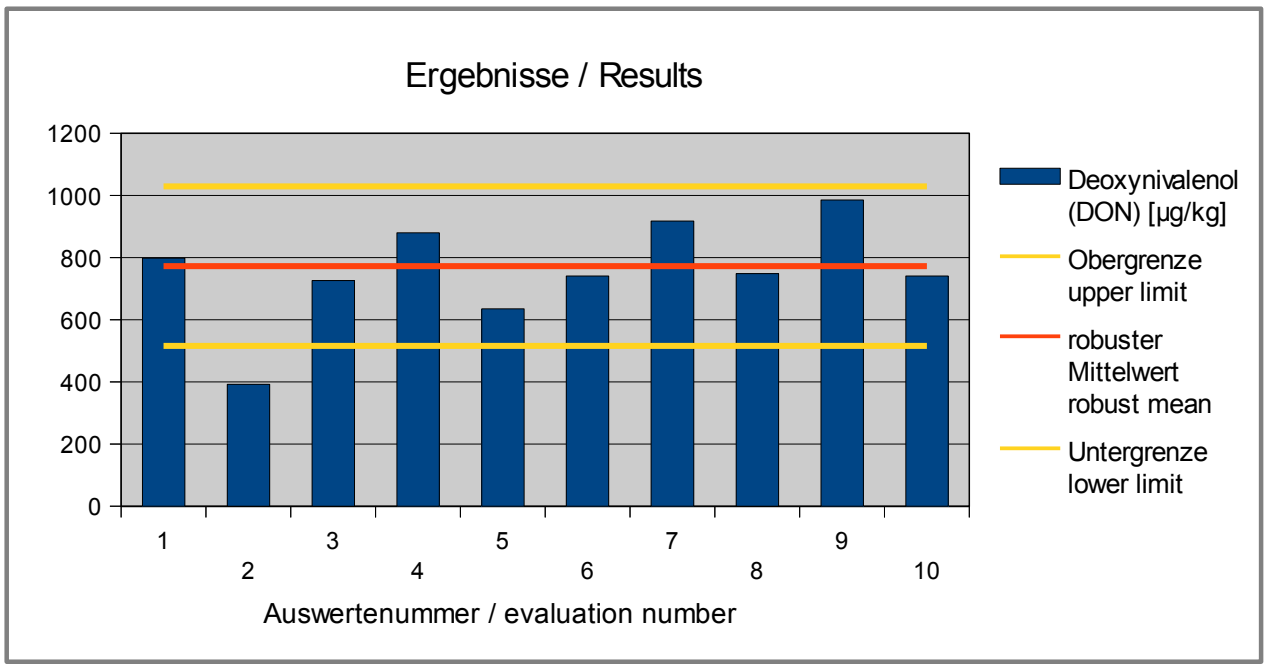


**Abb. / Fig. 1:** Ergebnisse/ Results Deoxynivalenol

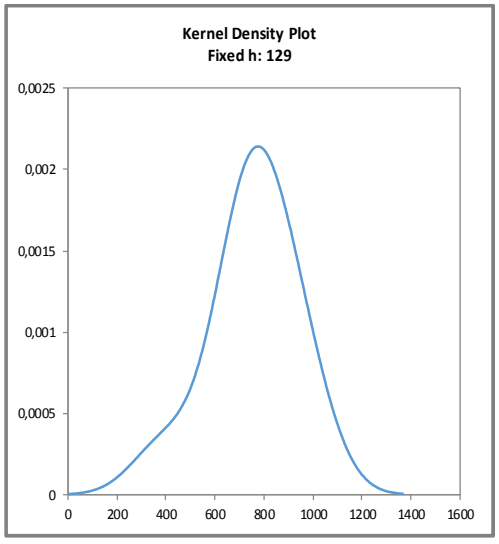


**Abb. / Fig. 2:**
Kerndichte-Schätzung der Ergebnisse (mit h = $\sigma_{pt}$ von Xpt (129 µg/kg)

Kernel density plot of results with h = $\sigma_{pt}$ *of* $X_{pt}$ (129 µg/kg)

*Comment:*
The kernel density shows a symmetrical distribution of results with a slight side peak at 400 µg/kg, due to the result outside the target range.

