DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

23/2014

GMO - Screening qualitative:

5 Samples with positive/negative amounts of GMO-Maize (Bt11) or GMO-Soya (RR)

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Content

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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 validity of the particular testing method.
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.

2. Realisation

2.1 Test material

The test materials are 5 different mixtures of common in commerce foods from European and US-American suppliers (s. table 1).
The ingredients were mixed, homogenized and portioned to approximately 10 g.
The materials were tested for homogeneity.

2.2 Test

One portion of each of the 5 test materials was sent to every participating laboratory in the 20th week of 2014. The testing method was optional. The tests should be finished at June 27th 2014 the latest.

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out along with the samples. The results given as positive/negative were evaluated with respect to each tested parameter.
Queried and documented were the indicated results and details of the test methods like specifity, test kit manufacturer and hints about the procedure.
All participants submitted their results in time.

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Table 1: Composition of DLA-samples

DLA-Sample	Ingredients (per 100 g)	GMO-Content Maize	GMO-Content Soya
1	Cereal-Milk-Pap-Powder (100 g) Ingredients: Rice flour, skimmed milk powder, whey powder partially demineralized, palm oil maltodextrin, **maize flour**, buckwheat whole flour, sorghum whole flour, calcium carbonate, Vitamin B1, Vitamin A, Vitamin D Ingredients per 100 g: Protein 13 g, Carbohydrates 64 g, Fat 16 g	-	-
2	Maize Flour, European-Supplier (85 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 77 g, Fat 1 g Maize Flour, USA-Supplier (15 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 9 g, Carbohydrates 79 g, Fat 0 g	- positive (bt11-Maize experimental)	- -
3	Meal Replacement, Dietetic Food (93,1 g) Ingredients: **Soyprotein-Isolate (40%),** honey, skimmed milk powder, yoghurt powder, soybean oil, maltodextrin, milk protein, emulgator: soy lecithin, antioxidation agent E304, E307, inulin, di-potassium phosphate, natural aroma, tri-calcium phosphate, magnesium hydroxide, vegetable oil, ascorbic acid, ferric di-phosphate, sweetener: sucralose, dl-alpha-tocopherylacetate, nicotinamide, zinc oxide, calcium-d-pantothenate, manganese-(II)-sulfate, pyridoxin hydrochloride, thiamine mononitrate, riboflavine, Vitamin B2, retinylacetate, copper carbonate, folic acid, sodium selenite, potassium iodide, d-biotin, cholecalciferol, cyanocobalamine Ingredients per 100 g: Protein 42 g, Carbonate 31 g, Fat 6,1 g Soya Chunks, USA-Supplier (6,9 g) Ingredients: **Soybean Flour** Nutrients per 100 g: Protein 47 g, Carbohydrates 17 g, Fat 0,8 g	-	positive (RR-Soya experimental)
4	Potato Flour (93,1 g) Ingredients: Potato flour Ingredients per 100 g: Protein 0,6 g, Carbohydrates 83 g, Fat 0,1 g Soyprotein-Product, European Supplier (6,9 g) Ingredients: **Soyprotein isolate**, dextrines, sweetener: xylit, vitamines and minerals Nutrients per 100 g: Protein 44 g, carbohydrates 18 g, fatt 3,6 g	-	-
5	Potato Flour (93,1 g) Ingredients: Potato flour Ingredients per 100 g: Protein 0,6 g, Carbohydrates 83 g, Fat 0,1 g	-	-

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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, GMO-Soya (RR), Lectin-DNA, GMO-Maize (bt11), Maize-DNA and other DNA results.
The numbers and percentage of positive and negative results are given at the end of each table. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

For every participant a qualitative valuation is made with respect to the consensus results. Therefore the number and percentage of "correct" results of consensus results is given.

