

Evaluation Report

proficiency test

DLA ptGMS1 (2020)

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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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 or feed samples from European and US-American suppliers (s. table 1). The raw materials were crushed, sieved (mesh $<400 \, \mu m$, $<600 \, \mu m$ and $<1,5 \, mm$), mixed and homogenized. The composition of the samples is given in table

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-Con- tent Maize	GMO-Con- tent Soya
1	Complete feed for laying hens (100 g) Ingredients: Wheat, maize, soy extraction flour, calcium carbonate, sunflower extraction flour, refining fatty acids, alfalfa meal, monocalcium phosphate, sodium chloride, sodium carbonate and other additives Nutrients per 100 g: Crude protein 17 g, crude fiber 4,8 g, crude fat 5,0 g, crude ash 13 g		positive (GMO-Soya experimental)
2	Wheat flour Typ 405 (79 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g Maize flour, USA Supplier (11 g) Ingredients: Maize flour Nutrients per 100 g: Protein 9 g, Carbohydrates 79 g, Fat 0 g Maize flour, European Supplier (10 g) Ingredients: Maize flour Nutrients per 100 g: Nutrients per 100 g:	positive (GMO-Maize experimental)	-
	Protein 7,5 g, Carbohydrates 77 g, Fat 1 g		
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 (100 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g	-	-
5	Wheat flour Typ 405 (80 g) Ingredients: Wheat Nutrients per 100 g: Protein 11 g, Carbohydrates 72 g, Fat 1,0 g	-	-
	Maize flour, European Supplier (10 g) Ingredients: Maize flour 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 \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 2-5 showed probabilities of 96%, 78%, 99% and 93%, 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 0,6, 0,9, 0,5 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 0,45 - 0,48 (18°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 20th week of 2020. The testing method was optional. The tests should be finished at Juli 24th 2020 the latest (extended).

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

DLA ptGMS1 - 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 22 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 if \geq 75% positive or negative results are available 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	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 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	positive	positive	positive	negative	positive	4/5 (80%)	4/5 (80%)	
2	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
3	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
4	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	Sample 5 positive < LOD
6	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
8	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
9	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
13	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
15	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
16	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
17	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
18	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
19	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
21	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
22	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	

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

<u>Comments:</u>

For all 5 samples consensus values with four times 100% and once 95% positive or negative results were obtained, respectively.

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	positive	positive	positive	negative	positive	4/5 (80%)	4/5 (80%)	Sample 5 positive traces
2	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
3	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
4	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	Sample 5 positive < LOD
6	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
8	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
9	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
11	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
13	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	positive	negative	negative	4/5 (80%)	4/5 (80%)	
15	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
16	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
17	negative	positive	positive	negative	negative	4/5 (80%)	4/5 (80%)	
18	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
19	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
21	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
22	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	20	22	22	0	1
Number negative	2	0	0	22	21
Percent positive	91	100	100	0	5
Percent negative	9	0	0	100	95
Consensus value	positive	positive	positive	negative	negative
Spiking	positive	positive	positive	negative	negative

For all 5 samples consensus values with two times 100%, once 95% and once 91% positive or negative results were obtained, respectively.

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	-	-	-	-	-			
2	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
3	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
4	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
6	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	positive	positive	negative	negative	4/5 (80%)	4/5 (80%)	
8	positive	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	
9	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	positive	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	
11	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	-	-	-	-	-			
13	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	negative	negative	negative	negative	3/5 (60%)	3/5 (60%)	no positive sample detected
15	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
16	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
17	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
18	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
19	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
21	positive	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	
22	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	19	1	16	0	0
Number negative	1	19	4	20	20
Percent positive	95	5	80	0	0
Percent negative	5	95	20	100	100
Consensus value	positive	negative	positive	negative	negative
Spiking	positive	negative	positive	negative	negative

Comments:

For all 5 samples consensus values with two times 100%, two times 95% and once 80% 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

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	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
2	-	-	-	-	-			
3	-	-	-	-	-			
4	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
5	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	Sample 5 positive < LOD
6	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	positive	negative	positive	negative	negative	4/5 (80%)	4/5 (80%)	
14	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
17	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
18	-	-	-	-	-			
19	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
21	-	-	-	-	-			
22	positive	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	13	12	13	0	0
Number negative	0	1	0	13	13
Percent positive	100	92	100	0	0
Percent negative	0	8	0	100	100
Consensus value	sensus value positive		positive	negative	negative
Spiking	positive	positive	positive	negative	negative

<u>Comments:</u>

For all 5 samples consensus values with four times 100% and once 92%positive or negative results were obtained, respectively.

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	-	-	-	-	-			
2	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	positive	positive	positive	negative	negative	3/3 (100%)	5/5 (100%)	Sample 1 at LOD
6	negative	positive	negative	negative	negative	3/3 (100%)	3/5 (60%)	
7	negative	positive	negative	negative	negative	3/3 (100%)	3/5 (60%)	
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	-	-	-	-	-			
14	negative	positive	negative	negative	negative	3/3 (100%)	3/5 (60%)	
15	-	-	-	-	-			
16	positive	positive	negative	negative	negative	3/3 (100%)	4/5 (80%)	
17	-	-	-	-	-			
18		-	-	_				
19	positive	positive	positive	negative	negative	3/3 (100%)	5/5 (100%)	
20	negative	positive	negative	negative	negative	3/3 (100%)	3/5 (60%)	
21	-	-	-	-	-			
22	negative	positive	positive	negative	negative	3/3 (100%)	4/5 (80%)	

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

Comments:

For the samples 2, 4 and 5 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).