**Ergebnisse der teilnehmenden Institute:**
**Results of Participants:**

| Auswerte-nummer<br>Evaluation number | Deoxynivalenol (DON) [µg/kg] | Abweichung [µg/kg]<br>Deviation [µg/kg] | z-Score ($\sigma_{pt}$) | z-Score (Info) | Hinweis<br>Remark |
|---|---|---|---|---|---|
| 1 | 798 | 25,4 | 0,20 | 0,15 | |
| 2 | 392 | -380 | -3,0 | -2,2 | |
| 3 | 726 | -46,6 | -0,36 | -0,28 | |
| 4 | 880 | 107 | 0,84 | 0,64 | |
| 5 | 636 | -137 | -1,1 | -0,81 | |
| 6 | 741 | -31,6 | -0,25 | -0,19 | |
| 7 | 918 | 145 | 1,1 | 0,86 | |
| 8 | 749 | -23,8 | -0,19 | -0,14 | |
| 9 | 986 | 213 | 1,7 | 1,3 | |
| 10 | 741 | -32,1 | -0,25 | -0,19 | |

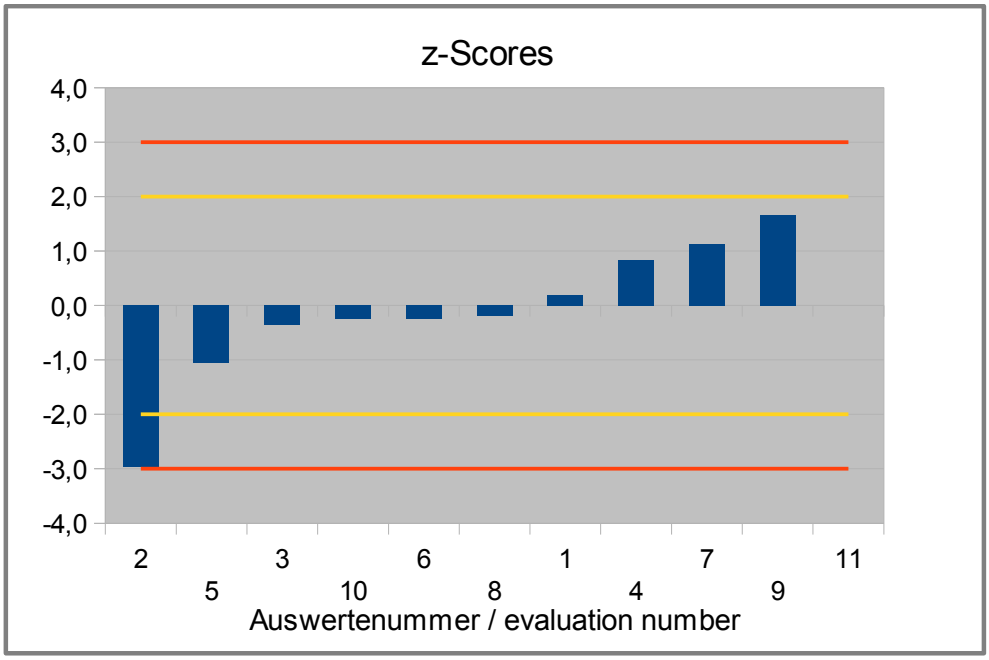


Abb. / Fig. 3: Z-Scores Deoxynivalenol

## 4.2 Zearalenone in µg/kg

**Vergleichsuntersuchung / Proficiency Test**

| **Kenndaten** | |
|---|---|
| *Anzahl der Messergebnisse* | 6 |
| *Anzahl der Ausreißer* | |
| Mittelwert | 84,5 |
| Median | 36,9 |
| **Robuster Mittelwert ($X_{pt}$)** | **44,4** |
| **Robuste Standardabweichung (S*)** | **17,4** |
| *Anzahl mit 2 Wiederholmessungen* | 5 |
| Wiederholstandardabweichung ($S_r$) | 5,52 |
| Variationskoeffizient ($VK_r$) | 14,1% |
| Vergleichsstandardabweichung ($S_R$) | 10,4 |
| Variationskoeffizient ($VK_R$) | 26,5% |
| *Zielkenndaten:* | |
| **Zielstandardabweichung $\sigma_{pt}$** | **9,78** |
| Zielstandardabweichung (zur Information) | 6,73 |
| **Untere Grenze des Zielbereichs** | **24,9** |
| **Obere Grenze des Zielbereichs** | **64,0** |
| *Quotient S*/$\sigma_{pt}$* | *1,8* |
| *Standardunsicherheit $U_{(Xpt)}$* | *8,87* |
| *Quotient $U_{(Xpt)}/\sigma_{pt}$* | *0,91* |
| *Ergebnisse im Zielbereich* | *5* |
| *Prozent im Zielbereich* | *83,3%* |

