Evaluation Report

proficiency test

DLA 33/2019

GMO-Screening I (qualitative):

5 Samples with positive/negative amounts of p-35S, t-NOS, p-FMV, CP4 EPSPS, 35S-Pat, Cry1Ab/Ac / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (23 September 2019) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
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: 23 September 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
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.

Inhalt / Content

1. Introduction

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

2. Realisation

2.1 Test material

The test materials are 5 different mixtures of common in commerce food samples from European, US-American and Asian suppliers (s. table 1). The raw materials were crushed, sieved (mesh <250 µm to <600 µm), mixed and homogenized. The composition of the samples is given in table 1.

Before homogenization microtracer particles were added in order to check the accuracy of mixing. After homogenization during bottling aliquots were taken for microtracer analysis (s. 2.1.1).

After homogenisation the samples were portioned to approximately 10 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

DLA-Sample	Ingredients (per 100 g)	GMO-Content Maize	GMO-Content Soya
1	Wheat flour Typ 405 (100 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g	-	-
2	Wheat flour Typ 405 (90 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g Soya flour, Vietnamese Supplier (10 g) Ingredients: Soya**bean**, grounded	- -	- positive (GMO-Soya experimental)
3	Wheat flour Typ 405 (90 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g Soya flour, European Supplier (7,5 g) Ingredients: **Soya flour toasted** Nutrients per 100 g: Protein 37 g Soya Chunks, USA Supplier (2,5 g) Ingredients: **Soya flour** Nutrients per 100 g: Protein 47 g, Carbohydrates 17 g, Fat 0,8 g	- - -	- - positive (GMO-Soya experimental)
4	Wheat flour Typ 405 (80 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g Maize Semolina, European Supplier (13 g) Ingredients: **Maize Semolina** Nutrients per 100 g: Protein 7,5 g, Carbohydrates 77 g, Fat 1 g Maize flour, USA Supplier (7,0 g) Ingredients: **Maize flour** Nutrients per 100 g: Protein 9 g, Carbohydrates 79 g, Fat 0 g	- - positive (GMO-Maize experimental)	- - -
5	Wheat flour Typ 405 (80 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g Maize Semolina, European Supplier (10 g) Ingredients: **Maize Semolina** Nutrients per 100 g: Protein 7,5 g, Carbohydrates 77 g, Fat 1 g Soya flour, European Supplier (10 g) Ingredients: **Soya flour toasted** Nutrients per 100 g: Protein 37 g	- - -	- - -

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 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 samples 2-5 showed probabilities of 45%, 100%, 79% and 94%, respectively. 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]. This gave HorRat values of 1,2, 0,4, 0,9 and 0,7, respectively. 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 PT samples was approx. 0,48 (23°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 the test materials (sample 1 to 5) were sent to every participating laboratory in the 21th week of 2019. The testing method was optional. The tests should be finished at Juli 05th 2019 the latest.

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

DLA 33/2019 - GMO-Screening I (qualitative): 5 Samples with positive/ negative amounts of p-35S, t-NOS, p-FMV, CP4 EPSPS, 35S-Pat, Cry1Ab/Ac / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)
There are 5 different test samples which possibly contain the above mentioned parameters. The indication of results and evaluation will be done exclusively qualitative (positive/negative). Results for specific sequences, screening sequences and other events can be analyzed.

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 sent by email or were available on our website. The results given as positive/negative were evaluated.
Queried and documented were the indicated results and details of the test methods like specificities, 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 21 participants submitted their results in time.

3. Evaluation

The evaluation of the GMO-screening proficiency test was done exclusively qualitative.

The results are presented for all 5 test samples in separate tables for each parameter p-35S, t-NOS, p-FMV, CTP2-CP4 EPSPS, 35S-Pat, Cry1Ab/Ac as well as GMO-Maize (Bt11, MIR604), Maize-DNA and GMO-Soya (RR GTS 40-3-2, RR2 MON89788), Lectin-DNA and other DNA.

