Proficiency test for ergot sclerotia in cereals

EURLPT-MP05 (2020)

D.P.K.H. Pereboom, J.B.G.M. Hedemann, C.P.A.F. Smits, M. de Nijs, L.W.D. van Raamsdonk

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Wageningen, April 2021

WFSR report 2021.002

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D.P.K.H. Pereboom, J.B.G.M. Hedemann, C.P.A.F. Smits, M. de Nijs, L.W.D. van Raamsdonk, 2021. *Proficiency test for ergot sclerotia in cereals; EURLPT-MP05 (2020).* Wageningen, Wageningen Food Safety Research, WFSR report 2021.002. 34 pp.; 5 fig.; 2 tab.; 21 ref.

Project number: WOT-02-001-064 1297362201-EURLMP
Project title: EURL mycotoxins & plant toxins 2020 (EURLMP 1.3. EURLPT-MP05 SCL)
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This report can be downloaded for free at https://doi.org/10.18174/541805 or at www.wur.eu/food-safety-research (under WFSR publications).

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Preface

A Proficiency Test (PT) was organised by the EURL mycotoxins & plant toxins in food and feed in cooperation with IAG section Feed Microscopy on the detection of ergot sclerotia in cereals. The National Reference Laboratories on mycotoxins & plant toxins in food and feed (NRLs) of the EU Member States and EFTA countries, as well as IAG members were invited to participate. The NRLs were welcome to pass the invitation to the official laboratories (OLs).

In the view of the EU harmonised legal limits for ergot sclerotia in food and feed, two different spike levels have been used for preparing the samples. A procedure for individual spiking of each sample was followed due to the situation that granular impurities are non-uniformly distributed in granular matrices.

The weight of each sample material and the weight and number of the spiked sclerotia were recorded for each individual sample in order to verify the performance of each participant. Every participant received one sample of 2 kg, either contaminated at a low (400 mg/kg) (24 samples) or at high (800 mg/kg) level (24 samples). Due to the situation that the amount of sample material observed has a major influence on the reliability of the result of the examination, comparability of different methods and strategies is lacking. Therefore, the participants were given the suggestion to apply the method described by the EURL mycotoxins & plant toxins. This PT was designed in compliance with the requirements of ISO 17043:2010.

In short, the EURL mycotoxins & plant toxins method is based on total sample material of 2 kg, divided in four equal portions of approximately 500 grams, and examination of two portions. The contamination level, expressed as mg sclerotia/kg sample, calculated from these two portions is the final result in the situation that this level does not exceed a fixed analytical threshold (60% of the legal limit). The threshold limit proposed in the framework of this PT is 600 mg/kg, 60% of the legal limit for feed. The remaining two portions need to be examined when the initial contamination level exceeds the analytical threshold. To allow proper statistical analysis of the results, the participants were asked to report the contamination level on the initial results of the first two portions, and, regardless of the excess of the analytical threshold, also to examine the two other portions. The report of an indication was requested whether or not this analytical threshold was exceeded.

Forty-eight laboratories, including 22 NRLs (representing 17 Member States (MS), one EFTA country and one Candidate MS), 20 laboratories from IAG members (representing 6 Member States and Switzerland) and six OLs (representing Germany and the UK) participated in the EURLPT-MP05 on the quantitative determination of sclerotia in cereals (rye) (Annex 1).

The results of forty-seven laboratories were included for the evaluation of the results. Results from one participant were excluded from evaluation since the results were not submitted correctly. Forty-four participants submitted results for each quarter of the sample, two participants for two quarter samples and participant submitted one result for the whole sample. The results were assessed for the reported contamination level in mg/kg versus the spiked level by calculating the contamination using the submitted data for the individual sample quarters or the reported value over the 2 kg sample. For the number of sclerotia(-part) counted and for the decision on the threshold value.

The way of implementation of the EURLMP method by participants was verified by calculating the standard deviation of the four portion weights, by comparing the contamination level after two (AVG-2) and after all four (AVG-4) portions as calculated by the participants with the individual results for each of the four portions, and by checking the reported indication of excess of the threshold.

A share of 32 participants obtained values for the standard deviation of the portion weights below 10 mg/kg. Higher values were calculated from the results of 10 participants. Five participants reported

exactly 500 grams or 505 grams for all four portion weights, which would result in a standard deviation of zero. The reported levels after two portions and after all four portions have been verified by means of a calculation based on the reported portion results. A deviation of less than 1 mg/kg can be due to rounding off to a result without decimals. A deviation equal to or exceeding 1 mg/kg would point to a calculation error. This was encountered in the reports of nine (AVG-2) and five (AVG-4) participants.

The deviation between the spiked amounts and the reported results based on the examination of two portions (AVG-2 based on 1 kg) is considerably larger than reported after the examination of all four portions (AVG-4 based on the entire 2 kg of sample material).

Forty of the 47 participants showed satisfactory results. Seven participants reported final contamination levels outside the interval 95%-105%, three participants reported higher weights, and four participants reported lower weights than added.

The participants were furthermore evaluated for number of sclerotia and decision after examination of two quarters of the samples. A total of 38 of 47 participants (81%) reported numbers of sclerotia detected in the 2 kg sample within the two units difference. Five participants reported a difference larger than two units of sclerotia with the spiked number, one participant reported more than two units above the added number and four participants reported more than two units under the added number. Two participants reported no results for the number of sclerotia and two participants reported results for two quarters.

Forty-three participants (91%) did correctly indicate the decision after examination of two subsamples.

With regard to the evaluation, the participants showed in general a good performance.

1 Introduction

Some of the first documented cases of food poisoning caused by infested plant ingredients are the consumption of bread from cereals infested by ergot sclerotia (spore bodies of the mould *Claviceps purpurea*) in north Norway early 17th Century (Alm, 2003) and in New England in the late 17th Century (the Salem witchcraft trials; Woolf, 2000), among other cases (Scott, 2009). The pattern of symptoms of the intoxication was already known as Saint Anthony's fire from the Middle Ages (Lee, 2009a) and is currently indicated as ergotism (Mulder et al., 2012). The causing substances, ergot alkaloids, show a varying content in individual sclerotia (Lorenz and Hoseney, 1979; EFSA, 2005; EFSA, 2012). Notwithstanding this, the monitoring of ergot sclerotia is an effective measure for prevention, and the detection of sclerotia is still the target of the official control.

Fungal infections by *C. purpurea* are most commonly found in rye, triticale, wheat, barley, oat and some genera of grass, in decreasing order of their probability. The fungus replaces the developing grain or seed with a characteristic dark coloured body (sclerotium) with the shape of the original cereal grain, containing the alkaloids. Sclerotia are harvested together with the cereal grains or grass seeds and may thus lead to contamination of cereal-based food and feed products with ergot alkaloids. Ergotism is still an important human health and veterinary problem (EFSA, 2017; Gupta, 2018).

The legal limits for presence of sclerotia in cereals are different for feed and food. European Directive 2002/32/EC (European Commission, 2002) sets a maximum allowed amount of 1000 mg/kg sclerotia in unground cereals for animal feed application. The current limit in unprocessed cereals with the exception of maize and rice intended for use as food ingredient is 500 mg/kg (Regulation (EC) 1881/2006 (European Commission, 2006), amended by Regulation (EU) 2015/1940 (European Commission, 2015)).

Proficiency Tests (PTs) are being organised to provide participants the opportunity to evaluate their performance for the detection of the target. Proficiency testing is an important element in quality control (Regulation (EU) 2017/625 (European Union, 2017); ISO/IEC 17025:2017). Organisation of PTs is one of the tasks of the European Union Reference Laboratories (EURLs) (Regulation (EU) 2017/625). This PT is being organised by the EURL mycotoxins & plant toxins in cooperation with IAG section Feed Microscopy (https://www.iag-micro.org/). The primary goal is to document the proficiency of the National Reference Laboratories (NRLs). Simultaneously, official laboratories (OLs) and IAG members were also welcomed to participate.

There are several methods published for the detection of ergot sclerotia in cereals, most notably by CEN (CEN, 2018), IAG (IAG, 2008), and the German organisation VDLUFA (VDLUFA, 2007). These methods differ primarily in the amount of sample material examined. This ranges from 250 grams in the CEN and IAG procedures to 2 kg in Annex II of Regulation (EC) 152/2009 (European Commission, 2009). The smaller the examined amount of sample material, the higher the probability that the analysed portion is not representative for the original aggregate sample. This deviation is expected to be higher at lower contamination levels. The background to this situation is described in the *Quality Guidelines for visual monitoring methods* (in preparation). Therefore, WFSR has developed a procedure based on the examination of a fixed amount of material in a prescribed strategy allowing quality control of the performance. This method was adopted by the EURL (EURLMP, 2020). Proficiency testing principally allows the application of modified or alternative methods, which are intended to examine the target of the PT. In the situation that the amount of material observed has a major influence on the reliability of the result of the examination, comparability of different methods and strategies is lacking. Therefore, the participants were recommended to apply the EURL method.

