Evaluation Report
proficiency test

DLA ptGMF (2021)

GMO-Determination in Feed: (qualitative + quantitative): GMO-Soya (RR and RR2), GMO-Maize (bt11 and TC1507) and GMO-Rape (GT73)

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

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 31 January 2022
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Gehaltsprüfung der EP-Parameter As part of the present proficency test the following services were subcontracted: Quantification of PT-parameter(s)
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Contents

1. Introduction

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

2. Realisation

2.1 Test material

The test material consists of two different commercially available feeds for laying hens from European suppliers (see Table 1). One feed was labeled non-GMO (sample A), while the other feed (sample B) contains ingredients from GMO soya and GMO maize products. In addition, reference materials from GMO maize and GMO rape seeds were added to sample B.

The respective feed raw materials were crushed and sieved (mesh <1,5 mm), the other reference materials were added to sample B and then homogenized. The composition of samples is shown in Table 1.

Before homogenization microtracer particles were added to check the homogeneity of the mixture. During filling, aliquots were taken for the microtracer analysis (see 2.1.1).

After homogenization the samples were filled in portions of approx. 10 g in metallized PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**GMO-Content**
Complete feed for laying hens (without GMO ingredients) Ingredients: Maize, wheat, steam-heated soybean meal, calcium carbonate, barley, lucerne meal, Ca-Na-phosphate, vegetable fatty acids, oats, wheat bran, sodium chloride and nutritional and technological additives Nutrients per 100 g: Crude Protein 17 g, Crude Fat 3,4 g, Crude Fiber 3,6 g, Crude Ash 11 g	100 g/100 g	-	-
Complementary feed for laying hens (with GMO ingredients*) Ingredients: Maize*, soybean meal*, steam-heated, calcium carbonate, Ca-Na-phosphate, wheat gluten, wheat bran, sodium chloride, vegetable fatty acids, vegetable oils as well as nutritional and technological additives Nutrients per 100 g: Crude Protein 19 g, Crude Fat 3,0 g, Crude Fiber 3,9 g, Crude Ash 17 g	-	89,2 g/100 g	positive
Rape seed feed pellets Ingredients: Rape (press residue)	-	9,9 g/100 g	-
GMO Rape seed GT73/RT73 Reference material: 100% GMO-Rape	-	0,51 g/100 g	positive
GVO Maize Bt11 Reference material: 100% GMO-Maize		0,32 g/100 g	positive

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT sample B showed a probability of 99%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. A HorRat value of 0,54 was obtained. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the samples were approx. 0,54 (19°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A and B sample were sent to every participating laboratory in the 38th week of 2021. The testing method was optional. The tests should be finished at 19th November 2021 the latest.

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

*There are 2 different test samples with possible contents of the parameters **GMO soy (GTS 40-3-2 RoundUp-Ready and MON89788 RR2Yield), maize (bt11 maize and TC1507 Herculex I)** and **GMO rape (GT73 RoundUp-Ready)** in ground feed. The parameters can be analyzed qualitatively and quantitatively. The presence of other GMO events is not excluded. The results are given as **positive / negative** or as the concentration in **percentage (%)** of the respective GMO proportion of the total proportion of the relevant plant species (e.g. GMO proportion GTS 40-3-2 per total soy content).*

Note: Please store samples at 2 - 10°C on arrival!

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
On one hand the results given as positive/negative and on the other hand the indicated results of the GMO events were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, limit of quantifications, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 11 participants submitted at least one result.

3. Evaluation

The evaluation of GMO proficiency tests have shown, that the quantitative results do not follow a symmetric normal distribution. The distribution of results is shifted to higher results. One reason is the multiplicative error contribution by PCR-methods. In contrast the logarithmized results show a normal distribution. Therfore for the determination of the robust mean and z-scores the logarithmized results were used [22, 23, 24].

The PCR results were valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted **logarithmized results** (log-data) was used as the assigned value (X_{pt}) („consensus value from participants"). The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. Afterwards the assigned value X_{pt} is expressed in the laboratory analytical unit percent (%-data) by subsequent delogarithmic calculation.
In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

Single results giving values outside the measuring range of the participating laboratory or given as „0" are not considered for statistical evaluation (e.g. results given as > 10 % and < 0,1 %, respectively) [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S*) of the submitted **logarithmized results** (log-data) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

3.3 Exclusion of results and outliers

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

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

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

Results are tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.4 Target standard deviation (for proficiency assessment)

A target range for quantitative GMO-determinations was proposed by Powell & Owen (2002) and Thompson et al. (2006) by the principle of „fitness for purpose" and the „perceived best practice" [22, 24].

The target range is calculated as defined below:

Lower Limit $x_{min} = X_{pt} / f$ Upper Limit $x_{max} = f * X_{pt}$

and the target standard deviation (σ_{pt}):

$$\sigma_{pt} = 1/z \ (\log f)$$

with
f = factor or quotient for a satisfactory target range
z = ±2 (z-score-limits)

In the present proficiency test a factor of 2,0 was choosen as a suitable target range. Thus the **target standard deviation** σ_{pt} (log-data) is 0,15 with a z-Score of ± 2,0. This target standard deviation is in the range of the „perceived best pratice" proposed by Powell & Owen (2002) and Thompson et al. (2006) obtained by evaluation of several proficiency tests.

Target range:

Lower Limit $x_{min} = X_{pt} / 2,0$ Upper Limit $x_{max} = 2,0 * X_{pt}$

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation σ_{pt} (log-data) the logarithmized result x_i (log-data) of the participant is deviating from the assigned value (X_{pt}) [3].

Participants' z-scores are derived from:

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

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

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

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

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

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(xpt)}$), all values as logarithmic values (log-data) [3].

The calculation is performed by:

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

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

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

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

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

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

3.8 Standard uncertainty and traceability

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

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

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$, the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

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

4. Results

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

In the first table the characteristics are listed:

Statistic Data	**[log-data]**	**[%-data]**
Number of results		
Number of outliers		
Mean		
Median		
Robust Mean (X_{pt})		
Robust standard deviation (S*)		
Target range:		
Target standard deviation σ_{pt}		
lower limit of target range		
upper limit of target range		
Quotient S/σ_{pt}*		
Standard uncertainty $U_{(Xpt)}$		
Results in the target range		
Percent in the target range		

In the table below, the results of the participating laboratories are given:

Auswerte-nummer / **Evaluation number**	**GMO Event [%]**	**Abweichung [%]** / **Deviation [%]**	**GMO Event [log data]**	**Abweichung [log]** / **Deviation [log]**	**z-Score** (σ_{pt})	**Hinweis** / **Remark**

4.1 Proficiency Test GMO-Maize Bt11

Qualitative evaluation of the results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[%]	pos/neg	[%]	Agreement with consensus value	
1	negative		positive	1,70	2/2 (100%)	
2	negative		positive		2/2 (100%)	
3						
4	negative	-	positive	1,70	2/2 (100%)	
5	negative	-	positive		2/2 (100%)	
6	negative		positive	3,70	2/2 (100%)	
7	negative		positive	2,72	2/2 (100%)	
8	negative	<0,10	positive	0,44	2/2 (100%)	
9	negative		positive	2,70	2/2 (100%)	
10	negative	<0,1	positive		2/2 (100%)	
11	negative	<0,1	positive	2,40	2/2 (100%)	

	Sample A		Sample B	
Number positive	0		10	
Number negative	10		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Comment:
For samples A and B there are consensus values of 100% negative and positive results each.

