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# Report on the 2008 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, regarding the Determination of Deoxynivalenol in a Cereal Product and a Test Solution

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**Summary**

A proficiency test was conducted by the Community Reference Laboratory for Mycotoxins with 33 European National Reference Laboratories (NRLs) for Mycotoxins and 2 Laboratory from candidate countries, thus a total of 35 participants. Test materials were a deoxynivalenol (DON) solution in acetonitrile and three cereal test materials. Laboratories determined the DON content by either enzyme linked immuno sorbent assay (ELISA), gas chromatography (GC) or reverse-phase high-performance liquid-chromatography (RP-HPLC). One NRL did not report any results.

Applying the Horwitz equation as a basis for the target standard deviation (19% in the case of this proficiency test), 27 out of the remaining 34 laboratories reported values within the z-score limit of 2 after recovery correction of the result for the DON-positive sample. Twenty-five laboratories reported results within a z-score limit of 1. Thus, 79 % of the participating laboratories performed satisfactorily in the proficiency test. No z-scores were calculated for the blank material.

## Introduction

In 2006 the Institute for Reference Materials and Measurements (IRMM) in Geel was designated as Community Reference Laboratory (CRL) for Mycotoxins by the Directorate General for Health and Consumers (DG SANCO). One of the main responsibilities of the CRL is to organise comparative testing to benchmark and harmonise the measurement capabilities of National Reference Laboratories (NRLs) working in the same field. Therefore, the CRL for Mycotoxins together with the network of NRLs agreed to conduct the proficiency test (PT) in 2008 (PT2008) as follow up action to the PT it organised in 2006 and 2007. The topic of the PT2008 was the determination of deoxynivalenol (DON) in cereals.
Test materials in this study were maize based cereal flours, either free or naturally contaminated with DON. The batch was tested for homogeneity using an ANOVA based experimental design and found sufficiently homogeneous. The stability of the test material was not tested explicitly, as the material was intended to be used shortly after preparation in the PT and previous studies on the fate of DON in dry cereal based products did not indicate any reason for the need of short term stability testing.

## Methodology

Each participant received one ampoule containing the DON test solution and three cereal materials;

- 2 coded test materials with a level of DON unknown to the participants of which one contained DON at a level less than 20 ng/g and was considered as blank in the following
- 1 material marked as "blank" for recovery experiment (<20 ng/g DON).
- 1 ampoule of a test solution of "DON in acetonitrile" with a concentration of DON unknown to the participants.

Participants were asked to measure the two coded cereal test materials and the test solution for DON, and to spike the blank cereal material with their own calibrant, reporting the spiking level and amount found to obtain recovery information. The detailed instructions as sent to the participants are included in the annex.
All graphs were made with SigmaPlot 9.01. Results were gathered via electronic forms using Adobe Life-cycler. Z-scores were calculated using Microsoft Excel®.
As basis for the z-scoring, the predicted Horwitz standard deviation was used, which was in this case 19% (z-score = 1)

**Results and Discussion:**

*Assignment of consensus values*

The DON test solution was produced from a Certified Reference Material (CRM) calibrant solution by dilution (IRMM-315). The assigned value of the prepared test solution (the coverage factor k=2 corresponding to a level of confidence of about 95 %) was 0.304 ± 0.015 µg/mL. A full report on the production and certification of the initial CRM calibrant, which was used to prepare the calibrant solution, is available from the IRMM[1].
For the DON positive test material, which was naturally contaminated, the median of the values submitted by the laboratories was chosen as the assigned value. This is a rather straight forward and easy method and does not significantly differ from results calculated by other methods, such as robust means (see Table 1). Prior computation extreme values have been taken out (Lab-ID 133).

**Table 1**: Calculation of assigned values[2]:

| Parameter | Uncorrected result [µg/kg] | Recovery corrected result [µg/kg] |
|---|---|---|
| Median | 283 | 304 |
| A15 mean | 287 | 306 |
| H15 mean | 290 | 307 |

For each tested material the individual z-scores (cereal material) respectively the %-deviation from the assigned value (test solutions) are listed together with all single results submitted by the participants in **Table 2 - 3** in the annex.

**Figure 1** depicts the ranking of the results of the participating laboratories for DON in the cereal product (containing DON) prior recovery correction and prior calibrant correction, in ascending order. The assigned value is depicted by a black line. The limit for a z-score of z = |2| is indicated by red lines. For 26 of the laboratories the reported value fell within the z-score limit of 2. Eight laboratories reported values outside this limit. No further evaluation was made on this result, as only the recovery corrected result is of relevance for evaluation. The assigned value was calculated out of the median of all submitted results with the exception of the result from participant ID 133.

*Correction for recovery*

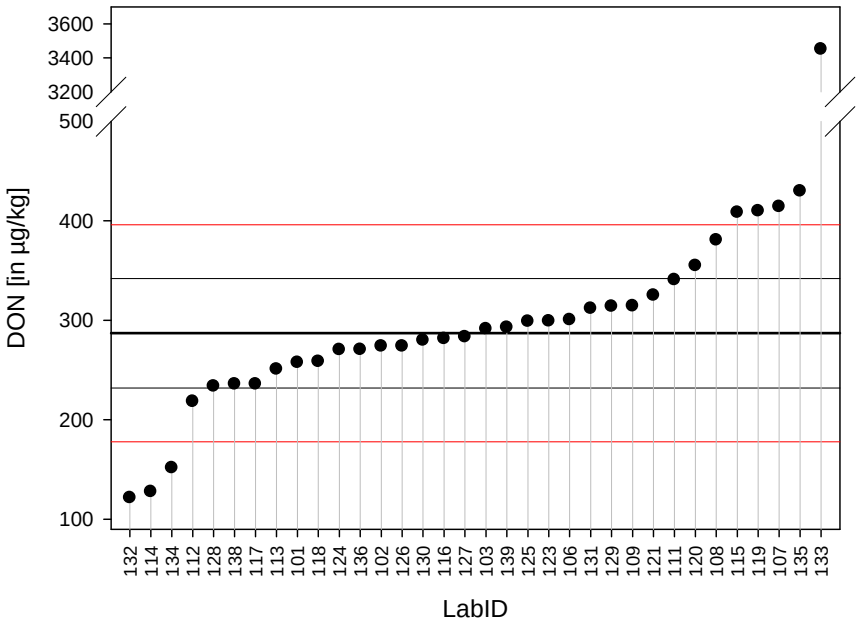
The effect of recovery correction on the results can be seen in **Figure 2**. As a result of this, 27 of the laboratories had values within the z-score limit of 2. Seven laboratories reported values outside this limit. Consequently, no improvement of the overall population performance based on the z =|2| limit can be observed by taking into account recovery. However, on a z =|1| limit an improvement can be observed as 22 laboratories fell within that limit prior recovery correction, whereas 25 did after correction. The assigned value was calculated out of the median of all submitted results (after recovery correction) with the exception of the result from participant ID 133.

[1] https://irmm.jrc.ec.europa.eu/rmcatalogue/detailsrmcatalogue.do?referenceMaterial=I-0315%2B%2B%2B%2B%2B%2B
[2] A15 and H15 means have been calculated according to "Analytical Methods Committee, Analyst, 1989, 114, 1489", using an excel sheet available form the Royal Chemists Society. See here also for more details on these parameters.

