Report on the 2012 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

Determination of DON, ZON, T-2 and HT-2 in Cereals

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2012
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1. Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the European Union Reference Laboratory (EU-RL) for Mycotoxins. One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs).

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of deoxynivalenol (DON), zearalenone (ZON), T-2 and HT-2 in cereal samples.

The two test items were naturally contaminated cereal-based animal feed. The two materials were procured by the IRMM and dispatched to the participants in May 2012. Each participant received two sachets containing approximately 100 g of test material each.

Thirty-five participants from 27 countries registered for the exercise. Thirty four (Sample A) and 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

The assigned values, established by exact-matching double isotope dilution mass spectrometry, were 605 μg/kg (Sample A) and 282 μg/kg (Sample B) for DON, and 445 and 28 μg/kg for ZON. The uncertainties of the respective assigned values were 49 and 26 μg/kg, and 16 and 4 μg/kg.

Participants were invited to report the uncertainty of their measurements. This was done by the majority of laboratories.

Laboratory results for DON and ZON were rated with z-scores and zeta-scores in accordance with ISO 13528 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.

Only z-scores for DON and ZON were used for an evaluation of an underperformance. In total about 95 % of the attributed z-scores were below an absolute value of two for these two mycotoxins, which indicated that most of the participants performed satisfactory or better.

Due to lack of legislative limits and inconclusive data on the assigned values neither z-scores nor zeta-scores were calculated at the moment for T-2 and HT-2.

2. Introduction

Figure 1: Chemical structures of the analytes in the proficiency test

[Chemical structures of DON, ZON, T-2, and HT-2 are shown here.

a) DON
b) ZON
c) T-2
d) HT-2]
Fusarium fungi species produce a heterogeneous variety of mycotoxins such as trichothecenes and myco-oestrogens.

The most abundant trichothecenes are deoxynivalenol (DON, vomitoxin, type B) [Figure 1a], produced by *F. graminearum* and *F. culmorum*, T-2-toxin and HT-2-toxin (T-2, HT-2, type A) [Figure 1c-d], produced by *F. poae*, *F. langsethiae* and *F. sporotrichioides*. These are mainly contaminating cereals like wheat, barley and maize used as food and feed. T-2 can be metabolised into HT-2. Emesis, reduced weight gain and other gastrointestinal disorders are the most sensitive functional manifestations of the type B trichothecenes, while immunotoxicity, cytotoxicity and neurotoxicity are caused by the type A trichothecenes [1], [2].

The structure of myco-oestrogens (zearalenone and derivatives) resembles oestradiol as it has high oestrogenic activity causing hyperoestrogenism in animals and humans. An oestrogenic response is induced by several organisms, resulting in common symptoms as infertility, vulval oedema and testicular atrophy. Zearalenone (ZON) [Figure 1b] is mainly produced by *F. graminearum* and *F. culmorum*, consequently co-occurrence with DON and wide geographical spread is described. The production, mainly in maize, wheat, oats, barley, depends on environmental conditions and is favoured by high humidity and low temperature [1], [2].

DON, ZON and T-2 are ordered in category 3 (not classified relating to carcinogenicity for humans) by the IARC [3].


### 3. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [7], one of the core duties of the EU-RL is to organise interlaboratory comparison tests (ILCs) for the benefit of staff from NRLs. The scope of this ILC was to test the competence of the appointed NRLs to determine the amount of DON, ZON, T-2, HT-2 in cereal samples.

IRMM organised a proficiency test on DON in 2008 [8] and on T-2/HT-2 in 2009 [9] in cereal products. This year's PT was the first one to be conducted for the determination of ZON.

All invited laboratories were free to use their method of choice. The methodologies used for the determination of these mycotoxins range from high-performance liquid chromatography (HPLC) with various detection systems, over gas chromatography and enzyme linked immunosorbant assays (ELISA). The most common approach in EU member states is however HPLC with mass selective detection.

The ILC was designed and the reported data were processed along the lines of the International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories [10].

As accredited according to ISO 17043 PT provider, EURL-Mycotoxins performed the assessment of the measurement results on the basis of requirements laid down in legislation and followed administrative and logistic procedures of ISO 17043 [11].

#### 3.1. Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed.

### 4. Time frame

The ILC was agreed upon by the NRL network at the sixth EU-RL Mycotoxins workshop held on 7 April 2011. Specific details of the exercise were refined during the seventh EU-RL Mycotoxins workshop held on 26-27 April 2012 and the planned ILC was published on the IRMM web page [12]. The exercise was open for registration on 3 May 2012 [Annex 13.1]. The samples were dispatched to the participants on 30 May 2012 [Annex 13.2]. Reporting deadline was 5 July 2012.
5. Material

5.1. Preparation
The test materials used in this study were prepared by Eurofins WEJ, Hamburg, Germany. The materials were provided milled to a particle size < 500 μm.
The composition of the test materials and the percent content are the following:
- Sample A: soya (16%), sugar beet (8%), maize gluten (18%), bean (8%), rice (24%), oat (26%)
- Sample B: rye (25%), wheat (17%), maize (17%), oat (8%), rice (33%)

5.2. Homogeneity
To verify the homogeneity of the test materials 10 units per material Sample A and Sample B were selected at random. Two independent determinations per unit were performed with an LC-MS/MS based method, which has been validated at a collaborative trial organised by the EU-RL Mycotoxin group. The measurement batch order was randomised. Sufficient homogeneity was assumed if the between-sample variance ($s_{\text{sam}}^2$) was smaller than a critical factor (c) [10].
The between-sample variance ($s_{\text{sam}}^2$) and the within-sample variance ($s_{\text{an}}^2$) were obtained from one-way analysis of variance (ANOVA). The allowable variance ($\sigma_{\text{all}}^2$) was calculated as $(0.3 \cdot \sigma_p)^2$ from the Horwitz equation modified by Thompson [13].

Annex 13.3 lists the details of the homogeneity tests for the two materials. For all materials the between-sample variance ($s_{\text{sam}}^2$) was smaller than the critical factor (c) and, therefore, sufficient homogeneity was assumed.

5.3. Stability
The amount of DON, ZON, T-2 and HT-2 in the test materials were monitored (n=20) over a period of two years (from August 2009 until August 2011) because the material was used as QC-sample. No indication of any degradation was found and the material is considered to be stable.

5.4. Distribution
All samples were packed in cardboard boxes and sent to the participant via DHL express mail. One set of material was sent to every participant. The test materials were dispatched to the participants by IRMM on 30 May 2012. The samples were mostly received within 24 hours after dispatch.

Each participant received:
a) two packages containing approximately 100 g of test materials,
b) an accompanying letter with instructions on sample handling and reporting [Annex 13.2],
c) a sample receipt form [Annex 13.4] and
d) a registration key for the reporting interface.

The materials were shipped at room temperature; storage upon arrival was required to be at -18°C until the analysis was performed. Based on previous experience a short period of 1-2 days without cooling imposes no harm for the material, for storage above -18°C over a longer period of time no stability information is available.

6. Instructions to participants
The laboratories were asked to report the recovery corrected value and the measurement uncertainty in μg/kg, the coverage factor and the recovery in %.
The results were to be reported in a special online form for which each participant received an individual access code. A specific questionnaire was attached to this online form. The questionnaire was intended to
provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in Annex 13.5.

7. Reference values and their uncertainties

Assigned values and their uncertainties for the test samples were established by 'Exact-matching Double Isotope Dilution Mass Spectrometry' at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [14]. The details of the procedure can be found in the report of the NRL PT from 2011.

8. Evaluation of results

8.1. General observations

Thirty-five laboratories, NRL’s from twenty-seven MS (two different NRLs for food and feed for eight MS) registered to the PT [Figure 2] and all of them sent back results.

34 (Sample A) & 34 (Sample B) sets of results were reported for deoxynivalenol, 33 & 32 for zearalenone, 32 & 28 for T-2 and 30 & 28 for HT-2.