For samples 1 and 3 no consensus values 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	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
5	-	-	-	-	-			
6	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
7	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
8	-	-	-	-	-			
9	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
14	negative	positive	negative	negative	negative	4/5 (80%)	5/5 (100%)	
15	-	-	-	-	-			
16	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
17	-	positive	negative	-	-	2/2 (100%)	2/2 (100%)	
18	-	-	-	-	-			
19	-	-	-	-	-			
20	positive	positive	negative	negative	negative	5/5 (100%)	4/5 (80%)	
21	-	-	-	-	-			
22	-	-	-	-	-			

	Sample 1	Sample 2 Sample 3		Sample 4	Sample 5	
Number positive	7	9	0	0	0	
Number negative	1	0	9	8	8	
Percent positive	88	100	0	0	0	
Percent negative	13	0	100	100	100	
Consensus value	positive	positive	negative	negative	negative	
Spiking	positive	positive	negative	negative	negative	

Comments:

For all 5 samples consensus values with four times 100% and once 88%positive or negative results were obtained, respectively.

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	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	negative	positive	positive	negative	negative	4/5 (80%)	4/5 (80%)	
7	-	-	-	-	-			
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
18	-	-	-	-	-			
19	positive	positive	negative	negative	negative	4/5 (80%)	4/5 (80%)	
20	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
21	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
22	-	-	-	-	-			

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	1	7	1	0	0
Number negative	6	0	6	7	7
Percent positive	14	100	14	0	0
Percent negative	86	0	86	100	100
Consensus value	negative	positive	negative	negative	negative
Spiking	negative	positive	negative	negative	negative

<u>Comments:</u>

For all 5 samples consensus values with three times 100% and twice 86%positive or negative results were obtained, respectively.

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	-	-	-	-	1			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	-	-	-	-	-			
7	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	1			
9	-	-	-	-	1			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	no positive sample detected
15	-	-	-	-	-			
16	-	-	-	-	-			
17	-	positive	negative	-	-	2/2 (100%)	2/2 (100%)	
18	-	-	-	-	-			
19	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
20	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
21	-	-	-	-	-			
22	-	-	-	-	-			

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

Comments:

For all 5 samples consensus values with four times 100% and once 83% positive or negative results were obtained, respectively.

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	-	-	-	-	-			
3	1	-	-	-	1			
4	positive	positive	positive	negative	positive	4/5 (80%)	4/5 (80%)	
5	-	-	-	-	-			
6	positive	positive	negative	negative	negative	4/5 (80%)	4/5 (80%)	
7	-	-	-	-	-			
8	-	-	-	-	-			
9	-	-	-	-	-			
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	negative	positive	negative	negative	positive	4/5 (80%)	4/5 (80%)	
14	-	-	-	-	-			
15	-	-	-	-	-			
16	positive	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
17	-	positive	-	-	-	1/1 (100%)	1/1 (100%)	
18	-	-	-	-	-		,	
19	positive	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
20	positive	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
21	-	-	-	-	-			
22	-	-	-	-	-			

	Sample 1	Sample 2 Sample 3		Sample 4	Sample 5	
Number positive	5	7	1	0	5	
Number negative	1	0	5	6	1	
Percent positive	83	100	17	0	83	
Percent negative	17	0	83	100	17	
Consensus value	sensus value positive		negative	negative	positive	
Spiking	positive	positive	negative	negative	positive	

For all samples consensus values with twice 100% and three times 83%positive or negative results were obtained, respectively.

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	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
8	-	-	-	-	-			
9	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	negative	negative	positive	negative	negative	4/5 (80%)	4/5 (80%)	
15	-	-	-	-	-			
16	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
17	negative	negative	positive	negative	negative	4/5 (80%)	4/5 (80%)	
18	-	-	-	_	-			
19	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
21	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
22	-	-	-	_	-			

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

Comments:
For all 5 samples consensus values with four times 100% and once 80% positive or negative results were obtained, respectively.

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	-	-	-	-	-			
3	-	-	-	-	-			
4	-	-	-	-	-			
5	-	-	-	-	-			
6	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	positive	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	
8	-	-	-	-	-			
9	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
14	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
15	-	-	-	-	-			
16	-	-	-	-	-			
17	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
18	-	-	-	-	-			
19	positive	negative	positive	negative	negative	5/5 (100%)	5/5 (100%)	
20	positive	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	
21	-	-	-	-	-			
22	-	-	-	-	_			

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

Comments:

For all 5 samples consensus values with four times 100% and once 75% positive or negative results were obtained, respectively.

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	-	-	-	-	-			
3	-	-	-	-	-			
4	positive	positive	positive	positive	positive	3/3 (100%)	3/5 (60%)	
5	-	-	-	-	-			
6	positive	negative	positive	negative	positive	3/3 (100%)	5/5 (100%)	
7	-	-	-	-	-			
8	-	-	-	-	-			
9	positive	positive	positive	positive	positive	3/3 (100%)	3/5 (60%)	
10	-	-	-	-	-			
11	-	-	-	-	-			
12	-	-	-	-	-			
13	positive	negative	positive	negative	positive	3/3 (100%)	5/5 (100%)	
14	-	-	-	-	-			
15	-	-	-	-	-			
16	positive	positive	positive	positive	positive	3/3 (100%)	3/5 (60%)	Sample 2 + 4 very small amounts
17	positive	-	positive	-	-	2/2 (100%)	2/2 (100%)	
18	-	-	-	-	-			
19	positive	negative	positive	negative	positive	3/3 (100%)	5/5 (100%)	
20	positive	negative	positive	negative	positive	3/3 (100%)	5/5 (100%)	
21	positive	negative	positive	positive	positive	3/3 (100%)	4/5 (80%)	
22	-	-	-	-	-			

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	9	3	9	4	8
Number negative	0	5	0	4	0
Percent positive	100	38	100	50	100
Percent negative	0	63	0	50	0
Consensus value	positive	none	positive	none	positive
Spiking	positive	negative	positive	negative	positive

Comments:

For the samples 1, 3 and 5 consensus values with 100% positive or negative results were obtained, respectively.

The consensus values are in agreement with the addition of the ${\sf GMO-containing}$ ingredients (spiking).

For samples 2 and 4 no consensus values with $\geq 75\%$ positive or negative results were obtained.