Quantitative evaluation GMO-Maize Bt11:

Sample B

Statistic Data	**[log-data]**	**[%-data]**
Number of results	7	7
Number of outliers	–	–
Mean	0,274	1,88
Median	0,380	2,40
Robust Mean (x_{pt})	**0,328**	**2,13**
Robust standard deviation (S*)	**0,205**	**-**
Target range:		
Target standard deviation σ_{pt}	**0,15**	**-**
lower limit of target range	**-**	**1,06**
upper limit of target range	**-**	**4,26**
Quotient S/σ_{pt}*	*1,4*	–
Standard uncertainty $U_{(xpt)}$	*0,097*	–
Results in the target range	**-**	*6*
Percent in the target range	**-**	*86%*

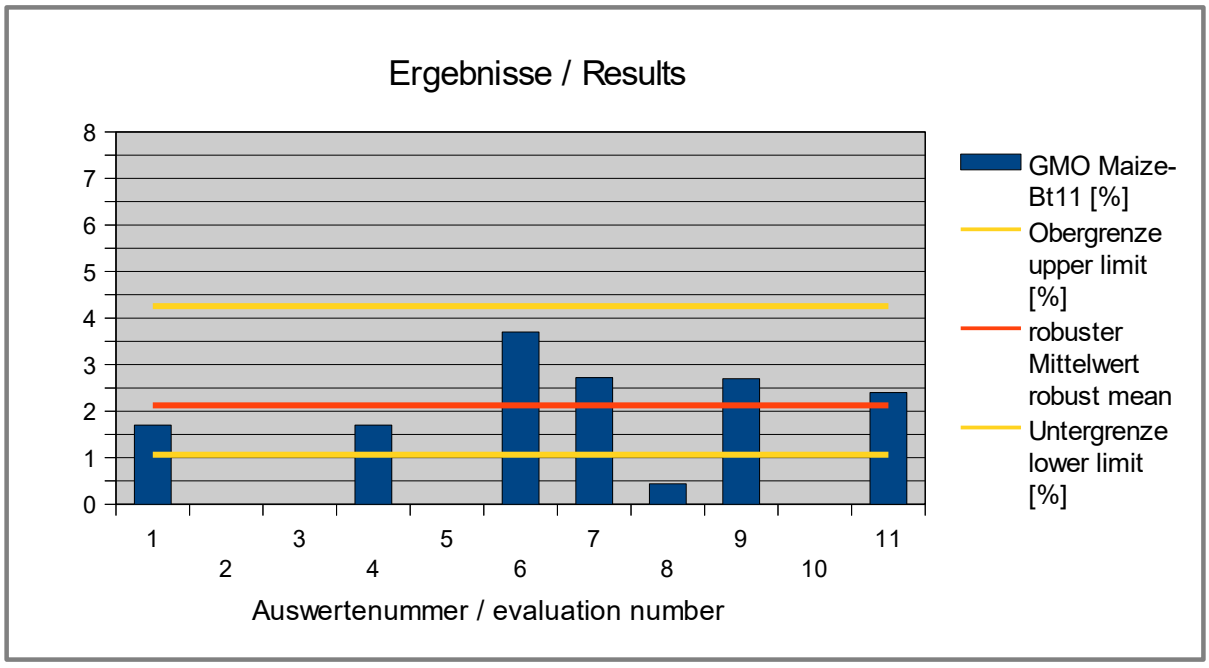

Abb. / Fig. 1: Ergebnisse GVO-Mais Bt11 **/** Results GMO-Maize Bt11

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	GMO Maize-Bt11 [%]	Abweichung [%] Deviation [%]	GMO Maize-Bt11 [log]	Abweichung [log] Deviation [log]	z-Score (σ_{pt})	Hinweis Remark
1	1,70	-0,428	0,230	-0,098	-0,65	
2						
3						
4	1,70	-0,428	0,230	-0,098	-0,65	
5						
6	3,70	1,57	0,568	0,240	1,6	
7	2,72	0,592	0,435	0,107	0,71	
8	0,44	-1,69	-0,357	-0,685	-4,6	
9	2,70	0,572	0,431	0,103	0,69	
10						
11	2,40	0,272	0,380	0,052	0,35	

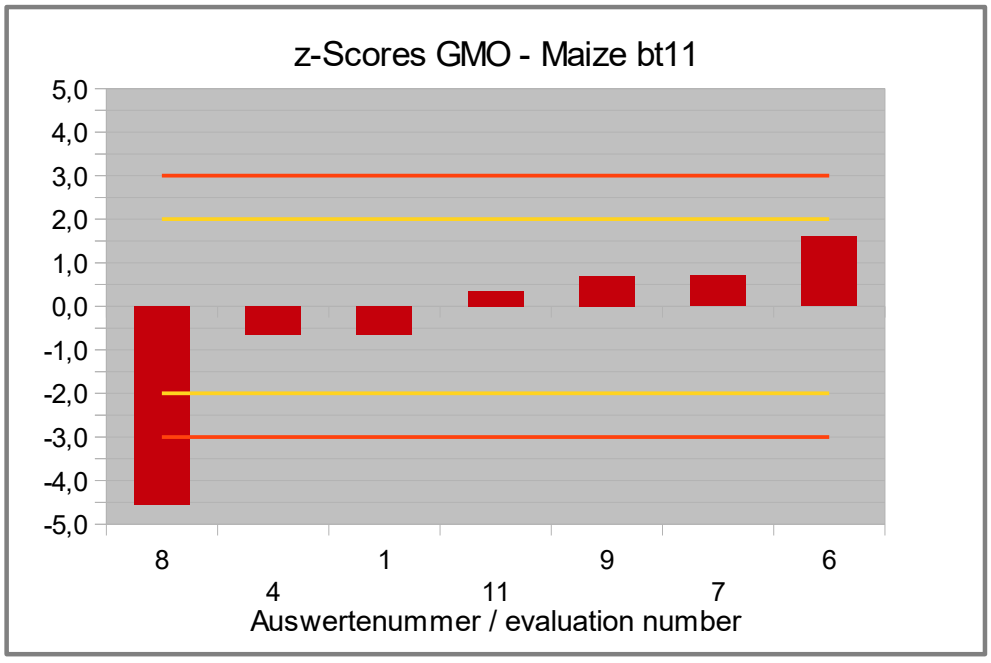

Abb. / Fig. 2: z-Scores GVO-Mais Bt11 / GMO-Maize Bt11

4.2 Proficiency Test GMO-Maize TC1507 (Herculex I)

Qualitative evaluation of the results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[%]	pos/neg	[%]	Agreement with consensus value	
1	negative		positive	0,30	2/2 (100%)	
2	negative		positive		2/2 (100%)	
3						
4						
5	negative	-	positive		2/2 (100%)	
6	negative		positive	0,33	2/2 (100%)	
7	negative		positive	0,50	2/2 (100%)	
8	negative	<0,10	negative	<0,10	1/2 (50%)	no positve sample identified
9						
10	negative	<0,1	positive	0,20	2/2 (100%)	
11	negative	<0,1	positive	0,20	2/2 (100%)	

	Sample A		Sample B	
Number positive	0		7	
Number negative	8		1	
Percent positive	0		88	
Percent negative	100		13	
Consensus value	negative		positive	

Comment:
There are consensus values of 100% negative results for sample A and 88% positive results for sample B.