8.2. Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta (ζ) scores in accordance with ISO 13528 [15] and the International Harmonised Protocol [10].

\[
z = \frac{x_{\text{lab}} - X_{\text{ref}}}{\sigma_p}
\]

Equation 1.

\[
\zeta = \frac{x_{\text{lab}} - X_{\text{ref}}}{\sqrt{u_{\text{lab}}^2 + u_{\text{ref}}^2}}
\]

Equation 2.

where:

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

\(\sigma_p\) was calculated using the Horwitz equation:

- for analyte concentrations < 120 ppb (ZON Sample B, T-2 Sample A, T-2 Sample B, HT-2 Sample B)

\[
\sigma_p = 0.22 \cdot c
\]

Equation 3.

- for analyte concentrations ≥ 120 ppb ≤ 13.8% (DON Sample A, DON Sample B, ZON Sample A, HT-2 Sample A)

\[
\sigma_p = 0.02 \cdot c^{0.8495}
\]

Equation 4.

where:

- \(c\) = concentration of the measurand (assigned value, \(X_{\text{ref}}\)) expressed as a dimensionless mass ratio, e.g. 1 ppb = 10^{-9}, 1 ppm = 10^{-6}

The z score compares the participant’s deviation from the reference value with the target standard deviation accepted for the proficiency test, \(\sigma_p\). The z-score is interpreted as:
The zeta (ζ) score provides an indication of whether the participant’s estimate of 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 values.

The interpretation of the zeta score is similar to the interpretation of the z-score:

\[
\begin{align*}
|\zeta| & \leq 2 & \text{satisfactory result} \\
2 < |\zeta| & \leq 3 & \text{questionable result} \\
|\zeta| & > 3 & \text{unsatisfactory result}
\end{align*}
\]

An unsatisfactory |ζ|-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory |ζ|-score indicated an uncertainty which is not consistent with the laboratory’s deviation from the reference value.

**8.3. Laboratory results and scoring**

Statistical evaluation of the results was performed using MS Excel.

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528 [15] by application of a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [16].

As a result z-scoring and zeta-scoring was only made for DON and ZON and is in line with the planning to only benchmark results submitted for DON and ZON, unsatisfactory z-scores will results in a corrective action for these two mycotoxins.

The results from the T-2 and HT-2 measurements are nonetheless summarized (for information only) without any z-scoring or further evaluation. This will be done once sufficient experimental data or other evidence can lead to a sound scientific explanation of the discrepancy between IDMS certification and consensus value. The findings will be published as an addendum to this report and shall be discussed with the NRLs at the next possible occasion.

The results as reported by the participants were summarised in Table 2,4,6,8 together with the z-scores and zeta-scores. Summary of the statistical evaluation for each analyte and test sample are presented in Tables 1,3,5,7.

Figures 2-9 provide for each analyte/matrix combinations the individual laboratories values and their uncertainty as reported.
### Table 1: Summary statistics for the deoxynivalenol (DON)

<table>
<thead>
<tr>
<th></th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of results</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Range of results (μg/kg)</td>
<td>391.6-897</td>
<td>86.5-448.9</td>
</tr>
<tr>
<td>Median of results of participants (μg/kg)</td>
<td>583.9</td>
<td>266</td>
</tr>
<tr>
<td>Mean of results of participants (μg/kg)</td>
<td>5870</td>
<td>2679</td>
</tr>
<tr>
<td>Robust mean of results of participants (μg/kg)</td>
<td>573.3</td>
<td>267.2</td>
</tr>
<tr>
<td>Assigned value (μg/kg)</td>
<td>605</td>
<td>282</td>
</tr>
<tr>
<td>Expanded uncertainty (k=2) of the assigned value (μg/kg)</td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td>Robust standard deviation (σ̂) (μg/kg)</td>
<td>109</td>
<td>37</td>
</tr>
<tr>
<td>Target standard deviation (fitness for purpose) (μg/kg)</td>
<td>104.4</td>
<td>54.6</td>
</tr>
<tr>
<td>Number (percentage) of results of</td>
<td>1 (3%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td></td>
<td>[</td>
<td>z</td>
</tr>
<tr>
<td>Number (percentage) of results of</td>
<td>11 (32%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td></td>
<td>[</td>
<td>z</td>
</tr>
</tbody>
</table>

### Table 2: Results of analysis, z-scores and zeta-scores for deoxynivalenol (DON)

(The meaning of colors: green – satisfactory, yellow – questionable, red – unsatisfactory result)

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>SAMPLE A</th>
<th></th>
<th>SAMPLE B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Result [μg/kg]</td>
<td>z-score</td>
<td>zeta-score</td>
<td>Result [μg/kg]</td>
</tr>
<tr>
<td>101</td>
<td>897</td>
<td>2.8</td>
<td>2.6</td>
<td>279</td>
</tr>
<tr>
<td>102</td>
<td>668</td>
<td>0.6</td>
<td>0.9</td>
<td>295</td>
</tr>
<tr>
<td>103</td>
<td>512</td>
<td>-0.9</td>
<td>-1.4</td>
<td>246</td>
</tr>
<tr>
<td>104</td>
<td>495</td>
<td>-1.1</td>
<td>-2.3</td>
<td>106.8</td>
</tr>
<tr>
<td>105</td>
<td>800</td>
<td>19</td>
<td>3.8</td>
<td>400</td>
</tr>
<tr>
<td>106</td>
<td>596.5</td>
<td>-0.1</td>
<td>-0.2</td>
<td>448.9</td>
</tr>
<tr>
<td>107</td>
<td>502.7</td>
<td>-1.0</td>
<td>-2.0</td>
<td>86.5</td>
</tr>
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<td>108</td>
<td>605</td>
<td>0.0</td>
<td>0.0</td>
<td>255</td>
</tr>
<tr>
<td>109</td>
<td>792.4</td>
<td>18</td>
<td>3.1</td>
<td>321.94</td>
</tr>
<tr>
<td>110</td>
<td>507</td>
<td>-0.9</td>
<td>-1.2</td>
<td>266</td>
</tr>
<tr>
<td>111</td>
<td>590.5</td>
<td>-0.1</td>
<td>-0.3</td>
<td>275.1</td>
</tr>
<tr>
<td>112</td>
<td>391.6</td>
<td>-2.0</td>
<td>-3.4</td>
<td>213.6</td>
</tr>
<tr>
<td>113</td>
<td>720</td>
<td>11</td>
<td>0.9</td>
<td>340</td>
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<tr>
<td>114</td>
<td>577</td>
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<td>-0.3</td>
<td>260</td>
</tr>
<tr>
<td>115</td>
<td>491</td>
<td>-1.1</td>
<td>-1.2</td>
<td>240</td>
</tr>
<tr>
<td>116</td>
<td>593.4</td>
<td>-0.1</td>
<td>-0.1</td>
<td>264.4</td>
</tr>
<tr>
<td>117</td>
<td>578</td>
<td>-0.3</td>
<td>-0.3</td>
<td>268</td>
</tr>
<tr>
<td>118</td>
<td>522</td>
<td>-0.8</td>
<td>-1.4</td>
<td>245</td>
</tr>
<tr>
<td>119</td>
<td>795.3</td>
<td>18</td>
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</tr>
<tr>
<td>120</td>
<td>431</td>
<td>-17</td>
<td>-4.9</td>
<td>241</td>
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<tr>
<td>121</td>
<td>428.3</td>
<td>-17</td>
<td>-3.3</td>
<td>268.4</td>
</tr>
<tr>
<td>122</td>
<td>651.1</td>
<td>0.4</td>
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<td>286.4</td>
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<td>123</td>
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<td>-0.2</td>
<td>252.2</td>
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<td>124</td>
<td>771.2</td>
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<td>2.6</td>
<td>327.5</td>
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<tr>
<td>125</td>
<td>590</td>
<td>-0.1</td>
<td>-0.1</td>
<td>286</td>
</tr>
<tr>
<td>126</td>
<td>642</td>
<td>0.4</td>
<td>0.5</td>
<td>280</td>
</tr>
<tr>
<td>127</td>
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<td>-3.0</td>
<td>348</td>
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<tr>
<td>128</td>
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<td>-1.2</td>
<td>256.67</td>
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<td>129</td>
<td>661</td>
<td>0.5</td>
<td>1.7</td>
<td>297</td>
</tr>
<tr>
<td>130</td>
<td>598</td>
<td>-0.1</td>
<td>-0.1</td>
<td>229</td>
</tr>
<tr>
<td>131</td>
<td>581.5</td>
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<td>-0.3</td>
<td>248.5</td>
</tr>
<tr>
<td>132</td>
<td>No result</td>
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<td></td>
<td>No result</td>
</tr>
<tr>
<td>133</td>
<td>505.5</td>
<td>-10</td>
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<td>205.5</td>
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<tr>
<td>134</td>
<td>450</td>
<td>-1.5</td>
<td>-2.3</td>
<td>179</td>
</tr>
<tr>
<td>135</td>
<td>450</td>
<td>-1.5</td>
<td>-1.8</td>
<td>266</td>
</tr>
</tbody>
</table>