*Comments:*

Due to the relatively low variability of the results, a statistical evaluation was carried out despite <7 results (see also under 3.6).

The standard target deviation was evaluated using the model of Horwitz/ Thompson. The target standard deviation "for information" was calculated from values by perception [19/22].

The distribution of the results showed a normal variability. The quotient $S^*/\sigma_{pt}$ was 1,8. The robust standard deviation is comparable to those of prior PT´s (see 3.6.3). The comparability of results is given.

Repeatability- and reproducibility standard deviation are in the range of established values for the methods used (see 3.6.2).

The quotient $U(X_{pt})/\sigma_p$ (0,91) is increased, but is acceptable on the basis of the other characteristics and the use of different methods.

83,3% of the results were in the target area.

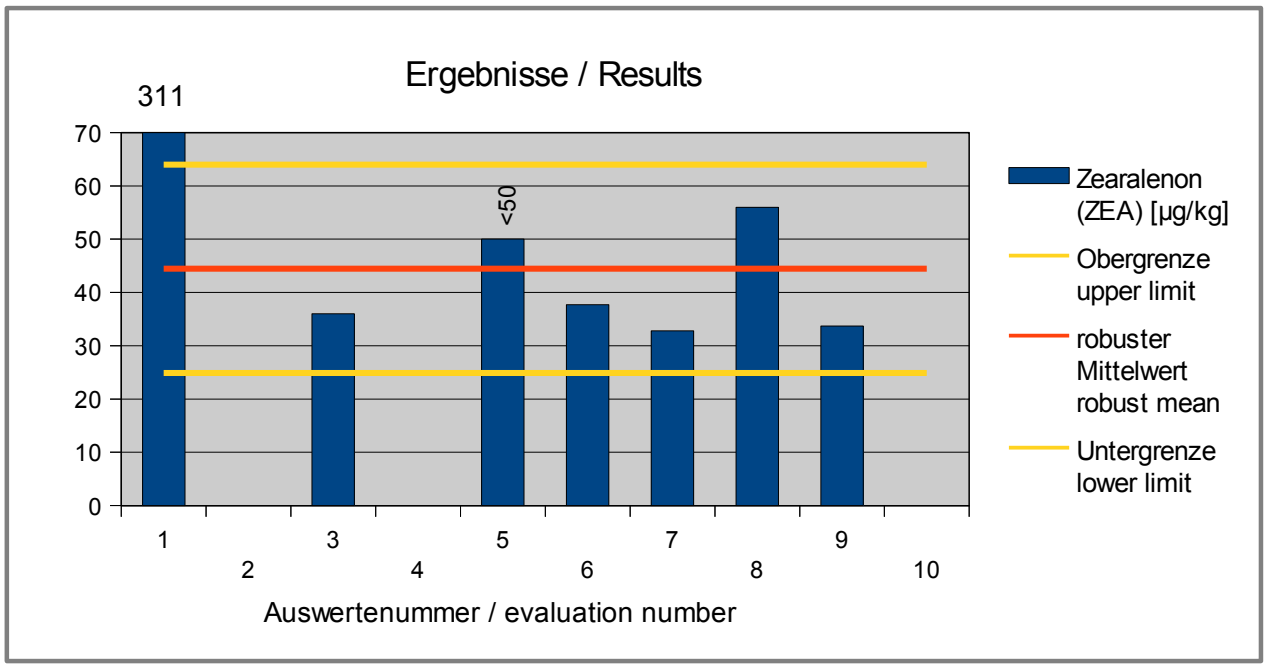


**Abb. / Fig. 4:** Ergebnisse/ Results Zearalenone

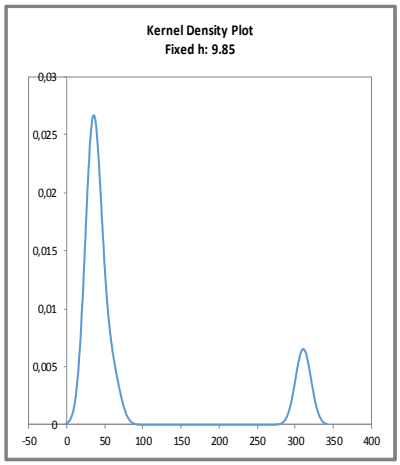


**Abb. / Fig. 5:**
Kerndichte-Schätzung der Ergebnisse (mit h = $\sigma_{pt}$ von Xpt (9,8 µg/kg)