3.1 Agreement with consensus values from participants

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

The qualitative evaluation of the results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the five PT-samples** with GMO-containing ingredients (see Tab. 1).
The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

4. Results

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

The participant results and evaluation are tabulated as follows:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive				
Number negative				
Percent positive				
Percent negative				
Consensus value				
Spiking				

4.1 Proficiency Test GMO

4.1.1 Results: p-35S-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
p-35S	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
2	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
3	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
4	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
5	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
6	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
7	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
8	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
9	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11a	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11b	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
12	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	
13	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
15	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
16	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
17	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
18	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	Sample 2 traces (<0,1%)
19	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
20	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
21	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	20	22	22	0
Number negative	22	2	0	0	22
Percent positive	0	91	100	100	0
Percent negative	100	9	0	0	100
Consensus value	negative	positive	positive	positive	negative
Spiking	negative	positive	positive	positive	negative

Comments:
For all 5 samples consensus values with four times 100% and once 91% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.2 Results: t-NOS-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
t-NOS	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
2	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
3	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
4	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
5	negative	positive	positive	positive	positive	4/5 (80%)	4/5 (80%)	
6	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	
7	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
8	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
9	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11a	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11b	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
12	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
13	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
15	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
16	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
17	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	
18	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	Sample 2 traces (<0,1%)
19	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
20	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
21	negative	negative	positive	negative	negative	3/5 (60%)	3/5 (60%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	18	22	21	1
Number negative	22	4	0	1	21
Percent positive	0	82	100	95	5
Percent negative	100	18	0	5	95
Consensus value	negative	positive	positive	positive	negative
Spiking	negative	positive	positive	positive	negative

Comments:
For all 5 samples consensus values with two times 100%, two times 95% and once 82% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.3 Results: p-FMV-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
P-FMV	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
2	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
3	-	-	-	-	-			
4	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
5	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
6	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
7	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
10	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
11	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
12	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
13	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
14	negative	positive	negative	negative	negative	4/4 (100%)	4/5 (80%)	
15	negative	negative	negative	negative	negative	3/4 (75%)	3/5 (60%)	
16	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
17	negative	positive	negative	negative	negative	4/4 (100%)	4/5 (80%)	
18	negative	negative	negative	negative	negative	3/4 (75%)	3/5 (60%)	Sample 2 and 3 traces (<0,1%)
19	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
20	negative	positive	positive	negative	negative	4/4 (100%)	5/5 (100%)	
21	negative	negative	negative	negative	negative	3/4 (75%)	3/5 (60%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	16	14	0	0
Number negative	19	3	5	19	19
Percent positive	0	84	74	0	0
Percent negative	100	16	26	100	100
Consensus value	negative	positive	none	negative	negative
Spiking	negative	positive	positive	negative	negative

Comments:

For the samples 1, 2, 4 and 5 consensus values with three times 100% and once 84% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).
With 74% positive results, just no consensus with ≥75% positive or negative results was obtained for sample 3.

4.1.4 Results: CTP2-CP4 EPSPS-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
CTP2-CP4 EPSPS	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	negative	positive	positive	negative	negative	4/5 (80%)	4/5 (80%)	
3	-	-	-	-	-			
4	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	Sample 2 positive (<0,1%), Sample 4 at LOD
5	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
6	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
7	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
8	negative	positive	positive	positive	positive	4/5 (80%)	4/5 (80%)	
9	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	-	-	-	-	-			
14	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	negative	positive	negative	positive	negative	4/5 (80%)	4/5 (80%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	10	10	10	1
Number negative	11	1	1	1	10
Percent positive	0	91	91	91	9
Percent negative	100	9	9	9	91
Consensus value	negative	positive	positive	positive	negative
Spiking	negative	positive	positive	positive	negative

Comments:
For all 5 samples consensus values with once 100% and four times 91% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.5 Results: 35S-Pat-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
35S-Pat	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	positive	positive	negative	3/3 (100%)	5/5 (100%)	
2	-	-	-	-	-			
3	-	-	-	-	-			
4	negative	negative	negative	negative	negative	2/3 (67%)	2/5 (40%)	Sample 2, 3, 4 positive (0,1%)
5	negative	positive	negative	positive	negative	3/3 (100%)	4/5 (80%)	
6	negative	negative	negative	positive	negative	3/3 (100%)	3/5 (60%)	
7	negative	positive	positive	positive	negative	3/3 (100%)	5/5 (100%)	
8	negative	positive	negative	positive	negative	3/3 (100%)	4/5 (80%)	
9	-	-	-	-	-			
10	negative	positive	positive	positive	negative	3/3 (100%)	5/5 (100%)	
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	negative	negative	positive	negative	3/3 (100%)	3/5 (60%)	
14	negative	negative	negative	positive	negative	3/3 (100%)	3/5 (60%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	negative	negative	negative	positive	negative	3/3 (100%)	3/5 (60%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	5	3	9	0
Number negative	10	5	7	1	10
Percent positive	0	50	30	90	0
Percent negative	100	50	70	10	100
Consensus value	negative	none	none	positive	negative
Spiking	negative	positive	positive	positive	negative

Comments:

For the samples 1, 4 and 5 consensus values with two times 100% and once 90% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).
For samples 2 and 3 no consensus with ≥75% positive or negative results were obtained.

