

Proficiency Tests

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Evaluation Report

proficiency test

DLA 33/2017

GMO-Screening qualitative:

**5 Samples with positive/negative amounts
of 35S, NOS, FMV, CTP2-CP4 EPSPS /
GMO-Maize (Bt11, MIR604) and GMO-Soya
(RR GTS 40-3-2, RR2 MON89788)**

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1st Correction 07/11/2017:

The qualitative valuation in chapter 4.1.7 Maize-DNA (Maize-specific) on page 16 has been corrected: In the column "Qualitative Valuation Agreement with Consensus Values" the results are related to 3 consensus values now. By mistake only 2 consensus values were taken previously.

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

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<i>EP-Koordinator</i> <i>PT-Coordinator</i>	Dr. Matthias Besler
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<i>EP-Bericht Freigabe</i> <i>PT-Report Authorization</i>	Dr. Matthias Besler (Technischer Leiter / Technical Manager) - <i>gezeichnet / signed M. Besler</i> Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - <i>gezeichnet / signed G. Wichmann</i> Datum / Date: 7 November 2017
<i>Unteraufträge</i> <i>Subcontractors</i>	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
<i>Vertraulichkeit</i> <i>Confidentiality</i>	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.

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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 mixtures 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	<p>Wheat flour Type 405 (80 g) Ingredients: Wheat Nutrients per 100 g: Protein 10 g, Carbohydrates 72 g, Fat 1,1 g</p> <p>Maize Semolina, European-Supplier (13 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 74 g, Fat 1 g</p> <p>Maize Flour, USA-Supplier (6,6 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 9 g, Carbohydrates 79 g, Fat 0 g</p>	- - positive (GM-Maize experimental)	- - -
2	<p>Wheat flour Type 405 (80 g) Ingredients: Wheat Nutrients per 100 g: Protein 10 g, Carbohydrates 72 g, Fat 1,1 g</p> <p>Maize Semolina, European-Supplier (10 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 74 g, Fat 1 g</p> <p>Soya Flour, European Supplier (10 g) Ingredients: Soya flour toasted Nutrients per 100 g: Protein 37 g</p>	- - -	- - -
3	<p>Wheat flour Type 405 (90 g) Ingredients: Wheat Nutrients per 100 g: Protein 10 g, Carbohydrates 72 g, Fat 1,1 g</p> <p>Soya Flour, European Supplier (7,5 g) Ingredients: Soya flour toasted Nutrients per 100 g: Protein 37 g</p> <p>Soya Chunks, USA-Supplier (2,5 g) Ingredients: Soybean Flour Nutrients per 100 g: Protein 47 g, Carbohydrates 17 g, Fat 0,8 g</p>	- - -	- - positive (GM-Soya experimental)
4	<p>Wheat flour Type 405 (90 g) Ingredients: Wheat Nutrients per 100 g: Protein 10 g, Carbohydrates 72 g, Fat 1,1 g</p> <p>Soya Flour, European Supplier (15 g) Ingredients: Soya flour toasted Nutrients per 100 g: Protein 37 g</p> <p>Soybeans, Asian Supplier (5,0 g) Ingredient: Soybeans</p>	- - -	- - positive (GM-Soya experimental)
5	<p>Wheat flour Type 405 (100 g) Ingredients: Wheat Nutrients per 100 g: Protein 10 g, Carbohydrates 72 g, Fat 1,1 g</p>	-	-

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 **micro-tracer 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 $\geq 5\%$ is equivalent to a good homogeneous mixture and of $\geq 25\%$ to an excellent mixture [14, 15].

The microtracer analysis of the present PT samples 1-4 showed probabilities of 70% to 99%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave HorRat values of 0,48 to 1,1. The results of microtracer analysis are given in the documentation. The PT sample 5 was not tested by microtracer analysis, because it contained neither soy nor maize products.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the samples (spoilage) and the content of EP-parameters (allergens) in a comparable matrix and water activity (a_w value <0.5). The stability of sample material is therefore given during the investigation period under consideration of given 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 22nd week of 2017. The testing method was optional. The tests should be finished at July 14th 2017 the latest.

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

DLA 33-2017 - GMO-Screening qualitative: 5 Samples with positive/negative amounts of Screening Targets 35S, NOS, FMV, CTP2-CP4 EPSPS / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)

There are 5 different test samples which possibly contain the 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 analysed.