2 PT Material

2.1 Scope of the PT

The PT was intended to give laboratories the opportunity to evaluate and demonstrate their performance for the detection of ergot sclerotia in cereal products. In the view of the different legal limits for food and feed, two different spike levels have been used for preparing the samples. Every participant of the PT received one sample, either contaminated at a low or a high level of sclerotia and were asked to report the results in mg/kg. In addition, the participants were additional asked to also report the results of the weight and count of sclerotia per quarter weight of the sample.

2.2 Material preparation

A total of 100 kg commercially obtained rye grains has been examined for the presence of (fragments of) ergot sclerotia and of mimicking mould particles in September 2020. As far as discovered, these particles have been removed. Other impurities such as broken grains, sprouted grains, other grain impurities and miscellaneous non-cereal material (Besatz; CEN 15587:2018) and moulded grains were not removed. The cleaned material was split in cereal samples of two kilograms of which the weight was recorded, and each cereal sample was placed in a firm plastic bag and labelled.

Ergot sclerotia were selected from the stock at Wageningen Food Safety Research (WFSR). Portions of ergot sclerotia, in whole or as fragments, were selected to form either a total of approximately 800 mg (concentration in the end-product 400 mg/kg) or approximately 1600 mg (concentration in the end-product 800 mg/kg) material per sample. The number of units (entire sclerotia and sclerotia fragments) and the exact total weight was documented for every sclerotia sample. The sclerotia samples obtained an individual code.

Every cereal sample was spiked with one portion of sclerotia, which was evenly distributed over the sample material by stirring in September 2020. The weight of each cereal sample, the total weight of the sclerotia spike and number of sclerotia (parts) spiked (indicated by a unique identification) were recorded for each final sample.

2.3 Sample identification

All final samples have been labelled uniquely. Each individual portion of spike material was uniquely labelled as well. The unique relation between every sample and the portion used for spiking has been documented.

The sample for the participants was randomly selected and coded using a web application designed for PTs. The code used was “2020/EURLPT MP/sclerotia/xxx”, in which the three digit number of the code was automatically generated by the web application. One sample was prepared for each laboratory consisting of one randomly selected sample. The codes of the samples for each sample set are presented in Annex 2.

2.4 Homogeneity study

In the approach of individually spiked samples the homogeneity among the samples was assured.

2.5 Stability of the materials

Specific classes of biological units, such as seeds or mould spore bodies, are by nature intended to withstand sturdy circumstances for a longer period of time. The WFSR stock of ergot sclerotia, stored for many years under constant conditions, and without daylight exposure, did not show any signs of degradation or wearing in the past. In this specific situation, cereal grains (seeds) and ergot sclerotia (spore bodies) were considered to be sufficiently stable for the period of the PT, which was approximately two months from material selection to the end of the reporting period.

3 Organisational details

3.1 Participants

Members of IAG section Feed Microscopy were invited to participate in the PT for ergot sclerotia in cereals in January 2020 in the framework of their annual schedule of PTs. NRLs were informed in Summer 2020. The invitation was sent to the NRLs and IAG members on July 20, 2020 (Annex 3). As always, NRLs can invite their OLs to participate. All respondents were registered as participants to the managing system of WFSR as used for all PTs organised by the WFSR organisation.

A total of 48 participants registered (Annex 1).

3.2 Material distribution and instructions

Each participant received one final sample of 2 kg. Each sample was packed in a cardboard box and all samples were dispatched by courier on Monday 28 September, 2020. Twenty-four samples of each of the two contamination levels (400 mg/kg and 800 mg/kg) were evenly distributed among the participants.

An instruction letter describing the requested analysis (Annex 4) was included in the packages as well as an acknowledgement of receipt form.

The participants were asked to store the samples until analysis according to their routine method. The deadline for submitting the results was fixed on Monday 9 November, 2020 allowing 6 weeks for the inspection. All samples were received in good order by the participants. A report form was provided where the four portion weights, numbers and weights of the spiked ergot sclerotia could be entered, together with the contamination level obtained after examination of two portions, an indication whether this result was below or exceeding the analytical threshold (no/yes), and the contamination level after examination of all four portions (Annex 5). The participants were asked to submit the results in two documents, namely the filled-out report form and a signed pdf version of that same report form.

Results were submitted within the deadline with one exception. This participant gave notice of the delay in time and the results were included.

4 Evaluation of the results

The statistical evaluation was carried out to gain insight in the performance of each participant, as well as the performance in relation to all participants. This evaluation had two components: (a) the procedure of reporting, the verification of the data collection, calculation of number of sclerotia (parts) counted, calculation of contamination levels and correct interpretation of the analytical threshold, and (b) the final performance of the participants in terms of deviation of the percentage of the contamination level (mg/kg) based on the results submitted for each portion sample.

4.1 Procedure of reporting

The report form provided cells for every single result, without the option of automatic calculation of the results based on two and four portions. The participants were asked to make their own calculations and express the result as mg/kg.

The EURL method includes an analytical threshold. All four portions have to be analysed when the contamination level obtained after the examination of two portions exceeds that analytical threshold. Participants were asked to report whether the obtained level after examination of two portions exceeded the analytical threshold (yes/no), since they were asked to examine all four portions. An analytical threshold of 600 mg/kg was fixed as 60% of the legal limit for ergot sclerotia in feed of 1000 mg/kg (Directive 2002/32/EC).

The participants were asked to submit the results in two documents, namely the filled-out report form and a signed pdf version of that same report form.

4.2 Verification of reported results

4.2.1 Subsample weight

To verify the data collection, the organiser used the individual subsample weight to establish the diversity in portion weights, and to assess the correct calculation of the results reported as based on two and on all four portion results.

4.2.2 Number of sclerotia/parts

According to ISO 17043:2010, Annex B.3.1.3 a), the deviation from the number or count of ergot sclerotia can be calculated as:

$$D = x - X \quad [1]$$

with x as reported value and X as assigned value.

For this PT it is rewritten as:

$$D = r_i - s_i \quad [2]$$

with r_i as the recovered number of sclerotia and s_i as the spiked number of sclerotia, both for sample i.

A deviation of up to 2 (fragments of) sclerotia was accepted as complying.

4.2.3 Calculation of the contamination level

Recovery is expressed as the percent Difference and is, according to ISO 17043:2010, Annex B.3.1.3 b), calculated as:

$$D_{\%} = \frac{x-X}{X} * 100 \quad [3]$$

with x as reported weight and X as assigned weight.

The version appropriate for this PT is expressed as:

$$D_{\%} = \frac{r_i-s_i}{s_i} * 100 \quad [4]$$

with r_i as the recovered weight of material and s_i as the spiked weight of material, both for sample i.

All participants were asked to report a final contamination level in mg based on the total sample weight in kg. Since the total amount of spiked material could be recovered from the sample, the broadly applied limit of 5% is used, resulting in an interval of 95%-105%.

4.2.4 Interpretation of the analytical threshold

The participants were asked to assess the threshold value after examination of two portions. The samples spiked at a low level (400 mg/kg) were expected to receive the indication "no" (excess of the analytical threshold) and the samples spiked at a high level (800 mg/kg) should get the indication "yes".

4.3 Performance expressed as contamination level

To assess the participants performances, the calculation of z-scores from the participants' results is the usual way of evaluating their performance. This procedure is based on the assumption that every sample is a draw from a homogeneous batch, and that the resulting data follow a normal distribution. In the practise of detection of visible units in a granular matrix, two circumstances prevent the application of this approach. First, samples have been spiked individually in order to avoid inhomogeneity issues in a large batch of spiked sample material. Secondly, a relatively small number of large, visible units, as present in a sample, follows a binomial distribution. With an increasing number of units, the distribution of hypothetical results tends to a normal distribution. The approach fits in the span of options as included in ISO 17043:2010, where clause 4.4.3 on homogeneity and stability intends to assure that every participant receives comparable test items. This intention is met by producing individually spiked samples. The comparable but still independent nature of the samples fit in the circumscription of the principle of proficiency testing in clause 3.7. The need of having comparable results is conform the requirement of clause 4.5.

The assigned value is a major element for calculating the performance of the participants. In the current approach, meeting the specific needs of visual inspection methods, every individual sample is documented with its own assigned value, more specific, the amount and weight of the spike portion, which does not have a value for uncertainty. This precise identification meets the requirements as set out in Annex B.2 of ISO 17043:2010.

Several procedures for calculating the performance statistics are presented in Annex B of ISO 17043:2010. In the view that z-scores cannot be calculated, an alternative is being used for the calculation of the recovery in terms of contamination level.