*Measurement of the test solution*

The dispersion of results concerning the test solution is shown in **Figure 3**. As there is little information or agreement how to set a target standard deviation for the measurement of pure calibrants, no such figure was used for calculating z-scores. However, it can be assumed that the simple measurement of a test solution should give a smaller dispersion of results than the results for the test material that require a more complex analytical treatment for generation. Due to lack of any agreed procedure, results are calculated in **Table 3** as deviation from the assigned value. Under the above mentioned assumption that this type of measurement is simpler and thus much less influenced by analytical procedures it can serve as a good indicator for the quality of the laboratory calibrant used in this PT.
An important aspect to consider is that there are only limited possibilities for routine laboratories to confirm the concentration and purity of DON calibrants (unlike for aflatoxins[3]). Thus, laboratories largely rely on the manufacturers' statements and, once prepared, on the stability of the solutions in their laboratory. Participants that reported results far outside the uncertainty range of the test solution, shall investigate the exact source for the deviation.

**Figure 1:** Plot of DON results in the naturally contaminated cereal product prior to recovery correction. The bold black line reflects the assigned value, red lines the z-score limit of z=|2|.




[3] See previous NRL PT reports from 2006 and 2007

**Figure 2:** Plot of DON results in the naturally contaminated cereal product after recovery correction. The bold black line reflects the assigned value, red lines the z-score limit of z=|2|.

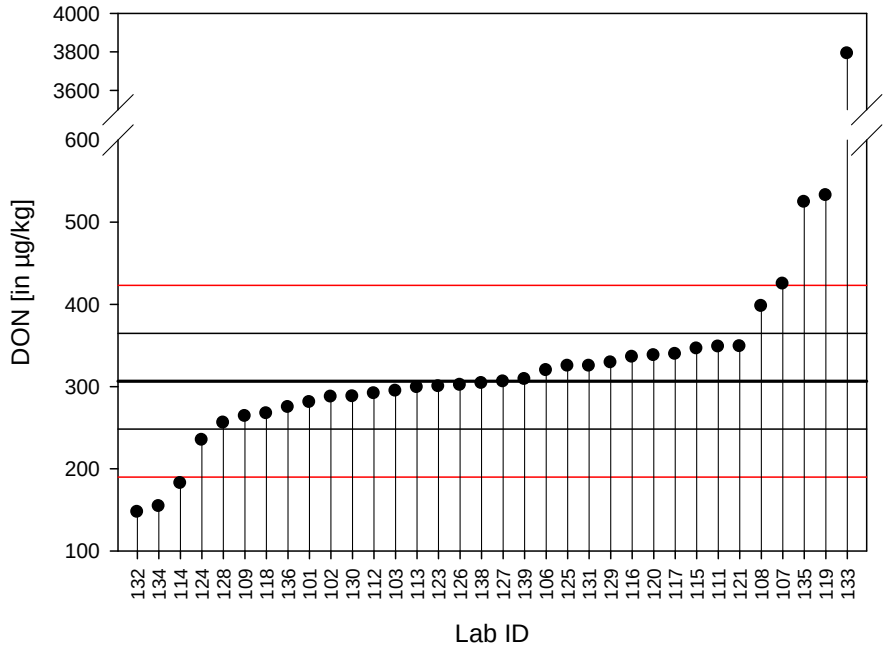
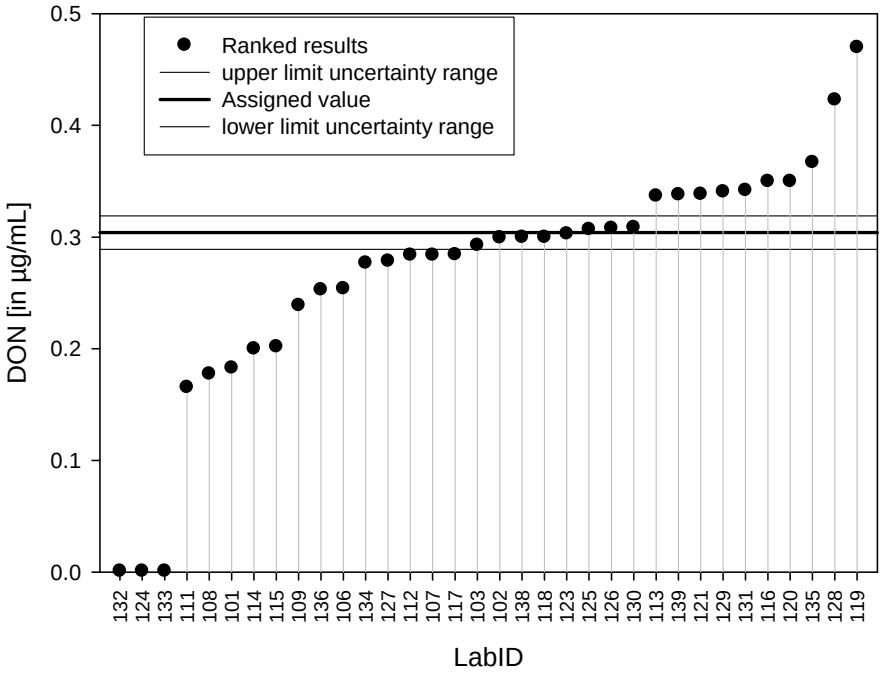


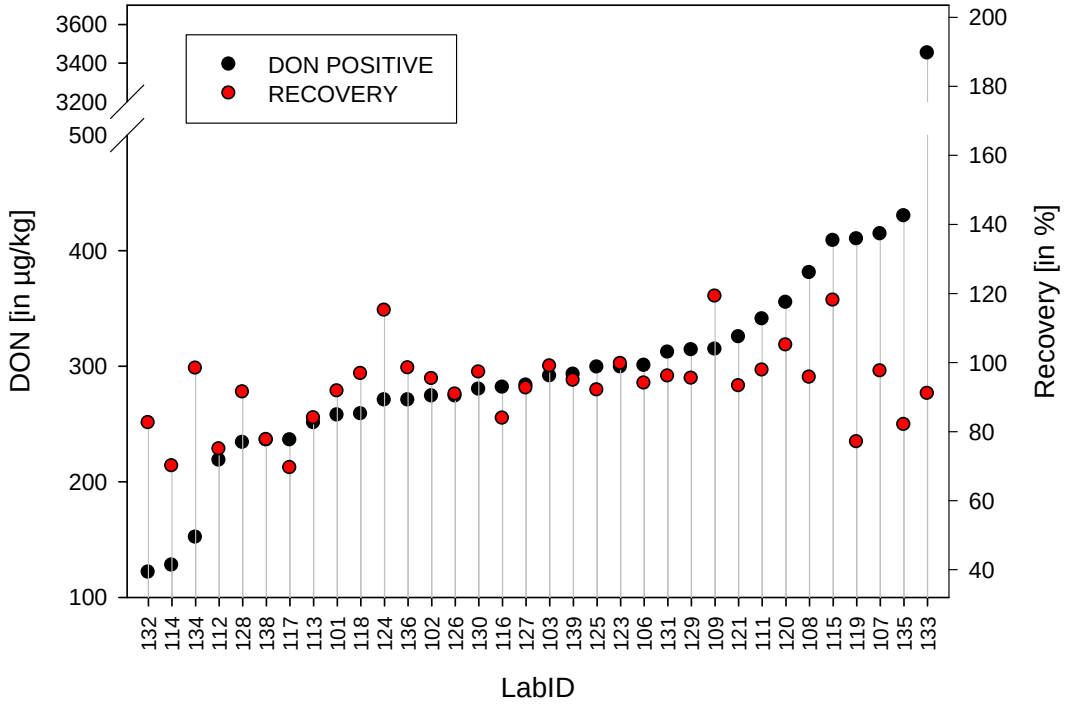

**Figure 3:** Plot of DON results in the test solution. The bold black line reflects the assigned value, the thin black lines the uncertainty range of the concentration of the solution.