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 22 participants submitted at least one result 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 35S, NOS, FMN, CTP2-CP4 EPSPS, GMO-Maize Bt11, GMO-Maize MIR604, Maize-DNA and GMO-Soya RR (GTS 40-3-2), GMO-Soya RR2 (MON89788), Lectin-DNA and other DNA.

3.1 Agreement with consensus values from participants

The qualitative evaluation of the 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 $\geq 75\%$ 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**. A consensus value is determined unless $\geq 75\%$ 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 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	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
	pos/neg	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	Sample 5
Number positive					
Number negative					
Percent positive					
Percent negative					
Consensus value					
Spiking					

4.1 Proficiency Test

4.1.1 Results: 35S-Screening-Sequence

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

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	22	0	21	19	0
Number negative	0	22	1	3	22
Percent positive	100	0	95	86	0
Percent negative	0	100	5	14	100
Consensus value	positive	negative	positive	positive	negative
Spiking	positive	negative	positive	positive	negative

Comments on results:

For all 5 samples consensus values with three times 100%, 95% and 86% 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: NOS-Screening-Sequence

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

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	22	1	21	17	0
Number negative	0	21	1	5	22
Percent positive	100	5	95	77	0
Percent negative	0	95	5	23	100
Consensus value	positive	negative	positive	positive	negative
Spiking	positive	negative	positive	positive	negative

Comments on results:

For all 5 samples consensus values with two times 100%, two times 95% and 77% 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: FMV-Screening-Sequence

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

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	1	0	15	18	0
Number negative	17	18	3	0	18
Percent positive	6	0	83	100	0
Percent negative	94	100	17	0	100
Consensus value	negative	negative	positive	positive	negative
Spiking	negative	negative	positive	positive	negative

Comments on results:

For all 5 samples consensus values with three times 100%, 94% and 83% positive or negative results were obtained, respectively. The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.4 Results: CTP2-CP4 EPSPS-Screening-Sequence

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	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
2	-	-	-	-	-			
3	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
4	-	-	-	-	-			
5	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
11	-	-	-	-	-			
12	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
13	-	-	-	-	-			
14	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
17	-	-	-	-	-			
18	-	-	-	-	-			
19	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
20	positive	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
21	-	-	-	-	-			
22	-	-	-	-	-			

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

Comments on results:

For all 5 samples consensus values with 100% positive or negative results were obtained.

The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.5 Results: GMO-Maize Bt11

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	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
2	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
3	-	-	-	-	-			
4	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	-	-	-	-	-			
14	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
22	-	-	-	-	-			

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

Comments on results:

For all 5 samples consensus values with 100% positive or negative results were obtained.

The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.6 Results: GMO-Maize MIR604

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	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	-	-	-	-	-			
14	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	positive	negative	negative	negative	negative	5/5 (100%)	5/5 (100%)	
22	-	-	-	-	-			

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

Comments on results:

For all 5 samples consensus values with 100% positive or negative results were obtained.

The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.7 Results: Maize-DNA (Maize-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Maize	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	positive	positive	positive	positive	negative	3/3 (100%)	3/5 (60%)	
2	negative	negative	negative	negative	negative	1/3 (33%)	3/5 (60%)	no positive sample identified
3	-	-	-	-	-			
4	-	-	-	-	-			
5	positive	positive	negative	negative	negative	3/3 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	positive	positive	positive	positive	negative	3/3 (100%)	3/5 (60%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	positive	positive	positive	positive	negative	3/3 (100%)	3/5 (60%)	
13	-	-	-	-	-			
14	positive	positive	negative	negative	negative	3/3 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	positive	positive	positive	positive	negative	3/3 (100%)	3/5 (60%)	
20	-	-	-	-	-			
21	negative	negative	negative	positive	negative	1/3 (33%)	2/5 (40%)	no positive sample identified
22	-	-	-	-	-			

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	6	6	4	5	0
Number negative	2	2	4	3	8
Percent positive	75	75	50	63	0
Percent negative	25	25	50	38	100
Consensus value	positive	positive	none	none	negative
Spiking	positive	positive	negative	negative	negative

Comments on results:

For samples 1, 2 and 5 consensus values with two times 75% positive and one time 100% negative results were obtained, respectively. The consensus values are in agreement with the addition of the maize-containing ingredients (spiking).