Kernel density plot of results with h = $\sigma_{pt}$ of $X_{pt}$ (9,8 µg/kg)

*Comment:*
The kernel density shows a symmetrical distribution of results with a side peak at 300 µg/kg, due to the result outside the target range.

**Ergebnisse der Teilnehmer:**
**Results of Participants:**

| Auswerte-nummer<br>Evaluation number | Zearalenon (ZEA) [µg/kg] | Abweichung [µg/kg]<br>Deviation [µg/kg] | z-Score ($\sigma_{pt}$) | z-Score (Info) | Hinweis<br>Remark |
|---|---|---|---|---|---|
| 1 | 311 | 267 | 27 | 40 | |
| 2 | | | | | |
| 3 | 36,0 | -8,45 | -0,86 | -1,3 | |
| 4 | | | | | |
| 5 | < 50 | | | | Limit of detection in target range |
| 6 | 37,7 | -6,75 | -0,69 | -1,0 | |
| 7 | 32,8 | -11,7 | -1,2 | -1,7 | |
| 8 | 56,0 | 11,5 | 1,2 | 1,7 | |
| 9 | 33,7 | -10,8 | -1,1 | -1,6 | |
| 10 | | | | | |

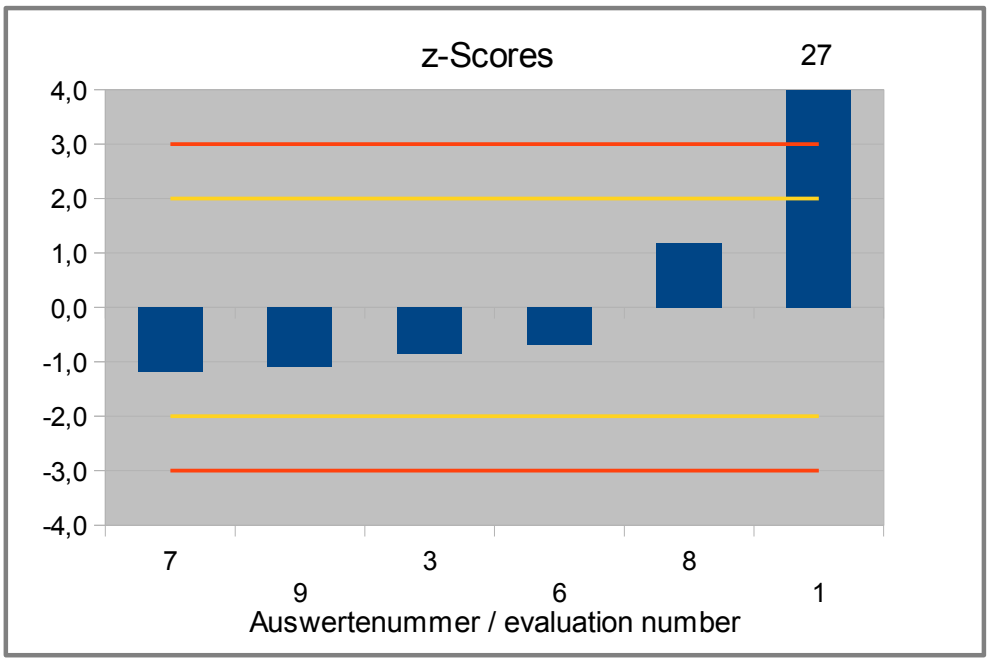


**Abb. / Fig. 6:** Z-Scores Zearalenone

# 5. Documentation

## 5.1 Details by participants

### 5.1.1 Primary data

Note: Information given in German was translated by DLA to the best of our knowledge (without guarantee of correctness).