All participants were asked to report on each individual subsample. The organisers used the data on the individual subsamples to calculate the recovery, using equation [2] with two or four entries (of subsamples) per sample. Since the total amount of spiked material could be recovered from the sample, the broadly applied limit of 5% is used, resulting in an interval of 95%-105%.

5 Assessment of participants' performance

5.1 Procedure of reporting

All 48 participants submitted reports, in most cases the two requested files. Three participants did only submit the Excel report form, without the signed pdf version. Nineteen of the other participants did not give the PDF file a name in the requested format. Participant PT9139 did only submit the PDF file without the Excel report form. Since the reported values for two portions and for all four portions were not given in the requested Excel format (mg/kg) the reported results could not be evaluated, and therefore the results of participant PT9139 were not included in the further evaluation. The other results (n=47) were considered eligible for further analysis, with 23 sets of results for low contaminated samples (400 mg/kg) and 24 sets of results for high contaminated samples (800 mg/kg). The data as reported by the participants in the Excel format report form are presented in Annex 6.

5.2 Verification of the reported results

Not all participants reported full sets of results. Some intermediate results lack in various combinations or are not fit for further verification. The numbers of participants included in each of the three parameters is therefore consequently different. The data for the verification are presented in Annex 7.

5.2.1 Subsample weight

One participant (PT9108) analysed the sample of 2 kg as one portion, and reported the final result without data concerning the subsamples. Four participants reported four portion weights which were not only equal to each other, but also of a weight of exactly 500 grams (PT9118, PT9124, PT 9138) or of 505 gram (PT9111) were reported. The results of these five participants were excluded from the verification of the reported values of the subsample weight.

The standard deviations of the weights of the portions as reported by 42 participants are presented in Figure 1. A majority of 32 participants (76%) obtained values for the standard deviation below 10 mg/kg (indicated in blue). Higher values were calculated from the results of 10 participants (indicated in yellow).

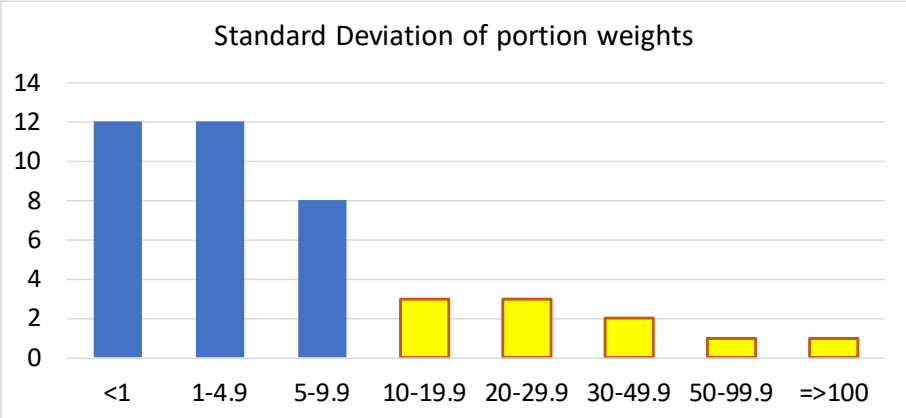


Figure 1 *Frequency distribution of the standard deviations of the portion weights (42 participants).*

5.2.2 Number of sclerotia/parts

Out of 47 participants, the results of 43 participants were evaluated for number of sclerotia. The two participants which examined only two of the four portions (PT9015 and PT9136) were, for obvious reasons, not reporting the full number of sclerotia present in the sample. A further two participants did not report sclerotia counts (PT9125 and PT9140). The results for these four participants are not included in the evaluation of the sclerotia numbers. The deviation between spiked numbers and reported numbers per participant (equation [2]) are shown in Figure 2.

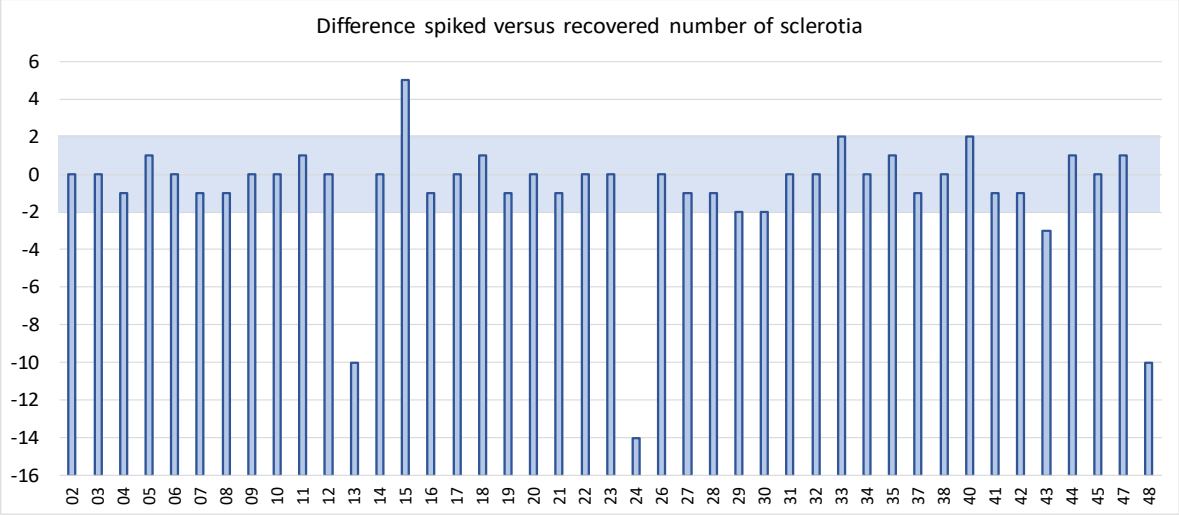


Figure 2 *Results for the counts of the sclerotia calculated with equation [2]. X-axis indicates the last two digits of the participant number. Y-axis indicates the deviation in number (43 participants).*

A total of 38 of 47 participants (81%) reported numbers of sclerotia detected in the 2 kg sample within the two units difference. Two participants analysed 2 quarters and two participants did not report the number of sclerotia (-parts) detected. Five participants reported a difference larger than 2 units, one participant reported more than two units above the added number and four participants reported more than two units under the added number. All cases of a deviating number of sclerotia apply to samples with a high contamination level (800 mg/kg).

5.2.3 Calculation of the contamination level

Forty-four of the 47 participants reported the requested results for weight of sclerotia in mg in the 2 kg sample. As discussed, participant PT9108 analysed the sample of 2 kg as one portion, and reported only the final result. The results of this participant are not included in the calculations for verification. Two participants (PT9015 and PT9136) obtained a result below the analytical threshold and did not report the results for the remaining two portions. This means that a set of 46 results are available for the calculation of the contamination levels over two subsamples (AVG-2) and 44 sets of results for the contamination level over all four subsamples (AVG-4).

The reported levels in mg/kg after two portions (AVG-2) and after all four portions (AVG-4) have been verified by means of re-calculation based on the reported subsample results. The difference between the levels reported by the participants and the calculated levels are presented in Figure 3. A deviation of less than 1 mg/kg can be due to rounding off to a result without decimals. A deviation equal to or exceeding 1 mg/kg would point to a calculation error. This was encountered in the reports of eight (AVG-2) and four (AVG-4) participants.

Three participants reported final results over four portions, which were clearly based on misinterpretations or miscalculations for being a factor 2 (PT9104; PT9105) or a factor 4 too high (PT9146). The levels calculated from the subsample results will be used in the further analysis, since

these subsample results are the best direct parameters for the performance of the participant in terms of analytical skills.

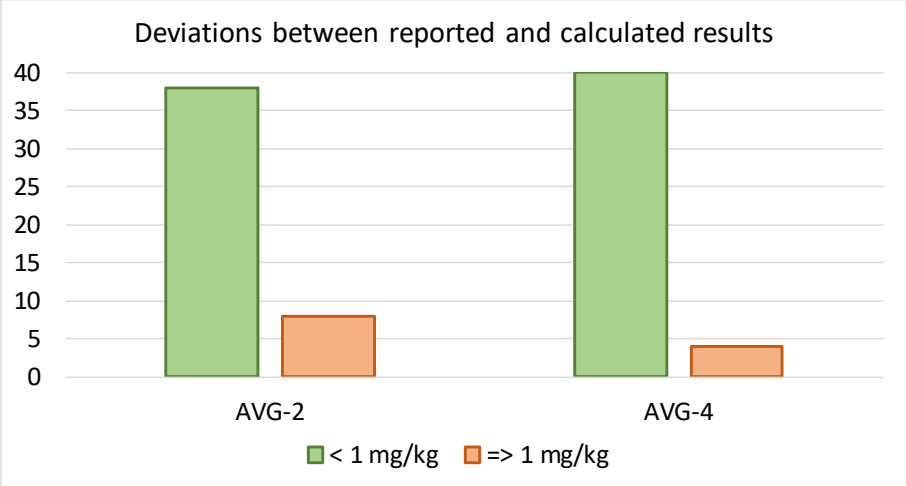


Figure 3 *Frequency distribution of the deviations in mg/kg between the reported and the calculated average levels, based on two portions (AVG-2) (46 participants) and based on all four portions (AVG-4) (44 participants).*

5.2.4 Interpretation of the analytical threshold level

Forty-three of the 47 participants (91%) did correctly indicate the decision after examination of two subsamples. Four of the 47 participants did not correctly indicate the exceeding of the analytical threshold after two portions (PT9130, PT9137, PT9138, PT9148). In all four cases a "no" was reported for a contamination level exceeding the threshold after examination of two portions. The correct result for the excess of the analytical threshold is a parameter for checking the implementation of the method, and 91% of all participants reported a correct indication. Diversified over contamination levels, 17% of the indications for the high contaminated samples were incorrect.