For samples 3 and 4 no consensus values of $\geq 75\%$ positive or negative results were obtained.

4.1.8 Results: GMO-Soya RR

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
GMO-Soya (RR)	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
2	negative	negative	positive	negative	negative	4/5 (100%)	4/5 (100%)	
3	-	-	-	-	-			
4	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
5	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	negative	-	positive	-	-	2/2 (100%)	2/2 (100%)	
11	-	-	-	-	-			
12	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
13	negative	-	positive	positive	-	3/3 (100%)	3/3 (100%)	
14	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
15	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
16	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	-	-	-	-	-			
20	-	-	-	-	-			
21	positive	negative	negative	negative	negative	2/5 (40%)	2/5 (40%)	no positive sample identified
22	negative	negative	positive	positive	-	4/4 (100%)	4/4 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	1	0	11	9	0
Number negative	11	10	1	2	9
Percent positive	8	0	92	82	0
Percent negative	92	100	8	18	100
Consensus value	negative	negative	positive	positive	negative
Spiking	negative	negative	positive	positive	negative

Comments on results:

For all 5 samples consensus values with two times 100% and 92%, respectively, and one time 82% positive or negative results were obtained, respectively.

The consensus values are in agreement with the addition of the GMO-containing ingredients (spiking).

4.1.9 Results: GMO-Soya RR2

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

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	0	9	9	0
Number negative	10	8	1	0	7
Percent positive	0	0	90	100	0
Percent negative	100	100	10	0	100
Consensus value	negative	negative	positive	positive	negative
Spiking	negative	negative	positive	positive	negative

Comments on results:

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

4.1.10 Results: Lectin-DNA (Soya-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Lectin	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	positive	positive	positive	positive	positive	3/5 (60%)	3/5 (60%)	
2	negative	negative	positive	positive	negative	5/5 (100%)	5/5 (100%)	
3	-	-	-	-	-			
4	-	-	-	-	-			
5	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
6	-	-	-	-	-			
7	-	-	-	-	-			
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
13	-	-	positive	positive	-	2/2 (100%)	2/2 (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	-	-	-	-	-			
17	-	-	-	-	-			
18	-	-	-	-	-			
19	negative	positive	positive	positive	negative	5/5 (100%)	5/5 (100%)	
20	-	-	-	-	-			
21	-	-	-	-	-			
22	-	-	-	-	-			

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	1	6	8	8	1
Number negative	6	1	0	0	6
Percent positive	14	86	100	100	14
Percent negative	86	14	0	0	86
Consensus value	negative	positive	positive	positive	negative
Spiking	negative	positive	positive	positive	negative

Comments on results:

For all 5 samples consensus values with two times 100% and three times 86% positive or negative results were obtained, respectively. The consensus values are in agreement with the addition of the soya-containing ingredients (spiking).

4.1.11 Results: Other Parameters (DNA)

Evaluation number	Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Remarks
	other DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	
9	AgroBorder II	positive (?)	negative	positive	positive	negative	
3a	BAR	negative	negative	negative	negative	negative	
5a	bar	negative	negative	negative	negative	negative	
10a	BAR	negative	negative	negative	negative	negative	
12	bar	positive	negative	negative	negative	negative	
20a	BAR	negative	negative	negative	negative	negative	
5b	cry1A(b/c)	positive	negative	negative	negative	negative	
12	CryIAb/Ac	positive	negative	negative	negative	negative	
5c	CV127	-	negative	negative	negative	-	
5d	DAS40278	negative	negative	-	-	-	
5e	DP32138	negative	negative	-	-	-	
5f	LY038	negative	negative	-	-	-	
10b	Mon810	positive	negative	negative	negative	-	
5g	MON87427	negative	-	-	-	-	
5h	MON87751	-	-	negative	negative	-	
5i	MON88017	positive	-	-	-	-	
5j	NK603	positive	-	-	-	-	
3b	NPTII	positive	negative	negative	negative	negative	
10c	NPTII	positive	negative	negative	negative	negative	
20b	NPTII	positive	negative	negative	negative	negative	
3c	PAT	positive	negative	negative	negative	negative	
10d	PAT	positive	negative	negative	negative	negative	
20c	PAT	positive	negative	positive	positive	negative	
15	Plant-DNA	positive	positive	positive	positive	positive	
5k	SAMS-HRA	negative	negative	negative	negative	negative	
5l	synPAT	positive	negative	negative	negative	negative	
10e	T25	positive	negative	negative	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).