Quantitative evaluation GMO-Maize TC1507 Herculex I:

Sample B

Statistic Data	**[log-data]**	**[%-data]**
Number of results	5	5
Number of outliers	0	0
Mean	-0,541	0,288
Median	-0,523	0,300
Robust Mean (X_{pt})	**-0,541**	**0,288**
Robust standard deviation (S*)	**0,189**	**-**
Target range:		
Target standard deviation σ_{pt}	**0,15**	**-**
lower limit of target range	**-**	**0,144**
upper limit of target range	**-**	**0,576**
Quotient S/σ_{pt}*	*1,3*	*-*
Standard uncertainty $U_{(Xpt)}$	*0,106*	*-*
Results in the target range	**-**	*5*
Percent in the target range	**-**	*100%*

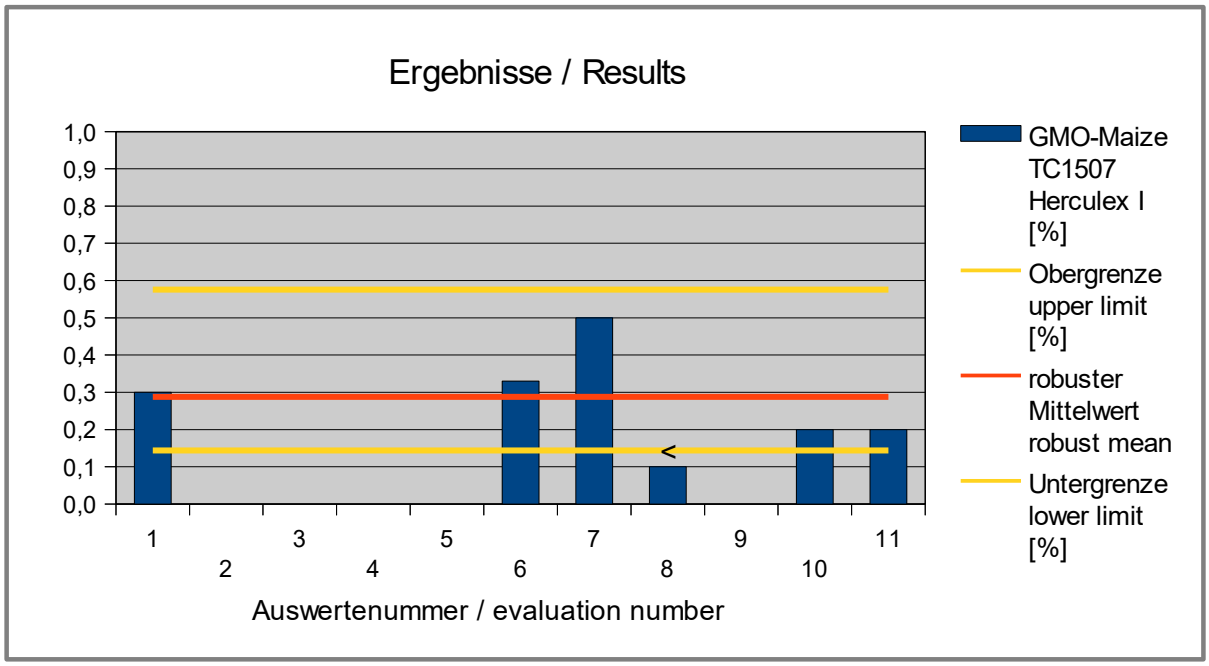

Abb. / Fig. 3: Ergebnisse GVO-Mais TC1507 **/** Results GMO-Maize TC1507

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	GMO-Maize TC1507 Herculex I [%]	Abweichung [%] Deviation [%]	GMO-Maize TC1507 Herculex I [log]	Abweichung [log] Deviation [log]	z-Score (σ_{pt})	Hinweis Remark
1	0,30	0,012	-0,523	0,018	0,12	
2						
3						
4						
5						
6	0,33	0,042	-0,481	0,059	0,39	
7	0,50	0,212	-0,301	0,240	1,6	
8	< 0,10					
9						
10	0,20	-0,088	-0,699	-0,158	-1,1	
11	0,20	-0,088	-0,70	-0,158	-1,1	

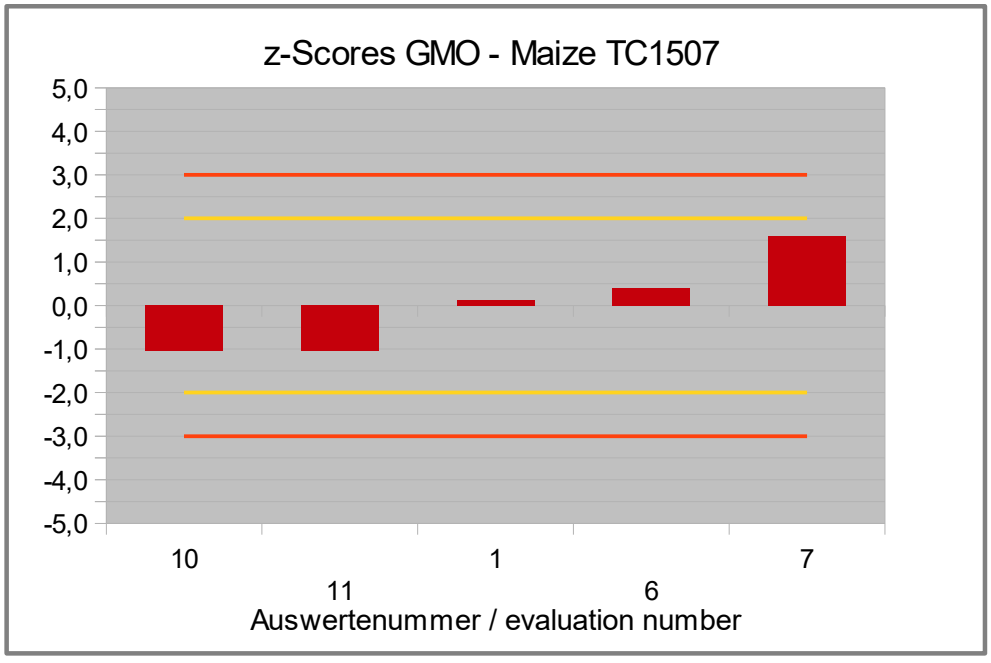

Abb. / Fig. 4: z-Scores GVO-Mais TC1507 / GMO-Maize TC1507

4.3 Proficiency Test GMO-Rape GT73 (RoundUp-Ready)

Qualitative evaluation of the results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[%]	pos/neg	[%]	Agreement with consensus value	
1	negative		positive	>1	2/2 (100%)	
2	negative		positive	2,03	2/2 (100%)	
3						
4	negative	-	positive	2,70	2/2 (100%)	
5	negative	-	positive	3,70	2/2 (100%)	
6	negative		positive	2,70	2/2 (100%)	
7	negative		positive	4,07	2/2 (100%)	
8	negative	<0,10	positive	2,95	2/2 (100%)	
9						
10	negative	<0,1	positive	2,10	2/2 (100%)	
11	positive	>0,9	positive	4,00	1/2 (50%)	

	Sample A		Sample B	
Number positive	1		9	
Number negative	8		0	
Percent positive	11		100	
Percent negative	89		0	
Consensus value	negative		positive	

Comment:
There are consensus values of 89% negative results for sample A and 100% positive results for sample B.