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	
12	ABII	positive	positive	positive	negative	negative	
5	bar	negative	negative	negative	negative	negative	
13	bar	negative	negative	negative	negative	negative	
17	bar	negative	negative	negative	negative	negative	
16	CaMV	positive	negative	negative	negative	negative	
6	NK603-Mais	negative	positive	negative	negative	negative	
17	NPTII	negative	positive	positive	positive	negative	
22	NPTII	negative	positive	negative	negative	negative	
9	pat	positive	positive	positive	negative	negative	
13	pat	positive	positive	positive	negative	negative	
16	pat	negative	positive	negative	negative	negative	Sample 1 + 3 slightly positivee <lod< td=""></lod<>
17	pat	negative	positive	negative	negative	negative	
9	Pflanzen-Nachweis	positive	positive	positive	positive	positive	
13	pnos-nptII	negative	negative	negative	negative	negative	
13	Raps spezifische DNA	positive	negative	negative	negative	negative	
6	Raps spezifische DNA	positive	negative	negative	negative	negative	
3	Soja A2704-12	positive	negative	positive	negative	negative	
9	GVO-Soja LL (A2704-12)	negative	negative	positive	negative	negative	
3	Soja A5547-127	negative	negative	negative	negative	negative	
3	Soja CV127-9	negative	negative	negative	negative	negative	
3	Soja DP305423-1	negative	negative	negative	negative	negative	
3	Soja DP356043-5	negative	negative	negative	negative	negative	
3	Soja MON87701-2	positive	negative	negative	negative	negative	

5. Documentation

5.1 Details by the participants

 $\underline{\text{Note:}}$ 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

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	20.5.20	DNA	<10 copies / PCR-reaction	GEN-IAL / EN ISO 21570	Machery-Nagel: NucleoSpin Food	Real Time PCR (GEN-IAL: genControl RT Triplex 35S/tNOS/EPSPS Kit)	
2	25.5.20	P35S/DNA	0,025%	Imegen Screening kit	CTAB	Real time PCR	
3	02.06.	35S	10 copies	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	
4	04.06.		5 copies	§64 LFGB L 00.00-122 (modified) (2008-06)	DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles	
5	18.6.20	p-35S	0.1 %	DIN EN ISO 21569:2013-08	mod. Wizard®-DNA-Clean-UP	Real Time PCR / 83 bp	sample -05: detectable but < 0,1%
6	03.06.	82 bp Amplikon	0,01	DIN EN ISO 21569: 2013-08	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	9.6.20	35S-CaMV Promotor	0,01%	SureFood® GMO SCREEN 35S/NOS/FMV (S2026)	SureFood® Add-on (S1055)	real-time PCR	K00
8	28.5.20		1%	SUREFOOD® GMO SCREEN 4PLEX 35S/NOS/FMV + IAC	omega E.Z.N.A. food DNA kit		
9	28.5.20	DNA	0.01%	genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey- Nagel	Real Time PCR	LOD: Specification of decimal places with "point"
10	2.6.20			biotecon GMO Screening kit	mericon food kit	real time pcr	
11				R-Biopharm, S2026:2017- 04 & QMAA-P-19:2018-08 (Multiplex-PCR)			
12			5 copies, 0,05 %	Gene Scan TR 35S/NOS/ABII IPC	Sure Food Prep Advancded r-Biopharm	Real-Time PCR, 45 cycles	
13	13.6.20			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target- Sequence 35S	0,01%	foodproof GMO Screening 1 LyoKit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15	2020-06-09/ 2020-07-17	Target- Sequence / -DNA	0,1%	Bioside / ISO 21569:2005/Amd 1:2013	Machery Nagel FOOD/PLANT extraction kit	Real time PCR	
16	19.6.20	Promotor des Cauliflower Mosaic Virus (CaMV35S)	0.1 %	ASU L 00.00-122 mod	Maxwell FFS Kit, 200 mg Einwaage		
17	08.07.2020.		0,05%	TaqMan GMO Screening Kit	Qiagen Dneasy Plant Kit	RT PCR	
18	14.7.20	Target- Sequenz	5 copies 5 mg/kg	R-Biopharm S2126	R-Biopharm S1052	Real-Time PCR	
19	28.5.20			L 00.00122 Ausgabe 06/2008			
20			0,1%	ASU L00.00-122	CTAB-Methode	Real Time PCR	Real Time PCR/50 cycles
21	2.7.20		0,10%	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	

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	20.5.20	DNA	<10 copies / PCR-reaction	GEN-IAL / EN ISO 21570	Machery-Nagel: NucleoSpin Food	Real Time PCR (GEN-IAL: genControl RT Triplex 35S/tNOS/EPSPS Kit)	Sample 5: tra- ces (at LOD of test kit)
2	25.5.20	TNOS/DNA	0,025%	Imegen Screening kit	CTAB	Real time PCR	
3	02.06.	nos	10 copies	GEN-IAL genControl RT-Tri- plex IV p35S / NOS / pFMV, incl. IC	Congen SureFood PREP Basic extraction kit	Real Time PCR, 45 cycles, reference material ERM-BF 410dp	
4	04.06.		10 copies	§64 LFGB L 00.00-122 (modified) (2008-06)	DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles	
5	18.6.20	t-Nos	0.1 %	DIN EN ISO 21569:2013-08	mod. Wizard®-DNA-Clean-UP	Real Time PCR / 83 bp	sample -05: detectable but < 0,1%
6	03.06.	87 bp Ampli- kon	0,01	DIN EN ISO 21569: 2013-08	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	9.6.20	NOS Termina- tor	0,01%	SureFood® GMO SCREEN 35S/NOS/FMV (S2026)	SureFood® Add-on (S1055)	real-time PCR	K00
8	28.5.20		1%	SUREFOOD® GMO SCREEN 4PLEX 35S/NOS/FMV + IAC	omega E.Z.N.A. food DNA kit		
9	28.5.20	DNA	0.01%	genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey- Nagel	Real Time PCR	LOD: Specifica- tion of decimal places with "point"
10	2.6.20			biotecon GMO Screening kit	mericon food kit	real time pcr	
11				R-Biopharm, S2026:2017- 04 & QMAA-P-19:2018-08 (Multiplex-PCR)			
12			5 copies, 0,05 %	Gene Scan TR 35S/NOS/ABII IPC	Sure Food Prep Advancded r-Biopharm	Real-Time PCR, 45 cycles	
13	13.6.20			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target-Se- quence T- NOS	0,01%	foodproof GMO Screening 1 LyoKit, Biotecon Diagno- stics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cy- cle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15	2020-06-09/ 2020-07-17	Target-Se- quence / -DNA	0,1%	Bioside / ISO 21569:2005/Amd 1:2013	Machery Nagel FOOD/PLANT extraction kit	Real time PCR	
16	19.6.20	Terminator des Agrobac- terium tume- faciens (nos)	0.1 %	ASU L 00.00-122 mod	Maxwell FFS Kit, 200 mg Einwaage		
17	08.07.2020.		0,05%	TaqMan GMO Screening Kit	Qiagen Dneasy Plant Kit	RT PCR	
18	14.7.20	Target-Se- quenz	5 copies 5 mg/kg	R-Biopharm S2126	R-Biopharm S1052	Real-Time PCR	
19	28.5.20			L 00.00122 from 06/2008			
20			0,1%	ASU L00.00-122	CTAB-Methode	Real Time PCR	Real Time PCR/50 cycles
21 22	02.07.20 9.7.20	DNA	0,10% 0,1%	In House Method SureFood Congen S2026	Magnetic Bead Extraction As per Kit Instructions	Gel Electrophoresis As Per Kit Instructions	