The results are written as reported by the laboratories.
This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported. The red line corresponds to Xref, the blue lines mark the boundary of the reference interval (Xref ± 2u_ref), and the green lines that of the target interval (Xref ± 2σ).
Table 3: Summary statistics for the zearalenone (ZON)

<table>
<thead>
<tr>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of results</td>
<td>33</td>
</tr>
<tr>
<td>Range of results μg/kg</td>
<td>267-585</td>
</tr>
<tr>
<td>Median of results of participants μg/kg</td>
<td>462.2</td>
</tr>
<tr>
<td>Mean of results of participants μg/kg</td>
<td>449.7</td>
</tr>
<tr>
<td>Robust mean of results of participants μg/kg</td>
<td>457.8</td>
</tr>
<tr>
<td>Assigned value μg/kg</td>
<td>445</td>
</tr>
<tr>
<td>Expanded uncertainty (k=2) of the assigned value μg/kg</td>
<td>16</td>
</tr>
<tr>
<td>Target standard deviation (fitness for purpose) μg/kg</td>
<td>80.4</td>
</tr>
<tr>
<td>Number (percentage) of results of</td>
<td>z</td>
</tr>
<tr>
<td>Number (percentage) of results of</td>
<td>ζ</td>
</tr>
</tbody>
</table>

Table 4: Results of analysis, z-scores and zeta-scores for zearalenone (ZON)
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>SAMPLE A</th>
<th>z-score</th>
<th>zeta-score</th>
<th>SAMPLE B</th>
<th>z-score</th>
<th>zeta-score</th>
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</thead>
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<tr>
<td>101</td>
<td>528</td>
<td>1.0</td>
<td>1.3</td>
<td>23</td>
<td>-0.8</td>
<td>-1.4</td>
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<td>102</td>
<td>354</td>
<td>-1.1</td>
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<td>25.7</td>
<td>-0.4</td>
<td>-0.7</td>
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<td>103</td>
<td>579</td>
<td>1.7</td>
<td>1.9</td>
<td>32</td>
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<td>104</td>
<td>406</td>
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<td>105</td>
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The results are written as reported by the laboratories.
Figure 4: EU-RL Mycotoxins PT 2012: Zearalenone in cereals - Sample A
Certified value: Xref = 445 μg/kg; Uref = 16 μg/kg (k=2); σ = 80.4 μg/kg

This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported. The red line corresponds to Xref, the blue lines mark the boundary of the reference interval (Xref ± 2σ), and the green lines that of the target interval (Xref ± 2σ).

Figure 5: EU-RL Mycotoxins PT 2012: Zearalenone in cereals - Sample B
Certified value: Xref = 28 μg/kg; Uref = 4 μg/kg (k=2); σ = 6.2 μg/kg

This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported. The red line corresponds to Xref, the blue lines mark the boundary of the reference interval (Xref ± 2σ), and the green lines that of the target interval (Xref ± 2σ).
Table 5: Summary statistics for the T-2

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<tr>
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<tr>
<td>Range of results (µg/kg)</td>
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<td>Median of results of participants (µg/kg)</td>
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<tr>
<td>Mean of results of participants (µg/kg)</td>
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<td>Robust standard deviation ((\hat{\sigma})) (µg/kg)</td>
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<td>Target standard deviation (µg/kg)</td>
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Table 6: Results of analysis (T-2)

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The results are written as reported by the laboratories.
This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
Table 7: Summary statistics for the HT-2

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<tr>
<td>Robust mean of results of participants μg/kg</td>
<td>156.6</td>
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<td>Robust standard deviation ( $\hat{\sigma}$ ) μg/kg</td>
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<td>Target standard deviation μg/kg</td>
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Table 8: Results of analysis (HT-2)

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The results are written as reported by the laboratories.
Figure 8: EU-RL Mycotoxins PT 2012: HT-2 in cereals - Sample A

This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.

Figure 9: EU-RL Mycotoxins PT 2012: HT-2 in cereals - Sample B

This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
8.4. Evaluation of the questionnaire

All laboratories that reported results, in total thirty four participants, supplied their filled in questionnaire. Summary of the answers is presented in the Annex 13.6.

General overview of the reported answers showed that participants used mainly three techniques – HPLC-DAD, HPLC-FLD and LC-MS/MS - for obtaining the results for different mycotoxins.

For the determination of T-2 and HT-2, most of the laboratories (80%) used LC-MS/MS. HPLC-FLD was applied for ZON by 73% of the participants. Regarding the analysis of DON, LC-MS/MS and HPLC-DAD techniques were used equally.

Fifty percent of the participants used Biopure standard for the determination of DON, 47% for ZON, 62% for T-2 and 61% for HT-2.

Most of the laboratories analysed 50-150 samples or more for DON and ZON, but less than 50 samples for T-2 and HT-2 annually. Eighty-nine percent of the NRLs are accredited for the analysis of DON, 80% for ZON and only 51% for both T-2 and HT-2.

For the recovery estimation nearly all of the participants used a "Standard solution to blank” method. Details about the applied methodology for different analytes – extraction, clean up, overnight stop, etc. - are presented in Annex 13.6. No statistically relevant information could be obtained that linked performance results with answers on methodology, overnight stop etc.

All participants found the instructions adequate and regarding the registration-reporting interface the EU-RL received mostly good reviews.

9. Conclusions

34 (Sample A) & 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

Most of the participants performed satisfactory or better than the minimal performance criteria required. The performance of most NRLs was very good and better compared with a previous PT for DON [8] organised by the EU-RL. This was the first PT conducted for the determination of ZON and the results of most participants were outstanding.

Zeta-scores were not as good as the z-scores, which indicate that the respective participants should review their uncertainty estimation.

It was noted that the consensus values and the certified values match for DON and ZON, but not for T-2 and HT-2 toxins. IRMM has dedicated itself to investigate the reason for this difference as it has shown in previous PTs that IDMS certification is a method with many assets for the generation of assigned values in PTs.
10. Acknowledgements

The organizers of the study would like to thank Franz Ulberth and Beatriz de la Calle for their support.

The laboratories participating in this exercise, listed in Table 9, are also kindly acknowledged.