#### 5.1.1.1 Deoxynivalenol

| Teilnehmer | Probe I DLA Nr. | Probe II DLA Nr. | Datum der Analyse | Ergebnis | Ergebnis Probe I | Ergebnis Probe II | Bestimmungs-grenze | Angabe inkl. Wiederfindung | Wiederfin-dungsrate |
|---|---|---|---|---|---|---|---|---|---|
| Participant | Sample I DLA-No | Sample II DLA-No | Date of analysis | Result | Result Sample I | Result Sample II | Limit of determina-tion | Recovery included | Recovery Rate |
| | | | day/month | µg/kg | µg/kg | µg/kg | µg/kg | yes/ no | in % |
| 1 | 24 | 32 | 03.01.19 | 798 | 795 | 801 | 50 | no | |
| 2 | 1 | 55 | 18.12.18 | 392,3 | 378,1 | 406,5 | 25 | no | / |
| 3 | | | 14.12.18 | 726 | 722 | 730 | 15 | yes | 100 |
| 4 | 27 | 29 | 03.12.18 | 880 | 890 | 870 | 600 | yes | 103 |
| 5 | 15 | 41 | 17.12.18 | 635,705 | 648,7 | 622,71 | 222 | no | |
| 6 | 23 | 33 | 17.12. | 741 | 750 | 731 | 50 | yes | 85,7 |
| 7 | 9 | 47 | 11.12.2018 | 917,8 | 933,4 | 902,3 | 100 | yes | 24,5 |
| 8 | 7 | 49 | 28.11.18 | 748,82 | 730,84 | 766,8 | | no | |
| 9 | 12 | 44 | 28.11. | 985,6 | 952,4 | 1018,7 | 100.0 | yes | 95.0 |
| 10 | 19 | 37 | 11.12.18 | 740,5 | 783 | 698 | 101,6 | yes | 102,5 |

#### 5.1.1.2 Zearalenone

| Teilnehmer | Probe I DLA Nr. | Probe II DLA Nr. | Datum der Analyse | Ergebnis | Ergebnis Probe I | Ergebnis Probe II | Bestimmungs-grenze | Angabe inkl. Wiederfindung | Wiederfin-dungsrate |
|---|---|---|---|---|---|---|---|---|---|
| Participant | Sample I DLA-No | Sample II DLA-No | Date of analysis | Result | Result Sample I | Result Sample II | Limit of determina-tion | Recovery included | Recovery Rate |
| | | | day/ month | µg/kg | µg/kg | µg/kg | µg/kg | yes/ no | in % |
| 1 | 24 | 32 | 20.12.18 | 311 | 308 | 313 | 50 | no | |
| 2 | | | | | | | | | |
| 3 | 39 | 32 | 14.12.18 | 36 | 39 | 32 | 3 | yes | 100 |
| 4 | | | | | | | | | |
| 5 | 15 | 41 | 18.12.18 | <50 | <50 | <50 | <50 | no | |
| 6 | 23 | 33 | 04.12. | 37,7 | 37,9 | 37,2 | 5 | yes | 94,1 |
| 7 | 9 | 47 | 11.12.18 | 32,8 | 37,5 | 28,2 | 15 | yes | 39 |
| 8 | 7 | 49 | 18.12.18 | 55,99 | 49,6 | 62,38 | | no | |
| 9 | 12 | 44 | 10.12. | 33.7 | 34.8 | 32.5 | 7.0 | yes | 101.8 |
| 10 | | | | | | | | | |