4.1.6 Results: Cry1Ab/Ac-Screening-Sequence

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Cry1Ab/Ac	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
7	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
10	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
11	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
14	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	-	-	-	-	-			not clear

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	0	0	8	0
Number negative	8	8	8	0	8
Percent positive	0	0	0	100	0
Percent negative	100	100	100	0	100
Consensus value	negative	negative	negative	positive	negative
Spiking	negative	negative	negative	positive	negative

Comments:
For all 5 samples consensus values with 100% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.7 Results: GMO-Maize Bt11

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
GMO-Maize (Bt11)	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	negative	negative	negative	negative	negative	4/4 (100%)	4/5 (80%)	
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	negative	negative	positive	negative	4/4 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	negative	negative	negative	positive	negative	4/4 (100%)	5/5 (100%)	
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	negative	negative	positive	negative	4/4 (100%)	5/5 (100%)	
14	negative	negative	negative	positive	negative	4/4 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	negative	negative	positive	negative	negative	3/4 (75%)	3/5 (60%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	0	1	4	0
Number negative	6	6	5	2	6
Percent positive	0	0	17	67	0
Percent negative	100	100	83	33	100
Consensus value	negative	negative	negative	none	negative
Spiking	negative	negative	negative	positive	negative

Comments:

For the negative samples consensus values with three times 100% and once 83% negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).
With 67% positive results, just no consensus with ≥75% positive or negative results was obtained for sample 4.

4.1.8 Results: GMO-Maize MIR604

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
GMO-Maize (MIR604)	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	negative	positive	positive	negative	4/5 (80%)	4/5 (80%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
14	negative	negative	negative	positive	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	-	-	-	-	-			not clear

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	0	1	4	0
Number negative	4	4	3	0	4
Percent positive	0	0	25	100	0
Percent negative	100	100	75	0	100
Consensus value	negative	negative	negative	positive	negative
Spiking	negative	negative	negative	positive	negative

Comments:
For all 5 samples consensus values with four times 100% and once 75% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.9 Results: Maize-DNA (Maize-specific)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Maize specific DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	negative	negative	positive	positive	positive	5/5 (100%)	4/5 (80%)	
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	positive	positive	positive	positive	4/5 (80%)	3/5 (60%)	
8	negative	negative	positive	positive	positive	5/5 (100%)	4/5 (80%)	
9	negative	negative	positive	positive	positive	5/5 (100%)	4/5 (80%)	
10	negative	negative	negative	positive	positive	4/5 (80%)	5/5 (100%)	
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	negative	positive	positive	positive	5/5 (100%)	4/5 (80%)	
14	negative	negative	negative	positive	positive	4/5 (80%)	5/5 (100%)	
15	-	-	-	-	-			
16	negative	negative	positive	positive	positive	5/5 (100%)	4/5 (80%)	
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	-	-	-	-	-			not clear

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	1	6	8	8
Number negative	8	7	2	0	0
Percent positive	0	13	75	100	100
Percent negative	100	88	25	0	0
Consensus value	negative	negative	positive	positive	positive
Spiking	negative	negative	negative	positive	positive

Comments:
For all samples consensus values with three times 100%, once 88% and once 75% positive or negative results were obtained, respectively.
With the exception of sample 3, the consensus values are in agreement with the addition of the maize-containing ingredients (spiking). The presence of traces of maize in sample 3 can not be excluded.