Table 9: Participating laboratories

<table>
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</thead>
<tbody>
<tr>
<td>AGES GmbH</td>
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<tr>
<td>CODA-CERVA, Chemical Safety Food Chain</td>
<td>Belgium</td>
</tr>
<tr>
<td>Central Laboratory for Chemical Testing and Control, Control of Mycotoxins</td>
<td>Bulgaria</td>
</tr>
<tr>
<td>Department Of Agriculture, Analytical Laboratories Section</td>
<td>Cyprus</td>
</tr>
<tr>
<td>State General Laboratory, Food Contamination Laboratory</td>
<td>Cyprus</td>
</tr>
<tr>
<td>Czech Agriculture and Food Inspection Authority</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>Central Institute for Testing and Supervising in Agriculture (UKZUZ)</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>Ministry of Food, Agriculture and Fisheries; Danish Veterinary and Food Adm.</td>
<td>Denmark</td>
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<tr>
<td>DTU Food, Food Chemistry</td>
<td>Denmark</td>
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<tr>
<td>Agricultural Research Centre, Lab For Residues and Contaminants</td>
<td>Estonia</td>
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<td>Finnish Food Safety Authority (Evira), Chemistry and Toxicrology Unit</td>
<td>Finland</td>
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<td>Finnish Customs Laboratory</td>
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<td>Laboratoire de l'INPS, Mycotoxines</td>
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<td>Federal Institute For Risk Assessment -BFR</td>
<td>Germany</td>
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</tr>
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</table>

11. Abbreviations

ANOVA Analysis of variance
DON Deoxynivalenol
EC European Commission
ELISA Enzyme linked immunosorbant assays
EU European Union
EU-RL European Reference Laboratory
FLD Fluorescent detection
HPLC High-performance liquid chromatography
IAC Immunoaffinity column
IDMS Isotope Dilution Mass Spectrometry
ILC Interlaboratory Comparison
IRMM Institute for Reference Materials and Measurements
ISO International Organisation for Standardisation
IUPAC International Union for Pure and Applied Chemistry
JRC Joint Research Centre
LOD Limit of Detection
LOQ Limit of Quantification
NRL National Reference Laboratory
PT Proficiency Test
ZON Zearalenone
12. References


[13] Thompson, M., Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 2000, 125, 385-386


13. Annexes

13.1. Opening of registration

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Madam/Sir,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of:

- deoxynivalenol (DON),
- zearalenone (ZON),
- T-2 and
- HT-2 in cereals.

This proficiency test (PT) was announced during the last EU-RL Mycotoxins workshop. More details on the PT design will be communicated upon sample dispatch.

The EU-RL Mycotoxins would like to inform you that, according to Regulation (EC) No 882/2004, the participation of activities organized by the EU-RL is mandatory for the NLs.

The participation is free of charge.

Confidentiality of the participants and their results are guaranteed.

Registration of participants is open until midnight of 1st May, 2012.

Dispatch of the PT materials is foreseen to be at the end of May and will be announced in advance.

In order to register, laboratories must:

1. Enter the details online:
   https://e-crm.jrc.ec.europa.eu/RegistrationWeb/registration/registration.do?sid=Comparison&cid=100

2. Print the completed form (approved and confirmed version) when the system asks to do so, sign it and stamp it with your company stamp

3. Send it to the EU-RL Mycotoxins members indicated below:

   The PT coordinator is:

   Zoltan KUNSPORT
   Tel: +32 14 571 313
   Fax: +32 14 571 783
   Email: JRC-IRMAC-CRL-MYCOTOX@jrc.europa.eu

   Deadline for reporting will be the 29th June. You will receive the link for entering the results upon reception of the PT samples.

   A detailed outline of the PT will accompany the PT sample parcel; anyhow we would like to encourage you to contact us in case you seek further clarification.

   Please contact us at the email address:

   JRC-IRMAC-CRL-MYCOTOX@jrc.europa.eu

   With kind regards,

   Zoltan Kunairo

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

   Cc: Frans Veerkamp, Franz Ulberth, Beatrice De La Calle, Jorg Stoka

   Telephone: direct line (32-94) 571 328. Fax: (32-94) 571 783
   E-mail: jrc-irmac@jrc.ec.europa.eu
13.2. Accompanying letter

Print out the final pdf and return the signed and stamped report sheet NOT later than 5th July 2012 to:

Zoltan Kunungi
JRC-HRM-TQG
EURL Mycotoxins
Rehovutzweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: jrc-immt-tqg-mycotoxin@ec.europa.eu

In case of questions please do not hesitate to contact us.

Zoltan Kunungi
(on behalf of the Operating Manager of the EU-RL Mycotoxins)
Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Jozef Straka

Dear Participant,

Please read the following information carefully before starting any analysis. If there are additional questions, do not hesitate to contact us by either phone or e-mail (see details below).

The 2012 PT aims to:
Assess the content in two naturally contaminated test samples (labeled as "Sample A", "Sample B"). You will be asked to report the recovery corrected value (µg/kg), including your recovery (%) and measurement uncertainty (µg/kg) for a coverage factor of 2 (k=2).

Please confirm the parcel's receipt by fax or e-mail immediately, by using the "Materials receipt form". If any material is damaged, please request new material immediately.

The materials are shipped at room temperature, storage however should be at -18°C until the analysis is performed. A short period of 1-2 days without cooling is no harm for the material, but a longer period of storage above -18°C shall be avoided.

The password key for this interface is included in the parcel with the test materials. When you enter the code please pay attention to the capital letters!
### 13.3. Homogeneity test

<table>
<thead>
<tr>
<th>Material</th>
<th>Analyte</th>
<th>$s^2_{\text{sam}}$</th>
<th>$s^2_{\text{an}}$</th>
<th>$\sigma^2_{\text{all}}$</th>
<th>N</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample A</strong></td>
<td>DON</td>
<td>805</td>
<td>421</td>
<td>543</td>
<td>10</td>
<td>1450</td>
</tr>
<tr>
<td></td>
<td>ZON</td>
<td>626</td>
<td>336</td>
<td>669</td>
<td>10</td>
<td>1600</td>
</tr>
<tr>
<td></td>
<td>T-2</td>
<td>0.282</td>
<td>10.6</td>
<td>8.84</td>
<td>10</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>HT-2</td>
<td>212</td>
<td>131</td>
<td>117</td>
<td>10</td>
<td>354</td>
</tr>
<tr>
<td><strong>Sample B</strong></td>
<td>DON</td>
<td>118</td>
<td>31.8</td>
<td>45.2</td>
<td>10</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>ZON</td>
<td>0</td>
<td>1.36</td>
<td>1.02</td>
<td>10</td>
<td>3.29</td>
</tr>
<tr>
<td></td>
<td>T-2</td>
<td>0</td>
<td>1.48</td>
<td>0.188</td>
<td>10</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>HT-2</td>
<td>0.964</td>
<td>1.34</td>
<td>3.14</td>
<td>10</td>
<td>7.26</td>
</tr>
</tbody>
</table>

$s^2_{\text{sam}}$ – between-sample variance  
$s^2_{\text{an}}$ – analytical or within-sample variance  
$\sigma^2_{\text{all}}$ – allowable between-sample variance  
N – number of units tested  
c – critical value, equal to 0.3*target SD for the PT, according to ISO 13528, Annex B
13.4. Acknowledgement of receipt form

PROFICIENCY TESTING MATERIALS RECEIPT FORM

<table>
<thead>
<tr>
<th>Name:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Institute:</td>
<td></td>
</tr>
<tr>
<td>Address:</td>
<td></td>
</tr>
<tr>
<td>Member State:</td>
<td></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Date of the receipt</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All items have been received undamaged</td>
<td>YES ☐ / NO ☐</td>
</tr>
<tr>
<td>If NO, please list damaged items:</td>
<td></td>
</tr>
</tbody>
</table>

Contents of the parcel

a) 2 test materials for analysis:
   - Sample A
   - Sample B

b) An envelope with documents:
   - A copy of instructions
   - Participation code
   - Questionnaire

Please fax or e-mail the completed form to:

Zoltan Kunsagi
JRC-IRMM-PSQ
EURL Mycotoxins
Rietseweeg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: zoltan.kunsagi@ec.europa.eu

Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.
E-mail: jco-irmm-crl-mycotox@ec.europa.eu

Signature / Stamp:
### 13.5. Questionnaire

**Milk questionnaire**

Comparison for PT 2012 DON, ZON, T-2, HT-2

Please fill in your results and send them back to the questions. Print the final pdf and return the signed and stamped copy by fax +32 14 571 783 or by e-mail to JRC-IRMM-CRL-MYCOTOX@ec.europa.eu