## 5.1.2 Analytical methods

### 5.1.2.1 Deoxynivalenol

| Teilnehmer | Methodenangabe | Hinweise zu Probenvorbereitung und -aufarbeitung | Hinweise zur Messmethode | Kalibrierung und Referenzmaterial | Wiederfindung wurde mit gleicher Matrix bestimmt | Methode akkreditiert nach ISO/IEC 17025 | Sonstige Hinweise |
|---|---|---|---|---|---|---|---|
| **Particiipant** | **Method description** | **Notes to sample preparation** | **Notes to analytical method** | **Calibration and reference material** | **Recovery** with same matrix | **Method** accredited ISO/IEC 17025 | **Further Remarks** |
| | | | | | yes/ no | yes/ no | |
| 1 | LC-MS/MS | | | | yes | no | |
| 2 | Competitive direct enzyme-linked immunosorbent assay (CD-ELISA) - Veratox DON HS Quantitative test | solid/liquid extraction | / | / | no | no | / |
| 3 | PA_A-403 | | LC-MS/MS | KG + IS | no | yes | |
| 4 | ELISA method | | fast DON of r-boppharm | Bonner Enquete 2014 | yes | yes | |
| 5 | r-biopharm Ridascreen FastDON (Art. no. R5901) | | | | | no | |
| 6 | §64 LFGB F0034 | Immuno columns | HPLC-UV | LGC B-MYC 0320 DLA22-2017 | yes | yes | |
| 7 | Rosa Fast5 DON Quantitative Test for Feed and Grain | 10 g / 50 mL water | | positive control 1000 ppb | yes | yes | |
| 8 | ASU L 15.00-9:2014-02 | | | | | yes | |
| 9 | ASU §64 LFGB L15.00-9 | Extraction with Water - Purification with Immunoaffinity Columns | HPLC/DAD | ext. Standard | yes (rye flour) | yes | |
| 10 | | | | Biopure | yes | yes | |

### 5.1.2.2 Zearalenone

| Teilnehmer | Methodenangabe | Hinweise zu Probenvorbereitung und -aufarbeitung | Hinweise zur Messmethode | Kalibrierung und Referenzmaterial | Wiederfindung wurde mit gleicher Matrix bestimmt | Methode akkreditiert nach ISO/IEC 17025 | Sonstige Hinweise |
|---|---|---|---|---|---|---|---|
| **Participant** | **Method description** | **Notes to sample preparation** | **Notes to analytical method** | **Calibration and reference material** | **Recovery** with same matrix | **Method** accredited ISO/IEC 17025 | **Further Remarks** |
| | | | | | yes/ no | yes/ no | |
| 1 | HPLC-Rf | | | | yes | no | |
| 2 | | | | | | | |
| 3 | PA_A-403 | | LC-MS/MS | KG + IS | no | yes | |
| 4 | | | | | | | |
| 5 | r-biopharm Ridascreen FastZEA (Art. no. R5502) | | | | | no | |
| 6 | VDLUFA Bd III 19.9.2 | Immuno columns | LC-MS/MS | Biopure S02029 DLA22-2017 | yes | yes | |
| 7 | Rosa Fast5 ZEAR Quantitative Test for Feed and Grain | 10 g / 20 mL 70% methanol | | positive control 150-300 ppb | yes | yes | |
| 8 | ASU L 15.01/02-2:2006-12 | | | | | yes | |
| 9 | ASU §64 LFGB L48.02-3 | Extraction with ACN/water (75/25) - Purification with immunoaffinity columns | HPLC/FLD | ext. Standard | yes (corn flour) | yes | |
| 10 | | | | | | | |

## 5.2 Homogeneity

### 5.2.1 Homogeneity testing before PT

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis.**

**DLA 22-2018**

| | | |
|---|---|---|
| Weight whole sample | 3,005 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 17,8 | mg/kg |

**Results of analyses**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 10,07 | 69 | 13,7 |
| 2 | 10,02 | 73 | 14,6 |
| 3 | 10,02 | 78 | 15,6 |
| 4 | 9,97 | 74 | 14,8 |
| 5 | 10,01 | 63 | 12,6 |
| 6 | 10,01 | 88 | 17,6 |
| 7 | 10,08 | 81 | 16,1 |
| 8 | 10,11 | 89 | 17,6 |
| 9 | 10,00 | 82 | 16,4 |
| 10 | 9,98 | 90 | 18,0 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 10 | |
| Degree of freedom | 9 | |
| Mean | 78,7 | Particle |
| Standard deviation | 8,99 | Particle |
| $\chi^2$ (CHI-Quadrat) | 9,25 | |
| **Probbility** | **41** | % |
| Recovery rate | 88 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 10 | |
| Mean | 15,7 | mg/kg |
| Standard deviation | 1,79 | mg/kg |
| rel. Standard deviation | 11,4 | % |
| Horwitz Standard deviation | 10,6 | % |
| **HorRat value** | **1,1** | |
| Recovery rate | 88 | % |