see following pages

5.1.1 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
35S	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 electrophoresis / cycles / amplificate length / reference material	
1	07.12.17	p35S	< 5 DNA copies/ Ct 36	GEN-IAL/PB-137 ed. III z dnia 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1.80-2.0(A260/A280)/100 µl	Real time PCR; 45 cycles/ 82Bp/GEN-IAL reference material(maize- and soymix)	
2	12.07.17		0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3	14.07.17			MI.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6	26.06.17			Biotecon	Biotecon foodproof Sample Preparation Kit S 400061; Biotecon foodproof GMO Screening Kit R 30217	RealTime PCR	
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC Art. No. S2126	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8	04.07.17		<0,1%	in house method, Kit IEH-Laboratory	Silica-column based DNA-extraction	Multiplex-PCR, Gel electrophoresis, 35 cycles	
9			5 copies	Congen, Sure Food GMO Screen 35SNOS/FMV, GenS-cran, GMO Screen RT 35S/NOS/ABI IIPC		Real Time PCR	
10	07.06.		5 copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC, S2126, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11	04.06.17	< or > 0.9%	5	R-Biopharm	NucleoSpinFood	real time PCR; CRM : ERM-BF411D	sample 4: positive <0.9%
12	04.07.17	Promotor of Cauliflower Mosaic Virus (CaMV35S)	20 haploide Genomcopies	§64 LFGB L 00.00-122 (2008-06)	Extraction by Maxwell FFS Kit		
13	29/06	35S	ct-value 45	GEN-IAL genControl RT Triplex IV 35S/nos/FMV/IC	Congen SureFood PREP Basic Extraction kit	Real Time PCR, 45 cycles, Referenzmaterial ERM-BF 410 dn	
14	09.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15			0,02% (for RR-Soya-DNA)	ASU 00.00 122 / 06 2008	modified CTAB-method with clean-up	RealTime PCR	
16	19.06.17	-	0,01	Biotecon - Screening LyoKit1+2	Biotecon Sample Prep.Kit3	Real Time PCR	Due to the unknown mactrices several events are possible
17	15.06.17			GEN-IAL		Real Time PCR	
18	07.06.17	35S/NOS Screening	30	biotecon	DNA Extraction Biotecon	Real Time PCR Light Cyclor	
19	08.06.17				CTAB	Real-Time PCR	
20	05.06.17	35S	0,001	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22			copies 10 / ct-value 40	in House Method		Real Time PCR	

5.1.2 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
NOS	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	Tnos	< 5 DNA copies /Ct 36	GEN-IAL/PB-137 ed. III z dnia 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/ 84Bp/Real time PCR; 45 cycles/ 82Bp/GEN-IAL reference material(maize- and soymix)	
2	13.07.17		0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3	14.07.17			MI.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6	26.06.17			Biotecon	Biotecon foodproof Sample Preparation Kit S 400061; Biotecon foodproof GMO Screening Kit R 30217	RealTime PCR	
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC Art. No. S2126	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8	04.07.17		<0,1%	in house method, Kit IEH-Laboratory	Silica-column based DNA-extraction	Multiplex-PCR, Gel electrophoresis, 35 cycles	
9			5 copies	Congen, Sure Food GMO Screen 35SNOS/FMV, GenScran, GMO Screen RT 35S/NOS/ABII IPC		Real Time PCR	
10	07.06.		5 copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC, S2126, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11	04.06.17	< or > 0.9%	5	R-Biopharm	NucleoSpinFood	real time PCR; CRM : ERM-BF411D	sample 4: positive <0.9%
12	04.07.17	Terminator of Agrobacterium tumefaciens (nos)	20 haploid genomic copies	§64 LFGB L 00.00-122 (2008-06)			
13	29/06	nos	ct-Wert 45	GEN-IAL genControl RT Triplex IV 35S/nos/FMV/IAC	Congen SureFood PREP Basic Extraction kit	Real Time PCR, 45 cycles, Reference material ERM-BF 410 dn	
14	09.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15			0,03% (für RR-Soja-DNA)	ASU 00.00 122 / 06 2008	modified CTAB-method with clean-up	RealTime PCR	
16	19.06.17	-	0,01	Biotecon - Screening LyoKit1+2	Biotecon Sample Prep.Kit3	Real Time PCR	Due to the unknown mactrices several events are possible
17	15.06.17			GEN-IAL		Real Time PCR	
18	07.06.17	35S/NOS Screening	31	biotecon	DNA Extraction Biotecon	Real Time PCR Light Cyclor	
19	08.06.17				CTAB	Real-Time PCR	
20	05.06.17	NOS	0,001	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22			copies 10 / ct-Wert 40	in House Method		Real Time PCR	