*Correlation between recovery and uncorrected value*

One parameter to examine proper recovery estimation is to correlate the recovery with the recovery-uncorrected result for the cereal test material. This correlation can be regarded as an indicator whether the reported recovery values have been correctly estimated under the test conditions applied by the participants in this PT. Both scales (left legend = uncorrected result for the test material; right legend = reported recovery) are scaled so that 100% recovery matches with the assigned value from the recovery corrected result (**Figure 4**).
If the reported recovery rate and the recovery-uncorrected result are displayed in close proximity to each other in Figure 4 (red and black points), the correction for recovery of the result of the test material has a positive effect on the z-score as the correction improves the result. In case that recovery values were incorrectly estimated the scenario inverses resulting in enlarged z-scores after recovery correction. As an example, results improve for Lab-ID 112 after recovery correction compared with the recovery uncorrected result (**Figure 1 → Figure 2**) due to the well corresponding values for both results. This is the opposite for Lab-ID 124, where a previously good z-score got poorer after recovery correction.
This highlights the importance of correctly estimating recovery values and in addition might set the basis for the discussion how recovery values should be generated to improve analytical results. One aspect that becomes apparent is that in general the dispersion of recovery values is less than the one for the unknown sample. This could indicate that recovery estimates are much more optimistic (with regards to a target value of 100%) than they are in reality, assuming that a correct correction for recovery should lead harmonised values. One explanation for this might be that for "blind" measurements no target value is known and thus this value is subject to acceptance "as is". For recovery values a target value exists (usually 100%) and any large deviation (even if reflecting the correct performance of the method at that particular moment) might be subject to doubt. As a result repetition and/or correction is made until a sufficient closeness to the expected target value is achieved.

**Figure 4**: Correlation between recovery rate and recovery-uncorrected value for the DON positive sample. Black points (●) display the amount reported for the uncorrected DON positive material (left ordinate) and red points (●) the reported recoveries. Both ordinates are scaled to match the recovery corrected consensus value with a 100 % recovery.



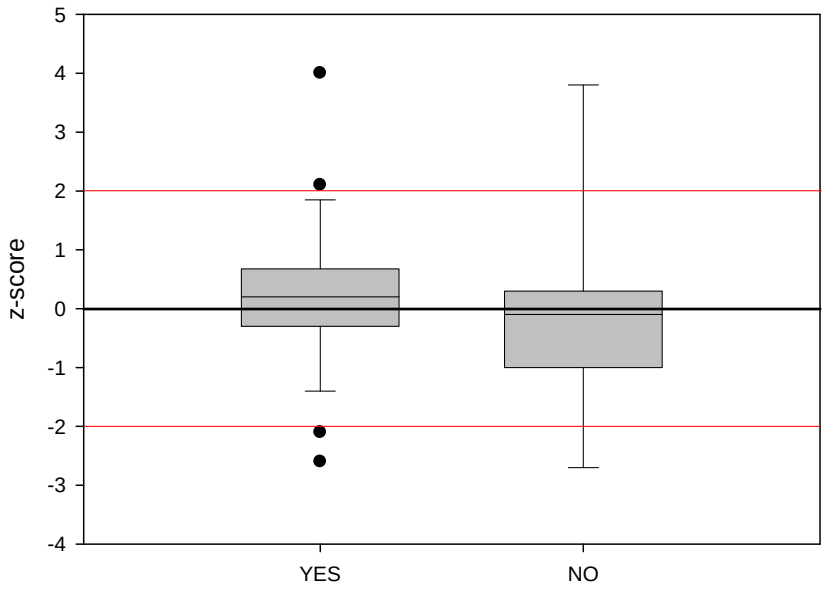

*Evaluation of the comments in the questionnaire*

With few exceptions the pool of participants had more than two years of experience in the analysis of DON. Concerning the procedures applied the following comments should be noticed. In some cases participants reported that the analysis was carried out within two consecutive days which means that the analysis was interrupted (over night) at various stages (LabID 111, 118, 124, 128, 138 and 139). No effect, such as general under or overestimation, of the result could however be attributed to such an interruption. This indicates that clean-up and chromatography can be performed at different days, without negatively affecting the results. Even a storage of the extracts (MeCN-water) for a 6 weeks period resulted in acceptable results (LabID 124).
Most laboratories used peak area for signal reporting and valley-to-valley integration settings in combination with visual inspection to verify correctness of integration. Most integration settings can be regarded as robust, as only in a few cases chromatograms needed to be manually re-integrated. The reported number of re-integrated peaks was around 1 - 4 for the whole set of analyses; in once case up to 20. As no further information was given on these circumstances, the need for visual inspection can only be a recommendation. In the case of 20 re-integrations (LabID 115) this figure demonstrates the minimum amount of chromatograms generated for this PT. As long as these re-integrated chromatograms refer to the overall measurements (including calibration) this is acceptable from the point of the amount of work that was dedicated to the analysis of the PT materials.
Most participants found the instructions and the e-Form for reporting adequate, however a few reported problems or mentioned short-comings, which were however not always qualitatively described. All comments will be taken into consideration for design of documents/and procedures in the next PT.

Laboratories were asked to report if they are accredited according to ISO 17025 for this type of analysis and both pools (accredited and not accredited) were compared in a box plot (**Figure 5**). Independently of the status of accreditation, the two pools performed similarly, not allowing for any

conclusion on the possible correlation between the status of accreditation and the quality of the reported result.
One important comment was to allow the participants more freedom in the way of producing recovery values.

**Figure 5**: Comparison of status of accreditation for the tested parameter

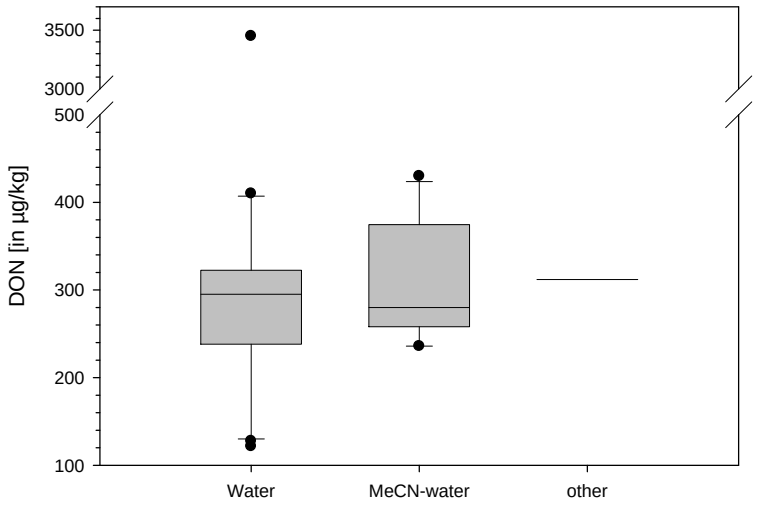



The left box-plot shows the dispersion of results for accredited laboratories (YES) and the right box-plot that of those not accredited for DON. Red horizontal lines indicate the z-score limit of 2. Result of LabID 133 is not displayed.