5.3 Evaluation of performance

The data for the evaluation of the performance are presented in Annex 8. An overview of the overall performance for each participant in this PT is given in Annex 9.

5.3.1 Performance expressed as contamination level

One parameter is used for the evaluation of the performance of the participants: contamination level expressed as mg/kg and calculated from the submitted results per subsample.

All results, including those based on the examination of two portions or the sample as a whole, are included in the evaluation of the performance of the 47 participants. Figure 4 shows the results, calculated with equation [4].

Forty of the 47 participants (85%) reported levels within the interval. Seven participants reported levels outside the interval 95%-105%, three participants reported higher weights, and four participants reported lower weights than added. The highest excess was 97.8%, apparently based on biased reporting of the portion results (PT9105).

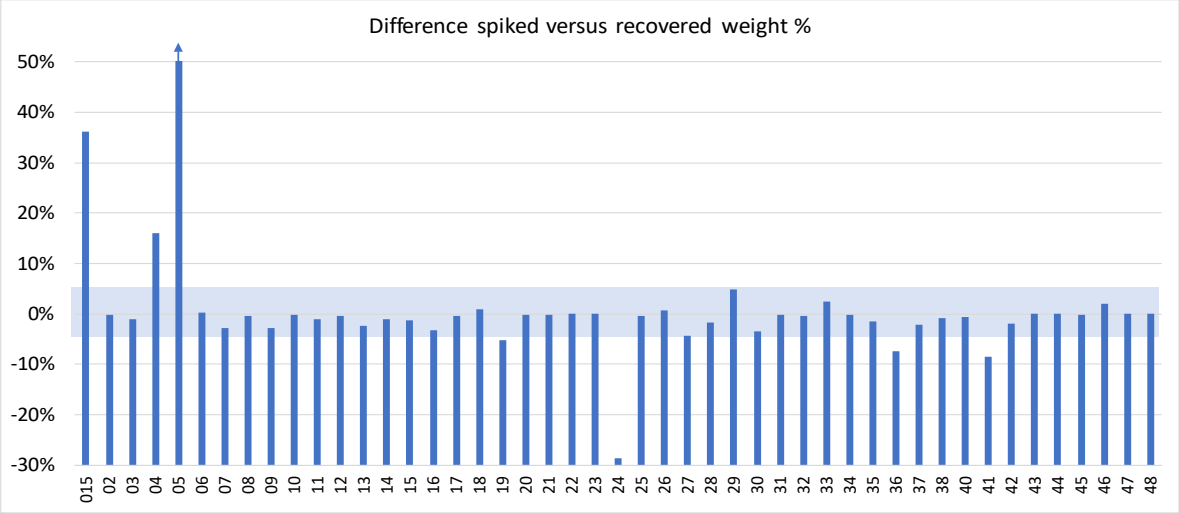


Figure 4 *Results of the participants for the contamination levels calculated with equation [2]. X-axis indicates the last two digits of the participant number. Y-axis indicates the percentage difference between the spiked level and the reported level. Arrow indicates an excess of more than 50% (47 participants).*

5.3.2 Relation between contamination level and number of sclerotia reported

A relation could theoretically be expected between the number of sclerotia found and the contamination level reported: reporting a lower contamination level in mg/kg could be associated with reporting a lower number of sclerotia than added to the sample. A total of 43 participants reported sets of both parameters (sclerotia count and contamination level). This relation is shown in Table 1. Participant PT9124 reported a number of 27 sclerotia, a deficit of 14 compared to the added number, and a contamination level of 570.5 mg/kg for a correct contamination level of 800 mg/kg. Both values indicate a comparable lower reported contamination level. Deviating levels of -8.5%, -5.3%, 16.0% and 97.8% for contamination level have been reported in combination with a correct number of sclerotia (+/- 2). Deviations in number of sclerotia include -10, -10, -3 and +5 sclerotia combined with a correctly reported level of contamination (within the interval 95%-105%).

Table 1 *Combination of results*

Reported values		Number of sclerotia	
		Correct	Incorrect
Contamination level	Correct	34	4
	Incorrect	4	1

5.3.3 Performance related to contamination level

Samples with two different spike levels (400 mg/kg and 800 mg/kg) were evenly distributed among the participants. Overviews of the results are presented in Table 2 and Figure 5. The box and whisker plots are based on the difference between the correct and reported results per sample, calculated with equation [2]. The deviation in the results based on the examination of two portions (1 kg) is considerably larger than reported after the examination of all four portions (entire 2 kg of sample material). The latter results are derived in a situation that all sample material has been examined and varying amounts of target material were not left outside the examination. Large deviations appear to occur in the situation when only two portions were examined, due to the large inhomogeneity of the sample material. This inhomogeneity in absolute figures (mg/kg) is consequently larger at the high contamination level (800 mg/kg) than found among the results at the lower level (400 mg/kg). The good results after examination of all four portions proves the performance of the participants.

Table 2 *Results for all participants separated for the low contaminated (LOW: 400 mg/kg) and the high contaminated (HIGH: 800 mg/kg) sample, after 2 portions and after all 4 portions examined. All values in mg/kg.*

	LOW-2	LOW-4	HIGH-2	HIGH-4
Average	3.8	-2.7	0.1	-13.1
Standard deviation	91.2	10.8	154.8	55.0
Minimum	-169.0	-34.4	-285.9	-229.5
Maximum	202.0	19.0	240.3	128.8

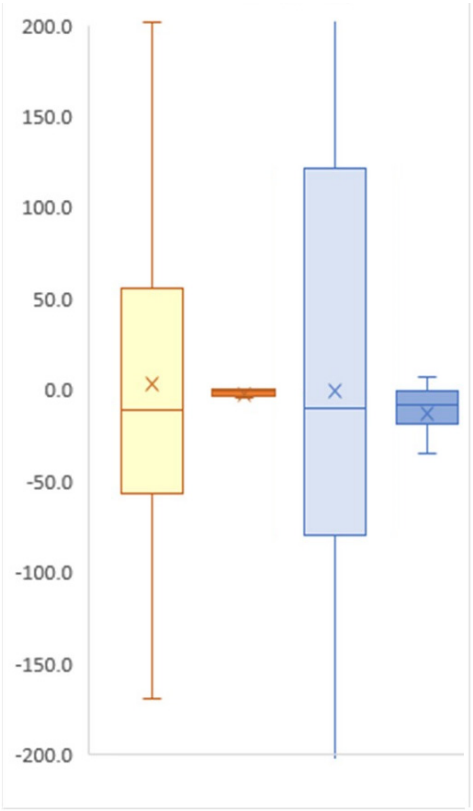


Figure 5 *Box and whisker plots of the difference between correct and reported contamination levels (in mg/kg) for the pooled results of all participants, as indicated in Table 2. Box: P_{25}-P_{75} interval, whiskers: $P_{2.5}$-$P_{97.5}$ interval, horizontal line in box: median, cross: average. The whiskers for the results of the high contaminated sample after examination of two portions (light blue box) are cut off.*

6 Conclusions

Forty-eight laboratories, including 22 NRLs (representing 17 MS, one EFTA country and one Candidate MS), 20 laboratories from IAG members (representing 6 Member States and Switzerland) and 6 OLs (representing Germany and the UK) participated in the EURLPT-MP05 on the quantitative determination of sclerotia in cereals (rye). The participants were evaluated for the quantification of sclerotia in the sample in mg/kg. An additional evaluation was carried out for the number of sclerotia (-part) counted and for the decision on the threshold value. The results of 47 laboratories were included for the evaluation of the results.