#### Submission Form

1. How many samples does your laboratory analyse for the following mycotoxins per year?

<table>
<thead>
<tr>
<th>Question/Response table</th>
<th>DON</th>
<th>HT-2</th>
<th>T-2</th>
<th>ZON</th>
<th>Info</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) &lt;50</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>b) 50–150</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>c) 151–500</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>d) 500c</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

2. Which food or feed matrices does your laboratory analyse for DON, ZON, T-2 and HT-2 on a routine basis? (maximum 3) *

3. Are you accredited for the determination of those mycotoxins from cereals?

<table>
<thead>
<tr>
<th>Question/Response table</th>
<th>DON</th>
<th>HT-2</th>
<th>T-2</th>
<th>ZON</th>
<th>Info</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accredited for:</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

4. Proficiency test samples: DON in cereals

4.1. Please indicate the LOD for DON of the method used (µg/kg).

4.2. Please indicate the LOQ for DON of the method used (µg/kg).

5. Proficiency test samples: ZON in cereals

5.1. Please indicate the LOD for ZON of the method used (µg/kg).

6. Proficiency test samples: T-2 in cereals

6.1. Please indicate the LOD for T-2 of the method used (µg/kg).

6.2. Please indicate the LOQ for T-2 of the method used (µg/kg).

7. Proficiency test samples: HT-2 in cereals

7.1. Please indicate the LOD for HT-2 of the method used (µg/kg).

7.2. Please indicate the LOQ for HT-2 of the method used (µg/kg).

8. What is your main procedure for recovery estimation? *

   - a) Internal Standard to Extract
   - b) Internal Standard to Sample
   - c) Standard solution to Blank
   - d) other

8.1. If other please specify! *

9. During the analysis did you include an overnight step? *

   - a) Yes
   - b) No

9.1. If YES please state for which samples and at what stage of the analysis.

10. Please indicate the sample amount (in grams) for extraction. *

11. What was the solvent to sample ratio used during extraction (in ml/g)? *

12. What was the extraction solvent used? *
13. What was the extraction mode (e.g. blending or shaking)? *

14. What was the extraction time? *

15. What type of clean up methodology was used (e.g. immunosynthesis column)? *

16. If you used immunosynthesis columns...
16.1. ... please specify the manufacturer of the immunosynthesis columns you used during the analysis!

17. What type of detection method did you use? *
   - a) HPLC-FLD
   - b) LC-MS/MS
   - c) other

17.1. If HPLC-FLD, please specify your method (type of column, injection volume, mobile phase etc.)! *

17.2. If LC-MS/MS, please specify your method! *

17.3. If other, please specify the type of your method! *

18. How did you integrate the signals?
   - Automatic
   - Manual

19. Did you encounter any problems during the analysis? *
   - a) Yes
   - b) No

19.1. IF YES, what were the specific problems and to which samples do they apply? *

20. Did you notice any unusual observations which, however, did not seem to have any effect on the results? *
   - a) Yes
   - b) No

20.1. IF YES, what were these observations and to which samples do they apply? *

21. Did you find the instructions distributed for this PT adequate? *
   - a) Yes
   - b) No

21.1. If NO, which parts do you think can improve? *

22. What is your opinion about the registering/reporting format of this interface?

23. Any other comments you wish to address?
## 13.6. Experimental details

Results and method performance characteristics for DON

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>Technique</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Coverage factor</th>
<th>Recovery [%]</th>
<th>LOD [μg/kg]</th>
<th>LOQ [μg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>LC-MS/MS</td>
<td>897</td>
<td>279</td>
<td>2</td>
<td>70</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>102</td>
<td>LC-MS/MS</td>
<td>668</td>
<td>295</td>
<td>2</td>
<td>95</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>103</td>
<td>HPLC</td>
<td>512</td>
<td>246</td>
<td>2</td>
<td>100</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>104</td>
<td>LC-MS/MS</td>
<td>495</td>
<td>1068</td>
<td>2</td>
<td>100.5</td>
<td>86</td>
<td>21.5</td>
</tr>
<tr>
<td>105</td>
<td>HPLC</td>
<td>800</td>
<td>400</td>
<td>2</td>
<td>78.8</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>106</td>
<td>HPLC</td>
<td>596.5</td>
<td>448.9</td>
<td>2</td>
<td>56.8</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>107</td>
<td>LC-MS/MS</td>
<td>502.7</td>
<td>86.5</td>
<td>2</td>
<td>102</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>108</td>
<td>LC-MS/MS</td>
<td>605</td>
<td>255</td>
<td>2</td>
<td>91</td>
<td>35</td>
<td>64</td>
</tr>
<tr>
<td>109</td>
<td>HPLC</td>
<td>792.4</td>
<td>321.94</td>
<td>2</td>
<td>66.88</td>
<td>52</td>
<td>156</td>
</tr>
<tr>
<td>110</td>
<td>HPLC</td>
<td>507</td>
<td>266</td>
<td>2</td>
<td>90</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>111</td>
<td>HPLC</td>
<td>590.5</td>
<td>275.1</td>
<td>2</td>
<td>88.7</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>112</td>
<td>HPLC</td>
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<td>213.6</td>
<td>2</td>
<td>95</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>113</td>
<td>GC-MS</td>
<td>720</td>
<td>340</td>
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<td>83</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>114</td>
<td>LC-MS/MS</td>
<td>577</td>
<td>260</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>115</td>
<td>LC-MS/MS</td>
<td>491</td>
<td>240</td>
<td>2</td>
<td>109</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>116</td>
<td>LC-MS/MS</td>
<td>593.4</td>
<td>324.4</td>
<td>2</td>
<td>69.2</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>117</td>
<td>HPLC</td>
<td>578</td>
<td>268</td>
<td>2</td>
<td>96</td>
<td>20</td>
<td>50</td>
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<td>2</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>119</td>
<td>LC-MS/MS</td>
<td>795.3</td>
<td>325.9</td>
<td>2</td>
<td>89.2</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>120</td>
<td>HPLC</td>
<td>431</td>
<td>241</td>
<td>2</td>
<td>79</td>
<td>11</td>
<td>3.2</td>
</tr>
<tr>
<td>121</td>
<td>HPLC</td>
<td>428.3</td>
<td>268.4</td>
<td>2</td>
<td>90.9</td>
<td>15</td>
<td>49.5</td>
</tr>
<tr>
<td>122</td>
<td>HPLC</td>
<td>651.1</td>
<td>284.4</td>
<td>2</td>
<td>94.75</td>
<td>not determined</td>
<td>50</td>
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<td>HPLC/DAD</td>
<td>586.2</td>
<td>252.2</td>
<td>2</td>
<td>99</td>
<td>25</td>
<td>80</td>
</tr>
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<td>HPLC</td>
<td>771.2</td>
<td>327.5</td>
<td>2</td>
<td>89</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>125</td>
<td>GC-MS</td>
<td>590</td>
<td>286</td>
<td>2</td>
<td>96</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>126</td>
<td>HPLC</td>
<td>642</td>
<td>280</td>
<td>2</td>
<td>79.5</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>127</td>
<td>LC-MS/MS</td>
<td>420</td>
<td>348</td>
<td>2</td>
<td>94</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>128</td>
<td>LC-MS/MS</td>
<td>558.77</td>
<td>256.67</td>
<td>2</td>
<td>98.58</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td>129</td>
<td>LC-MS/MS</td>
<td>661</td>
<td>297</td>
<td>2</td>
<td>107</td>
<td>19</td>
<td>57</td>
</tr>
<tr>
<td>130</td>
<td>GC-MS</td>
<td>596.2</td>
<td>229</td>
<td>2</td>
<td>91</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>131</td>
<td>LC-MS/MS</td>
<td>581.5</td>
<td>248.5</td>
<td>2</td>
<td>93.5</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>132</td>
<td>No result</td>
<td>No result</td>
<td>No result</td>
<td>No result</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>LC-MS/MS</td>
<td>505.5</td>
<td>202.2</td>
<td>2</td>
<td>100</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>134</td>
<td>LC-MS</td>
<td>450</td>
<td>179</td>
<td>2</td>
<td>85.4</td>
<td>35</td>
<td>115.0</td>
</tr>
<tr>
<td>135</td>
<td>LC-MS/MS</td>
<td>450</td>
<td>266</td>
<td>2</td>
<td>109</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>
### Results and method performance characteristics for ZON