Quantitative evaluation GMO-Rape GT73:

Sample B

Statistic Data	**[log-data]**	**[%-data]**
Number of results	8	8
Number of outliers	0	0
Mean	0,468	2,94
Median	0,451	2,82
Robust Mean (X_{pt})	**0,468**	**2,94**
Robust standard deviation (S*)	**0,134**	**-**
Target range:		
Target standard deviation σ_{pt}	**0,15**	**-**
lower limit of target range	**-**	**1,47**
upper limit of target range	**-**	**5,87**
Quotient S/σ_{pt}*	*0,89*	*-*
Standard uncertainty $U_{(Xpt)}$	*0,059*	*-*
Results in the target range	**-**	*8*
Percent in the target range	**-**	*100%*

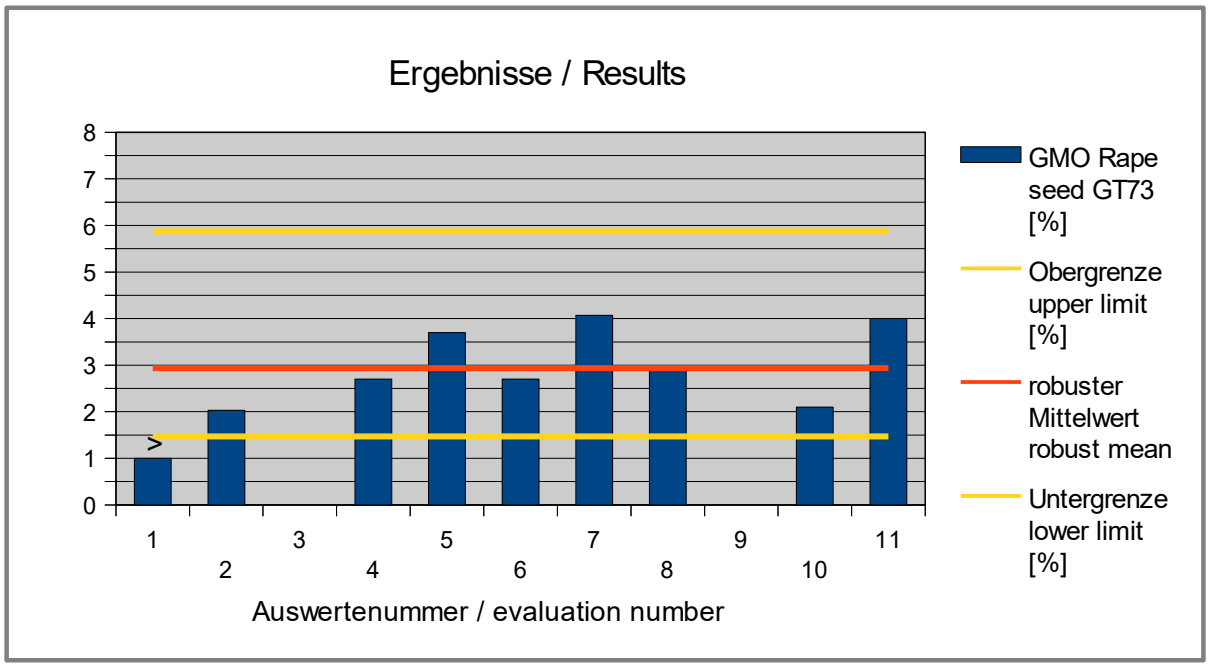

Abb. / Fig. 5: Ergebnisse GVO-Raps GT73 / Results GMO-Rape GT73

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	GMO Rape seed GT73 [%]	Abweichung [%] Deviation [%]	GMO Rape seed GT73 [log]	Abweichung [log] Deviation [log]	z-Score (σ_{pt})	Hinweis Remark
1	> 1,00					
2	2,03	-0,906	0,307	-0,160	-1,1	
3						
4	2,70	-0,236	0,431	-0,036	-0,24	
5	3,70	0,764	0,568	0,100	0,67	
6	2,70	-0,236	0,431	-0,036	-0,24	
7	4,07	1,13	0,610	0,142	0,95	
8	2,95	0,014	0,470	0,002	0,01	
9						
10	2,10	-0,836	0,32	-0,146	-0,97	
11	4,00	1,06	0,60	0,134	0,90	

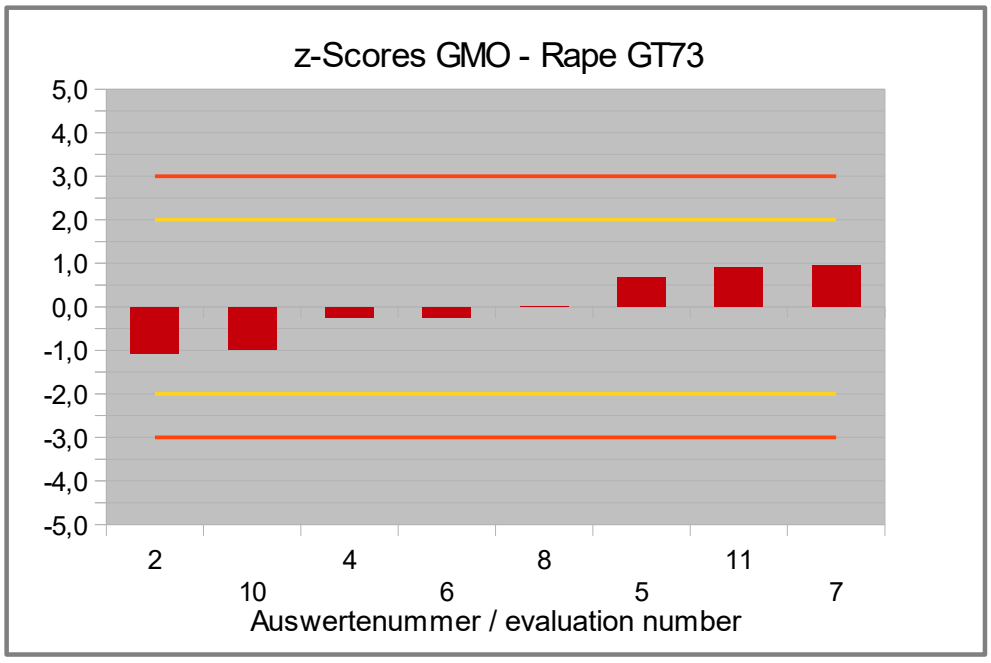

Abb. / Fig. 6: z-Scores GVO-Raps GT73 / GMO-Rape GT73

4.4 Proficiency Test GMO-Soya GTS 40-3-2 (RoundUp-Ready)

Qualitative evaluation of the results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[%]	pos/neg	[%]	Agreement with consensus value	
1	positive	<0,1	positive	>2	2/2 (100%)	Sample A: positive <0,1
2	negative	<0,01	positive	47,5	2/2 (100%)	
3	negative	-	positive	46,9	2/2 (100%)	
4						
5	negative	-	positive	>10	2/2 (100%)	
6	positive	<0,10	positive	16,0	2/2 (100%)	Sample A: positive <0,1
7	negative		positive	11,7	2/2 (100%)	
8	negative	<0,10	negative	<0,10	1/2 (50%)	no positive sample identified
9	negative		positive	20,0	2/2 (100%)	
10	negative	<0,1	positive	21,0	2/2 (100%)	
11	negative	<0,1	positive	13,0	2/2 (100%)	

	Sample A		Sample B	
Number positive	2		9	
Number negative	8		1	
Percent positive	20		90	
Percent negative	80		10	
Consensus value	negative		positive	

Comment:
There are consensus values of 80% negative results for sample A and 90% positive results for sample B.
For the two positive results for sample A, levels of < 0,1% were reported by the participants, so their qualitative assessment was valuated as an agreement for sample A.

Quantitative evaluation GMO-Soya GTS 40-3-2 (for Information):

The quantitative results showed a very heterogeneous distribution. Due to the small number of results, the evaluation was therefore purely informative.