5.1.3 p-FMV-Screening-Sequence

1 2 25.5.20 3 02.06. 4 04.06. 5 18.6.20 6 03.06. 7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-1 16 24.6.20	5.5.20 02.06. 04.06. 8.6.20 03.06. 0.6.20 8.5.20	-DNA 20 PFMV/DNA 6. FMV 6. FMV 6. 20 p-FMV 78 bp Amplikon 20 34S-FMV Promotor	number of copies / % / ct-value 0,025% 10 copies 5 copies 0,01 % 0,01%	Imegen Screening kit GEN-IAL genControl RT- Triplex IV p35S / NOS / pFMV, incl. IC §64 LFGB L 00.00-148 (modified) (2014-02) DIN EN ISO 21569:2013-08 ASU §64 L 00. 00-148: 2014-02	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount CTAB Congen SureFood PREP Basic extraction kit DNeasy Mericon Food Kit;Qiagen	e.g real time PCR / gel electro- phoresis / cycles / amplificate length / reference material Real time PCR Real Time PCR, 45 cycles Real Time PCR, TaqMan Universal-AB/ThermoFisher,	
2 25.5.20 3 02.06. 4 04.06. 5 18.6.20 6 03.06. 7 9.6.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-064 2020-07- 16 24.6.20	02.06. 04.06. 8.6.20 03.06. 0.6.20	6. FMV 6. 20 p-FMV 78 bp Amplikon 34S-FMV Promotor	10 copies 5 copies 0.1 % 0,01	GEN-IAL genControl RT- Triplex IV p35S / NOS / pFMV, incl. IC §64 LFGB L 00.00-148 (modified) (2014-02) DIN EN ISO 21569:2013-08 ASU §64 L 00. 00-148:	Congen SureFood PREP Basic extraction kit DNeasy Mericon Food Kit;Qiagen	Real Time PCR, 45 cycles Real Time PCR, TaqMan Universal-AB/ThermoFisher,	
3 02.06. 4 04.06. 5 18.6.20 6 03.06. 7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-16 24.6.20	02.06. 04.06. 8.6.20 03.06. 0.6.20	6. FMV 6. 20 p-FMV 78 bp Amplikon 34S-FMV Promotor	10 copies 5 copies 0.1 % 0,01	GEN-IAL genControl RT- Triplex IV p35S / NOS / pFMV, incl. IC §64 LFGB L 00.00-148 (modified) (2014-02) DIN EN ISO 21569:2013-08 ASU §64 L 00. 00-148:	Congen SureFood PREP Basic extraction kit DNeasy Mericon Food Kit;Qiagen	Real Time PCR, 45 cycles Real Time PCR, TaqMan Universal-AB/ThermoFisher,	
4 04.06. 5 18.6.20 6 03.06. 7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07- 16 24.6.20	94.06. 8.6.20 93.06. 9.6.20 8.5.20	6. p-FMV 78 bp Amplikon 34S-FMV Promotor	5 copies 0.1 % 0,01	Triplex IV p35S / NOS / pFMV, incl. IC §64 LFGB L 00.00-148 (modified) (2014-02) DIN EN ISO 21569:2013-08 ASU §64 L 00. 00-148:	extraction kit DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher,	
5 18.6.20 6 03.06. 7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-16 24.6.20	8.6.20 03.06. 0.6.20 8.5.20	20 p-FMV 3. 78 bp Amplikon 34S-FMV Promotor	0.1 %	(modified) (2014-02) DIN EN ISO 21569:2013-08 ASU §64 L 00. 00-148:		Universal-AB/ThermoFisher,	
6 03.06. 7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-4 2020-07- 16 24.6.20	03.06. 0.6.20 8.5.20	6. 78 bp Amplikon 34S-FMV Promotor	0,01	ASU §64 L 00. 00-148:		45 cycles	
7 9.6.20 8 28.5.20 9 16.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-16 24.6.20	9.6.20 8.5.20	Amplikon 34S-FMV Promotor			mod. Wizard®-DNA-Clean-UP	Real Time PCR / 82 bp	
8 28.5.20 9 16.6.20 10 2.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-4 2020-07- 16 24.6.20	8.5.20	20 Promotor	0,01%		CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
9 16.6.20 10 2.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-4 2020-07- 16 24.6.20		20		SureFood® GMO SCREEN 35S/NOS/FMV (S2026)	SureFood® Add-on (S1055)	real-time PCR	K00
10 2.6.20 11 12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-16 24.6.20	6.6.20		1%	SUREFOOD® GMO SCREEN 4PLEX 35S/NOS/FMV + IAC	omega E.Z.N.A. food DNA kit		
11		20 DNA	0.003%	in House Method	Genomic DNA from food, Macherey- Nagel	Real Time PCR	LOD: Specification of decimal places with "point"
12 13 13.6.20 14 23.6.20 15 2020-06-1 2020-07- 16 24.6.20	2.6.20	20		biotecon GMO Screening kit	mericon food kit	real time pcr	
13 13.6.20 14 23.6.20 15 2020-06-1 2020-07-1 16 24.6.20				R-Biopharm, S2026:2017- 04 & QMAA-P-19:2018-08 (Multiplex-PCR)			
14 23.6.20 15 2020-06- 2020-07- 16 24.6.20							
15 2020-06-1 2020-07- 16 24.6.20	3.6.20	20		Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
16 24.6.20	3.6.20	FMV	0,01%	foodproof GMO Screening 1 LyoKit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
		7-17 Sequence / -DNA	0,1%	Bioside / ISO 21569:2005/Amd 1:2013	Machery Nagel FOOD/PLANT extraction kit	Real time PCR	
17 00 07 00	4.6.20	Promotor des Feigenwurz Mosaik Virus (pFMV)	0.1 %	ASU L 00.00-148 mod.	Maxwell FFS Kit, 200 mg sample wheight		
17 08.07.20		020.	0,05%	TaqMan GMO Screening Kit	Qiagen Dneasy Plant Kit	RT PCR	
18 14.7.20	07.2020.	20 Target- Sequence	5 copies 5 mg/kg	R-Biopharm S2126	R-Biopharm S1052	Real-Time PCR	
19 28.5.20		20		L 00.00-148 Ausgabe 02/2014			
20	4.7.20		0,1%	L00.00-148	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21 03.07.2 22 9.7.20	4.7.20 8.5.20	20	0,10% 0,1%	In House Method sureFood Congen S2026	Magnetic Bead Extraction As per Kit Instructions	Gel Electrophoresis As Per Kit Instructions	