| Lab Code | Technique | Sample A |  | Sample B |  | Coverage factor | Recovery [%] | LOD [μg/kg] | LOQ [μg/kg] |
|----------|-----------|----------|  |----------|  |                |              |             |             |
| 101      | LC-MS/MS  | 528      | 24 | 23       | 24 | 2              | 97           | 10          | 20          |
| 102      | HPLC      | 554      | 70.8| 25.7     | 5.14| 2              | 92           | 2           | 10          |
| 103      | HPLC      | 579      | 141 | 32       | 12  | 2              | 72           | 5           | 10          |
| 104      | LC-MS/MS  | 406      | 60.5| <25      | 24 | 2              | 80           | 1           | 2           |
| 105      | HPLC      | 414      | 35  | 33       | 2.84| 2              | 102.7        | 6           | 11          |
| 106      | HPLC      | 444.1    | 55.5| 37.5     | 4.7 | 2              | 110          | 1           | 3           |
| 107      | LC-MS/MS  | 482.7    | 95.5| 33.2     | 6.6 | 2              | 97           | 10          | 20          |
| 108      | LC-MS/MS  | 489      | 196 | 23       | 9   | 2              | 97           | 1           | 2           |
| 109      | HPLC      | 393.93   | 71  | 22.13    | 6.67| 2              | 104.81       | 10          | 20          |
| 110      | HPLC      | 404      | 121 | 29       | 9   | 2              | 90           | 3           | 10          |
| 111      | HPLC      | 475.5    | 85.6| 20.5     | 3.7 | 2              | 104.2        | 3           | 10          |
| 112      | HPLC      | 493      | 123.3| 30.2     | 7.6 | 2              | 99           | 2           | 5           |
| 113      | HPLC      | <3       |     |          |    |                |              | 80          | 3           | 6           |
| 114      | LC-MS/MS  | 514      | 226 | 30       | 13  | 2              | 100          | 10          | 10          |
| 115      | HPLC      | 481      | 173.2| 30.5     | 8.2 | 2              | 106          | 0.5         | 10          |
| 116      | LC-MS/MS  | 585      | 321.2| 39.6     | 11.6| 2              | 86           | 6           | 20          |
| 117      | HPLC      | 476      | 143 | 30.3     | 9.1 | 2              | 91           | 2           | 5           |
| 118      | HPLC      | 310      | 62  | <25      |    |                |              | 120         | 8           | 25          |
| 119      | HPLC      | 456.4    | 78  | 29.2     | 7.6 | 2              | 89.4         | 0.3         | 3           |
| 120      | HPLC      | 504      | 10  | 32       | 10  | 2              | 95           | 0.9         | 2.7         |
| 121      | HPLC      | 433      | 71.9| 26.4     | 4.4 | 2              | 91           | 10          | 33          |
| 122      | HPLC      | 472.1    | 23.6| 52.1     | 1.6 | 2              | 101.91       | not determined | 20        |
| 123      | HPLC:FLD  | 414.3    | 103.6| 30.9     | 15.5| 2              | 72           | 5           | 15          |
| 124      | HPLC      | 481.6    | 72.2| 24.5     | 3.7 | 2              | 96           | 6.5         | 20          |
| 125      | HPLC      | 414      | 124.2| 29       | 8.7 | 2              | 100          | 5           | 15          |
| 126      | HPLC      | 493      | 79  | 31.5     | 5   | 2              | 98.9         | 2           | 4           |
| 127      | LC-MS/MS  | 267      | 3   | 26.5     | 3   | 2              | 72           | 5           | 2           |
| 128      | HPLC      | 465.56   | 49.35| 32.82    | 1.48| 2              | 95           | 0.22        | 0.43        |
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| 130      | HPLC      | 428      | 74  | 28       | 5   | 2              | 79.5         | 20          | 50          |
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| 132      | HPLC      | 462.2    | 75  | 29.5     | 6.9 | 2              | 99.4         | 5           | 15          |
| 133      | LC-MS/MS  | 349.7    | 139.9| 25       | 10  | 2              | 79           | 10          | 25          |
| 134      | LC/MS     | 436      | 152.6| 33       | 11.6| 2              | 98           | 45          | 15          |
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## Results and method performance characteristics for T-2

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How many samples does your laboratory analyse for the following mycotoxins per year?

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>DON</th>
<th>ZON</th>
<th>T-2</th>
<th>HT-2</th>
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<td>&lt;50</td>
</tr>
</tbody>
</table>
Which food or feed matrices does your laboratory analyse for DON, ZON, T-2 and HT-2 on a routine basis the most? (maximum 3)

Are you accredited for the determination of these mycotoxins from cereals?