Sample B

Statistic Data	**[log-data]**	**[%-data]**
Number of results	7	7
Number of outliers	0	0
Mean	1,34	21,7
Median	1,30	20,0
Robust Mean (X_{pt})	**1,34**	**21,7**
Robust standard deviation (S*)	**0,281**	**-**
Target range:		
Target standard deviation σ_{pt}	**0,15**	**-**
lower limit of target range	**-**	**10,9**
upper limit of target range	**-**	**43,4**
Quotient S/σ_{pt}*	*1,9*	-
Standard uncertainty $U_{(Xpt)}$	*0,133*	-
Results in the target range	**-**	*5*
Percent in the target range	**-**	*71%*

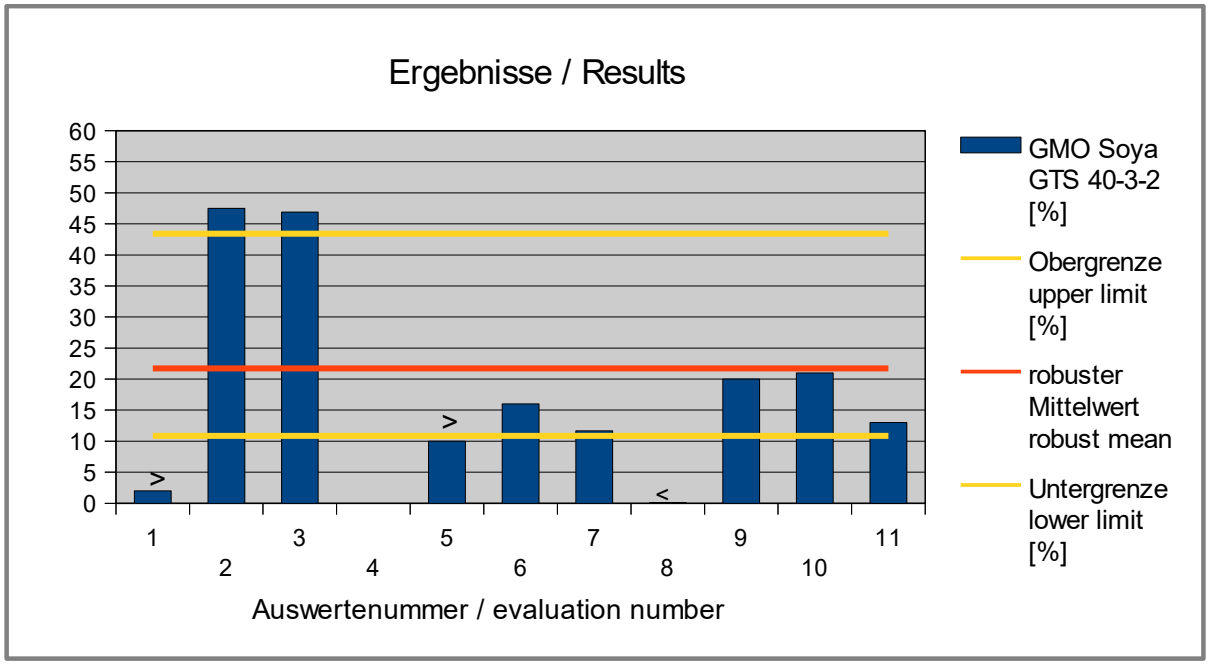

Abb. / Fig. 7: Ergebnisse GVO-Soja GTS 40-3-2 **/** Results GMO-Soya GTS 40-3-2

The following evaluation was purely informative

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	GMO Soya GTS 40-3-2 [%]	Abweichung [%] Deviation [%]	GMO GMO Soya GTS 40-3-2 [log]	Abweichung [log] Deviation [log]	z-Score (σ_{pt})	Hinweis Remark
1	> 2,00					
2	47,5	25,8	1,68	0,340	2,3	
3	46,9	25,2	1,67	0,335	2,2	
4						
5	> 10,0					
6	16,0	-5,70	1,20	-0,132	-0,88	
7	11,7	-10,0	1,07	-0,269	-1,8	
8	< 0,10					
9	20,0	-1,70	1,30	-0,036	-0,24	
10	21,0	-0,735	1,32	-0,015	-0,10	
11	13,0	-8,70	1,11	-0,223	-1,5	

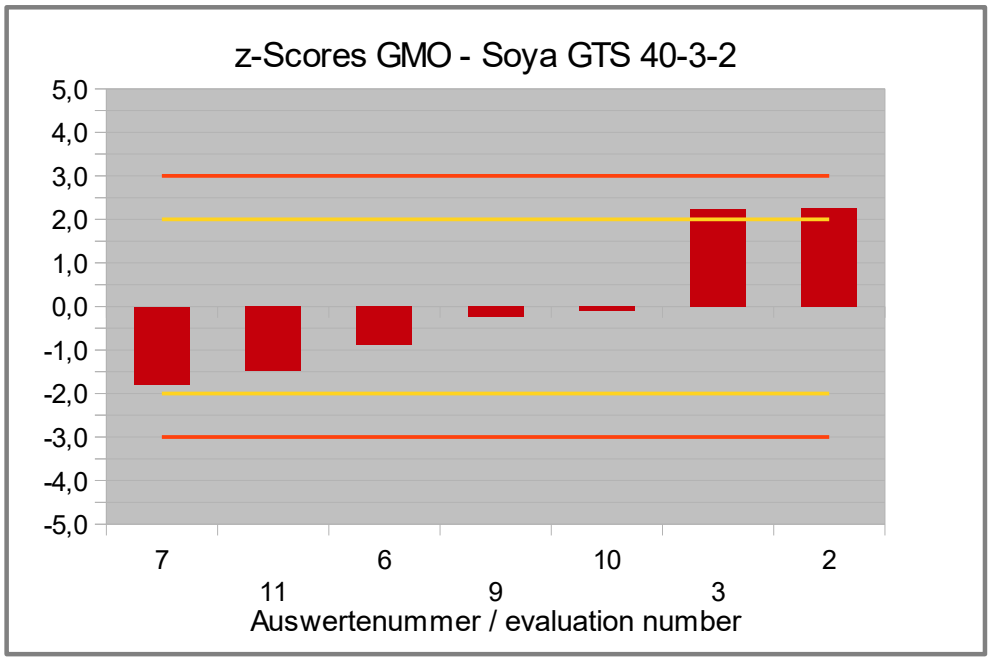

Abb. / Fig. 8: z-Scores GVO-Soja GTS 40-3-2 **/** GMO-Soya GTS 40-3-2

4.5 Proficiency Test GMO-Soya MON89788 (RR2Yield)

Qualitative evaluation of the results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[%]	pos/neg	[%]	Agreement with consensus value	
1	positive	<0,1	positive	>1,4	2/2 (100%)	Sample A: positive <0,1
2	negative		positive	34,3	2/2 (100%)	
3	negative		positive	12,2	2/2 (100%)	
4			-	-		
5	negative		positive	>10	2/2 (100%)	
6	positive	<0,10	positive	33,0	2/2 (100%)	Sample A: positive <0,1
7	negative		positive	23,5	2/2 (100%)	
8	negative	<0,10	positive	0,45	2/2 (100%)	
9	negative		positive	45,0	2/2 (100%)	
10	negative	<0,1	positive	18,4	2/2 (100%)	
11	negative	<0,1	positive	50,0	2/2 (100%)	

	Probe A		Probe B	
Number positive	2		10	
Number negative	8		0	
Percent positive	20		100	
Percent negative	80		0	
Consensus value	negative		positive	

Comment:
There are consensus values of 80% negative results for sample A and 100% positive results for sample B.
For the two positive results for sample A, levels of < 0,1% were reported by the participants, so their qualitative assessment was valuated as an agreement for sample A.