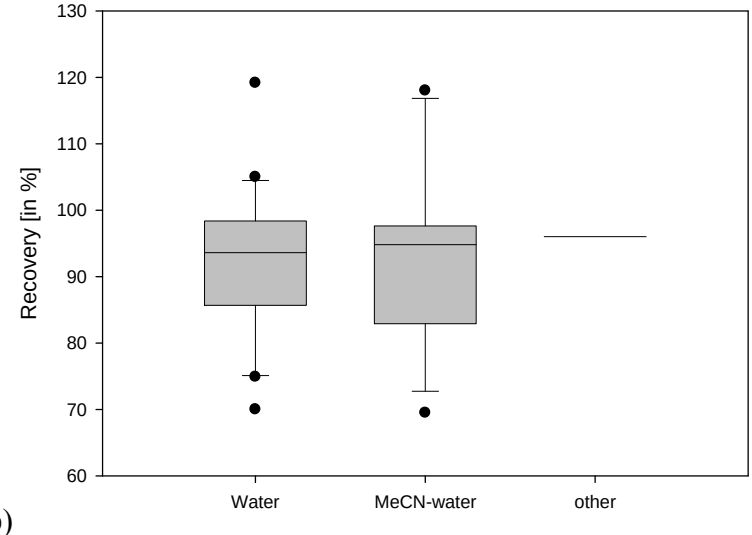
*Comparison of methodological parameters*

In addition to the information on the estimation of the performance of a participant, a PT can under certain circumstance offer additional information on methodological parameters such as extraction, clean-up, etc. In the following, different parameters are grouped and compared. The comparisons are visualised with Box and Whisker plots. As an example **Figure 6** shows the comparison of the main two different extraction solvents that are commonly used for the analysis of DON. Apparently water, as an extraction solvent is comparable to MeCN-water mixtures.

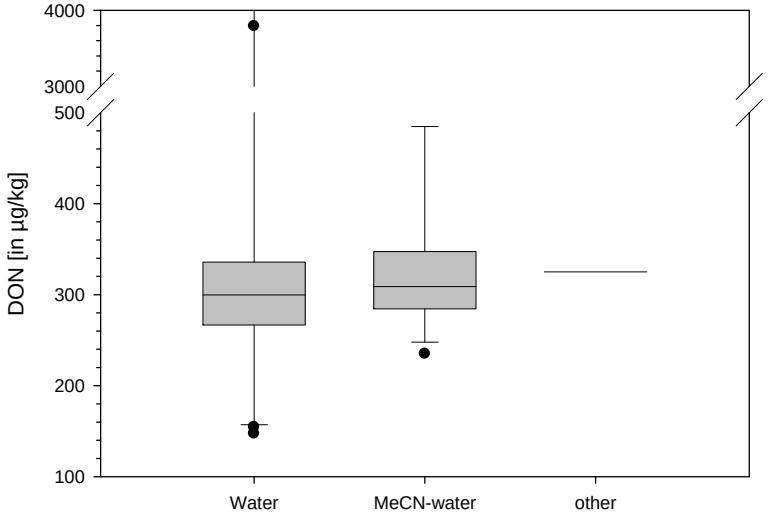
**Figure 6**: Box and Whisker plots of different extraction solvents used by participants of the PT. The data refer to the uncorrected value of DON (a), the recovery reported (b), and the recovery corrected results (c).




**6a**)




**6b**)

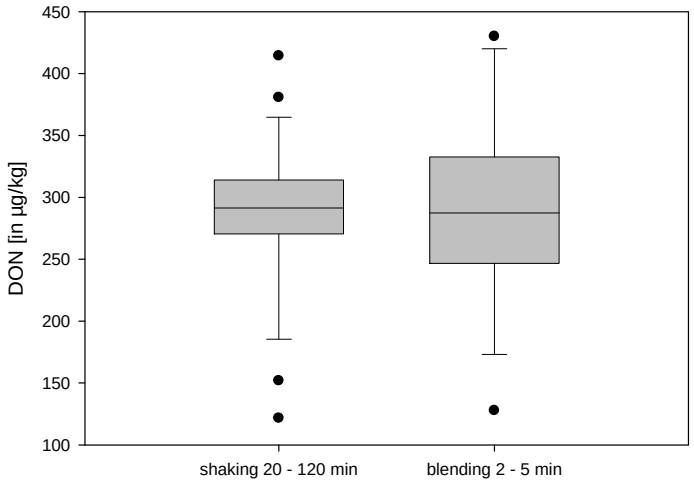



**6c**)

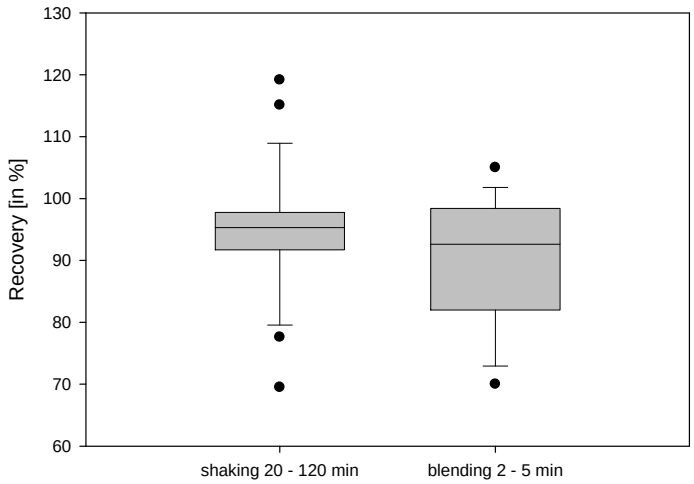
Another parameter that apparently varies in laboratories is the extraction mode by either shaking or blending. Shaking requires a longer time, but offers the processing of several samples simultaneously and minimises the risk of cross contamination. Blending is often preferred when just a low number or

single samples need to be analysed. A comparison of data shows (**Figure 7**) that both extraction principles are equivalent.

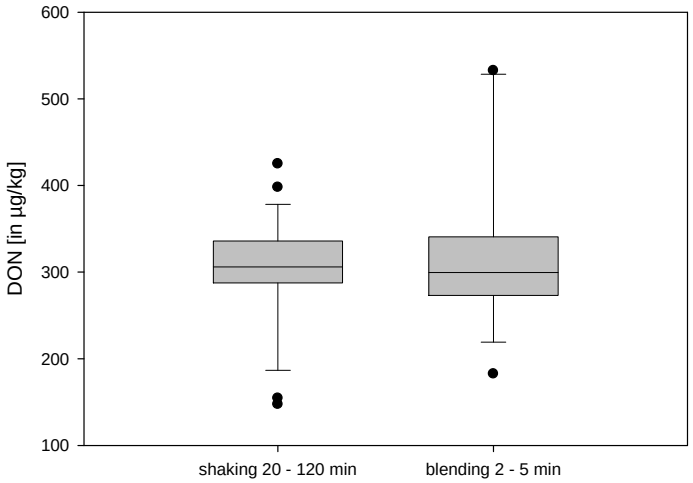
**Figure 7**: Box and Whisker plots illustrating the effect of different extraction procedures used by participants of the PT on the measured DON values. The data refer to the uncorrected value of DON (6a), the recovery reported (6b) and the recovery corrected result (6c). In the top and bottom chart results from Lab 133 were taken out as they were far out of scale (>3000 µg/kg).




**7a**)




**7b**)




**7c)**

Other interesting parameters to compare are the clean-up procedures (**Figure 8**) or the determination methodology used (**Figure 9**). **Figure 8** indicates that the three clean-up procedures used (immunoaffinity clean-up, solid phase extraction or even no clean-up) achieve similar results. The same applies to the comparison of detection methodologies (**Figure 9**).