5.1.3 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
FMV	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	p34S	< 5 DNA copies/Ct 36	GEN-IAL/PB-137 ed. III z dnia 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/105 Bp/Real time PCR; 45 cycles/ 82Bp/GEN-IAL reference material(maize- and soymix)	
2	12.07.17		0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3	14.07.17			MI.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
4			0,0001				
5	20.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6	26.06.17			Biotecon	Biotecon foodproof Sample Preparation Kit S 400061; Biotecon foodproof GMO Screening Kit R 30217	RealTime PCR	
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC Art. No. S2126	extraction mit SureFood® PREP Basic Art. No. S1052	-	-
8	04.07.17		<0,1%	in house method, Kit IEH-Laboratory	Silica-column based DNA-extraction	Multiplex-PCR, Gel electrophoresis, 35 cycles	
9			5 copies	Congen, Sure Food GMO Screen 35SNOS/FMV		Real Time PCR	
10	07.06.		5 copies	SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC, S2126, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11	04.06.17	< or > 0.9%	5	R-Biopharm	NucleoSpinFood		sample 3 and 4: positive <0.9%
12	04.07.17	Promotor of Figwort Mosaic Virus (pFMV)	10 haploid genomic copies	ASU L 00.00-148 (2014-02)			
13	29/06	FMV	ct-value 45	GEN-IAL genControl RT Triplex IV 35S/nos/FMV/IC	Congen SureFood PREP Basic extraction kit	Real Time PCR, 45 Cycles, Reference material ERM-BF 410 dn	
14	09.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15			0,003% (für RR-Soya-DNA)	ASU 00.00 148 / 02 2014	modified CTAB-method with clean-up	RealTime PCR	
16	19.06.17	-	0,01	Biotecon - Screening LyoKit1+2	Biotecon Sample Prep.Kit3	Real Time PCR	Due to the unknown matrices several events are possible
17							
18							
19							
20	05.06.17	FMV	0,001	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
21	06.06.17	Target-Sequence	50 Cyclen	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22			copies 10 / ct-value 40	in House Method		Real Time PCR	

5.1.4 CTP2-CP4 EPSPS-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
CTP2-CP4 EPSPS	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	CTP2-CP4 EPSPS	< 5 DNA copies/Ct 36	GEN-IAL/ PB-137 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,8-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/ 88 Bp	
2							
3	14.07.17			MI.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
4			0,0001				
5	15.06.17		0,0001	Hausverfahren	CTAB-NucleoSpin	RealTime-PCR, 45 Zyklen	
6							
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO SCREEN 4plex BAR/NPTII/PAT/CTP2:CP4 EPSPS Art. No. S2127	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10	07.06.		5 copies	SureFood® GMO SCREEN 4plex BAR/NPTII/PAT/CTP2:CP4EPSPS, S2127, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11							
12	04.07.17	Transition from CTP2 to CP4-EPSPS-Gene	10 haploid genomic copies	§64 LFGB L 00.00-125 (2009-06)			
13							
14	12.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15							
16	19.06.17	-	0,01	Biotecon - Screening LyoKit1+2	Biotecon Sample Prep.Kit3	Real Time PCR	Due to the unknown matrices several events are possible
17							
18							
19	08.06.17				CTAB	Real-Time PCR	
20	05.06.17	CTP2-CP4 EPSPS	0,001	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
21							
22							

5.1.5 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
GMO-Maiz (Bt11)	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 electrophoresis / cycles / amplicate length / reference material	
1	13.07.17	Bt11-corn (Agrisure CB)	< 0,1 %/Ct 36	GEN-IAL/PB-168 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles;	
2	12.07.17		0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3							
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6							
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO QUANT Bt11 Corn Art. No. S2016	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10							
11							
12							
13							
14	13.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15							
16							
17							
18							
19							
20							
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22							