## *5.2 Sample cover letter: Information on the Proficiency Test (PT)*

Before the PT, the participants are given the following information in the sample cover letter:

### Information on the Proficiency Test (PT)

| | |
|---|---|
| *PT number* | ***DLA 22-2018*** |
| *PT name* | ***DON + Zearalenon in Cereals*** |
| *Sample matrix** | *Samples I + II:* ***Cornmeal*** |
| *Number of samples and sample amount* | *2 identical samples I + II, 50 g each.* |
| *Storage* | *Samples I + II: cooled 2 - 10°C* |
| *Intentional use* | *Laboratory use only (quality control samples)* |
| *Parameter* | *quantitative:* ***Deoxynivalenol*** *(DON),* ***Zearalenone*** *(ZEA)* |
| *Methods of analysis* | *Analytical methods are optional* |
| *Notes to analysis* | *The analysis of PT samples should be performed like a routine laboratory analysis.*<br>*In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.* |
| *Result sheet* | *The results for sample I and II as well as the final results calculated as mean of the double determination (samples I and II) should be filled in the result submission file. The recovery rates, if carried out, has to be included in the calculation.* |
| *Units* | *µg/kg* |
| *Number of significant digits* | *at least 2* |
| *Further information* | *For information please specify:*<br>– *Date of analysis*<br>– *DLA-sample-numbers (for sample I and II)*<br>– *Limit of detection*<br>– *Assignment incl. Recovery*<br>– *Recovery with the same matrix*<br>– *Method is accredited* |
| *Result submission* | *The result submission file should be sent by e-mail to:*<br>***pt@dla-lvu.de*** |
| *Deadline* | ***the latest 04th January 2019*** |
| *Evaluation report* | *The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.* |
| *Coordinator and contact person of PT* | *Dr. Gerhard Wichmann* |

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

## 6. Index of participant laboratories

| Teilnehmer/ Participant | Ort/ Town | Land/ Country |
|---|---|---|
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Croatia |
| | | Croatia |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |

*[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]*

*[The address data of the participants were deleted for publication of the evaluation report.]*

## 7. Index of literature


1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with microtracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.

14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.

15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH

16. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln

17. EU-VO 519/2014 zur Änderung der Verordnung (EG) Nr. 401/2006 hinsichtlich der Probenahmeverfahren für große Partien, Gewürze und Nahrungsergänzungsmittel, der Leistungskriterien für die Bestimmung von T-2-Toxin, HT-2-Toxin und Citrinin sowie der Screening-Methoden für die Analyse (v. 16. Mai 2014)

18. ASU §64 LFGB L 15.00-9 (entspricht DIN EN 15891/2010): Bestimmung von Deoxynivalenol in Getreide, Getreideerzeugnissen und Säuglings- und Kleinkindernahrung auf Getreidebasis; HPLC-Verfahren (Februar 2014)

19. ASU §64 LFGB L 15.01/02-2: Bestimmung von Zearalenon in Weizen und Roggen (Dezember 2006)

20. ASU §64 LFGB L 16.01-8: Bestimmung von Zearalenon in Gerstenmehl, Maismehl und Weizenmehl (Januar 2011)

21. ASU §64 LFGB L 16.02-1: Bestimmung von Zearalenon in Maisgrieß (Januar 2011)

22. ASU §64 LFGB L 48.02-3: Bestimmung von Zearalenon in Säuglings- und Kleinkindernahrung (Januar 2011)

23. EU VO 1881/2006 zur Festsetzung der Höchstgehalte für bestimmte Kontaminanten in Lebensmitteln/ setting maximum levels for certain contaminants in foodstuffs (16.12.2006)