4.1.10 Results: CMO-Soya RR (GTS 40-3-2)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
GMO-Soya RR (GTS 40-3-2)	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	positive	positive	positive	negative	4/5 (80%)	4/5 (80%)	
8	-	-	-	-	-			
9	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11a	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11b	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	negative	negative	positive	negative	negative	4/5 (80%)	4/5 (80%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	7	8	1	0
Number negative	8	1	0	7	8
Percent positive	0	88	100	13	0
Percent negative	100	13	0	88	100
Consensus value	negative	positive	positive	negative	negative
Spiking	negative	positive	positive	negative	negative

Comments:
For all 5 samples consensus values with three times 100% and two times 88% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.11 Results: GMO-Soya RR2 (MON89788)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
GMO-Soya RR2 (MON89788)	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11a	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11b	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	positive	positive	-	-	2/2 (100%)	2/2 (100%)	
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	negative	positive	negative	negative	negative	4/5 (80%)	4/5 (80%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	10	9	0	0
Number negative	9	0	1	9	9
Percent positive	0	100	90	0	0
Percent negative	100	0	10	100	100
Consensus value	negative	positive	positive	negative	negative
Spiking	negative	positive	positive	negative	negative

Comments:
For all 5 samples consensus values with four times 100% and once 90% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.12 Results: Lectin-DNA (Soya-specific)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Lectin DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	-	-	-	-	-			
2	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
7	negative	positive	positive	positive	positive	4/5 (80%)	4/5 (80%)	
8	negative	positive	positive	positive	positive	4/5 (80%)	4/5 (80%)	
9	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
10	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
11	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	negative	positive	positive	negative	positive	5/5 (100%)	5/5 (100%)	
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	-	-	-	-	-			

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	10	10	2	10
Number negative	10	0	0	8	0
Percent positive	0	100	100	20	100
Percent negative	100	0	0	80	0
Consensus value	negative	positive	positive	negative	positive
Spiking	negative	positive	positive	negative	positive

Comments:
For all 5 samples consensus values with four times 100% and once 80% positive or negative results were obtained, respectively.
The consensus values are in agreement with the addition of the Soya-containing ingredients (spiking).

4.1.13 Results: Other Parameters (DNA)

Qualitative valuation of results

Evaluation number	Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Remarks
	further DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	
3	ABII	negative	positive	positive	positive	negative	
4	bar	negative	negative	negative	negative	negative	
5a	bar	negative	negative	negative	negative	negative	
6a	bar	negative	negative	negative	negative	negative	
8	bar	negative	negative	negative	negative	negative	
9a	CaMV/BMV -Wildtyp	-	negative	negative	negative	-	
6b	P-nos nptII	negative	negative	negative	negative	negative	
9b	Plant-actin	positive	positive	positive	positive	positive	
11	Plants proof	positive	positive	positive	positive	positive	
14a	Mon88017	-	-	-	positive	-	
14b	NK603	-	-	-	positive	-	
5b	NPTII	negative	negative	negative	positive	negative	

5. Documentation

5.1 Details by the participants

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

5.1.1 p-35S-Screening-Sequence

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1	19.06.19	DNA		Microbiologique, Inc.; R-Biopharm, S2026:2017-04	Extraction	Real Time PCR	
2	04.07.19						
3	13.06.19			GeneScan			
4	28.06.19	p-35S	0.1 %	DIN EN ISO 21569:2013-08	mod. Wizard®-DNA-Clean-UP System	Real Time PCR / 83 Bp	
5	08.06.19	target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 1	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
6	06.06.19		30.12.99	ISO 21569:2005/Amd1:2013	CTAB	realtime PCR / 40 cycles / CRM Bt11	ISO 21569:2005/Amd1:2013
7	04.07.19			L-00.00-122(06/2008)	Nucleospin(R)Food	Real Time PCR	
8	02.07.19		0.1 %	BVL L 00.00-122:2008	Nucleospin Food	Real Time PCR / 45 cycles	
9	25.06.		5 copies	§64 LFGB L00.00-122	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	4.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11a	29.05.19	DNA	0.02 %	ASU §64 Method 00.00 122	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
11b	07.06.19	DNA	0.01%	genControl® RT-Triplex-35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey-Nagel	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
12	01.07.19	35S Promotor	≤ 5 DNA copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV/IAC	SureFood® PREP Basic	Real Time PCR, 45 Cycles	-
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15	19.06.19	p-35S CaMV		biotecon	biotecon	real time PCR 50 cycles , ligeth cycler 96	
16	12.06./19.06.2019	Target-Sequence	5 copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC	SureFood® PREP Basic Art. No. S1052	real Time PCR, lt. Manual	
17	29.05.19			GEN-IAL		Real Time PCR	
18	28/05	35S	< 0,1%	GEN-IAL genControl RT-Triplex IV p35S / NOS / pFMV, incl. IC	Congen SureFood PREP Basic Extraction-kit	Real Time PCR, 45 Cycles, reference material ERM-BF 410dp	*traces (< 0,1 %)
19	31.05.19	-	5 DNA-Copies	r-biopharm / L00.00-118, -119, -121 und -148	Extraction control by detection of plant DNA with PCR	Real Time PCR	-
20	28.05.19	P35S/DNA	30.12.99	Imegen	CTAB	Real time PCR	
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 2	DNA Extratcion with commercial kit	Real Time PCR 50 Cycles	