5.1.6 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
GMO-Maize MIR604	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 electrophoresis / cycles / amplicate length / reference material	
1							
2							
3							
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6							
7	07.06.17	-	≤ 0,01 %	in House Method	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10							
11							
12							
13							
14	13.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15							
16							
17							
18							
19							
20							
21	06.06.17	Target-Sequence	50 Cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22							

5.1.7 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
Maize	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 electrophoresis / cycles / amplicate length / reference material	
1	13.07.17	Corn-reference.	< 0,1 %/Ct 36	GEN-IAL/PB-168 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/ MON810 ERM-BF413gk	
2	12.07.17	Invertase	0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3							
4			0,0001				
5	06.07.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6							
7	07.06.17	-	≤ 4 mg/kg	SureFood® GMO Plant 4plex Corn/Soya/Canola+IAC Art. No. S2158	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10							
11							
12	29.06.17	Maize-specific DNA-Sequence	25 haploid genomic copies	§64 LFGB L 00.00-105 Annex C3 (2014-02)		hmg-Gene	samples 3+4 not suitable for analysis of gm maize due to less extractable of maize-specific DNA to amplify
13							
14	27.06.17			r-biopharm	FFS-Kit, Promega	Real Time PCR	
15							
16							
17							
18							
19	08.06.17				CTAB	Real-Time PCR	
20							
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22							

5.1.8 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
GMO-Soya RR	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	RoundapReady (GTS 40-3-2)	< 0,1 %/Ct 36	GEN-IAL/PB-168 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/ RR 0,5% ERM BF4 10c	
2	13.07.17		0,0010	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3							
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6							
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO ID Roundup Ready Soya Art. No. S2030	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10	06.07.		5 copies / < 0,1%	SureFood® GMO QUANT RR Soja, S2014, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11							
12	03.07.17	Soybean GTS 40-3-2 (Roundup Ready) genetically modified	50 haploid genomic copies	\$64 LFGB L 00.00-105 (2014-02)		junction region between CTPGene and 35S-Promotor	
13	04.07.17			GEN-IAL genControl RT GMO Soy Kit	Congen SureFood PREP Basic Extraction kit	Real Time PCR, 45 cycles	
14	13.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15			0,05% (for RR-Soya-DNA)	ASU 00.00 105 / 12 2006	modified CTAB-method with clean-up	RealTime PCR	
16							
17							
18							
19							
20							
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22			copies 5 / ct-value 40	in House Method		Real Time PCR	

5.1.9 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
GMO-Soya RR2	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	RoundapReady2 (MON89788)	< 0,1 %/Ct 36	GEN-IAL/PB-168 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/	
2							
3							
4			0,0001				
5	15.06.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR; 45 cycles	
6							
7	07.06.17	-	≤ 5 DNA-copies	SureFood® GMO ID RR2Y Soya Art. No. S2034	Extraction with SureFood® PREP Basic Art. No. S1052	-	-
8							
9							
10	06.07.		5 copies / < 0,1%	SureFood® GMO QUANT RR2Y Soja, S2029, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
11							
12							
13	04.07.17			GEN-IAL genControl RT GMO Soy Kit	Congen SureFood PREP Basic extraction kit	Real Time PCR, 45 cycles	
14	13.06.17			GEN-IAL	FFS-Kit, Promega	Real Time PCR	
15			0,002% (für RR2-Soya-DNA)	JRC QT-EVE-GM-006	modified CTAB-method with clean-up	RealTime PCR	
16							
17							
18							
19							
20							
21	06.06.17	Target-Sequence	50 cycles	Biotecon Diagnostics	foodproof Extraction Kit / Lyo Kit	foodproof Detection Kit, BIOTECON Diagnostics	
22			copies 5 / ct-Wert 40	in House Method		Real Time PCR	

5.1.10 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
Lectin	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 electrophoresis / cycles / amplicate length / reference material	
1	07.12.17	soy-lectin	< 0,1 %/Ct 36	GEN-IAL/PB-168 ed. III; 15.01.2016	Silica membrane spin technology/proteinase K/ 3-steps of DNA purification/ 1,80-2,0(A260/A280)/100 µl	Real time PCR; 45 cycles/RR 0,5% ERMBF410c	
2	13.07.17		0,001	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
3							
4							
5	06.07.17		0,0001	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
6							
7	-	-	-	-	-	-	-
8							
9							
10							
11							
12	29.06.17	Soya-specific DNA Sequence	5 haploid genomic copies	§64 LFGB L 00-00-105 Annex B1 (2014-02)			
13	04.07.17			GEN-IAL genControl RT GMO Soy Kit	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 Cycles	
14	27.06.17			r-biopharm	FFS-Kit, Promega	Real Time PCR	
15			0,015 (for Soya-DNA)	ASU 00.00 105 / 12 2006	modifiziertes CTAB-Verfahren mit clean-up	RealTime PCR	
16							
17							
18							
19	08.06.17				CTAB	Real-Time PCR	
20							
21							
22							