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>Analysed matrices on a routine basis</th>
<th>Accredited</th>
<th>DON</th>
<th>ZON</th>
<th>T-2</th>
<th>HT-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>wheat and wheat products, maize and maize products</td>
<td>√</td>
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<tr>
<td>102</td>
<td>corn, wheat, mixed feed</td>
<td>√</td>
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</tr>
<tr>
<td>103</td>
<td>cereals</td>
<td>√</td>
<td></td>
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<tr>
<td>104</td>
<td>feed for poultry, feed for swine</td>
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<tr>
<td>105</td>
<td>Feed-DON, ZON, T-2</td>
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<tr>
<td>106</td>
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<td>√</td>
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<tr>
<td>107</td>
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<td>108</td>
<td>cereals, feed, straw</td>
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<td>109</td>
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<tr>
<td>111</td>
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<td>112</td>
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<td>cereals (wheat, barley, oats), feed mixtures</td>
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<td>varied feed for cattle, pigs and poultry + ingredients</td>
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<tr>
<td>116</td>
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<td>food, cereals, cereal flour, pasta</td>
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<td>flour, cereals, baby food</td>
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<td>121</td>
<td>cereals, breakfast cereals, pasta</td>
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<td>maize, other cereal products, cereal-based baby foods</td>
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<td>we are accredited for DON, HT-2 and T-2 in flour and oat (not ZON)</td>
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<td>feed material, compound feedingstuffs for all species</td>
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<td>raw cereals, feed pellets</td>
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<tr>
<td>Lab Code</td>
<td>Sample amount (g)</td>
<td>Solvent to sample ratio</td>
<td>Extraction solvent</td>
<td>Extraction mode</td>
<td>Extraction time</td>
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<tr>
<td>101</td>
<td>25 g</td>
<td>1.28</td>
<td>MeOH+AcCN:H2O (3:1:3.5 v/v)</td>
<td>blending</td>
<td>3 min</td>
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<tr>
<td>102</td>
<td>25 g</td>
<td>100:15</td>
<td>acetonitrile/water (75:25)</td>
<td>shaking</td>
<td>2 hours</td>
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<tr>
<td>103</td>
<td>DON, ZON, 5 g</td>
<td>T2-HT2: 25 g</td>
<td>acetonitrile/water (75:25) for ZON, water for DON, methanol/water (90:10) for T2-HT2</td>
<td>Ultra-Turrax</td>
<td>2 min for deoxynivalenol and zearalenone, 3 min for T2-HT2</td>
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<td>104</td>
<td>1 g</td>
<td>8</td>
<td>ethyl acetate</td>
<td>shaking</td>
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<td>105</td>
<td>DON: 25 g</td>
<td>ZON: 5 g</td>
<td>DON 200 ml water</td>
<td>blending</td>
<td>5 min</td>
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<td>106</td>
<td>DON: 20 g</td>
<td>T2, HT2: 4 g</td>
<td>PEG+water (DON); MeOH+w+acetic ac. (T-2, HT-2)</td>
<td>blending</td>
<td>3 min (DON), 2 min (ZON), 3 min (T-2, HT-2)</td>
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<tr>
<td>107</td>
<td>25 g</td>
<td>8</td>
<td>water for DON, ACN/water for ZON, MeOH/water for T2 HT2</td>
<td>blending</td>
<td>3 min</td>
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<td>10 g</td>
<td>5</td>
<td>AcN-H2O-HCOOH</td>
<td>shaking</td>
<td>1 h</td>
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<td>109</td>
<td>5 g</td>
<td>5 g (40 ml for DON; 5 g/25 ml for ZON, T2, HT2)</td>
<td>DON: distillated water</td>
<td>shaking</td>
<td>20 minutes</td>
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<td>DON: 25 g</td>
<td>T2, HT2: 2 g</td>
<td>DON: 40 g</td>
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<td>5 g</td>
<td>25 g, 5 g/T2 HT2: 8</td>
<td>DON: H2O+PEG</td>
<td>shaking</td>
<td>2 hours</td>
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<td>112</td>
<td>6 g</td>
<td>5 ml/g</td>
<td>For DON - water, 5 ml/g</td>
<td>shaking</td>
<td>1 hour (DON), 30 min (ZON)</td>
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<td>ZON: 20 g</td>
<td>T2, HT2: 2.5 g</td>
<td>ZON: CH3CN: H2O: T2, HT2: Ethyl Acetate</td>
<td>shaking</td>
<td>120 min (DON, HT2, T2)</td>
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<td>114</td>
<td>2.5 g</td>
<td>4</td>
<td>Acetonitrile / Water / Formic Acid = 84:16/1</td>
<td>shaking</td>
<td>2 h</td>
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<td>DON, T2, HT2: 20 g</td>
<td>ZON: 25 g</td>
<td>Trics (DON, T2, HT2)</td>
<td>Trics ACN/H2O 84:16</td>
<td>5 minutes</td>
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<td>116</td>
<td>10 g - 0.1 g</td>
<td>4 ml/g</td>
<td>B14:16 Acetonitrile/Water</td>
<td>shaking</td>
<td>3 min</td>
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<td>117</td>
<td>25 g</td>
<td>4</td>
<td>ZON: Acetonitrile-water, HT-2 and T-2: Methanol-water</td>
<td>shaking</td>
<td>120 min</td>
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<td>118</td>
<td>25 g for DON, 5 g for ZON, 2 g for T-2 &amp; HT-2</td>
<td>8 ml/g for DON, 4 ml/g for ZON, 5 ml/g for T-2 &amp; HT-2</td>
<td>acetonitrile/water (84/16, v/v) for ZON, acetonitrile/water/acetic acid (79/21, v/v) for T-2 &amp; HT-2</td>
<td>vortex-mixing and shaking</td>
<td>0.5 h for DON, 1.5 h for T2 &amp; HT2</td>
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<td>DON, HT2, 12.5 g</td>
<td>ZON: 25 g</td>
<td>8 (DON), 4 (ZEA, HT-2/7)</td>
<td>water (DON)</td>
<td>AcN/Acetone:75%/25% (ZEA)</td>
<td>AcN/Acetone:80:20% (HT-2/7)</td>
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<td>DON: 15 g</td>
<td>2 g</td>
<td>DON: H2O, ZON- MEOH/H2O 75:25; T2-HT2- MEOH/H2O 90:10</td>
<td>DON- shaking, ZON - shaking, T2-HT2 - blending</td>
<td>DON: 20m, ZON- 60m, T2-HT2 - 2m</td>
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<td>DON: 20 g</td>
<td>ZON: 25 g</td>
<td>ACN/H2O</td>
<td>blending and shaking</td>
<td>3 min</td>
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<td>122</td>
<td>DON: 25 g</td>
<td>ZON: 5 g</td>
<td>DON UPW; ZON 75:25 ACN/UPW; T-2 and HT-2 Ethyl acetate</td>
<td>DON Blender, ZON Blender: T-2 and HT-2</td>
<td>DON 2 min (ZON), 2 min (T-2 and HT-2), 30 min (DON)</td>
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<td>10</td>
<td>DON/H2O, ZON/MeOH/H2O, T-2/MeOH/H2O</td>
<td>shaking and sonication</td>
<td>60 min</td>
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<td>DON: 20 g</td>
<td>ZON: 20 g</td>
<td>100:10 ml/g ZON 50:20 ml/g ZON</td>
<td>blending DON, shaking</td>
<td>3 min ZON, 30 min ZON</td>
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<td>125</td>
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<td>acetonitrile/water/B14:16 v/v</td>
<td>stirring</td>
<td>2 hours</td>
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<td>25 g</td>
<td>4</td>
<td>DON, ZEA Acetonitrile 84 %, T2, HT2: Acetonitrile H20:HAc 79:20:1</td>
<td>shaking</td>
<td>30 min</td>
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<td>127</td>
<td>5 g</td>
<td>4</td>
<td>Acetonitrile/water</td>
<td>shaking</td>
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<td>128</td>
<td>ZON: 12.5 g</td>
<td>HT2: 10 g</td>
<td>Acetonitrile/water / (75:25) for ZON, Acetonitrile / water (84/16) for DON, HT2</td>
<td>blending for ZON HT2 and T2, shaking for DON</td>
<td>3 minutes for ZON HT2 and T2, one hour for DON</td>
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</tr>
<tr>
<td>129</td>
<td>10 g</td>
<td>40:10</td>
<td>Acetonitrile/Water: B14:16</td>
<td>shaking</td>
<td>2 hours</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>DON, T2, HT2:10g</td>
<td>10 g</td>
<td>Acetonitrile/water/B14:16 v/v</td>
<td>shaking</td>
<td>1 hour</td>
<td></td>
</tr>
<tr>
<td>131</td>
<td>DON, T2, HT2:10g</td>
<td>10 ml/g</td>
<td>Acetonitrile/water/B14:16 v/v</td>
<td>shaking</td>
<td>1 hour</td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>10 g</td>
<td>190:20</td>
<td>Med/HT Water (75:25)</td>
<td>shaking</td>
<td>2 hours</td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>4 g</td>
<td>100%</td>
<td>ACN 80%, HAc 1%, water 19%</td>
<td>shaking (overhead)</td>
<td>1 hour</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>DON, T2, HT2: 20 g</td>
<td>ZON: 25 g</td>
<td>Trics ACN/H2O 84:16</td>
<td>Trics ACN/H2O 75:25</td>
<td>blending</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>
What type of clean up methodology was used (e.g. immunoaffinity column)?
If you used immunoaffinity columns please specify the manufacturer of the immunoaffinity columns you used during the analysis!
What is your main procedure for recovery estimation?
During the analysis did you need to include any over night stop?
How did you integrate the signals?