**Figure 8**: Comparison of different clean-up procedures on the uncorrected DON result.

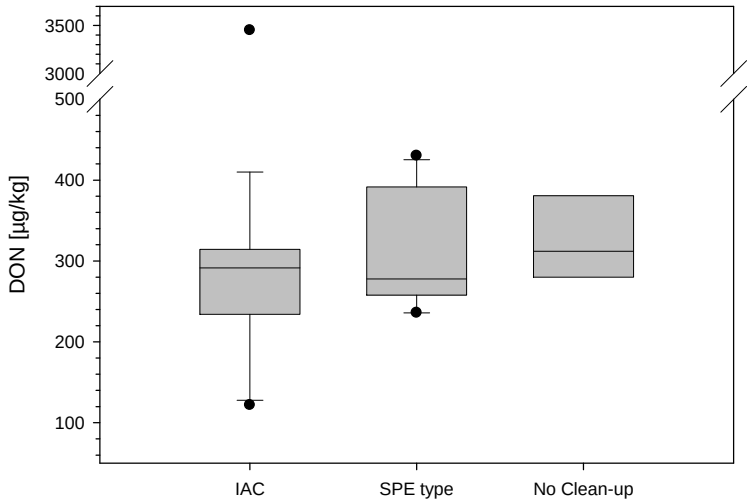
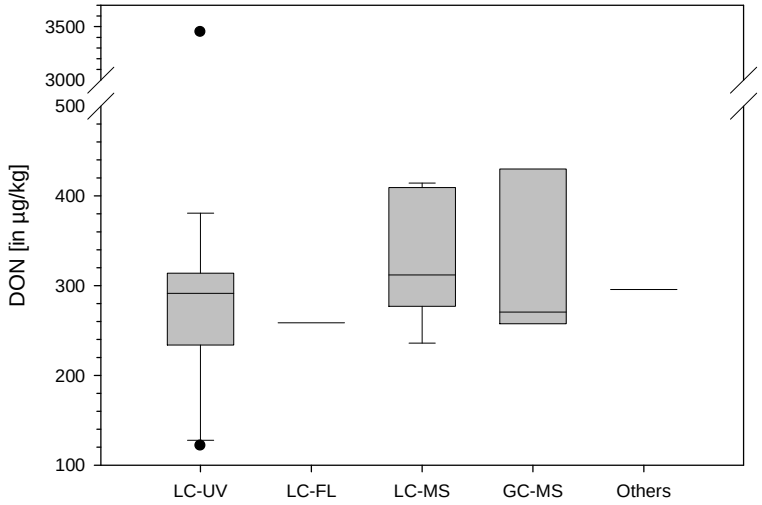


**Figure 9**: Comparison of detection methodology used on the uncorrected result:




## Conclusion

According to the results obtained during this proficiency test,

- 79 % of the participating laboratories are able to determine DON in cereal in a satisfactory manner;
- The importance of recovery correction for the harmonisation of results could be demonstrated by putting recovery uncorrected results with the reported recovery into correlation.
- During extraction, water performs equally well as MeCN-water mixtures, and shaking performs equally well to blending.
- All three types of detection methodology applied by the laboratories performed equally well (LC-UV, LC-MS and GC-MS).
- Non-accredited laboratories performed equally well to accredited laboratories.
- It is proposed that the seven participants whose z-scores exceeded an absolute value of 2 participate in a follow-up PT for DON in cereals.

**Acknowledgments:**
The authors wish to thank Anne-Mette Jensen, Piotr Robouch, Pieter Dehouck and Franz Ulberth for valuable comments.

# Annex

**Table 2**: Individual results as reported by the participants:

| Laboratory code | PMC | PM | BLC | BL | TS | SP+ | SPF | REC |
|---|---|---|---|---|---|---|---|---|
| 101 | D-030 | 257.6 | D-130 | 10.3 | 0.183 | 300.0 | 275.0 | 91.7 |
| 102 | D-024 | 274.0 | S-111 | 0.0 | 0.2995 | 199.4 | 190.0 | 95.3 |
| 103 | D-023 | 291.4 | D-115 | >50.0 | 0.293 | 200.0 | 197.7 | 98.9 |
| 106 | D-004 | 300.5 | D-106 | >70.0 | 0.254 | 250.0 | 235.0 | 94 |
| 107 | D-035 | 414.3 | D-122 | 29.3 | 0.284 | 200.0 | 204.9 | 97.5 |
| 108 | D-012 | 380.7 | D-114 | 26.9 | 0.178 | 200.0 | 191.4 | 95.7 |
| 109 | D-037 | 314.5 | D-116 | 84.9 | 0.239 | 300.0 | 357.5 | 119.17 |
| 111 | D-032 | 340.7 | D-127 | 0.0 | 0.1656 | 300.0 | 293.3 | 97.77 |
| 112 | D-050 | 218.5 | D-110 | 0.0 | 0.284 | 262.5 | 196.5 | 74.9 |
| 113 | D038 | 250.9 | D145 | 0.0 | 0.337 | 257.2 | 215.7 | 83.9 |
| 114 | D021 | 127.7 | D119 | 0.0 | 0.200 | 272.0 | 190.3 | 70 |
| 115 | D-049 | 408.5 | D-144 | 4.6 | 0.202 | 100.0 | 118.0 | 118 |
| 116 | D-027 | 281.6 | D-129 | 6.9 | 0.350 | 290.8 | 243.9 | 83.8 |
| 117 | D-045 | 236.0 | D-121 | 0.0 | 0.284 | 300.0 | 208.5 | 69.5 |
| 118 | D-026 | 258.6 | D-120 | 2.2 | 0.300 | 190.4 | 184.2 | 96.74 |
| 119 | D- 041 | 410.0 | D-139 | 143.9 | 0.470 | 288.0 | 223.0 | 77 |
| 120 | D-011 | 355.0 | D-118 | 13.0 | 0.350 | 200.0 | 209.9 | 105 |
| 121 | D-018 | 325.2 | D-102 | 7.2 | 0.3385 | 209.1 | 194.9 | 93.2 |
| 123 | D-033 | 299.2 | D-131 | 8.3 | 0.303 | 200.8 | 200.1 | 99.65 |
| 124 | D-046 | 270.5 | D-140 | 9.0 | 0.000 | 250.0 | 287.7 | 115.1 |
| 125 | D22 | 299.0 | D124 | 6.6 | 0.307 | 300.0 | 277.0 | 92 |
| 126 | D-015 | 274.0 | D-108 | >25.0 | 0.308 | 229.0 | 208.0 | 90.8 |
| 127 | D-044 | 283.4 | D-109 | 0.0 | 0.2787 | 200.4 | 185.5 | 92.6 |
| 128 | D031 | 233.9 | D113 | 0.0 | 0.423 | 199.7 | 182.6 | 91.4 |
| 129 | D-020 | 314.0 | D-104 | 0.0 | 0.341 | 250.0 | 238.5 | 95.4 |
| 130 | D-040 | 280.0 | D-126 | 0.0 | 0.3087 | 100.0 | 97.2 | 97.2 |
| 131 | D-025 | 312.0 | D-117 | 0.0 | 0.342 | 300.0 | | 96 |
| 132 | D-036 | 121.6 | D-125 | 0.0 | 0.000 | 160.0 | 132.0 | 82.5 |
| 133 | D-009 | 3451.0 | B-073 | 157.0 | 0.000 | 200.0 | 182.0 | 91 |
| 134 | D-028 | 151.8 | D-103 | 0.0 | 0.277 | 150.0 | 147.4 | 98.3 |
| 135 | D-039 | 430.0 | D-041 | 30.0 | 0.367 | 300.0 | 246.0 | 82 |
| 136 | D017 | 270.6 | D107 | 0.0 | 0.253 | 170.0 | 167.3 | 98.41 |
| 138 | D-005 | 235.9 | D-112 | 0.0 | 0.300 | 172.8 | 134.1 | 77.6 |
| 139 | D-010 | 292.8 | D-132 | 0.0 | 0.3381 | 217.4 | 206.1 | 94.80 |

**PMC** = DON positive material container code, **PM** = DON positive material [µg/kg], **BLC** = DON coded blank container code, **BL** = DON blank [µg/kg], **TS** = test solution [µg/mL], **SP+** = spiking level [µg/kg], **SPF** = spike recovered [µg/kg] and **REC** = recovery value [%].