Quantitative evaluation GMO-Soya MON89788:

Sample B

Statistic Data	**[log-data]**	**[%-data]**
Number of results	7	7
Number of outliers	1	1
Mean	1,45	28,0
Median	1,52	33,0
Robust Mean (X_{pt})	**1,45**	**28,0**
Robust standard deviation (S*)	**0,249**	**-**
Target range:		
Target standard deviation σ_{pt}	**0,15**	**-**
lower limit of target range	**-**	**14,0**
upper limit of target range	**-**	**56,0**
Quotient S/σ_{pt}*	*1,7*	-
Standard uncertainty $U_{(Xpt)}$	*0,118*	-
Results in the target range	**-**	*6*
Percent in the target range	**-**	*86%*

° *without outliers (result no. 8)*

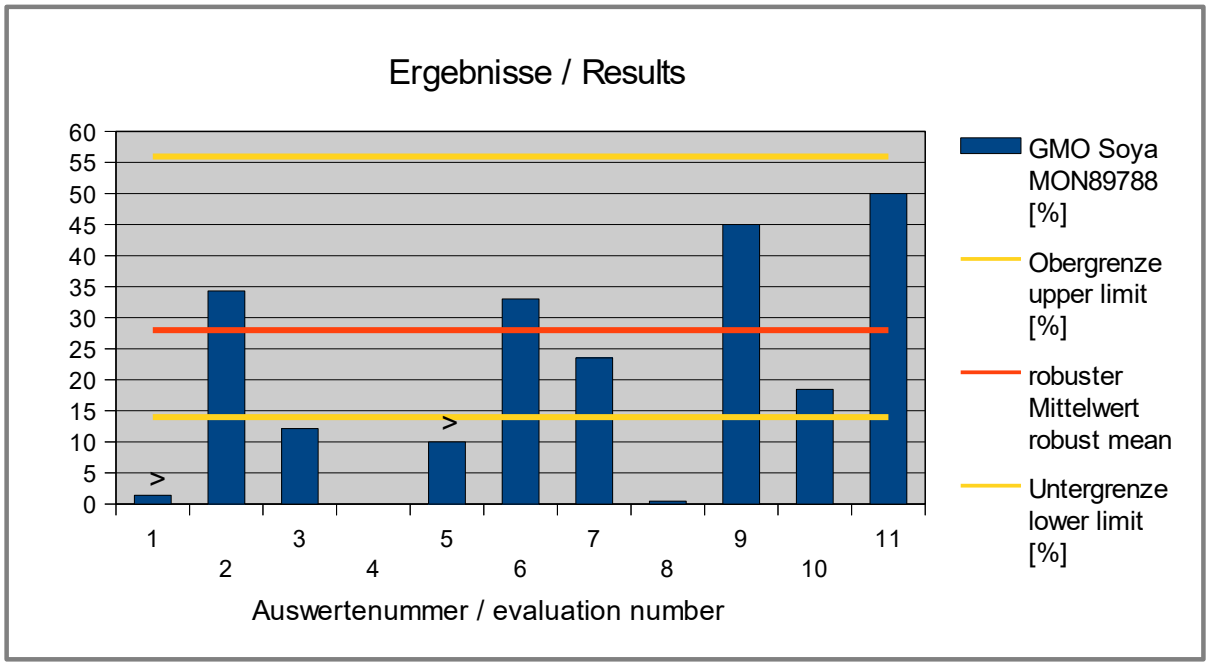

Abb. / Fig. 9: Ergebnisse GVO-Soja MON89788 / Results GMO-Soya MON89788

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	GMO Soya MON89788 [%]	Abweichung [%] Deviation [%]	GMO Soya MON89788 [log]	Abweichung [log] Deviation [log]	z-Score (σ_{pt})	Hinweis Remark
1	> 1,4					
2	34,3	6,32	1,54	0,088	0,59	
3	12,2	-15,83	1,08	-0,362	-2,4	
4						
5	> 10,0					
6	33,0	5,02	1,52	0,072	0,48	
7	23,5	-4,44	1,37	-0,075	-0,50	
8	0,45					outlier excluded
9	45,0	17,02	1,65	0,206	1,4	
10	18,4	-9,55	1,27	-0,181	-1,2	
11	50,0	22,020	1,70	0,252	1,7	

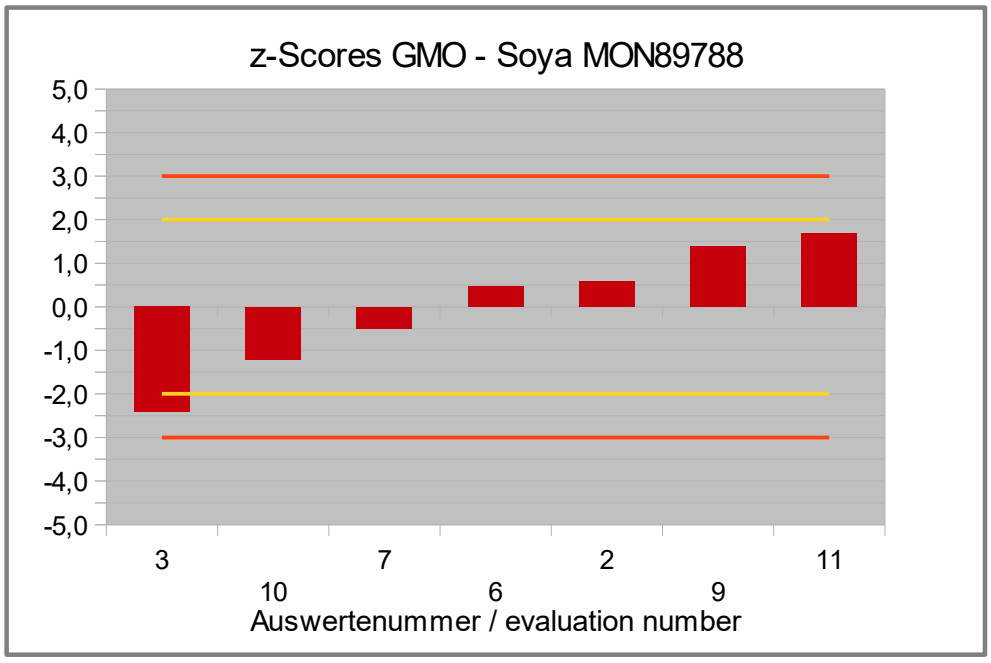

Abb. / Fig. 10: z-Scores GVO-Soja MON89788 / GMO-Soya MON89788

4.6 Participant z-Scores: overview table

Z-Scores for the assigned values from participants' results (consensus values)

Evaluation number	GMO-Maize Bt11	GMO-Maize TC1507	GMO-Rape GT73	GMO-Soya GTS 40-3-2	GMO-Soya MON89788
				informativ	
1	-0,65	0,12			
2			-1,1	2,3	0,59
3				2,2	-2,4
4	-0,65		-0,24		
5			0,67		
6	1,6	0,39	-0,24	-0,88	0,48
7	0,71	1,6	0,95	-1,8	-0,50
8	-4,6		0,01		
9	0,69			-0,24	1,4
10		-1,1	-0,97	-0,10	-1,2
11	0,35	-1,1	0,90	-1,5	1,7

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

5.1 Details by the participants

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

5.1.1 GMO-Maize Bt11

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Negative			Positive		1,70%	0,01%	0,10%		
2	11.10.21	negative			positive			0,01			
3	-										
4	27.09.21	negative	-	-	positive	-	1,7	5 Kopien	0,20	30	Bt11 Mais (SYN-BTØ11-1)
5		negative		-	positive			0.1		40	
6	29.09.21	negative			positive	1:2 verd. 385	3,7		0,1	1,03	
7	05.10.21	negative			positive		2,72	0,01	0,1	20	
8	19.11.21	negative		<0,10%	positive		0,44	15 cop	0,1	39	
9		negative			positive		2,7		0,10	50	
10	28.10.21	negative	o.A.	<0,1%	positive	o.A.	o.A.	0,01	0,1	25	IVS/pat construct
11	27.10.2021	negative	not detectable	<0.1	positive	not detectable	2,40%	0,10%	0,10%		