5.1.2 t-NOS-Screening-Sequence

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1	19.06.19	DNA		Microbiologique, Inc.; R-Biopharm, S2026:2017-04	Extraction	Real Time PCR	
2	04.07.19						
3	13.06.19			GeneScan			
4	28.06.19	t-NOS	0.1 %	DIN EN ISO 21569:2013-08	mod. Wizard®-DNA-Clean-UP System	Real Time PCR / 95 Bp	
5	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 1	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
6	06.06.19		30.12.99	ISO 21569:2005/Amd1:2013	CTAB	realtime PCR / 40 cycles / CRM Bt11	ISO 21569:2005/Amd1:2013
7	04.07.19			L-00.00-122(06/2008)	Nucleospin(R)Food	Real Time PCR	
8	05.07.19		0.1 %	BVL L 00.00-122:2008			
9	25.06.		10 Copies	§64 LFGB L00.00-122	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	4.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11a	07.06.19	DNA	0.01 %	genControl® RT-Triplex-35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey-Nagel	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
11b	29.05.19	DNA	0.03 %	ASU §64 Method 00.00 122	in house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
12	01.07.19	NOS Terminator	≤ 5 DNA Copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV/IAC	SureFood® PREP Basic	Real Time PCR, 45 Cyclen	-
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15	19.06.19	t-NOS		biotecon	biotecon	real time PCR 50 cycles , ligeth cycler 96	
16	12.06./19.06.2020	Target-Sequenz	5 Copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC	SureFood® PREP Basic Art. No. S1052	real Time PCR, lt. Manual	
17	29.05.19			GEN-IAL		Real Time PCR	
18	28/05	nos	< 0,1%	GEN-IAL genControl RT-Triplex IV p35S / NOS / pFMV, incl. IC	Congen SureFood PREP Basic Extraction kit	Real Time PCR, 45 Cycles, reference material ERM-BF 410dp	*traces (< 0,1 %)
19	31.05.19	-	5 DNA-Copies	r-biopharm / L00.00-118, -119, -121 und -148	Extraction controll by detection of plant DNA with PCR	Real Time PCR	-
20	28.05.19	TNOS/DNA	30.12.99	Imegen	CTAB	Real time PCR	
21	27.05.19	35S/NOS/FMV Screening	50 Cycles	GMO 5 Target Screening 1 and 3	DNA Extratcion with commercial kit	Real Time PCR 50 Cycles	

5.1.3 p-FMV-Screening-Sequence

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1	19.06.19	DNA		Microbiologique, Inc.; R-Biopharm, S2026:2017-04	Extraction	Real Time PCR	
2	04.07.19						
3							
4	28.06.19	p-FMV	0.1 %	DIN EN ISO 21569:2013-08	mod. Wizard®-DNA-Clean-UP System	Real Time PCR / 82 Bp	
5	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 1	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
6	06.06.19		30.12.99	ISO 21569-5:2016	CTAB	realtime PCR / 40 cycles / CRM H7-1	ISO 21569-5:2016
7	04.07.19			L-00.00-148(02/2014)	Nucleospin(R)Food	Real Time PCR	
8	-						
9	25.06.		5 copies	§64 LFGB L00.00-124	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	4.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11	29.05.19	DNA	0.003 %	ASU §64 Method 00.00 148	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
12	01.07.19	FMV Promotor	≤ 5 DNA copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV/IAC	SureFood® PREP Basic	Real Time PCR, 45 Cycles	-
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15	19.06.19	p-FMV		biotecon	biotecon	real time PCR 50 cycles , ligeth cycler 96	
16	12.06./19.06 .2021	Target-Sequenz	5 copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC	SureFood® PREP Basic Art. No. S1052	real Time PCR, lt. Manual	
17	29.05.19			GEN-IAL		Real Time PCR	
18	28/05	FMV	< 0,1%	GEN-IAL genControl RT-Triplex IV p35S / NOS / pFMV, incl. IC	Congen SureFood PREP Basic Extraction kit	Real Time PCR, 45 cycles, reference material ERM-BF 410dp	*traces (< 0,1 %)
19	31.05.19	-	5 DNA-copies	r-biopharm / L00.00-118, -119, -121 und -148	Extrcktion controll by detection of plant DNA with PCR	Real Time PCR	-
20	28.05.19	PFMV/DNA	30.12.99	Imegen	CTAB	Real time PCR	
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 4	DNA Extratcion with commercial kit	Real Time PCR 50 cycles	