**Table 3**: Performance parameters for the recovery corrected DON result and the test solution:

| Laboratory code | z-score | %-deviation test solution |
|---|---|---|
| 101 | -0.4 | -39.8 |
| 102 | -0.3 | -1.5 |
| 103 | -0.2 | -3.7 |
| 106 | 0.3 | -16.4 |
| 107 | 2.1 | -6.6 |
| 108 | 1.6 | -41.6 |
| 109 | -0.7 | -21.4 |
| 111 | 0.8 | -45.5 |
| 112 | -0.2 | -6.6 |
| 113 | -0.1 | 10.9 |
| 114 | -2.1 | -34.2 |
| 115 | 0.7 | -33.6 |
| 116 | 0.6 | 15.1 |
| 117 | 0.6 | -6.4 |
| 118 | -0.6 | -1.3 |
| 119 | 4.0 | 54.6 |
| 120 | 0.6 | 15.1 |
| 121 | 0.8 | 11.3 |
| 123 | -0.1 | -0.3 |
| 124 | -1.2 | -99.7 |
| 125 | 0.4 | 1.0 |
| 126 | 0.0 | 1.3 |
| 127 | 0.0 | -8.3 |
| 128 | -0.8 | 39.1 |
| 129 | 0.4 | 12.1 |
| 130 | -0.3 | 1.5 |
| 131 | 0.4 | 12.5 |
| 132 | -2.7 | -99.7 |
| 133 | 60.4 | -99.7 |
| 134 | -2.6 | -8.9 |
| 135 | 3.8 | 20.7 |
| 136 | -0.5 | -16.8 |
| 138 | 0.0 | -1.3 |
| 139 | 0.1 | 11.2 |

The calculated z-scores refer to the **recovery corrected value**.
Underreported values for the content of the test solutions are indicated as negative figures.

**Table 4a**: Evaluation of the Questionnaire:

| Lab ID\ Question | Analytical experience with DON determination | Time of experience | Number of samples per year | Evaporation time [minutes] | Spiking solvent |
|---|---|---|---|---|---|
| 101 | YES | 6-12 months | 51-100 | 60 | MeCN |
| 102 | NO | 1-5 months | 05-050 | 30 | MeCN |
| 103 | YES | > 2 years | 101-500 | 40 | MeCN |
| 106 | YES | > 2 years | 51-100 | 120 | other |
| 107 | YES | > 2 years | 101-500 | 20 | MeCN |
| 108 | YES | 1-2 years | 101-500 | 10 | MeOH |
| 109 | YES | 6-12 months | 05-050 | 15 | MeCN |
| 111 | YES | > 2 years | >500 | 1000 | MeCN |
| 112 | YES | > 2 years | 51-100 | 30 | MeCN |
| 113 | YES | > 2 years | 05-050 | 45 | MeCN |
| 114 | YES | > 2 years | 51-100 | 1 | Water |
| 115 | YES | > 2 years | 05-050 | 60 | MeCN |
| 116 | YES | > 2 years | 05-050 | 30 | MeCN |
| 117 | YES | 1-2 years | 101-500 | 1 | MeCN |
| 118 | YES | > 2 years | 51-100 | 30 | MeCN |
| 119 | YES | > 2 years | 05-050 | 60 | MeCN |
| 120 | YES | > 2 years | 101-500 | 60 | MeCN |
| 121 | YES | > 2 years | 101-500 | 45 | MeCN |
| 123 | YES | > 2 years | 51-100 | 20 | MeOH |
| 124 | YES | > 2 years | 51-100 | 10 | MeCN |
| 125 | YES | 1-2 years | 51-100 | 30 | other |
| 126 | YES | > 2 years | >500 | 30 | MeCN |
| 127 | YES | 1-2 years | 05-050 | 720 | MeOH |
| 128 | YES | 1-2 years | 05-050 | 30 | mobile phasis (water/MeOH/ACN) |
| 129 | YES | > 2 years | 101-500 | 90 | other |
| 130 | YES | > 2 years | >500 | 60 | MeCN |
| 131 | YES | > 2 years | 101-500 | 15 | MeOH |
| 132 | NO | xxx | 05-050 | 180 | other (ETHANOL) |
| 133 | YES | > 2 years | 05-050 | 60 | MeCN |
| 134 | YES | > 2 years | 05-050 | 30 | other |
| 135 | YES | > 2 years | 05-050 | 30 | MeCN |
| 136 | YES | > 2 years | 51-100 | 180 | other |
| 138 | YES | > 2 years | 51-100 | 30 | other, chloroform |
| 139 | YES | > 2 years | 51-100 | 30 | MeCN |

**Table 4b**: Evaluation of the Questionnaire:

| Lab ID\ Question | **Have there been any over night stops in the course of analysis?** |
|---|---|
| 101 | no |
| 102 | no |
| 103 | no |
| 106 | no |
| 107 | no |
| 108 | no |
| 109 | no |
| 111 | for all solid samples; between clean up and silylation |
| 112 | no |
| 113 | no |
| 114 | no |
| 115 | no |
| 116 | no |
| 117 | no |
| 118 | The final extracts of samples D - 120 and D - 026 were kept overnight (in refrigerator)and injected the following day. |
| 119 | D-139 after extraction |
| 120 | no |
| 121 | no |
| 123 | no |
| 124 | All samples. Due to the instrumental problems (that were observed only after summer vacations), the whole set had to be re-analysed 6 weeks after the original extraction. During this time, all extracts had been stored at +4 degrees. |
| 125 | no |
| 126 | no |
| 127 | After spiking samples were left overnight to evaporate |
| 128 | Extraction solutions were kept overnight before application to IA-columns |
| 129 | no |
| 130 | no |
| 131 | no |
| 132 | no |
| 133 | no |
| 134 | no |
| 135 | no |
| 136 | no |
| 138 | HPLC analysis performed the day after preparation for all samples |
| 139 | Day 1: extraction, clean-up, evaporating to dryness<br>Day 2: dissolving in the mobile phase, HPLC/UV running |

**Table 4c**: Evaluation of the Questionnaire:

| Lab ID\ Question | Methodology | Clean-up | Detector | Extraction mode |
|---|---|---|---|---|
| 101 | GC | MycoSep | Mass Spec (Single Quad) | Shaking |
| 102 | LC | Immunoaffinity | UV-absorption | Shaking |
| 103 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 106 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 107 | LC | MycoSep | Mass Spec (Triple Quad) | Shaking |
| 108 | ELISA | No clean-up | UV-absorption | Shaking |
| 109 | LC | Immunoaffinity | UV-absorption | Shaking |
| 111 | GC | MycoSep | Other = ECD | Other = stirring |
| 112 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 113 | Other | Immunoaffinity | Other | Blending (e.g. Turrax) |
| 114 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 115 | LC | Other | Mass Spec (Triple Quad) | Other |
| 116 | LC | MycoSep | Mass Spec (Triple Quad) | Shaking |
| 117 | LC | MycoSep | Mass Spec (Triple Quad) | Shaking |
| 118 | LC | MycoSep | Fluorescence | Blending (e.g. Turrax) |
| 119 | LC | Immunoaffinity | Mass Spec (Single Quad) | Blending (e.g. Turrax) |
| 120 | LC | Immunoaffinity | Mass Spec (Triple Quad) | Blending (e.g. Turrax) |
| 121 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 123 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 124 | GC | MycoSep | Mass Spec (Single Quad) | Shaking |
| 125 | LC | Immunoaffinity | UV-absorption | Shaking |
| 126 | LC | Other | Mass Spec | Shaking |
| 127 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 128 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 129 | LC | Immunoaffinity | UV-absorption | Shaking |
| 130 | LC | No clean-up | Mass Spec (Triple Quad) | Shaking |
| 131 | LC | No clean-up | Mass Spec (Triple Quad) | Shaking |
| 132 | LC | Immunoaffinity | UV-absorption | Shaking |
| 133 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 134 | LC | Immunoaffinity | UV-absorption | Shaking |
| 135 | GC | Dispersive "SPE" | Mass Spec (Single Quad) | Blending (e.g. Turrax) |
| 136 | LC | Immunoaffinity | UV-absorption | Blending (e.g. Turrax) |
| 138 | LC | Other, MultiSep and immunoaffinity | UV-absorption | Shaking |
| 139 | LC | MycoSep | UV-absorption | Shaking |