5.1.4 CTP2-CP4 EPSPS-Screening Sequenz

1 20.5 2 3 4 04.0 5 18.6 6 03.0 7 10.6 8 9 28.5 10 11 12 12 13 17.	y/Month 0.5.20 04.06. 8.6.20 03.06. 0.6.20	Target-Sequence / -DNA DNA DNA CTP2-CP4 EPSPS 88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS DNA	number of copies / % / ct-value <10 copies / PCR-Reaktion 5 copies 0.1 % 0,1 0,01%	Manufacturer / Official Method GEN-IAL / EN ISO 21570 §64 LFGB L 00.00-125 (modified) (2009-06) genControl®RT Triplex V, GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CryIAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex-35S/NOS/EPSPS Kit, GEN-IAL GmbH Gen-ial	e.g. Extraction / enzymes / clean-up / DNA quality / DNA amount Machery-Nagel: NucleoSpin Food DNeasy Mericon Food Kit;Qiagen mod. Wizard®-DNA-Clean-UP CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel CTAB, Proteinase K, FFS-Kit Promega	Real Time PCR / 88 bp Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	Sample 3 weakly positive (<loq; "point"<="" -05:="" 0,1%="" <="" but="" decimal="" detectable="" k01="" lod:="" loq="100copies)" of="" places="" sample="" specification="" th="" with=""></loq;>
2 3 4 04.0 5 18.6 6 03.0 7 10.6 8 9 28.5 10 11 12 13 17.	04.06. 8.6.20 03.06. 0.6.20	CTP2-CP4 EPSPS 88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	PCR-Reaktion 5 copies 0.1 % 0,1 0,01%	§64 LFGB L 00.00-125 (modified) (2009-06) genControl*RT Triplex V, GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex-355/NOS/EPSPS Kit, GEN-IAL GmbH	DNeasy Mericon Food Kit;Qiagen mod. Wizard®-DNA-Clean-UP CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	genControl RT Triplex 35S/tNOS/EPSPS Kit) Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles Real Time PCR / 88 bp Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	weakly positive (<loq; -05:="" 0,1%="" <="" but="" decimal="" detectable="" k01="" lod:="" loq="100copies:" of="" places<="" sample="" specification="" td=""></loq;>
3 4 04.0 5 18.6 6 03.0 7 10.6 8 9 28.5 10 11 12 13 17.	8.6.20 03.06. 0.6.20 8.5.20	88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0.1 % 0,1 0,01%	(modified) (2009-06) genControl®RT Triplex V, GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CryIAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	mod. Wizard®-DNA-Clean-UP CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	Universal-AB/ThermoFisher, 45 cycles Real Time PCR / 88 bp Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	weakly positive (<loq; -05:="" 0,1%="" <="" but="" decimal="" detectable="" k01="" lod:="" loq="100copies" of="" places<="" sample="" specification="" td=""></loq;>
4 04.0 5 18.6 6 03.0 7 10.6 8 9 28.5 10 11 12 13 17.	8.6.20 03.06. 0.6.20 8.5.20	88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0.1 % 0,1 0,01%	(modified) (2009-06) genControl®RT Triplex V, GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CryIAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	mod. Wizard®-DNA-Clean-UP CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	Universal-AB/ThermoFisher, 45 cycles Real Time PCR / 88 bp Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	weakly positive (<loq; -05:="" 0,1%="" <="" but="" decimal="" detectable="" k01="" lod:="" loq="100copies" of="" places<="" sample="" specification="" td=""></loq;>
5 18.6 6 03.0 7 10.6 8 9 28.5 10 11 12 13 17.	8.6.20 03.06. 0.6.20 8.5.20	88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0.1 % 0,1 0,01%	(modified) (2009-06) genControl®RT Triplex V, GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CryIAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	mod. Wizard®-DNA-Clean-UP CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	Universal-AB/ThermoFisher, 45 cycles Real Time PCR / 88 bp Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	weakly positive (<loq; -05:="" 0,1%="" <="" but="" decimal="" detectable="" k01="" lod:="" loq="100copies" of="" places<="" sample="" specification="" td=""></loq;>
6 03.0 7 10.6 8 9 28.5 10 11 12 13 17.	03.06.	88 bp Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0,1	GEN-IAL ASU §64 L 00.00-154: 2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey- Nagel	Real Time PCR (Taqman), 45 cycles real-time PCR Real Time PCR	detectable but < 0,1% K01 LOD: Specification of decimal places
7 10.6 8 9 28.5 10 11 12 13 17.	8.5.20	Amplikon transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0,01%	2014-08 SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	Mericon Food Kit; 100 ng/rxn. SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	real-time PCR Real Time PCR	LOD: Specification of decimal places
7 10.6 8 9 28.5 10 11 12 13 17.	8.5.20	transition of CTP2 to Herbicide Tolerance Gene CP4 EPSPS	0,01%	SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	SureFood® Add-on (S1055) Genomic DNA from food, Macherey-Nagel	real-time PCR Real Time PCR	LOD: Specification of decimal places
8 9 28.5 10 11 12 13 17.	8.5.20	CTP2 to Herbicide Tolerance Gene CP4 EPSPS	,	4plex BAR/PAT/ CrylAb/lac/ CTP2:CP4 EPSPS (S2128) genControl® RT-Triplex- 35S/NOS/EPSPS Kit, GEN-IAL GmbH	Genomic DNA from food, Macherey- Nagel	Real Time PCR	LOD: Specification of decimal places
9 28.5 10 11 12 13 17.		DNA	0.01%	35S/NOS/EPSPS Kit, GEN-IAL GmbH	Nagel		Specification of decimal places
10 11 12 13 17.		DNA	0.01%	35S/NOS/EPSPS Kit, GEN-IAL GmbH	Nagel		Specification of decimal places
11 12 13 17.	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
12 13 17.	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
13 17. 14 23.6	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14 23.6	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
15	3.6.20	target- Sequence CTP2-CP4- EPSPS	0,01%	foodproof GMO Screening 2 LyoKit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
			-				
16 24.6	4.6.20	transition of CTP2 to CP4- EPSPS-Gene		ASU L 00.00-125 mod.	Maxwell FFS Kit, 200 mg Einwaage		
17 10.07.	07.2020.		0,05%	GMO Screen 4 plex(BAR,NPTII,PAT,CTP2: CP4 EPSPS)	Qiagen Dneasy Plant Kit	RT PCR	
18							
19 28.5				L 00.00-125 Ausgabe 12/2008			
20	8.5.20		1			1	Real Time
21 22 9.7.	8.5.20		0,1%	ASU L00.00-125	CTAB-Method	Real Time PCR	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							
2							
3							
4	-						
5	18.6.20	35S-Pat	0.1 %	genControl®RT Triplex V, GEN-IAL	mod. Wizard®-DNA-Clean-UP	Real Time PCR / 108 bp	Sample -01: at LOD
6	03.06.	111 bp Amplicon	0,1	ASU §64 L 00.00-154: 2014-09	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	10.6.20	PAT Gen	0,01%	SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/CTP2:CP4 EPSPS (S2128)	SureFood® Add-on (S1055)	real-time PCR	K01
8							
9							
10							
11							
12 13							
13							
14	23.6.20	target- Sequence P- 35S-pat	0,01%	foodproof GMO Screening 2 LyoKit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are Iysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	25.6.20	transition of CaMV35S to pat-Gene	0.1 %	ASU G 30.40-1 mod.	Maxwell FFS Kit, 200 mg sample weight		Sample 1 and 3 high Ct-values > contents ~ 0,1 %
17	-						
18							
19	9.6.20			QL-ELE-00-025			
20			0,1%	G 30.40-14	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21							
22	9.7.20	DNA	0,1%	sureFood Congen S2127	As per Kit Instructions	As Per Kit Instructions	