4. Results

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

The results of the participants are given in tables as indicated below:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 4	Qualitative Valuation	Remarks
Parameter	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus results	

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4.1 Test

4.1.1 Results: 35S-Screening-Sequence

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
35S	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
1	negative	positive	positive	positive	negative	4/5 (80%)	
2	negative	positive	positive	negative	negative	5/5 (100%)	
3	negative	positive	positive	negative	negative	5/5 (100%)	
4	negative	positive	positive	negative	negative	5/5 (100%)	
5	negative	positive	positive	negative	negative	5/5 (100%)	
6	negative	positive	positive	negative	negative	5/5 (100%)	
7	negative	positive	positive	negative	negative	5/5 (100%)	
8	negative	positive	positive	negative	negative	5/5 (100%)	
9	negative	positive	positive	negative	negative	5/5 (100%)	
10	negative	positive	positive	positive	negative	4/5 (80%)	
11	negative	positive	positive	negative	negative	5/5 (100%)	
12	negative	positive	positive	negative	negative	5/5 (100%)	
13	negative	positive	positive	negative	negative	5/5 (100%)	
14	negative	positive	positive	negative	negative	5/5 (100%)	
15	negative	positive	positive	negative	negative	5/5 (100%)	
16	negative	positive	positive	negative	negative	5/5 (100%)	
17	negative	positive	positive	pos/neg	negative	4/5 (80%)	Sample 4: valuated as not in agreement
18	negative	positive	positive	negative	negative	5/5 (100%)	
20	negative	positive	positive	negative	negative	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	19	19	2	0
Number negative	19	0	0	16	19
Percent positive	0	100	100	11	0
Percent negative	100	0	0	89	100
Consensus	negative	positive	positive	negative	negative

Comments on results:

For all 5 samples consensus results with four times 100% and one time 89% positive or negative results were obtained.

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4.1.2 Results: NOS-Screening-Sequence

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
NOS	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
1	negative	positive	positive	positive	negative	4/5 (80%)	
2	negative	positive	positive	negative	negative	5/5 (100%)	
3	negative	positive	positive	negative	negative	5/5 (100%)	
4	negative	positive	positive	negative	negative	5/5 (100%)	
5	negative	positive	positive	negative	negative	5/5 (100%)	
6	negative	positive	positive	negative	negative	5/5 (100%)	
7	negative	positive	positive	negative	negative	5/5 (100%)	
8	negative	positive	positive	negative	negative	5/5 (100%)	
9	negative	positive	positive	negative	negative	5/5 (100%)	
10	negative	positive	positive	positive	negative	4/5 (80%)	
11	negative	positive	positive	negative	negative	5/5 (100%)	
12	negative	positive	positive	negative	negative	5/5 (100%)	
13	negative	positive	positive	negative	negative	5/5 (100%)	
14	negative	positive	positive	negative	negative	5/5 (100%)	
15	negative	positive	positive	negative	negative	5/5 (100%)	
16	negative	positive	positive	negative	negative	5/5 (100%)	
17	negative	positive	positive	pos/neg	negative	4/5 (80%)	Sample 4: valuated as not in agreement
18	negative	positive	positive	negative	negative	5/5 (100%)	
20	negative	positive	positive	negative	negative	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	19	19	2	0
Number negative	19	0	0	16	19
Percent positive	0	100	100	11	0
Percent negative	100	0	0	89	100
Consensus	negative	positive	positive	negative	negative

Comments on results:

For all 5 samples consensus results with four times 100% and one time 89% positive or negative results were obtained.

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4.1.3 Results: GMO-Soya (RR-Round-Up-Ready-Soya)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
RR-Soya	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
3	negative	negative	positive	negative	negative	5/5 (100%)	
4	negative	negative	positive	negative	negative	5/5 (100%)	
5	negative	positive	positive	negative	negative	4/5 (80%)	
6	negative	negative	positive	negative	negative	5/5 (100%)	
9	negative	negative	positive	negative	negative	5/5 (100%)	
11	negative	negative	positive	negative	negative	5/5 (100%)	
12	negative	negative	positive	negative	negative	5/5 (100%)	
13	negative	negative	negative	negative	negative	4/5 (80%)	
14	negative	negative	positive	negative	negative	5/5 (100%)	
15	negative	negative	positive	negative	negative	5/5 (100%)	
17	negative	pos/neg	positive	negative	negative	4/5 (80%)	Sample 2: valuated as not in agreement
19	negative	negative	positive	negative	negative	5/5 (100%)	
20	negative	negative	positive	negative	negative	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	1	12	0	0
Number negative	13	11	1	13	13
Percent positive	0	8	92	0	0
Percent negative	100	92	8	100	100
Consensus	negative	negative	positive	negative	negative