**Table 4d**: Evaluation of the Questionnaire:

| Lab ID\ Question | Extraction time | Extraction solvent | Extraction solvent ratio | Overnight steps | Problems during analysis |
|---|---|---|---|---|---|
| 101 | 60 | MeCN-Water | 84:16 | NO | NO |
| 102 | 20 | Water only | pure | NO | NO |
| 103 | 3 | Water only | | NO | NO |
| 106 | 3 | Water only | pure | NO | NO |
| 107 | 60 | MeCN-Water | 84:16 | NO | YES |
| 108 | 10 | Water only | pure | NO | YES |
| 109 | 30 | Water only | | NO | NO |
| 111 | 120 | MeCN-Water | 84:16 | YES | NO |
| 112 | 3 | Water only | pure | NO | NO |
| 113 | 3 | Water only | | NO | NO |
| 114 | 2 | Water only | | NO | NO |
| 115 | 60 | MeCN-Water | 84:16 | NO | NO |
| 116 | 120 | MeCN-Water | 84:16 | NO | NO |
| 117 | 60 | MeCN-Water | 84:16 | NO | NO |
| 118 | 3 | MeCN-Water | 84:16 | YES | NO |
| 119 | 2 | Water only | pure | YES | NO |
| 120 | 3 | Water only | pure | NO | NO |
| 121 | 3 | Water | PEG | NO | NO |
| 123 | 3 | Water only | xxx | NO | NO |
| 124 | 120 | MeCN-Water | 84:16 | YES | YES |
| 125 | 30 | Water only | | NO | NO |
| 126 | 120 | MeCN-Water | 86:14 | NO | YES |
| 127 | 2 | Water only | | YES | NO |
| 128 | 2 | Water only | | YES | NO |
| 129 | 120 | Water only | pure | NO | NO |
| 130 | 120 | MeCN-Water | 80:20 | NO | NO |
| 131 | 120 | Other | other | NO | NO |
| 132 | 0 | Water only | pure | NO | NO |
| 133 | 3 | Water only | | NO | NO |
| 134 | 30 | Water only | | NO | NO |
| 135 | 5 | MeCN-Water | 84:16 | NO | NO |
| 136 | 2 | Water only | | NO | NO |
| 138 | 30 | MeCN-Water | 84:16 | YES | NO |
| 139 | 60 | MeCN-Water | 84:16 | YES | NO |

**Table 4e**: Evaluation of the Questionnaire:

| Lab ID\ Question | observations | Signal reporting | Integration by | Visual integration check | # of reintegrated peaks | Integration settings |
|---|---|---|---|---|---|---|
| 101 | NO | PEAK SIGNALS | AREA | NO | | horizontal baseline |
| 102 | NO | PEAK SIGNALS | HEIGHT | YES | 6 | valley-to-valley |
| 103 | NO | PEAK SIGNALS | AREA | YES | 0 | Horizontal baseline |
| 106 | NO | PEAK SIGNALS | AREA | YES | 0 | valley to valley |
| 107 | YES | PEAK SIGNALS | AREA | YES | 2 | valley-to-valley |
| 108 | NO | ABS (ELISA) | | NO | 0 | xxx |
| 109 | NO | PEAK SIGNALS | AREA | NO | 0 | xxx |
| 111 | NO | PEAK SIGNALS | HEIGHT | YES | 0 | valley-to-valley |
| 112 | NO | PEAK SIGNALS | AREA | YES | 0 | Empower (Waters) "Apex" calculation |
| 113 | NO | PEAK SIGNALS | AREA | YES | 3 | horizontal baseline |
| 114 | NO | PEAK SIGNALS | HEIGHT | xxx | 0 | xxx |
| 115 | NO | PEAK SIGNALS | AREA | YES | 20 | valley-to-valley |
| 116 | NO | PEAK SIGNALS | AREA | YES | 4 | Valley to Valley |
| 117 | NO | PEAK SIGNALS | AREA | YES | 0 | valley -to-valley |
| 118 | NO | PEAK SIGNALS | AREA | YES | 2 | valley to valley |
| 119 | NO | PEAK SIGNALS | AREA | NO | 0 | horizontal baseline |
| 120 | NO | PEAK SIGNALS | AREA | YES | 2 | Horizontal baseline |
| 121 | NO | PEAK SIGNALS | AREA | YES | 0 | valley to valley |
| 123 | NO | PEAK SIGNALS | AREA | YES | 1 | valley-to-valley |
| 124 | NO | PEAK SIGNALS | HEIGHT | NO | 0 | xxx |
| 125 | NO | PEAK SIGNALS | AREA | NO | 0 | valley-to-valley |
| 126 | NO | PEAK SIGNALS | AREA | YES | 2 | tangential |
| 127 | YES | PEAK SIGNALS | AREA | YES | 1 | Horizontal baseline |
| 128 | NO | PEAK SIGNALS | AREA | xxx | 0 | valley to valley |
| 129 | YES | PEAK SIGNALS | AREA | YES | 0 | horizontal baseline |
| 130 | NO | PEAK SIGNALS | AREA | YES | 0 | xxx |
| 131 | NO | PEAK SIGNALS | AREA | YES | 0 | xxx |
| 132 | NO | Other | AREA | NO | 0 | horizontal baseline |
| 133 | NO | PEAK SIGNALS | AREA | YES | 0 | tangential |
| 134 | NO | PEAK SIGNALS | AREA | YES | 4 | HORIZONTAL BASELINE |
| 135 | NO | PEAK SIGNALS | AREA | YES | 0 | xxx |
| 136 | NO | PEAK SIGNALS | AREA | YES | 2 | Width, Threshold |
| 138 | NO | PEAK SIGNALS | AREA | YES | 0 | valley-to-valley |
| 139 | NO | PEAK SIGNALS | HEIGHT | YES | 0 | Horizontal baseline |

**Table 4f**: Evaluation of the Questionnaire:

| Lab ID\ Question | Instructions adequate | Problems with e-Form | Accreditation | Unusual observations |
|---|---|---|---|---|
| 101 | YES | YES | NO | |
| 102 | YES | NO | YES | |
| 103 | YES | NO | YES | |
| 106 | YES | NO | YES | |
| 107 | YES | NO | YES | At sample D-035 |
| 108 | YES | NO | YES | |
| 109 | YES | NO | YES | |
| 111 | YES | NO | YES | |
| 112 | YES | NO | YES | |
| 113 | YES | NO | NO | |
| 114 | YES | YES | YES | |
| 115 | YES | NO | YES | |
| 116 | YES | NO | YES | |
| 117 | YES | NO | YES | |
| 118 | YES | YES | YES | |
| 119 | YES | NO | YES | |
| 120 | YES | NO | NO | |
| 121 | YES | NO | YES | - |
| 123 | YES | NO | NO | |
| 124 | YES | YES | NO | |
| 125 | YES | NO | YES | |
| 126 | YES | YES | YES | |
| 127 | YES | NO | NO | When the standard was analysed as received no DON was detected. After blow-down and reconstitution in mobile phase DON was then detected as expected |
| 128 | YES | YES | NO | |
| 129 | NO | YES | YES | Blank B-079 and D-104 samples are more similar to maze than to cereal samples |
| 130 | YES | NO | YES | |
| 131 | YES | YES | YES | |
| 132 | YES | NO | NO | |
| 133 | YES | NO | YES | |
| 134 | YES | NO | YES | |
| 135 | YES | NO | NO | |
| 136 | YES | NO | YES | |
| 138 | YES | NO | YES | |
| 139 | YES | NO | YES | |