5.1.11 Other DNA-Sequences

Parameter	Evaluation number	Date of Analysis	Results given as	Limit of Detection	Test-Kit or Literature	Notes to Extraction	Notes to PCR-reaction	Further Remarks
						e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount	e.g. real time PCR / gel electrophoresis / cycles / amplicate length / reference material	
		Day/Month	Target-Sequence / -DNA	number of copies / % / ct-value	Manufacturer / Official Method			
AgroBorder II	9			10 copies	GenScran, GMO Screen RT 35S/NOS/ABII IPC		Real Time PCR	
BAR	3a	14.07.17			M.I.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
bar	5a	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
BAR	10a	07.06.		5 copies	SureFood® GMO SCREEN 4plex BAR/NPTII/PAT(CTP2:CP4EP5PS, S2127, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
bar	12	03.07.17	bar Gen	10 haploid genomic copies	§64 LFGB L 00.00-124 (2008-12)			
BAR	20a	05.06.17	BAR	0,10%	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
cry1A(b/c)	5b	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
CryIAb/Ac	12	04.07.17	cry1Ab/cry1Ac DNA-Sequenzen	5 haploid genomic copies	§64 LFGB L 15.06-3 (2013-08)			
CV127	5c	11.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
DAS40278	5d	11.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
DP32138	5e	13.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
LY038	5f	11.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
Mon810	10b	07.07.		5 copies / < 0,1%	SureFood® GMO QUANT MON810 Corn, S2019, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
MON87427	5g	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
MON87751	5h	13.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
MON88017	5i	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
NK603	5j	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
NPTII	3b	14.07.17			M.I.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
NPTII	10c	07.06.		5 copies	SureFood® GMO SCREEN 4plex BAR/NPTII/PAT(CTP2:CP4EP5PS, S2127, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
NPTII	20b	05.06.17	NPTII	0,10%	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
PAT	3c	14.07.17			M.I.114 Rev.0 2017	DNA extraction with commercial Kit	Real time PCR	
PAT	10d	07.06.		5 copies	SureFood® GMO SCREEN 4plex BAR/NPTII/PAT(CTP2:CP4EP5PS, S2127, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		
PAT	20c	05.06.17	PAT	0,10%	CONGEN SureFood GMO Screen	Extracted using CONGEN Prep Advanced Kit	Real Time PCR - 45 Cycles	
Planzen-DNA	15			0,02% (für Mais-DNA)	ASU 40.00 14 / 07 2012	modified CTAB-method with clean-up	RealTime PCR	
SAMS-HRA	5k	05.07.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
synPAT	5l	15.06.17		0,01%	in House Method	CTAB-NucleoSpin	RealTime-PCR, 45 cycles	
T25	10e	07.07.		5 copies / < 0,1%	SureFood® GMO QUANT T25 Corn, S2017, R-Biopharm	SureFood® PREP Basic, S1052, R-Biopharm		

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test

DLA 33-2017 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,09	105	41,3
2	5,01	97	38,7
3	5,21	101	38,8
4	5,06	92	36,4
5	5,05	105	41,6
6	5,02	102	40,6
7	5,05	100	39,6
8	5,01	103	41,1

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	101	Particles
Standard deviation	4,47	Particles
χ^2 (CHI-Quadrat)	1,39	
Probability	99	%
Recovery rate	148	%

Normal distribution

Number of samples	8	
Mean	39,8	mg/kg
Standard deviation	1,76	mg/kg
rel. Standard deviation	4,44	%
Horwitz standard deviation	9,19	%
HorRat-value	0,48	
Recovery rate	148	%

Microtracer Homogeneity Test

DLA 33-2017 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	20,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,14	50	19,5
2	4,96	61	24,6
3	5,08	54	21,3
4	4,97	63	25,4
5	5,15	51	19,8
6	5,08	65	25,6
7	5,09	55	21,6
8	5,06	55	21,7