Forty-three participants reported both of the results for number of sclerotia and the contamination level in mg/kg within the applied performance characteristics. Most participants reported combinations within the limits +/-2 units for sclerotia counts and 95%-105% for the contamination level (n=34). One participant reported lower results for both parameters. The other eight participants reported varying combinations of number of sclerotia and contamination level. This may suggest that errors have been made in retrieving the results and copying them into the report form.

The analysis of only two portions in the situation that this intermediate result is below the analytical threshold would not necessarily indicate unreliability of the reported result. Although the results after examination of only two parts of the total sample are less precise (5), the application of an analytical threshold of 60% of the legal limit intends to predict a contamination level for the total sample below that legal limit with a probability higher than 95%.

Forty of the 47 participants (85%) showed satisfactory, being the final results for the contamination level (mg/kg) within the interval of 95%-105%. Of the other seven, four participants reported a too low result and three participants reported a too high result.

The participants were furthermore evaluated for number of sclerotia and decision after examination of two quarters of the samples. A total of 38 of 47 participants (81%) reported numbers of sclerotia detected in the 2 kg sample within the two units difference. Five participants reported a difference larger than two units of sclerotia with the spiked number, in four cases an lower results. Two participants reported no results for the number of sclerotia and two participants reported results for two quarters.

Forty-three participants (91%) did correctly indicate the decision after examination of two subsamples.

With regard to the evaluation, the participants showed in general a good performance.

References

Alm, T., 2003. The witch trials of Finnmark, northern Norway, during the 17th century: evidence for ergotism as a contributing factor. Economic Botany 57: 403-416.

CEN, 2018. EN 15587 - Cereals and cereal products - Determination of Besatz in wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), rye (*Secale cereale* L.) and feed barley (*Hordeum vulgare* L.).

European Commission, 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Off. J. European Union, L 140, 30.5.2002, 10-21.

EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798 [158 pp.].

EFSA, 2005. Opinion of the scientific panel on contaminants in food chain on a request from the Commission related to ergot as undesirable substance in animal feed. The EFSA Journal 225: 1-27. https://www.efsa.europa.eu/en/efsajournal/pub/225.

EFSA, 2017. Human and animal dietary exposure to ergot alkaloids. The EFSA Journal 2017; 15(7): 4902. efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.4902.

EURLMP, 2020. EURLMP-method_006 (version 1). Determination of ergot sclerotia (*Claviceps purpurea* Tul.) in whole kernel cereals by visual screening EURL mycotoxins and plant toxins, Wageningen Food Safety Research. https://www.wur.nl/en/show/EURL-MP-method_006-Ergot-sclerotia-by-visual-screening-v1.htm.

European Commission, 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal L 54, 26.2.2009, p. 1–130.

European Commission, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal L 364, 20.12.2006, pp. 5-33.

European Commission, 2015. Commission Regulation (EU) 2015/1940 of 28 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia in certain unprocessed cereals and the provisions on monitoring and reporting. Official Journal L 283, 29.10.2015, p. 3-6.

European Union, 2017. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017. Official Journal L 95/1, 7.4.2017, p. 1–142.

Gupta, G.K., 2018. Illustrated Toxicology: With Study Questions. Academic Press.

IAG, 2008. Method for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff, IAG-Method A4. https://www.iag-micro.org/files/iag-a4_ergot.pdf.

ISO/IEC 17025:2017(E). 2017. General requirements for the competence of testing and calibration laboratories.

ISO/IEC 17043:2010, 2010. Conformity assessment - General requirements for proficiency testing.

Lee, M.R., 2009. The history of ergot of rye (*Claviceps purpurea*) I: From antiquity to 1900. J R Coll Physicians Edinb. 39: 179–184.

Lorenz, K. & Hoseney, R.C., 1979. Ergot on cereal grains, C R C Critical Reviews in Food Science and Nutrition 11: 311-354.

Mulder, P.P.J., Raamsdonk, L.W.D. van, Voogt, H.J., Brakel, M.W. van, Horst, G.M. van der, & Jong, J. de, 2012. Dutch survey ergot alkaloids and sclerotia in animal feeds. RIKILT Report 2012.005 [45 pp.].

Scott, P.M., 2009. Ergot alkaloids: extent of human and animal exposure. World Mycotoxin Journal 2: 141-149.

VDLUFA, 2007. Bestimmung von Mutterkorn in Futtermitteln. VDLUFA MB3-30.2.

Woolf, A., 2000. Witchcraft or mycotoxin? The Salem witch trials. J Toxicol Clin Toxicol. 38: 457-60.

Annex 1 List of participants

Country	Organisation
AUSTRIA*/**	Austrian Agency for Health and Food Safety (AGES)
BELGIUM**	Federal Laboratory for the Safety of the Food Chain
BULGARIA**	Laboratory of SGS Bulgaria
CROATIA**	Croatian Veterinary Institute
CROATIA*	A. Stampar Teaching Institute of Public Health
CYPRUS*	Feeding Stuffs Quality Control Laboratory - Analytical Laboratories Section
CYPRUS*	STATE GENERAL LABORATORY
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
CZECH REPUBLIC*	UKZUZ (Central Institute for Supervising and Testing in Agriculture
DENMARK*	Danish Veterinary and Food Administration
FINLAND*	Finnish Food Authority
FRANCE*	SCL
GERMANY	LTZ Augustenberg
GERMANY**	Staatliche Betriebsgesellschaft fur Umwelt und Landwirtschaft
GERMANY**	LUFA Speyer
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GERMANY**	CVUA-RRW
GERMANY**	LAVES- Feedinvestigation Institute
GERMANY**	LUFA Nord-West
GERMANY	Thuringer Landesamt fur landwirtschaft und Landlichen Raum (TLLLR)
GERMANY**	Bayerisches Landesamt fur Gesundheit und Lebensmittelsicherheit
GERMANY**	LB Hessisches Landeslabor (LHL)
GERMANY**	Landeslabor Berlin-Brandenburg
GERMANY	Landesuntersuchungsamt
GERMANY**	SGS Germany GmbH
GERMANY**	Thuringer Landesamt fur Landwirtschaft und Landlichen Raum
GERMANY**	Landsanstalt fur Landwirtschaft LLG
GERMANY**	LMS Agrarberatung GmbH - LUFA Rostock
HUNGARY*	National Food Chain Safety Office
ITALY**	MIPAAF-ICQRF-LABORATORIO DI MODENA
ITALY**	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta.
LUXEMBOURG*	Laboratoire national de Sante
NETHERLANDS**	TLR International Laboratory
NETHERLANDS**	ForFarmers
POLAND*	National Institute of Public Health - National Institute of Hygiene
ROMANIA*	Institute for Hygiene and Veterinary Public Health
ROMANIA*	Directia Sanitara Veterinara si pentru Siguranta Alimentelor (DSVSA) Bucuresti
SERBIA*	SP LABORATORIJA A.D.
SLOVAKIA*	State veterinary and food institute Dolny Kubin Veterinary and food institute in Kosice
SLOVENIA*	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
SPAIN*	SPANISH AGENCY FOR CONSUMER AFFAIRS, FOOD SAFETY AND NUTRITION
SWEDEN*	National Veterinary Institute, SVA
SWITZERLAND**	Agroscope - Agroscope - Swiss centre for research in agriculture and food sector
SWITZERLAND*	Kantonales Laboratorium Thurgau
UNITED KINGDOM*	FERA Science Ltd
UNITED KINGDOM	Aberdeen Scientific Services Laboratory
UNITED KINGDOM	Minton, Treharne & Davies Ltd
UNITED KINGDOM	Dundee City Council

* National Reference Laboratory of EU Member State.

** IAG

Annex 2 Codification of the samples

Participants code	Material A*
PT9015	169
PT9102	273
PT9103	699
PT9104	498
PT9105	175
PT9106	299
PT9107	588
PT9108	556
PT9109	172
PT9110	343
PT9111	630
PT9112	440
PT9113	147
PT9114	244
PT9115	851
PT9116	789
PT9117	362
PT9118	951
PT9119	701
PT9120	740
PT9121	167
PT9122	319
PT9123	546
PT9124	513
PT9125	349
PT9126	450
PT9127	126
PT9128	209
PT9129	531
PT9130	120
PT9131	997
PT9132	466
PT9133	458
PT9134	834
PT9135	255
PT9136	564
PT9137	854
PT9138	478
PT9139	814
PT9140	577
PT9141	604
PT9142	964
PT9143	237
PT9144	519
PT9145	214
PT9146	416
PT9147	180
PT9148	957

* All sample codes start with 2020/EURLPT MP/sclerotia/.