Comments on results:

For all 5 samples consensus results with three times 100% and two times 92% positive or negative results were obtained.

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4.1.4 Results: Lectin-DNA (Soya-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
Lectin	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
3	negative	negative	positive	positive	negative	4/4 (100%)	
4	negative	negative	positive	positive	negative	4/4 (100%)	
9	negative	negative	positive	positive	positive	3/4 (75%)	
11	negative	negative	positive	positive	negative	4/4 (100%)	
12	negative	negative	positive	positive	negative	4/4 (100%)	
13	negative	negative	positive	positive	negative	4/4 (100%)	
14	positive	negative	positive	positive	negative	4/4 (100%)	
15	negative	negative	positive	positive	negative	4/4 (100%)	
17	positive	positive	positive	positive	negative	3/4 (75%)	
20	positive	positive	positive	positive	negative	3/4 (75%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	3	2	10	10	1
Number negative	7	8	0	0	9
Percent positive	30	20	100	100	10
Percent negative	70	80	0	0	90
Konsenswert	keiner	negative	positive	positive	negative

Comments on results:

For 4 samples consensus results with two times 100%, one time 90% and one time 80% positive or negative results were obtained.
For sample 1 there were 70% negative results. Therefore no consensus value could determined for sample 1 and it was not included for the "qualitative valuation".

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4.1.5 Results: GMO-Maize (bt11-Maize)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
bt11 Maize	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
3	negative	positive	negative	negative	negative	5/5 (100%)	
6	negative	positive	negative	negative	negative	5/5 (100%)	
9	negative	positive	negative	negative	negative	5/5 (100%)	
11	positive	positive	positive	positive	negative	2/5 (40%)	
13	negative	positive	negative	negative	negative	5/5 (100%)	
14	negative	positive	negative	negative	negative	5/5 (100%)	
15	negative	positive	negative	negative	negative	5/5 (100%)	
17	negative	positive	negative	negative	negative	5/5 (100%)	

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

Comments on results:

For all 5 samples consensus results with two times 100% and three times 88% positive or negative results were obtained.

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4.1.6 Results: Maize-DNA (Maize-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Remarks
Maize DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreements with consensus value	
3	positive	positive	negative	negative	negative	3/3 (100%)	
9	positive	positive	positive	positive	negative	3/3 (100%)	
13	positive	positive	negative	negative	negative	3/3 (100%)	
14	positive	positive	positive	positive	negative	3/3 (100%)	
15	positive	positive	positive	positive	negative	3/3 (100%)	
17	positive	positive	positive	positive	negative	3/3 (100%)	
20	positive	positive	negative	negative	negative	3/3 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	7	7	4	4	0
Number negative	0	0	3	3	7
Percent positive	100	100	57	57	0
Percent negative	0	0	43	43	100
Konsenswert	positive	positive	none	none	negative

Comments on results:

For 3 samples consensus results with three times 100% positive or negative results were obtained.
For samples 3 and 4 there were 57% positive and 43% negative results. Therefore no consensus value could determined for samples 3 and 4 and they were not included for the "qualitative valuation".