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	04.06.		10 copies	§64 LFGB L 15.06-3 (modified) (2013-08)	DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles	
5							
6	03.06.	74 bp Amplicon	0,1	ASU L 15.06-3:2013	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	10.6.20	CrylAb/CrylAc Element	0,01%	SureFood® GMO SCREEN 4plex BAR/PAT/ CrylAb/lac/CTP2:CP4 EPSPS (S2128)	SureFood® Add-on (S1055)	real-time PCR	K01
8							
9	16.6.20	DNA	10 copies	in House-Method	Genomic DNA from food, Macherey- Nagel	Real Time PCR	LOD: Specification of decimal places with "point"
10							
11							
12							
13	17.6.			r-biopharm (Congen)	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target- Sequence Cry1Ab/Ac	0,01%	foodproof GMO Screening 2 LyoKit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	24.6.20	cry1Ab/cry1A c DNA- Sequences	5 haploide Genomic copies	ASU L 15.06-3 mod.	Maxwell FFS Kit, 200 mg Einwaage		
17	10.07.2020.		0,05%	GMO Screen 4 plex(BAR,PAT,CrylAb/lAc, CTP2:CP4 EPSPS)	Qiagen Dneasy Plant Kit	RTPCR	
18							
19							
20			0,1%	G 30.40-14	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21							
22							

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							
3							
4	-				-	-	
5							
6	04.06.	110 bp Amplicon	0,05	ASU L 15.06-3:2013	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	-						
8							
9							
10							
11							
12							
13	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target- Sequence	0,1%	foodproof SL GMO Bt11 Maize Detection Kit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	nicht durchgeführ t						
17	10.07.2020.		0.1%	QT/ZM/015	Qiagen Dneasy Plant Kit	RT PCR	
18			-,		, , , , , , , , , , , , , , , , , , ,		
19	28.5.20			QT-EVEZM-006			
20			0,1%	JRC 2008 Event specific Method QT-EVE-ZM-015	CTAB-Methode	Real Time PCR	Real Time PCR/50 cycles
21	20.07.20		0,10%	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
22							

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	10.6.20	MIR604 Maize (SYN-IR6Ø4- 5)	0,01%	in-house Method	SureFood [®] Add-on (S1055)	real-time PCR	
8		·					
9							
10							
11							
12							
13	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 Cycles	
14	23.6.20	target- Sequence	0,1%	foodproof SL GMO MIR604 Maize Detection Kit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	nicht durchgeführ t						
17	20.07.2020.		0,1%	QT/ZM/013	Qiagen Dneasy Plant Kit	RT PCR	
18							
19	28.5.20			QT-EVE-ZM-013			
20			0,1%	JRC 2007 Event specific Method QT-EVE-ZM-013	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21							
22							