* NWG Nachweisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3				
4	SureFood® GMO QUANT Bt11 Corn (S2016)	SureFood® Advanced Protokoll 2, Elution mit 100 µl (S1053)		K00
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	genControl First-Bt11-Maize Quant/GEN-IAL		Real Time PCR	
8	Eurofins	King Fisher Flex	Real-Time-PCR	
9	Brodmann et al; J. AOAC Int. 85 (2002)	Clean-up of DNA: CTAB-Wizard	real-time PCR, Ref.-Material: Fluka	
10	VDLUFA 2006	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Gel electrophoresis / 45 cycles / 210 bp, Proficiency test	
11		Macherey Nagel Nucleospin Food Method		

5.1.2 GMO-Maize TC1507 (Herculex I)

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Negative			Positive		0,30%	0,01%	0,10%		
2	11.10.21	negative			positive			0,01			
3	-										
4	-	-	-	-	-	-	-	-	-	-	-
5		negative		-	positive			0.1		40	
6	29.09.21	negative			positive	1:2 verd. 14	0,33		0,1	0,11	
7	5.10./27.10. 2021	negative			positive		0,5	0,01	0,1	20	
8	30.09.21	negative		<0,10%	negative		<0,10%	5 cop	0,1	56	
9											
10	27.10.	negative	o.A.	<0,1%	positive	o.A.	0,20	0,01	0,10	25	event specific (TC1507)
11	27.10.2021	negative	not detectable	<0.1	positive	not detectable	0,20%	0,10%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3				
4	-			
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	genControl First-TC1507-Maize Quant/GEN-IAL		Real Time PCR	
8	Eurofins	King Fisher Flex	Real-Time-PCR	
9				
10	EU database	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 109 bp; AOCS (TC1507)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.3 GMO-Rape GT73 (RoundUp-Ready)

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Negative			Positive		>1%	0,01	0,10		
2	13.10.21	negative			positive		2,03	0,01	0,1		
3	-										
4	27.09.21	negativ	-	-	positiv	-	2,7	5 copies	0,07	30	GT73 Raps (MON-ØØØ73-7
5		negative		-	positive		3.7	5 gene copies (A)/0.1 (B)	0.1	40	
6	29.09.21	negativ			positiv	1:2 verd. 1600	2,7		0,1	0,85	
7	05.10./26.10	negativ			positiv		4,07	0,01	0,1	20	
8	07.10.21	negativ		<0,10%	positiv		2,95	20 cop	0,1	56	
9											
10	23.10.21	negativ	o.A.	<0,1%	positiv	o.A.	2,10	0,01	0,1	25	cevent-specific (RT73)
11	27.10.2021	positiv	traces detected	>0.9	positiv	detectable	4%	0,10%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3				
4	SureFood® GMO QUANT GT73 Canola (S2061)	SureFood® Advanced Protokoll 2, Elution mit 100 µl (S1053)		K00
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	genControl First-RT73-Canola/GEN-IAL		Real Time PCR	
8	Eurofins	King Fisher Flex	Real-Time-PCR	
9				
10	EU database	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 89 bp; AOCS (GT73)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.4 GMO-Soya GTS 40-3-2 (RoundUp-Ready)

Auswerte-nummer	Datum der Analyse	Ergebnis Probe A	Ergebnis Probe A	Ergebnis Probe A	Ergebnis Probe B	Ergebnis Probe B	Ergebnis Probe B	NWG / LOD *	BG / LOQ *	MU*	Spezifität
	Tag/Monat	positiv / negativ	Kopien	%	positiv / negativ	Kopien	%	Kopien / %	Kopien / %	%	Target-Sequenz / -DNA
1		Positive		<0.1	Positive		>2%	0,01	0,10		
2	16.10.21	negative		< 0.01	positive		47,5	0,01	0,1		
3	21.10.21	negativ	-	-	positiv	-	46,89	0,05	0,15	-	GTS 40-3-2 construct
4	-	-	-	-	-	-	-	-	-	-	-
5		negative		-	positive		>10	0.1	0.1	40	
6	28.09.21	positiv	1:2 werd. 4 Kopien	<0,10	positiv	1:2 verd. 2100	16		0,1	4,85	
7	05.und25.10und8.11.	negativ			positiv		11,68	0,01	0,1	20	
8	30.09.21	negativ		<0,10%	negativ		<0,10%	25 cop	0,1	18	
9	01.11.21	negativ			positiv		20		0,10	50	
10	15.10.21	negativ	o.A.	<0,1%	positiv	o.A.	20,97	0,01	0,1	25	RRS1-construct
11	01.11.2021	negativ	nicht nachweisbar	<0.1	positiv	nachweisbar	13%	0,10%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3	Hausverfahren	Extraktion mittels Silicasäulen	real-time PCR	
4	-			
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	genControl First-RR-Soya Quant/GEN-IAL		Real Time PCR	
8	Biotecon	King Fisher Flex	Real-Time-PCR	
9	AllSoyA; Microsynth	Reinigung der DNA: Wizard	Multiplex real-time PCR, Ref.-Material: Fluka	
10	DIN EN ISO 21570/2003	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 108 bp; ERM413 (RRS1)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.5 GMO-Soya MON89788 (RR2Yield)

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Positive		<0.1	Positive		>2%	0,01	0,10		
2	16.10.21	negative		< 0.01	positive		47,5	0,01	0,1		
3	21.10.21	negative	-	-	positive	-	46,89	0,05	0,15	-	GTS 40-3-2 construct
4	-	-	-	-	-	-	-	-	-	-	-
5		negative		-	positive		>10	0.1	0.1	40	
6	28.09.21	positive	1:2 dil. 4 copies	<0,10	positive	1:2 dil. 2100	16		0,1	4,85	
7	05.und25.10und8.11.	negative			positive		11,68	0,01	0,1	20	
8	30.09.21	negative		<0,10%	negative		<0,10%	25 cop	0,1	18	
9	01.11.21	negative			positive		20		0,10	50	
10	15.10.21	negative	o.A.	<0,1%	positive	o.A.	20,97	0,01	0,1	25	RRS1-construct
11	01.11.2021	negative	not detectable	<0.1	positive	not detectable	13%	0,10%	0,10%		

* NWG Nachweisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3	in-house method	Extraction by silica columns	real-time PCR	
4	-			
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	genControl First-RR-Soya Quant/GEN-IAL		Real Time PCR	
8	Biotecon	King Fisher Flex	Real-Time-PCR	
9	AllSoyA; Microsynth	Reinigung der DNA: Wizard	Multiplex real-time PCR, Ref.-Material: Fluka	
10	DIN EN ISO 21570/2003	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 108 bp; ERM413 (RRS1)	
11		Macherey Nagel Nucleospin Food Method		

5.1.6 Reference Gene Maize

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Positive			Positive			5 copies			
2	11.10.21	positive			positive			0,01			
3	-										
4	27.10.21	positive	-		positive	-		0,01	-	30	Zea mays
5	01/10-21/10	positive			positive						
6					positive	1:2 dil. 9000					
7	04.10.21	positive			positive			0,01	0,1	20	
8											
9	08.11.21	positive			positive						hmg
10	27.10.	positive	o.A.		positive	o.A.		0,01	0,10	25	adh1 Gene
11	25.10.2021	positive	detectable		positive	detectable		0,10%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3				
4	SureFood® GMO Plant 4plex Corn/Soya/Canola+IAC (S2158)	SureFood® Advanced Protokoll 2, Elution mit 100 µl (S1053)		K01
5			realtimePCR	
6	Euroins GeneScan Technologies	CTAB-Extraktion/Promega-Wizard/Mobispin	RT-PCR/ 45 cycles	
7	First-Plant Triplex/GEN-IAL	Simplex Easy Spin Food DNA/GEN-IAL	Real Time PCR	
8				
9				
10	EU database	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 109 bp; ERM410 (MON810)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.7 Reference Gene Rape