5.1.4 CTP2-CP4 EPSPS-Screening Sequenz

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2	04.07.19						
3							
4	28.06.19	CTP2-CP4-EPSPS	0.1 %	„genControl® RT Triplex V bar/pat/EPSPS“ ; FA. GEN-IAL	mod. Wizard®-DNA-Clean-UP System	Real Time PCR / 88 Bp	Sample 02 was positive but < 0.1%; 04 positive at the LOD
5	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 2	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
6	06.06.19		30.12.99	ISO 21569:2005/Amd1:2013	CTAB	realtime PCR / 40 cycles / CRM H7-1	ISO 21569:2005/Amd1:2013
7	04.07.19			L-00.00-125(12/2008)	Nucleospin(R)Food	Real Time PCR	
8	02.07.19		0.1 %	BVL L 00.00-125:2008			Probe 5 Ct > 40
9	25.06.		5 copies	§64 LFGB L00.00-125	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11	07.06.19	DNA	0.01 %	genControl® RT-Triplex-35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey-Nagel	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
12	-						
13							
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16							
17							
18							
19	-						
20							
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 5	DNA Extraction with commercial kit	Real Time PCR 50 cycles	

5.1.5 35S-Pat-Screening Sequenz

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1	19.06.19	DNA		Microbiologique, Inc.	Extraction	Real Time PCR	
2							
3							
4	28.06.19	pat	0.1 %	„genControl® RT Triplex V bar/pat /EPSPS“ ; FA. GEN-IAL	mod. Wizard®-DNA-Clean-UP System	Real Time PCR/ 108 Bp	Sample 02, 03, 04 positive but < 0.1 %
5	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 2	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
6	06.06.19		30.12.99	ISO 21569-3:2015	CTAB	realtime PCR / 40 cycles / CRM TC1507	ISO 21569-3:2015
7	05.07.19			QL-ELE-00-025	Nucleospin(R)Food	Real Time PCR	
8	03.07.19		0.1 %	BVL G 30.40-1:2012			Sample 2 Ct > 40
9							
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11							
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16							
17							
18							
19	-						
20							
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 6	DNA Extraction with commercial kit	Real Time PCR 50 cycles	

5.1.6 Cry1Ab/AC-Screening Sequenz

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2							
3							
4							
5							
6	06.06.19		30.12.99	ISO 21569-6:2016	CTAB	realtime PCR / 40 cycles / CRM Bt11	ISO 21569-6:2016
7	04.07.19			QL-ELE-00-016	Nucleospin(R)Food	Real Time PCR	
8	-						
9	25.06.		10 copies	§64 LFGB L15.06-3	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Congen	FFS-Kit, Promega	Real Time PCR	
11	29.05.19	DNA	10 copies	ASU §64 Method 15.06 3	In-house method	Real Time PCR	
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16	12.06./19.06 .2020	Target-Sequence	0,05% based on MON87708	ASU L 15.06-3	SureFood® PREP Basic Art. No. S1052	real Time PCR, Biozxm Blue Sample qPCR Mix according to protocol	
17							
18							
19	-						
20							
21							not clear

5.1.7 GMO-Maize (Bt11)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2	04.07.19						
3							
4							
5							
6							
7	04.07.19				Nucleospin(R)Food	Real Time PCR	
8	-						
9							
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11							
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16							
17							
18							
19	-						
20							
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 6	DNA Extraction with commercial kit	Real Time PCR 50 cycles	

5.1.8 GMO-Maize (MIR604)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2							
3							
4							
5							
6							
7	04.07.19			QT-EVE-ZM-013	Nucleospin(R)Food	Real Time PCR	
8	-						
9							
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11							
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16							
17							
18							
19	-						
20							
21							not clear