Annex 3 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

Wageningen Food Safety Research

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

NRLs mycotoxins & plant toxins in food and feed

DATE
July 20, 2020

SUBJECT
Invitation EURL mycotoxins & plant toxins proficiency test ergot sclerotia in food and feed matrices (EURLPT-MP05)

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

VISITORS' ADDRESS
Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB WAGENINGEN

INTERNET
www.wur.nl/wfsr

CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31 (0) 614323017

EMAIL
pt.wfsr@wur.nl

Dear colleague,

The EURL mycotoxins & plant toxins in food and feed at Wageningen Food Safety Research (WFSR), will organize a proficiency test (PT) regarding ergot sclerotia in food and feed matrices (EURLPT-MP05). Members of the section Feedingstuff Microscopy of the International Association for Feedingstuff Analysis (IAG[1]) will be invited to participate in this PT.

This PT will focus on quantification of ergot sclerotia in regard to the current and upcoming legislation in food and feed products. Harmonised EU regulation for ergot sclerotia in cereals for food and feed is in place in the EU (Regulation (EC) No 1881/2006 (and amendments) and Directive 2002/32/EC (and amendments)) and updated EU regulation for food matrices is under preparation.

The primary goal of this PT is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of these compounds in food and feed matrices. According to Regulation (EU) 2017/625 all EU National Reference Laboratories (NRLs) mycotoxins & plant toxins in food and feed are mandatory to participate.

I would like to invite you to participate in this PT.

1. Test materials
   One test sample of cereals, representative for food and feed, will be provided. The test amount sent will be approximately 2 kg.

2. Shipment of test materials
   The test material will be sent by the end of September 2020. The distribution of the test materials will be announced by e-mail. The deadline for reporting is strict and will be six weeks after the date of shipment of the samples.

3. Scope of analysis
   The materials contain ergot sclerotia.

[1] https://www.iag-micro.org/

Wageningen Research Foundation/Wageningen Food Safety Research (WFSR) is part of Wageningen University & Research. WFSR carries out research and analysis contributing to the safety and reliability of food and feed. WFSR is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014, L235 and R013)).

4. Questionnaire
   A questionnaire will be sent electronically. In this questionnaire the particants will be asked to provide information about the laboratory method used. This information is necessary to conduct a more in depth analysis of the results obtained in this proficiency test.

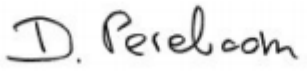
5. Report
   - The report of the proficiency test will be dispatched early in 2021.
   - Results of the proficiency test will be presented anonymously.
   - The follow-up protocol on proficiency test from DG Santé will be applied to the NRLs.

6. Additional information
   - WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
   - WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

7. Costs
   - Participation is free of charge for the NRLs.
   - Members of the IAG can participate. The participation fee is € 270,- (ex. VAT) as a compensation for the preparation and transportation of the samples.
   - Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee is € 270,- (ex. VAT) as a compensation for the preparation and transportation of the samples.
   - If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back before the 21th of August 2020 to: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiency test,



Diana Pereboom-de Fauw
Proficiency Tests

EURL mycotoxins & plant toxins in food and feed
Wageningen Food Safety Research
Wageningen
the Netherlands

Annex 4 Instruction letter

Dear Madam/Sir,

Thank you very much for your participation in the proficiency test regarding ergot sclerotia in food and feed matrices.

Your unique lab code number is:

The parcel shipped to you should contain:
- One test sample of cereals. The test material unit contains approximately 2 kg.

Instructions:
- After arrival the samples should be stored at room temperature in a dark place with low humidity. The matrix material has been treated for pests. Nevertheless, be aware of a good maintenance of the sample.
- Please fill in the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples by e-mail to pt.wfsr@wur.nl.
- Treat the test material as a sample for routine analysis. It is the intention to use only the method as available on the website of the EURL MP for mutual comparability of the participants' results in the framework of this PT. You can download the method from the website: EURL-MP-method_006 Ergot sclerotia by visual screening v1. This method is principally different from currently existing methods and is developed to meet the requirements of Regulation (EC) 152/2009 Annex II. The current draft of the new version of this Regulation includes this method.
- The method is based on the principle of dividing the sample in four portions of equal size, and the examination of two of these portions only if the result after examination of the first two portions exceeds the threshold. In the case of the current PT, all four portions will be examined, for reasons of the desired full statistical analysis.
- Please enter the details of your work procedure and report your results in the appropriate tabs enclosed in the file "Report form EURLPT-MP05 SCL 2020". The usual web application is not applicable. This report document will be send to you by e-mail.
- After completing the two forms "Procedure" and "Results" in the report document, the file has to be sent back in two ways:
  1.save the report file by using "Save as …", add your unique lab code to the end of name.
  2.print the form "Results", sign and scan as PDF file.
  Both the Excel file and the PDF file has to be sent by E-mail (pt.wfsr@wur.nl).
- The deadline for submitting test-results for this test is **the 9th of November, 2020.** Please note that this will be a strict deadline; results reported after the deadline will not be considered. The EURL should be contacted at least 2 weeks in advance, if for exceptional reasons the deadline cannot be met.

Please contact me in case you have any questions or need any assistance.

With kind regards,

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research (WFSR)
The Netherlands

Annex 5 Report form

Please complete all the orange cells.

EURL Myco- and Planttoxins proficiency test Ergot sclerotia 2020 (in cooperation with IAG section Feed Microscopy)

lab number				
portion number				
portion weight (grams)				
count of sclerotia (number)				
weight of sclerotia (mg)				
combined result over two portions (mg/kg)				
does the contamination level exceeds the threshold for feed (see Method EURL MP 006)?				
combined result over all four portions (mg/kg)				
Comment, if necessary				

Signature:

Date:

Annex 6 Sample composition and reported data

Sample description					Data reported																
	level	sample weight	spike number	spike weight	portion weight (g)				count				sclerotia weight (mg)				total 2 portions		total all 4 portions		> threshold
NR					1	2	3	4	1	2	3	4	1	2	3	4	count	mg/kg	count	mg/kg	
PT9015	L	2000.04	20	801	498.9	500.5	498.7	500.7	9	4	0	0	346.4	198.5	0	0	13	545.2			no
PT9102	L	2000.05	24	807	502.08	503.69	494.62	496.87	3	5	7	9	120.6	186.5	182.8	315	8	369.92	24	403.00	no
PT9103	H	2000	38	1607	496.5	513.4	493	497.7	5	13	10	10	140	650	360	440	18	782.3	38	794.8	yes
PT9104	H	2000.08	39	1613	500	500	500	200	10	10	11	7	487	438	467	198	20	925	38	1590	yes
PT9105	H	2000.04	41	1605	499.8	494.2	504.8	499.4	16	10	11	5	1208.4	622.4	875.6	465.7	26	1841.9	42	1587.5	yes
PT9106	L	2000.06	24	808	511.31	489.73	509.8	486.34	8	4	9	3	277.5	116	320.9	93.9	12	390	24	400.5	no
PT9107	L	2000.02	24	794	492.27	512.21	502.11	490.08	7	5	5	6	182.2	151.4	206.4	230.2	12	332	23	386	no
PT9108	L	2000.03	26	804	1995	0	0	0	25	0	0	0	798	0	0	0			25	400	no
PT9109	H	2000.03	39	1601	501.02	497.87	500.08	498.32	11	10	6	12	311.2	400.1	286.7	555.3	21	711.3	39	776.8	yes
PT9110	L	2000.03	23	797	500.27	501.43	505.14	492.14	5	2	10	6	143	86.9	380.6	184.4	7	230	23	397.65	no
PT9111	H	2000.08	41	1600	505	505	505	505	9	11	12	10	291.1	456.1	387.76	465.28	20	739.8	42	792.2	yes
PT9112	H	2000.1	45	1600	500.17	500.58	500.74	500.2	8	11	17	9	320	409	638	226	19	728	45	796	yes
PT9113	H	2000	41	1605	499.95	499.94	500.44	488.62	6	9	7	9	198.6	353.7	473.6	530.9	15	552.4	31	782.7	no
PT9114	L	2000.03	23	794	530.7	490.2	496.5	479.1	8	5	9	1	259	117.5	334.2	74	13	363.9	23	388.8	no
PT9115	H	2000.03	41	1611	493	504	510	495	9	6	22	9	258	260	704	368	15	520	46	794	no
PT9116	L	2000.08	23	796	495.25	496.79	495.64	502.68	9	5	4	4	313.2	171.4	113.2	168.7	14	488.48	22	385.11	no
PT9117	H	2000.06	42	1596	498.27	502.67	493	505.93	9	13	9	11	383	538	307	362	22	920.14	42	795.05	yes
PT9118	H	2000.04	41	1594	500	500	500	500	8	10	12	12	414	350	375	470	18	764	42	804.5	yes
PT9119	L	2000.09	24	791	498.69	500.89	500.61	499.16	10	6	5	2	286	162	203	98	16	448	23	375	no
PT9120	L	2000.07	24	800	500.02	500.07	500.08	499.61	6	5	7	6	188.3	156.6	273.6	179.6	11	344.86	24	398.95	no
PT9121	L	2000.08	24	791	499.9	500.1	499.8	500	3	7	8	5	96.7	242.5	288.6	162.3	10	339.2	23	395.1	no
PT9122	L	2000.07	23	795	500.23	500.29	500.05	499.79	5	7	6	5	140	257	229	170	12	397	23	398	no
PT9123	L	2000.1	23	803	510.64	473.51	518.13	495.63	6	5	4	8	260.9	189.6	233.4	117.7	11	432	23	401	no
PT9124	H	2000.03	41	1600	500	500	500	500	9	4	6	8	314	223	318	286	13	527	27	570.5	no
PT9125	H	2000.04	41	1604	499.83	499.73	499.8	499.76	0	0	0	0	549.34	341.22	453.22	251.57	0	890.95	0	798.03	yes