**Table 4g**: Evaluation of the Questionnaire:

| Lab ID\ Question | Any other comments? |
|---|---|
| 101 | |
| 102 | WE USSUALY ANALYSE DON BY ELISA, HPLC METHOD WAS ACCREDITED JUST IN AUGUST THIS YEAR |
| **103 - 111** | |
| 112 | Why not as XLS or DOC files? |
| 113 | The Laboratory is accredited for the analysis of aflatoxins B1,B2,G1,G2 and M1, ochratoxin, nitrates and lead, cadmium and mercury.<br>In above it would be useful if the nature of the matrix of samples was specified. |
| 114 | |
| 115 | |
| 116 | |
| 117 | |
| 118 | The extraction times were 3 minutes in ultraturrax followed by 60 minutes in orbital shaker |
| 119 | |
| 120 | |
| 121 | |
| 123 | The concentration of the sample D-131 was 8,3 ng/g , which is lower than the limit of quantification of the method 20-50 ng/g , depending on the sample type. We only wanted to give all the information we had. |
| 124 | |
| 125 | |
| 126 | |
| 127 | We hope to present our method for accreditation at our next Accreditation visit in Spring 2009 |
| 128 | Methods based on IA-HPLC-UV are not very robust and sensitive. NRL need assistance from CRL for development of an LC-MS/MS-method (maybe in the context of a multiresidue-method together with zearalenone and T-2/HT-2.. |
| 129 | |
| 130 | In Sample D-126 the DON content was < 25 µg/kg. This value could not be entered in the electronic form. The result for the test solution is in ng/ml!!<br>Using the LC-MS/MS method it is very important to have a blank which has the same composition as the samples, because we are dealing with matrix effects. Matrix matched standards were used for quantification. We did see a difference in the three samples, therefore sample D-040 was also analysed by the standard addition. Using this method we found 330.2 µg/kg. |
| 131 | Extraction solvent used was MeCN:H2O 84:16 with 1% Formic acid |
| **132 - 139** | |

**Table 4h**: Evaluation of the Questionnaire:

| Lab ID\ Question | Type of analytical problems |
|---|---|
| **101 - 106** | |
| 107 | LC-MS-MS sensitivity at DON toxin is low. |
| 108 | Sample cod D-114 has the same results like sample Blank(B-032); The sample cod 0017 we evaporated to 60 C degrees and reconstitution with the same volume of distillate water; |
| **109 - 123** | |
| 124 | All samples (see point 7). Additionally, for DON test solution no result could be delivered. Despite several independent attempts, the response for ISTD remained three times lower in test sample than for standards. The ampoule had been opened 6 weeks earlier and the solution stored at -18 degrees meanwhile. |
| 125 | |
| 126 | Ion suppression in case of D-015 sample. I needed to do a better separation, but the problem was solved. |
| **127 - 139** | |

**Table 3i**: Evaluation of the Questionnaire:

| Lab ID\ Question | Problems with e-Form? |
|---|---|
| 101 | It was not possible to put zero after decimal point (it was removed automatically). |
| **102 - 113** | |
| 114 | It is impossible to fill in results bellow LOQ. Symbols (<) and text are not allowed. |
| **115 - 117** | |
| 118 | It was not possible to reflect some results as < LOQ because the form only accepts figures (numbers). In question nr 9 has not been possible to reflect the real conditions (see part 23 .- comments)) |
| **119 - 123** | |
| 124 | See above (the result for DON test sample had to marked as 0, although it could not be analysed). |
| 125 | |
| 126 | The menus did not work |
| 127 | |
| 128 | impossible to report values as "<x" |
| 129 | We use different methods for analysis of DON, but in the form we can choose only one possibility |
| 130 | |
| 131 | It was not possible to report <RL results for the samples |
| **132 - 139** | |

**Table 4j**: Evaluation of the Questionnaire:

| Lab ID\ Question | Improvments? |
|---|---|
| **101 - 127** | |
| 128 | some contradictions between accompanying letter and reporting sheet |
| 129 | To leave more freedom in assessing our recovery |
| **130 - 139** | |

**Table 4k**: Evaluation of the Questionnaire:

| Lab ID\ Question | What is your opinion on the e-Form reporting? |
|---|---|
| 101 | Acceptable |
| 102 | IT IS GOOD |
| 103 | OK but could not submit. Sent as email attachment. |
| 106 | Electronic forms are quite good nevertheless, on line links could work better |
| 107 | This form is comfortable for use. |
| 108 | OK |
| 109 | very good |
| 111 | ok |
| 112 | Somewhat complicated. We have to follow your instruction step by step. |
| 113 | It is an easy and quick way to report the results |
| 114 | We can imagine that after improvement and changes would be useful for data collection. In such form is confusing , miss-leading and and not friendly. |
| 115 | Perfect! |
| 116 | It is easy and practical |
| 117 | OK |
| 118 | OK |
| 119 | normal |
| 120 | It is useful |
| 121 | OK |
| 123 | Reporting format was good. But in the instructions it was written "please fill out all fields" and also "The recovery rate will be calculated by us". We left the Recovery field empty. |
| 124 | Feasible, although no additional marks to numbers (e.g. text) could be used... |
| 125 | easy to use |
| 126 | Sometimes it does not work properly |
| 127 | Very convenient |
| 128 | It's OK! |
| 129 | it is imperfect |
| 130 | Easy |
| 131 | works OK |
| 132 | It is good and appropriated |
| 133 | OK |
| 134 | |
| 135 | Excellent |
| 136 | It is quite friendly |
| 138 | ok |
| 139 | Generally OK. Just one small fault on the RESULTS paper: test solution result was supposed to be reported as “ng/ml” – on the form it’s marked as “µg/ml” though (our result is as required in “ng/ml”). |

European Commission

**EUR 23787 EN – Joint Research Centre – Institute for Reference Materials and Measurements**
Title: Report on the 2008 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, regarding the Determination of Deoxynivalenol in a Cereal Product and a Test Solution

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**Abstract**
A proficiency test was conducted by the Community Reference Laboratory for Mycotoxins with 33 European National Reference Laboratories (NRLs) for Mycotoxins and 2 Laboratory from candidate countries, thus a total of 35 participants. Test materials were a deoxynivalenol (DON) solution in acetonitrile and three cereal test materials. Laboratories determined the DON content by either enzyme linked immuno sorbent assay (ELISA), gas chromatography (GC) or reverse-phase high-performance liquid-chromatography (RP-HPLC). One NRL did not report any results.

Applying the Horwitz equation as a basis for the target standard deviation (19% in the case of this proficiency test), 27 out of the remaining 34 laboratories reported values within the z-score limit of 2 after recovery correction of the result for the DON-positive sample. Twenty-five laboratories reported results within a z-score limit of 1. Thus, 79 % of the participating laboratories performed satisfactorily in the proficiency test. No z-scores were calculated for the blank material.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.


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