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>Clean up</th>
<th>If IAC: manufacturer</th>
<th>Recovery estimation</th>
<th>Over night stop</th>
<th>Integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>immunoaffinity</td>
<td>R-Biopharm</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>102</td>
<td>mixed bed column</td>
<td>Other: R-Biopharm</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>103</td>
<td>immunoaffinity column</td>
<td>R-Biopharm</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>104</td>
<td>phase separation</td>
<td>Internal Standard to Extract</td>
<td>No</td>
<td>Automatic</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>immunoaffinity columns</td>
<td>R-Biopharm Rhone DON YJ388/50, ZON YE 309/50, T2 YC 283/50</td>
<td>Other: CRM DON, CRM ZON, spiked sample for T2</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>106</td>
<td>IAC (DON, ZON)</td>
<td>Vicam: DonTest, Zeaalarat</td>
<td>Other: Standard solution to Sample</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>107</td>
<td>immunoaffinity column</td>
<td>R-Biopharm Rhone</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>108</td>
<td>MultiSep 226</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>DON prep, Easi extract Zearalenone, Easi extract T2</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>immuno-affinity for DON, ZON</td>
<td>R-Biopharm and Neogen</td>
<td>Standard solution to Blank</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>111</td>
<td>immunoaffinity columns</td>
<td>R-BIOPHARM RÔNE LTD</td>
<td>Standard solution to Blank</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>112</td>
<td>Immunoaffinity Columns</td>
<td>ROMER</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>113</td>
<td>IAC-column (ZON), MycoSep#227 (DON, HT-2, T-2)</td>
<td>Rhone Diagnostics (ZON)</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>114</td>
<td>no clean up</td>
<td>Standard solution to Blank</td>
<td>Yes</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>Trics - MYCOSEP ZON - Immunoaffinity column</td>
<td>R-Biopharm Rhone</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>116</td>
<td>solid phase filtration (Romer 226)</td>
<td>Internal Standard to Extract</td>
<td>No</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>immunoaffinity column</td>
<td>R-Biopharm Rhone Ltd</td>
<td>Internal Standard to Sample</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>118</td>
<td>Immunoaffinity SPE for DON, Immunoaffinity SPE for ZON, Strata-X SPE for T-2 &amp; HT-2</td>
<td>ROMER</td>
<td>Other: standard addition to sample prior to extraction</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>119</td>
<td>IAC (ZEA, DON) Bond Elute mycotoxin (HT-2/T-2)</td>
<td>R-BioPharm</td>
<td>Other: standard solution to sample</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>120</td>
<td>immunoaffinity column</td>
<td>DON: R-BIOPHARM DONPREP, ZON-R-BIOPHARM EASI-EXTRACT, T2+HT2-R-BIOPHARM EASI-EXTRACT</td>
<td>Internal Standard to Extract</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>121</td>
<td>immunoaffinity column</td>
<td>VICAM</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>122</td>
<td>Immunoaffinity column in all cases</td>
<td>R-Biopharm Rhone</td>
<td>Other: Spiking of samples</td>
<td>Yes</td>
<td>Manual</td>
</tr>
<tr>
<td>124</td>
<td>immunoaffinity column</td>
<td>R-BIOPHARM DON, VICAM ZON</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>127</td>
<td>MycoSep Columns</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>immunoaffinity columns for ZON, SPE for DON, HT2 and T2</td>
<td>VICAM for ZON, ROMER for DON, HT2 and T2</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>129</td>
<td>Mycosep columns no.225</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>Mycosep (DON, T2-HT2), immunoaffinity (ZON)</td>
<td>R-Biopharm Rhone</td>
<td>Internal Standard to Sample</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>131</td>
<td>MycoSep227Ttthe+Romer Labs and Easy Extract Zearalenon R Biopharm Rhone LTD</td>
<td>Easy Extract Zearalenon R Biopharm Rhone LTD</td>
<td>Internal Standard to Extract</td>
<td>No</td>
<td>Automatic</td>
</tr>
<tr>
<td>132</td>
<td>immunoaffinity column</td>
<td>VICAM</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>133</td>
<td>none</td>
<td>Other: standard additions curves (1g sub-samples spiked at 0-10-25-50-500-1000 μg/kg (plus 13-C IS to extract for matrix effect corrections)</td>
<td>Yes</td>
<td>Automatic</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>immunoaffinity column</td>
<td>R-Biopharm Rhone Ltd</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Manual</td>
</tr>
<tr>
<td>135</td>
<td>Trics – MYCOSEP ZON - Immunoaffinity column</td>
<td>R-Biopharm Rhone</td>
<td>Standard solution to Blank</td>
<td>No</td>
<td>Automatic</td>
</tr>
</tbody>
</table>
Did you encounter any problems during the analysis?
Did you notice any unusual observations which, however, did not seem to have any effect on the results?

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>Problems</th>
<th>Unusual observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>102</td>
<td>No</td>
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</tr>
<tr>
<td>103</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>104</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>105</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>106</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>107</td>
<td>Yes - Gel formation during T2 HT2 extraction with MeOH/H2O. Filtration not possible if not centrifuged first.</td>
<td>No</td>
</tr>
<tr>
<td>108</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>109</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>110</td>
<td>No</td>
<td>No</td>
</tr>
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</tr>
<tr>
<td>112</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>113</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>114</td>
<td>No</td>
<td>Yes - during extraction, sample material sticked to extraction tube</td>
</tr>
<tr>
<td>115</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>116</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>117</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>118</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>119</td>
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<td>120</td>
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<td>No</td>
</tr>
<tr>
<td>121</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>122</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>123</td>
<td>Yes - IAC - ZON unexpected very low recovery</td>
<td>No</td>
</tr>
<tr>
<td>124</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>125</td>
<td>No</td>
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<tr>
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<tr>
<td>134</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>135</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Did you find the instructions distributed for this PT adequate?
What is your opinion about the registering / reporting format of this interface?
Any other comments you wish to address?

<table>
<thead>
<tr>
<th>Lab Code</th>
<th>Instructions</th>
<th>Registering / reporting format</th>
<th>Any other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>Yes</td>
<td>very clear and time saving</td>
<td>The recovery factor value for T2 toxin (29%) is quite low (three replicates), but we decided to report anyway the results for T2 toxin.</td>
</tr>
<tr>
<td>102</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Yes</td>
<td>satisfied</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>Yes</td>
<td>good</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>Yes</td>
<td>lack of button 'save and return to main page'</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>Yes</td>
<td>adequate</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>Yes</td>
<td>Works well now (after some improvements)</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>Yes</td>
<td>Better than before</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>Yes</td>
<td>very good</td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>Yes</td>
<td>very feasible</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Yes</td>
<td>perfect</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Yes</td>
<td>Point. 3 Accreditation for DON and ZON is not yet accepted but it was not possible to send this report without any value in 3.</td>
<td>PT-tests are very important for us. This was the first time we analysed HT-2 and T-2 with LC-MS/MS.</td>
</tr>
<tr>
<td>118</td>
<td>Yes</td>
<td>Quite good</td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>Yes</td>
<td>too cumbersome</td>
<td>why is there still a need for a signed and a stamped report? who is not trusted? the lab or the https web site or both?</td>
</tr>
<tr>
<td>120</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>Yes</td>
<td>The question 3 of the questionnaire is not correct. Because if we are not accredited in any of the methods we cannot proceed the validation and submission of the questionnaire. So we had to tick at DON to proceed.</td>
<td>We are not accredited for the DON, ZON, T2, HT-2 methods of analysis</td>
</tr>
<tr>
<td>122</td>
<td>Yes</td>
<td>User friendly, no problems encountered</td>
<td>This form was designed with a single multianalyte method in mind!</td>
</tr>
<tr>
<td>123</td>
<td>Yes</td>
<td>The reporting format is very clear.</td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Yes</td>
<td>little bit too less place in point 22.</td>
<td>LCMS/MS: DON=453/213;HT2=168/45;T2=5629;ZON=492/32; LC DON=633/519 (sample A/B)</td>
</tr>
<tr>
<td>126</td>
<td>Yes</td>
<td>It is not clear how to get further from one page to another.</td>
<td></td>
</tr>
<tr>
<td>127</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>129</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>Yes</td>
<td>Registering format ok, problems with reporting format.</td>
<td></td>
</tr>
<tr>
<td>131</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>Yes</td>
<td>Good</td>
<td>The sample was not transported with refrigeration.</td>
</tr>
<tr>
<td>133</td>
<td>Yes</td>
<td>straightforward (except absence of 'return' button after filling in results and quest.)</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>Yes</td>
<td>OK!</td>
<td>Please, send blank sample (same matrix as sample) in the next PT too</td>
</tr>
<tr>
<td>135</td>
<td>Yes</td>
<td>OK</td>
<td></td>
</tr>
</tbody>
</table>
Abstract

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of deoxynivalenol (DON), zearalenone (ZON), T-2 and HT-2 in cereal samples.

Thirty-five participants from 27 countries registered for the exercise. 34 (Sample A) & 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

Only z-scores for DON and ZON were calculated and used for benchmarking and in total about 95 % of the attributed z scores were below an absolute value of two for these two mycotoxins, which indicated that most of the participants performed satisfactory or better.
As the Commission’s in-house science service, the Joint Research Centre’s mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new standards, methods and tools, and sharing and transferring its know-how to the Member States and international community.

Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security including nuclear; all supported through a cross-cutting and multi-disciplinary approach.