Sample description					Data reported												total 2 portions		total all 4 portions		> thres-hold
	level	sample weight	spike number	spike weight	portion weight (g)				count				sclerotia weight (mg)								
NR					1	2	3	4	1	2	3	4	1	2	3	4	count	mg/kg	count	mg/kg	
PT9126	H	2000.07	39	1599	501	501	493	503	14	10	9	6	558	445	379	227	24	1001	39	805	yes
PT9127	H	2000.05	42	1592	510.33	512.5	508.02	516.42	9	13	10	9	366.36	464.4	384.46	343.22	22	812.22	41	761.23	yes
PT9128	H	2000.08	40	1608	500.8	495.5	503.1	497.8	13	8	6	12	487.4	336.1	267.9	488	21	825.77	39	790.5	yes
PT9129	L	2000.07	25	793	502.54	499.33	415.05	503.17	10	3	5	5	337.8	180	160	120	13	516.83	23	415.5	no
PT9130	H	2000.04	41	1607	498.9228	500.5	500.1	500.02	13	10	8	8	622.8	355.9	307.1	263.3	23	979.3	39	774.7	no
PT9131	L	2000.04	23	805	485.93	471.6	521.9	520.41	5	3	8	7	176.5	123.4	310.4	193.2	8	313.2	23	401.8	no
PT9132	L	2000.07	24	801	492.37	499.47	499.9	506.55	5	4	6	9	197.6	177.8	127.4	294.9	9	379	24	399	no
PT9133	L	2000.07	23	801	501.69	508.36	498.97	489.77	8	4	8	5	246.2	96.3	346	130.8	12	339.1	25	409.9	no
PT9134	H	2000.06	38	1615	501.4	495	503	500.6	15	9	7	7	597	447	238	329	24	1047	38	806	yes
PT9135	H	2000.07	40	1611	500.01	500.01	500	499.91	9	14	12	6	334.6	440.9	453.6	357.8	23	775	41	793	yes
PT9136	L	2000.07	25	808	482.3	501.2	0	0	11	2	0	0	307.6	60	0	0	13	373.77			no
PT9137	H	2000	40	1602	511	538	469	480	8	11	6	14	317	440	210	597	19	722	39	783	no
PT9138	H	2000.01	39	1592	500	500	500	500	9	12	7	11	325	461	269	524	21	786	39	789.5	no
PT9139	L	2000.02	22	812																	
PT9140	L	2000.1	25	797	501	511.6	493.3	492.7	7	10	6	4	197.3	346	155.4	92.4	17	536.5	27	395.8	no
PT9141	L	2000.01	22	808	502.42	504.06	501.37	501.51	6	8	4	3	222.9	306.8	128.5	84.5	14	*526.3*	21	*369.6*	no
PT9142	H	2000.03	38	1610	480.75	505.5	534.81	478.04	11	11	10	5	592.2	409.8	409.5	164.7	22	1021	37	788	yes
PT9143	H	2000.06	41	1599	498.706	498.832	498.212	500.106	11	5	12	10	580	262	428	326	16	844.000	38	800	yes
PT9144	L	2000.1	23	799	505	494	495	504	8	4	8	4	237	145	321	96	12	382	24	400	no
PT9145	L	2000.05	24	793	500.19	500.05	500.67	499.22	8	10	3	3	241.1	357.5	93.6	98.9	18	598.46	24	395.52	no
PT9146	L	2000.02	25	802	604	478	492	422	0	0	0	0	217	181	219	200	0	804	0	1658	yes
PT9147	H	2000.03	41	1608	492.5	556.49	472.27	477.1	16	11	4	11	655.9	395	168.7	385.8	27	1001.82	42	803.36	yes
PT9148	H	2000.08	40	1605	500	500	500	498	5	7	10	8	215.7	342.5	598.8	446.6	12	814.2	30	802.52	no

Annex 7 Results: verification

Sample description			Verification					
	level	portion weight	AV-2			AV-4		
NR		SD	reported (g)	calculated (g)	difference (g)	reported (g)	calculated (g)	difference (g)
PT9015	L	0.906	545.2	545.2	0.0			
PT9102	L	3.702	369.92	305.3	64.6	403.0	403.0	0.0
PT9103	H	7.842	782.3	782.3	0.0	794.8	794.8	0.0
PT9104	H	129.904	925	925.0	0.0	1590.0	935.3	654.7
PT9105	H	3.751	1841.9	1841.9	0.0	1587.5	1587.5	0.0
PT9106	L	11.336	390	393.1	-3.1	400.5	404.7	-4.2
PT9107	L	8.788	332	332.1	-0.1	386.0	385.7	0.3
PT9108	L							
PT9109	H	1.282	711.3	712.1	-0.8	776.8	777.7	-0.9
PT9110	L	4.745	230	229.5	0.5	397.7	397.7	0.0
PT9111	H	0.000	739.8	739.8	0.0	792.2	792.2	0.0
PT9112	H	0.244	728	728.5	-0.5	796.0	795.8	0.2
PT9113	H	4.979	552.4	552.4	0.0	782.7	782.7	0.0
PT9114	L	19.265	363.9	368.8	-4.9	388.8	393.0	-4.2
PT9115	H	6.874	520	519.6	0.4	794.0	794.2	-0.2
PT9116	L	2.993	488.48	488.5	0.0	385.1	385.1	0.0
PT9117	H	4.855	920.14	920.1	0.0	795.1	795.1	0.0
PT9118	H	0.000	764	764.0	0.0	804.5	804.5	0.0
PT9119	L	0.933	448	448.2	-0.2	375.0	374.6	0.4
PT9120	L	0.195	344.86	344.9	0.0	399.0	399.1	-0.1
PT9121	L	0.112	339.2	339.2	0.0	395.1	395.1	0.0
PT9122	L	0.194	397	396.8	0.2	398.0	397.9	0.1
PT9123	L	17.041	432	457.8	-25.8	401.0	401.2	-0.2
PT9124	H	0.000	527	537.0	-10.0	570.5	570.5	0.0
PT9125	H	0.038	890.95	891.0	0.0	798.0	798.0	0.0

Sample description			Verification					
	level	portion weight	AV-2			AV-4		
NR		SD	reported (g)	calculated (g)	difference (g)	reported (g)	calculated (g)	difference (g)
PT9126	H	3.841	1001	1001.0	0.0	805.0	805.3	-0.3
PT9127	H	3.094	812.22	812.2	0.0	761.2	761.2	0.0
PT9128	H	2.889	825.77	826.6	-0.8	790.5	790.8	-0.3
PT9129	L	37.540	516.83	516.8	0.0	415.5	415.5	0.0
PT9130	H	0.585	979.3	979.3	0.0	774.7	774.7	0.0
PT9131	L	21.798	313.2	313.2	0.0	401.8	401.8	0.0
PT9132	L	5.017	379	378.5	0.5	399.0	399.2	-0.2
PT9133	L	6.673	339.1	339.1	0.0	409.9	409.9	0.0
PT9134	H	3.013	1047	1047.8	-0.8	806.0	805.5	0.5
PT9135	H	0.042	775	775.5	-0.5	793.0	793.5	-0.5
PT9136	L	9.450	373.77	373.8	0.0			
PT9137	H	27.042	722	721.6	0.4	783.0	782.8	0.2
PT9138	H	0.000	786	786.0	0.0	789.5	789.5	0.0
PT9139	L							
PT9140	L	7.636	536.5	536.5	0.0	395.8	395.8	0.0
PT9141	L	1.072	526.3	526.3	0.0	369.6	369.6	0.0
PT9142	H	22.883	1021	1016.0	5.0	788.0	788.5	-0.5
PT9143	H	0.699	844	844.1	-0.1	800.0	799.7	0.3
PT9144	L	5.025	382	382.4	-0.4	400.0	399.9	0.1
PT9145	L	0.522	598.46	598.5	0.0	395.5	395.5	0.0
PT9146	L	66.038	804	367.8	436.2	1658.0	409.3	1248.7
PT9147	H	33.690	1001.82	1001.8	0.0	803.4	803.4	0.0
PT9148	H	0.866	814.2	558.2	256.0	802.5	802.6	-0.1