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							
3							
4	03.06.		5 copies	§64 LFGB L 00.00-105 (modified) (2014-02)	DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles	Sample 3 weakly positive (<loq; LOQ=100 copies)</loq;
5							
6	04.06.	134 bp Amplicon	0,01	DIN EN ISO 21569: 2013-08	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7							
8							
9							
10							
11							
12							
13	17.6.			r-biopharm (Congen)	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 30 cycles	
14							
15							
16	18.6.20	hmg-Gene	25 haploide Genomic copies	ASU L 00.00-105 mod. (Annex C3)	Maxwell FFS Kit, 200 mg weight		
17	20.07.2020.		0,1%	QT/ZM/013 /015	Qiagen Dneasy Plant Kit	RT PCR	
18							
19	28.5.20			QT-EVE-ZM-006			
20			0,1%	L.00.00-105	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21							
22							

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	04.06.	84 bp Amplicon	0,01	DIN EN ISO 21569: 2013-09	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	10.6.20	GTS 40-3-2 Soya (MON-Ø4Ø32- 6)	0,01%	SureFood® GMO ID 4plex Soya II (S2162)	SureFood® Add-on (S1055)	real-time PCR	K01
8							
9	29.6.20	DNA	0.05%	in House Method	in House Method	Real Time PCR	Sample 1: 17% and sample 3: 24%; LOD: Specification of decimal places with "point"
10							
11							
12							
13	16.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target- Sequence	0,1%	foodproof SL GMO GTS40- 3-2 Soya Detection Kit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	19.6.20	transition of CTPGene to 35S-Promotor	0.1 %	ASU L 00.00-105 mod.	Maxwell FFS Kit, 200 mg sample weight		
17	13.07.2020.		0,1%	QT-EVE-GM-005	Qiagen Dneasy Plant Kit	RT PCR	
18							
19	28.5.20			QT-CON-00-003			
20			0,1%	L.00.00-105	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21	02.07.20		0,10%	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
22							

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							
3							
4	-				-	-	
5 6	04.06.	139 bp Amplicon	0,01	DIN EN ISO 21569: 2013-10	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7	10.6.20	MON89788 Soya (MON-89788- 1)	0,01%	SureFood® GMO ID 4plex Soya II (S2162)	SureFood® Add-on (S1055)	real-time PCR	K01
8							
9	29.6.20	DNA	0.0015%	in House Method	in House Method	Real Time PCR	Sample 1: 7,5% and sample 3: 0,09%; LOD: Specification of decimal places with "point"
10							•
11							
12							
13	16.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
14	23.6.20	target- Sequence	0,05%	foodproof GMO RR 2 Yield Soya Quantification Kit, Biotecon Diagnostics	Extraction with foodproof Sample Preparation Kit III, Biotecon Diagnostics Procedure: 1.Cells are lysed by incubation in the foodproof Sample Preparation Kit III extraction buffer. 2.Extracted DNA is digested in proteinase K to destroy endogenous nucleases and other proteins. 3.The DNA is applied to the included glass fiber filter and purified with the foodproof Sample Preparation Kit III wash buffer. 4. Purified DNA is then eluted using the foodproof Sample Preparation Kit III elution buffer and is ready to use.	Program for Light Cycler 96, Roche-Pre-incubation (1 cycle) Step 1: 37oC for 4 min Step 2: 95oC for 10min & Amplification (50 cycles) Step 1: 95oC for 5sec Step 2 (fluorescence detection) 60oC for 60 sec	
15							
16	nicht durchgeführ t						
17	13.07.2020.		0,1%	QT/GM/006	Qiagen Dneasy Plant Kit	RT PCR	
18							
19	28.5.20			QT-EVE-GM-006			
20			0,1%	JRC 2013 Event specific Method QT-EVE-GM-006	CTAB-Method	Real Time PCR	Real Time PCR/50 cycles
21							
22							

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							
3							
4	03.06.		5 copies	§64 LFGB L 00.00-105 (modified) C.2 (2014-02)	DNeasy Mericon Food Kit;Qiagen	Real Time PCR, TaqMan Universal-AB/ThermoFisher, 45 cycles	
5						- 1	
6	04.06.	81 bp Amplicon	0,01	DIN EN ISO 21569: 2013-09	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
7							
8							
9	28.5.20	DNA	0.015%	in House Method	in House Method	Real Time PCR	LOD: Specification of decimal places with "point"
10							'
11							
12							
13	17.6.			r-biopharm (Congen)	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 30 cycles	
14							
15							
16	18.6.20	lectin-Gene	5 haploide Genomic copies	ASU L 00.00-105 mod. (Annex B1)	Maxwell FFS Kit, 200 mg sample weight		Sample 2 + 4 very bad Ct- values, very small soya amount contained. Not suitable for analysis of GMC soya.
17	13.07.2020.		0,1%	QT-EVE-GM-005 and QT/GM/006	Qiagen Dneasy Plant Kit	RT PCR	
18							
19	9.6.20			QT-EVE-GM-006			
20			0,1%				
21	09.07.20		0,10%	In House Method	Magnetic Bead Extraction	Gel Electrophoresis	
22							