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Positive			Positive			5 copies			
2	11.10.21	positive			positive			0,01			
3	-										
4	27.09.21	positive	-		positive	-		5 copies	0,07	30	Brassica spp.
5	01/10-21/10	positive			positive						
6					positive	1:2 verd. 57 400					
7	04.10.	positive			positive			0,01	0,1	20	
8											
9											
10	22.10.21	positive (Spuren)	o.A.		positive	o.A.		0,01	0,1	25	cruciferin A
11	25.10.2021	positive	traces		positive	detectable		0,10%	0,10%		

* NWG Nachweisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3				
4	SureFood® GMO QUANT GT73 Canola (S2061)	SureFood® Advanced Protokoll 2, Elution mit 100 µl (S1053)		K00
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	First-Plant Triplex/GEN-IAL		Real Time PCR	
8				
9				
10	EU database	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 89 bp; AOCS (GT73)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.8 Reference Gene Soya

Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
	Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
1		Positive			Positive			5 copies			
2	11.10.21	positive			positive			0,01			
3	21.10.21	positive	-		positive	-		0,015 %	0,04%	-	Soyalectin-Gene
4	27.10.21	positive	-		positive	-		0,01	-	30	Glycine max
5	01/10-21/10	positive			positive						
6		positive	1:2 dil. 18 500 copies		positive	1:2 dil. 10 000					
7	04.10.21	positive			positive			0,01	0,1	20	
8											
9	01.11.21	positive			positive						Lectin
10	15.10.21	positive	o.A.		positive	o.A.		0,01	0,1	25	Lectin
11	25.10.2021	positive	detectable		positive	detectable		0,10%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
1				
2	According to kit manual - "Syntol"	DNA extraction by CTAB method	q-PCR	
3	Hausverfahren	Extraktion mittels Silicasäulen	real-time PCR	
4	SureFood® GMO Plant 4plex Corn/Soya/Canola+IAC (S2158)	SureFood® Advanced Protokoll 2, Elution mit 100 µl (S1053)		K01
5			realtimePCR	
6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
7	First-Plant Triplex/GEN-IAL		Real Time PCR	
8				
9				
10	L00.00-118_2014-02	CTAB, Prot. K, RNAse; Chloroform, Mericon Food Kit (Qiagen)	Real Time PCR / 45 Cycles / 108 bp; ERM413 (RRS1)	
11		Macherey Nagel Nucleospin Food Methode		

5.1.9 Other Parameters

Para-meter	Evaluation number	Date of Analysis	Result Sample A	Result Sample A	Result Sample A	Result Sample B	Result Sample B	Result Sample B	NWG / LOD *	BG / LOQ *	MU*	Specificity
		Day/Month	positive / negative	copies	%	positive / negative	copies	%	copies / %	copies / %	%	Target-Sequence / -DNA
GVO-Soya (A2704-12 LL-Soya)	3	11.10.21	negative	-	-	negative	-	-	0,04	0,02	-	A2704-12 construct
35S Promoter	6	29.09.21	positive			positive						
nos Terminator	6	29.09.21	positive			positive						
FMV Promoter	6	29.09.21	positive			positive						
GA 21	7	08.10./08.11.	negative			positive			0,01		20	
MON 810	7	05.10./26.10.	negative			positive			0,01		20	
MON89034	7	5.10./26.10.	negative			positive			0,01		20	
NK 603	7	05.10./26.10.	negative			positive			0,01		20	
T 25	7	08.10./11.8.	negative			positive			0,01		20	
Mon87701	11	21.11.2021	nicht analysiert	-	-	positive	detectable	40%	0,10%	0,10%		
pat	11	25.10.2021	positive	traces detectable	<0.1%	positive	detectable	3%	0,01%	0,10%		
cry1Ab/Ac	11	25.10.2021	positive	traces detectable	<0.1%	positive	detectable	35%	0,01%	0,10%		
ctp4epsps	11	29.10.2021	positive	traces detectable	<0.1%	positive	detectable	60%	0,01%	0,10%		
35S-P, NOS-T	11	29.10.2021	positive	traces detectable	<0.1%	positive	detectable	13%	0,01%	0,10%		

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncerta

Fortsetzung weitere Parameter

Para-meter	Evaluation number	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
		Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electrophoresis / cycles / amplificate length / reference material	
GVO-Soya (A2704-12 LL-Soya)	3	in-house method	Extraction by silica columns	real-time PCR	
35S Promoter	6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
nos Terminator	6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
FMV Promoter	6	Euroins GeneScan Technologies		RT-PCR/ 45 cycles	
GA 21	7	genControl First-GA21-Maize/GEN-IAL		Real Time PCR	
MON 810	7	genControl 4plex-Maize1 /GEN-IAL		Real Time PCR	Sample B: MIR 162-Maize positive 08.10.
MON89034	7	genControl 4plex-Maize1 /GEN-IAL		Real Time PCR	
NK 603	7	genControl 4plex-Maize1 /GEN-IAL		Real Time PCR	Sample B. MON87701-Soya positive 14.10.
T 25	7	genControl First-T25-Maize/GEN-IAL		Real Time PCR	
Mon87701	11		Macherey Nagel Nucleospin Food Method		Mon87751 negativ in Sample B
pat	11		Macherey Nagel Nucleospin Food Method		calculated against Bt11
cry1Ab/Ac	11		Macherey Nagel Nucleospin Food Method		calculated against Mon87701
ctp4epsps	11		Macherey Nagel Nucleospin Food Method		calculated against Mon89788
35S-P, NOS-T	11		Macherey Nagel Nucleospin Food Method		calculated against GTS40-3-2

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptGMF Sample B

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,9	mg/kg

Result of analysis

Sample	Weight (g)	Particle number	Particles [mg/kg]
1	5,02	67	26,7
2	4,95	67	27,1
3	5,01	74	29,5
4	4,98	70	28,1
5	4,98	63	25,3
6	5,03	66	26,2
7	5,02	66	26,3
8	5,01	72	28,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	68,1	Particle
Standard deviation	3,57	Particle
χ^2 (CHI-Quadrat)	1,31	
Probability	**99**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	27,2	mg/kg
Standard deviation	1,43	mg/kg
rel. Standard deviaton	5,24	%
Horwitz standard deviation	9,73	%
HorRat-value	**0,54**	
Recovery rate	91	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***DLA ptGMF - 2021***
PT name	***DLA ptGMF - GMO-Determination in Feed (qualitative + quantitative): GMO-Soya (RR and RR2), GMO-Maize (2 Events), GMO-Rape Seed / Canola (GT73)***
*Sample matrix**	***Samples A + B:*** *Feed for poultry (ground) / possible ingredients: maize, soy extraction meal, calcium carbonate, wheat, barley, oats, rapeseed pellets, sunflower extraction meal, alfalfa meal, wheat gluten feed, wheat bran, Ca-Na phosphate, sodium chloride, vegetable fatty acids, vegetable oil, minerals , vitamins and other additives*
Number of samples and sample amount	*2 different samples: 10 g each.*
Storage	*dry and dark at cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	***Qualtitative + quantitative:*** *GMO soy (GTS 40-3-2 RoundUp-Ready and MON89788 RR2Yield), maize (bt11 maize and TC1507 Herculex I) and GMO rape (GT73 RoundUp-Ready)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*For samples A + B, a qualitative and a quantitative result can be determined for each parameter and entered in the result file*
Units	*qualitative: positive / negative (detection limit: number of copies or percent)* *quantitative:% (proportion of GMO events per total soy, maize or rapeseed content)*
Number of significant digits	*at least 2 digits*
Further information	*Further information can be given in the result submission file.*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest November 19th 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SWITZERLAND
		Germany
		Germany
		FRANCE
		Germany
		Germany
		SWEDEN
		Germany
		Germany
		GEORGIA
		Germany

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

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

7. Index of references

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