5.1.9 Maize-DNA (Maize-specific)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2	04.07.19						
3							
4							
5							
6							
7	04.07.19			QT-EVE-ZM-013	Nucleospin(R)Food	Real Time PCR	
8	02.07.19						
9	01.07.		5 copies	§64 LFGB L00.00-105	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Congen	FFS-Kit, Promega	Real Time PCR	
11							
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16	12.06./19.06 .2020	Target-Sequence	-	ASU L 00.00-105	SureFood® PREP Basic Art. No. S1052	real Time PCR, Biozxm Blue Sample qPCR Mix according to protokol	Pract. LOD not determinded
17							
18							
19	-						
20							
21							not clear

5.1.10 GMO-Soya RR (GTS 40-3-2)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2							
3							
4							
5							
6							
7	04.07.19			ISO/FDIS 21570:2004	Nucleospin(R)Food	Real Time PCR	
8	-						
9	25.06.		5 copies	EURL-GMFF MON40-3-2 Soyabean	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11a	29.05.19	DNA	0.05%	ASU §64 Method 00.00 105	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
11b	21.06.19	DNA	8 copies	genControl® RT RR-Soya Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey-Nagel	Real Time PCR	
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16							
17							
18							
19	-						
20							
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 6	DNA Extraction wth commercial kit	Real Time PCR 50 cycles	

5.1.11 GMO-Soya RR2 (MON89788)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2	04.07.19						
3							
4							
5							
6							
7	04.07.19			QT-EVE-GM-006	Nucleospin(R)Food	Real Time PCR	
8	-						
9	25.06.		20 copies	EURL-GMFF MON89788 Soyabean	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Genial	FFS-Kit, Promega	Real Time PCR	
11a	29.05.19	DNA	0.0015 %	JRC (QT-EVE-GM-006)	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
11b	21.06.19	DNA	2 copies	genControl® RT RR2-Soya Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey-Nagel	Real Time PCR	
12	-						
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16	12.06./19.06 .2020	Target-Sequence	5 copies	SureFood® GMO QUANT RR2Y Soya	SureFood® PREP Basic Art. No. S1052	real Time PCR, according to Manual	
17							
18							
19	-						
20							
21	27.05.19	35S/NOS/FMV Screening	50 cycles	GMO 5 Target Screening 1 and 6	DNA Extraction with commercial kit	Real Time PCR 50 cycles	

5.1.12 Lectin-DNA (Soya-specific)

Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
	Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
1							
2	04.07.19						
3							
4							
5							
6	06.06.19			PD CEN/TS 15634-5:2016			PD CEN/TS 15634-5:2016
7	04.07.19			QT-EVE-GM-006	Nucleospin(R)Food	Real Time PCR	
8	02.07.19						
9	01.07.		5 copies	§64 LFGB L00.00-105 C.2	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
10	5.6.			Congen	FFS-Kit, Promega	Real Time PCR	
11	03.06.19	DNA	0.015 %	ASU §64 Method 00.00 105	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
12							
13						qPCR	
14	29.05.2019		0,01%		CTAB	RealTime PCR	
15							
16	12.06./19.06 .2020	Target-Sequence	5 copies	SureFood® GMO QUANT RR2Y Soya	SureFood® PREP Basic Art. No. S1052	real Time PCR, according to Manual	
17							
18							
19	-						
20							
21							

5.1.13 Other Parameter (DNA)

Parameter	Evaluati-on No.	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
		Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g real time PCR / gel electro-phoresis / cycles / amplificate length / reference material	
ABII	3	13.06.19			GeneScan			
bar	4	28.06.19	bar	0.1 %	„genControl® RT Triplex V bar/pat/EPSPS“ ; FA. GEN-IAL	mod. Wizard®-DNA-Clean-UP System	Real Time PCR / 60 Bp	
BAR	5a	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 2	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	
bar	6a	06.06.19		30.12.99	ISO 21569:2005/Amd1:2013	CTAB	realtime PCR / 40 cycles / CRM Bt176	ISO 21569:2005/Amd1:2013
bar	8	bar						
CaMV/BMV-Wildtype	9a	02.07.		no specification	Wolf et al., Eur Food Res Technol 210: 367-372	DNeasy Mericon Food 2g	conventional PCR, 50 cycles	
P-nos nptII	6b	06.06.19		30.12.99	ISO 21569-4:2016	CTAB	realtime PCR / 40 cycles / CRM EH92	ISO 21569-4:2016
Pflanzen-Actin	9b	25.06.		1 copy	Laube et al. Food Chemistry, 118: 979-986	DNeasy Mericon Food 2g	Real Time PCR, 45 cycles	
Pflanzen-Nachweis	11	29.05.19	DNA	0.02 %	In-house method	In-house method	Real Time PCR	Limit of Detection: Specification of decimal places with 'dot'
Mon88017	14a	29.05.2019		0,01%		CTAB	RealTime PCR	
NK603	14b	29.05.2019		0,01%		CTAB	RealTime PCR	
NPTII	5b	08.06.19	Target-Sequence / -DNA	0.1% w/w	SureFood GMO Screen 2	Surefood Prep Advanced Kit	Real-Time PCR / 45 Cycles	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 33-2019 Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	76	30,1
2	5,02	88	35,1
3	5,03	95	37,8
4	4,99	70	28,1
5	4,99	83	33,3
6	5,07	69	27,2
7	5,03	80	31,8
8	5,00	78	31,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	79,9	Particles
Standard deviation	8,84	Particles
χ^2 (CHI-Quadrat)	6,85	
Probability	**45**	%
Recovery rate	109	%