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
other events		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	12			5 copies, 0,05%	Gene Scan TR 35S/NOS/ABII IPC	Sure Food Prep Advancded r-Biopharm	Real-Time PCR, 45 cycles	
bar	5	18.6.20	bar	0.1 %	genControl®RT Triplex V, GEN-IAL	mod. Wizard®-DNA-Clean- UP	Real Time PCR / 60 bp	
bar	13	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
bar	17	10.07.2020.		0,05%	GMO Screen 4 plex(BAR,NPTII,PAT,CTP2:CP 4 EPSPS)	Qiagen Dneasy Plant Kit	RTPCR	
CaMV	16	24.6.20	Cauliflower Mosaik Virus- specific DNA- Sequence		ASU G 30.40-17	Maxwell FFS Kit, 200 mg sample weight		
NK603-Maize	6	04.06.		0,05	GMOMETHODS database; QT-EVE-ZM-008: 2005	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
NPTII	17	10.07.2020.		0,05%	GMO Screen 4 plex(BAR,NPTII,PAT,CTP2:CP 4 EPSPS)	Qiagen Dneasy Plant Kit	RTPCR	
NPTII	22	9.7.20		0,1%	sureFood Congen S2127	as per kit instructions	as per kit instructions	
pat	9	16.6.20	DNA	10 copies	in House Method	Genomic DNA from food, Macherey-Nagel	Real Time PCR	LOD: Specification of decimal places with "point"
pat	13	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
pat	16	25.6.20	pat-Gen	0.1 %	ASU G 30.40-14	Maxwell FFS Kit, 200 mg sample weight		Sample 1 + 3 weak positive si- gnals with con- tents < 0,1 %
pat	17	10.07.2020.		0,05%	GMO Screen 4 plex(BAR,NPTII,PAT,CTP2:CP 4 EPSPS)	Qiagen Dneasy Plant Kit	RTPCR	·
Plant detection	9	28.5.20	DNA	0.1%	in House Method	Genomic DNA from food, Macherey-Nagel	Real Time PCR	LOD: Specification of decimal places with "point"
pnos-nptll	13	17.6.			Gen-ial	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 45 cycles	
Rapeseed spe- cific DNA	13	17.6.			r-biopharm (Congen)	CTAB, Proteinase K, FFS-Kit Promega	real time PCR, 30 cycles	
Rapeseed spe- cific DNA	6	04.06.			GMOMETHODS database: 2011	CTAB, Prot. K, RNase A; Dneasy Mericon Food Kit; 100 ng/rxn.	Real Time PCR (Taqman), 45 cycles	
Soya A2704-12	3	05.06.		5 copies	GEN-IAL genControl RT Triplex Soya I	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	
GMO-Soya LL (A2704-12)	9	29.6.20	DNA	0.02%	in House Method	in House Method	Real Time PCR	LOD: Specification of decimal places with "point"
Soya A5547- 127	3	05.06.		15 copies	GEN-IAL genControl RT Triplex Soya I	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	
Soya CV127-9	3	05.06.		30 copies	GEN-IAL genControl RT Triplex Soya II	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	
Soya DP305423-1	3	05.06.		30 copies	GEN-IAL genControl RT Triplex Soya II	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	
Soya DP356043-5	3	05.06.		10 copies	GEN-IAL genControl RT Triplex Soya I	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	
Soya MON87701-2	3	05.06.		5 copies	GEN-IAL genControl RT Triplex Soya II	Congen SureFood PREP Basic Extraktionskit	Real Time PCR, 45 cycles	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test DLA -ptGM S1 Sample 1

Result of analysis

Cample	\Maight [g]	Particle	Particles
Sample	Weight [g]	number	[mg/kg]
1	5,02	75	29,9
2	5,01	83	33,1
3	4,98	82	32,9
4	4,97	73	29,4
5	5,01	78	31,1
6	5,01	82	32,7
7	4,98	84	33,7
8	4.97	87	35.0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	80,5	Particles
Standard deviation	4,85	Particles
χ² (CHI-Quadrat)	2,05	
Probability	96	%
Recovery rate	94	%

Normal distribution		
Number of samples	8	
Mean	32,2	mg/kg
Standard deviation	1,94	mg/kg
rel. Standard deviaton	6,0	%
Horwitz standard deviation	9,5	%
HorRat-value	0,6	
Recovery rate	94	%

Microtracer Homogeneity Test DLA -ptGMS1 Sample 2

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	67	26,5
2	5,00	81	32,4
3	5,03	70	27,8
4	4,97	67	27,0
5	5,02	75	29,9
6	4,96	77	31,0
7	5,03	75	29,8
8	5,02	85	33,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	74,6	Particles
Standard deviation	6,52	Particles
χ² (CHI-Quadrat)	3,99	
Probability	78	%
Recovery rate	124	%

Normal distribution		
Number of samples	8	
Mean	29,8	mg/kg
Standard deviation	2,60	mg/kg
rel. Standard deviaton	8,7	%
Horwitz standard deviation	9,6	%
HorRat-value	0,9	
Recovery rate	12/	0/2

Microtracer Homogeneity Test DLA -ptGMS1 Sample 3

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	79	31,8
2	5,03	75	29,8
3	5,03	75	29,8
4	4,97	75	30,2
5	5,00	79	31,6
6	5,03	84	33,4
7	4,99	81	32,5
8	5,01	73	29,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	77,6	Particles
Standard deviation	3,76	Particles
χ² (CHI-Quadrat)	1,27	
Probability	99	%
Recovery rate	114	%

Normal distribution		
Number of samples	8	
Mean	31,0	mg/kg
Standard deviation	1,50	mg/kg
rel. Standard deviaton	4,8	%
Horwitz standard deviation	9,5	%
HorRat-value	0,5	
Recovery rate	114	%

Microtracer Homogeneity Test DLA -ptGMS1 Sample 5

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	67	26,7
2	4,96	66	26,6
3	5,01	60	24,0
4	4,97	69	27,8
5	5,03	65	25,8
6	5,03	63	25,0
7	5,02	73	29,1
8	5,00	74	29,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	67,1	Particles
Standard deviation	4,83	Particles
χ² (CHI-Quadrat)	2,43	
Probability	93	%
Recovery rate	94	%

Normal distribution		
Number of samples	8	
Mean	26,8	mg/kg
Standard deviation	1,93	mg/kg
rel. Standard deviaton	7,2	%
Horwitz standard deviation	9,8	%
HorRat-value	0,7	
Recovery rate	94	%

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 ptGMS1
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
Last Deadline	the latest 24th July 2020
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	Alexandra Scharf MSc.

^{*} 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
		ITALY
		BELGIUM
		Germany
		GREAT BRITAIN
		Germany
		GREECE
		GREAT BRITAIN
		SERBIA
		Germany
		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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