Annex 8 Results: performance

A-priori data					Reported:				Calculated from portion data:				Recovery	
					over 2 portions	over 4 portions	above threshold		over 2 portions		over 4 portions		D number sclerotia	D% cont. level
NR	level	mg/kg	n sclerotia		mg/kg	mg/kg			count	mg/kg	count	mg/kg	n	% weight
PT9015	L	400.5	20		545.2		no		13	545.2				36.1%
PT9102	L	403.5	24		369.92	403	no		8	305.3	24	403.0	0	-0.1%
PT9103	H	803.5	38		782.3	794.8	yes		18	782.3	38	794.8	0	-1.1%
PT9104	H	806.5	39		925	1590	yes		20	925.0	38	935.3	-1	16.0%
PT9105	H	802.5	41		1841.9	1587.5	yes		26	1841.9	42	1587.5	1	97.8%
PT9106	L	404.0	24		390	400.5	no		12	393.1	24	404.7	0	0.2%
PT9107	L	397.0	24		332	386	no		12	332.1	23	385.7	-1	-2.8%
PT9108	L	402.0	26			400	no				25	400.0	-1	-0.5%
PT9109	H	800.5	39		711.3	776.8	yes		21	712.1	39	777.7	0	-2.8%
PT9110	L	398.5	23		230	397.65	no		7	229.5	23	397.7	0	-0.2%
PT9111	H	800.0	41		739.8	792.2	yes		20	739.8	42	792.2	1	-1.0%
PT9112	H	800.0	45		728	796	yes		19	728.5	45	795.8	0	-0.5%
PT9113	H	802.5	41		552.4	782.7	no		15	552.4	31	782.7	-10	-2.5%
PT9114	L	397.0	23		363.9	388.8	no		13	368.8	23	393.0	0	-1.0%
PT9115	H	805.5	41		520	794	no		15	519.6	46	794.2	5	-1.4%
PT9116	L	398.0	23		488.48	385.11	no		14	488.5	22	385.1	-1	-3.2%
PT9117	H	798.0	42		920.14	795.05	yes		22	920.1	42	795.1	0	-0.4%
PT9118	H	797.0	41		764	804.5	yes		18	764.0	42	804.5	1	0.9%
PT9119	L	395.5	24		448	375	no		16	448.2	23	374.6	-1	-5.3%
PT9120	L	400.0	24		344.86	398.95	no		11	344.9	24	399.1	0	-0.2%
PT9121	L	395.5	24		339.2	395.1	no		10	339.2	23	395.1	-1	-0.1%
PT9122	L	397.5	23		397	398	no		12	396.8	23	397.9	0	0.1%
PT9123	L	401.5	23		432	401	no		11	457.8	23	401.2	0	-0.1%
PT9124	H	800.0	41		527	570.5	no		13	537.0	27	570.5	-14	-28.7%
PT9125	H	802.0	41		890.95	798.03	yes			891.0		798.0		-0.5%

A-priori data					Reported:				Calculated from portion data:				Recovery	
					over 2 portions	over 4 portions	above threshold		over 2 portions		over 4 portions		D number sclerotia	D% cont. level
NR	level	mg/kg	n sclerotia		mg/kg	mg/kg			count	mg/kg	count	mg/kg	n	% weight
PT9126	H	799.5	39		1001	805	yes		24	1001.0	39	805.3	0	0.7%
PT9127	H	796.0	42		812.22	761.23	yes		22	812.2	41	761.2	-1	-4.4%
PT9128	H	804.0	40		825.77	790.5	yes		21	826.6	39	790.8	-1	-1.6%
PT9129	L	396.5	25		516.83	415.5	no		13	516.8	23	415.5	-2	4.8%
PT9130	H	803.5	41		979.3	774.7	no		23	979.3	39	774.7	-2	-3.6%
PT9131	L	402.5	23		313.2	401.8	no		8	313.2	23	401.8	0	-0.2%
PT9132	L	400.5	24		379	399	no		9	378.5	24	399.2	0	-0.3%
PT9133	L	400.5	23		339.1	409.9	no		12	339.1	25	409.9	2	2.4%
PT9134	H	807.5	38		1047	806	yes		24	1047.8	38	805.5	0	-0.2%
PT9135	H	805.5	40		775	793	yes		23	775.5	41	793.5	1	-1.5%
PT9136	L	404.0	25		373.77		no		13	373.8				-7.5%
PT9137	H	801.0	40		722	783	no		19	721.6	39	782.8	-1	-2.3%
PT9138	H	796.0	39		786	789.5	no		21	786.0	39	789.5	0	-0.8%
PT9139	L	406.0	22											
PT9140	L	398.5	25		536.5	395.8	no		17	536.5	27	395.8	2	-0.7%
PT9141	L	404.0	22		526.3	369.6	no		14	526.3	21	369.6	-1	-8.5%
PT9142	H	805.0	38		1021	788	yes		22	1016.0	37	788.5	-1	-2.1%
PT9143	H	799.5	41		844	800	yes		16	844.1	38	799.7	-3	0.0%
PT9144	L	399.5	23		382	400	no		12	382.4	24	399.9	1	0.1%
PT9145	L	396.5	24		598.46	395.52	no		18	598.5	24	395.5	0	-0.2%
PT9146	L	401.0	25		804	1658	yes		0	367.8	0	409.3		2.1%
PT9147	H	804.0	41		1001.82	803.36	yes		27	1001.8	42	803.4	1	-0.1%
PT9148	H	802.5	40		814.2	802.52	no		12	558.2	30	802.6	-10	0.0%

Annex 9 Overview performance per laboratory

Participant code	Number of sclerotia & interpretation analytical threshold Satisfactory performance*[a]	Contamination level Satisfactory performance*[b]
PT9015	1 out of 2**	0 out of 1**
PT9102	**2 out of 2**	**1 out of 1**
PT9103	**2 out of 2**	**1 out of 1**
PT9104	**2 out of 2**	0 out of 1
PT9105	**2 out of 2**	0 out of 1
PT9106	**2 out of 2**	**1 out of 1**
PT9107	**2 out of 2**	**1 out of 1**
PT9108	**2 out of 2**	**1 out of 1**
PT9109	**2 out of 2**	**1 out of 1**
PT9110	**2 out of 2**	**1 out of 1**
PT9111	**2 out of 2**	**1 out of 1**
PT9112	**2 out of 2**	**1 out of 1**
PT9113	1 out of 2	**1 out of 1**
PT9114	**2 out of 2**	**1 out of 1**
PT9115	1 out of 2	**1 out of 1**
PT9116	**2 out of 2**	**1 out of 1**
PT9117	**2 out of 2**	**1 out of 1**
PT9118	**2 out of 2**	**1 out of 1**
PT9119	**2 out of 2**	0 out of 1
PT9120	**2 out of 2**	**1 out of 1**
PT9121	**2 out of 2**	**1 out of 1**
PT9122	**2 out of 2**	**1 out of 1**
PT9123	**2 out of 2**	**1 out of 1**
PT9124	1 out of 2	0 out of 1
PT9125	1 out of 2***	**1 out of 1**
PT9126	**2 out of 2**	**1 out of 1**
PT9127	**2 out of 2**	**1 out of 1**
PT9128	**2 out of 2**	**1 out of 1**
PT9129	**2 out of 2**	**1 out of 1**
PT9130	1 out of 2	**1 out of 1**
PT9131	**2 out of 2**	**1 out of 1**
PT9132	**2 out of 2**	**1 out of 1**
PT9133	**2 out of 2**	**1 out of 1**
PT9134	**2 out of 2**	**1 out of 1**
PT9135	**2 out of 2**	**1 out of 1**
PT9136	1 out of 2**	0 out of 1**
PT9137	1 out of 2	**1 out of 1**
PT9138	1 out of 2	**1 out of 1**
PT9140	**2 out of 2**	**1 out of 1**
PT9141	**2 out of 2**	0 out of 1
PT9142	**2 out of 2**	**1 out of 1**
PT9143	1 out of 2	**1 out of 1**
PT9144	**2 out of 2**	**1 out of 1**
PT9145	**2 out of 2**	**1 out of 1**
PT9146	1 out of 2***	**1 out of 1**
PT9147	**2 out of 2**	**1 out of 1**
PT9148	0 out of 2	**1 out of 1**

*[a] = satisfactory performance = reported the number of sclerotia within the + / - 2 units & reported a correct interpretation of the analytical threshold

*[b] = satisfactory performance = delivered results on weight of sclerotia in mg/kg within the within the 95%-105% interval as calculated by the organiser from the submitted data per portion.

**examined two portions

***did not report number of sclerotia

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