Normal distribution		
Number of samples	8	
Mean	31,8	mg/kg
Standard deviation	3,52	mg/kg
rel. Standard deviaton	11,1	%
Horwitz standard deviation	9,51	%
HorRat-value	**1,2**	
Recovery rate	109	%

Microtracer Homogeneity Test
DLA 33-2019 Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,07	74	29,2
2	5,06	67	26,5
3	5,02	70	27,9
4	5,00	66	26,4
5	5,10	68	26,7
6	5,00	68	27,2
7	5,03	68	27,0
8	5,04	65	25,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	68,2	Particles
Standard deviation	2,66	Particles
χ^2 (CHI-Quadrat)	0,72	
Probability	**100**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	27,1	mg/kg
Standard deviation	1,05	mg/kg
rel. Standard deviaton	3,9	%
Horwitz standard deviation	9,74	%
HorRat-value	**0,40**	
Recovery rate	113	%

Microtracer Homogeneity Test
DLA 33-2019 Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	79	31,3
2	5,02	79	31,5
3	5,00	67	26,8
4	5,05	84	33,3
5	5,01	69	27,5
6	5,07	84	33,1
7	5,04	75	29,8
8	5,09	70	27,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	75,9	Particles
Standard deviation	6,53	Particles
χ^2 (CHI-Quadrat)	3,93	
Probability	**79**	%
Recovery rate	129	%

Normal distribution		
Number of samples	8	
Mean	30,1	mg/kg
Standard deviation	2,59	mg/kg
rel. Standard deviaton	8,60	%
Horwitz standard deviation	9,58	%
HorRat-value	**0,90**	
Recovery rate	129	%

Microtracer Homogeneity Test
DLA 33-2019 Sample 5

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	84	33,6
2	5,03	75	29,8
3	5,03	75	29,8
4	4,96	82	33,1
5	5,03	80	31,8
6	5,05	84	33,3
7	5,05	75	29,7
8	5,04	88	34,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	80,4	Particles
Standard deviation	5,08	Particles
χ^2 (CHI-Quadrat)	2,25	
Probability	**94**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	32,0	mg/kg
Standard deviation	2,02	mg/kg
rel. Standard deviaton	6,32	%
Horwitz standard deviation	9,50	%
HorRat-value	**0,67**	
Recovery rate	112	%

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 33-2019***
PT name	***GMO-Screening I (qualitative): 5 Samples with positive/negative amounts of p-35S, t-NOS, p-FMV, CP4 EPSPS, 35S-Pat, Cry1Ab/Ac / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)***
*Sample matrix**	*5 different Samples: possible ingredients: Products of soybean, maize and wheat flour and semolina*
Number of samples and sample amount	*5 different samples, 10 g each.*
Storage	*Samples: dry and dark at room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	***qualtitative: p-35S, t-NOS, p-FMV, CP4 EPSPS, 35S-Pat, Cry1Ab/Ac / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)***
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	*One result each should be determined for Samples 1-5 per parameter and filled in the result submission file..*
Units	*positive / negative (limit of detection: copies or percentage)*
Number of significant digits	*only qualitative*
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***
Deadline	***the latest 05th July 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		Germany
		Germany
		AUSTRIA
		Germany
		Germany
		Germany
		Germany
		Germany
		BELGIUM
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN
		Germany
		Germany
		VIETNAM
		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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