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	56,8	Particles
Standard deviation	6,17	Particles
χ^2 (CHI-Quadrat)	4,68	
Probability	70	%
Recovery rate	109	%

Normal distribution

Number of samples	8	
Mean	22,4	mg/kg
Standard deviation	2,43	mg/kg
rel. Standard deviation	10,9	%
Horwitz standard deviation	10,0	%
HorRat-value	1,1	
Recovery rate	109	%

Microtracer Homogeneity Test**DLA 33-2017 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	24,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	61	24,3
2	5,00	68	27,2
3	5,15	63	24,5
4	4,99	67	26,9
5	4,97	60	24,1
6	5,00	66	26,4
7	5,06	71	28,1
8	5,01	66	26,3

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	65,3	Particles
Standard deviation	3,74	Particles
χ^2 (CHI-Quadrat)	1,50	
Probability	98	%
Recovery rate	107	%

Normal distribution

Number of samples	8	
Mean	26,0	mg/kg
Standard deviation	1,49	mg/kg
rel. Standard deviation	5,74	%
Horwitz standard deviation	9,80	%
HorRat-value	0,59	
Recovery rate	107	%

Microtracer Homogeneity Test**DLA 33-2017 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	30,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	90	36,2
2	5,21	84	32,2
3	5,10	86	33,7
4	5,05	90	35,6
5	5,09	82	32,2
6	5,07	84	33,1
7	5,06	76	30,0
8	5,06	90	35,6

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	85,3	Particles
Standard deviation	5,39	Particles
χ^2 (CHI-Quadrat)	2,38	
Probability	94	%
Recovery rate	109	%

Normal distribution

Number of samples	8	
Mean	33,6	mg/kg
Standard deviation	2,12	mg/kg
rel. Standard deviation	6,32	%
Horwitz standard deviation	9,43	%
HorRat-value	0,67	
Recovery rate	109	%

5.3 Information on the Proficiency Test (PT)

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

<i>PT number</i>	DLA 33-2017
<i>PT name</i>	GMO-Screening qualitative: 5 Samples with positive/negative amounts of Screening Targets 35S, NOS, FMV, CTP2-CP4 EPSPS / GMO-Maize (Bt11, MIR604) and GMO-Soya (RR GTS 40-3-2, RR2 MON89788)
<i>Sample matrix*</i>	<i>Five different Samples: possible ingredients: Products of soybean, maize and wheat flour and semolina</i>
<i>Number of samples and sample amount</i>	<i>Five different samples, 10 g each.</i>
<i>Storage</i>	<i>Samples: dry and dark at room temperature (long term cooled 2 - 10°C)</i>
<i>Intentional use</i>	<i>Laboratory use only (quality control samples)</i>
<i>Parameter</i>	<i>qualitative: Target sequences 35S, NOS, FMV, CTP2-CP4 EPSPS as well as GMO-maize (Bt11, MIR604) and GMO-soya (RR GTS 40-3-2, RR2 MON89788)</i>
<i>Methods of analysis</i>	<i>Analytical methods are optional</i>
<i>Notes to analysis</i>	<i>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.</i>
<i>Result sheet</i>	<i>One result each should be determined for Samples 1-5 per parameter and filled in the result submission file..</i>
<i>Units</i>	<i>positive / negative (limit of detection: copies or percentage)</i>
<i>Number of significant digits</i>	<i>only qualitative</i>
<i>Further information</i>	<i>Further information can be given in the result submission file.</i>
<i>Result submission</i>	<i>The result submission file should be sent by e-mail to: pt@dla-lvu.de</i>
<i>Deadline</i>	the latest 14th July 2017
<i>Evaluation report</i>	<i>The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.</i>
<i>Coordinator and contact person of PT</i>	<i>Dr. Matthias Besler</i>

* Control of mixture homogeneity and qualitative testings are carried out by DLA. 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
		Germany
		AUSTRIA
		Germany
		Germany
		Germany
		ITALY
		Germany
		Germany
		Germany
		FRANCE
		Germany
		Germany
		Germany
		Germany
		Germany
		POLAND
		Germany
		Germany
		BELGIUM
		GREAT BRITAIN
		Germany
		GREAT BRITAIN

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswertebereichs nicht angegeben.]

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

7. Index of references

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