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4.1.7 Results: Other Parameters (DNA)

Evaluation number	Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	Other DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg
4	other			FMV: positive 7,9% RR-soya		
6	CTP2-CP4 epsps	negative	positive	positive	negative	negative
6	GVO-soya RR (Event GTS 40-3-2)	negative	negative	positive	negative	negative
6	GVO-soya RR 2 (MON89788)	negative	negative	positive	negative	negative
7	other	positive	positive	positive	positive	positive
8	other	negative (FMV)	negative	positive	negative (FMV)	negative (FMV)
9	other	FMV negative	FMV negative	positive	FMV negative	FMV negative
12	Chloroplasten-DNA	positive	positive	positive	positive	positive
15	EPSPS	negative	positive	positive	negative	negative
15	FMV	negative	negative	negative	negative	negative
15	Bt 176		negative	negative		
15	MON810		positive	positive		
15	MON863		positive	positive		
15	NK603		positive	positive		
15	TC1507		positive	positive		
16	other	negative	negative	positive	negative	negative
17	GVO-soya RR-2Y	negative	negative	positive	negative	
17	GVO-maize (MON863)		positive	negative	negative	
17	GVO-maize (NK603)		positive	positive	negative	
17	GVO-maize (T25)		positive	negative		
17	rice specific	positive	negative	negative	negative	negative
17	potato specific	negative	negative	negative	positive	positive
18	Screening FMV-P	negative	negative	positive	negative	negative
20	CTP2 CP4 EPSPS	negative	positive	positive	negative	negative

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5. Documentation

5.1 Details by participants about DNA-Extraction methods

5.1.1 35S-Screening Sequence

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1		GEN-IAL	
2	35S Promotor DNS (CaMV)	SureFood PREP Plant, SureFood GMO Screen 4plex	
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
4		SureFood GMO 4plex	Sure Food Prep Allergen; 150mg sample weight
5	Target - DNA	Biotecon Diagnostics	foodproof GMO Sample Preparation Kit
6		foodproof GMO-Screening Kit, Biotecon/ GenControl RT Triplex III-Kit, GEN-IAL	Genomic DNA from food, NucleoSpin Kit, Macherey and Nagel
7		ASU L00.00.31	modified CTAB-method with clean-up
8		Congen	Extraction with Sure Food PlantX, Screening with Sure Food GMO Screen 35S/NOS/FMV
9		Congen	Maxwell FFS Kit
10		real-time pcr	Biotecon plant extraction kit
11	target-Sequence	manufacturer	prepman ultra applyed biosystem-no clean up- taqman universal master mix applyed biosystem
12		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.	QIAamp® DNA Stool Mini Kit (Fa. Qiagen)
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	P-35S	Huber et al. 2013	In house (CTAB and Clean Up by innuPrep Micro Kit)
16	35S	R-Biopharm	R-Biopharm, SureFood PREP Plant X, according to manual
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food
18		Testkit Fa. Congen, ASU L00.00-118	Testkit Fa. Congen
20	Promotor of Cauliflower Mosaic Virus (CaMV35S),	Höhne et al (2002)	Machery & Nagel Nucleo Spin Food Kit, 200 mg sample weight

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5.1.2 NOS-Screening Sequence

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1		GEN-IAL	
2	A. tumefaciens NOS Terminator DNS	SureFood PREP Plant, SureFood GMO Screen 4plex	
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
4		SureFood GMO 4plex	Sure Food Prep Allergen; 150mg sample weight
5	Target - DNA	Biotecon Diagnostics	foodproof GMO Sample Preparation Kit
6		foodproof GMO-Screening Kit, Biotecon/ GenControl RT Triplex III-Kit, GEN-IAL	Genomic DNA from food, NucleoSpin Kit, Macherey and Nagel
7		ASU L00.00.31	modified CTAB-method with clean-up
8			
9		Congen	Maxwell FFS Kit
10			
11	target-Sequence	manufacturer	prepman ultra applyed biosystem-no clean up- taqman universal master mix applyed biosystem
12		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.	QIAamp® DNA Stool Mini Kit (Fa. Qiagen)
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	T-nos	Huber et al. 2013	In house (CTAB and Clean Up by innuPrep Micro Kit)
16	NOS	R-Biopharm	R-Biopharm, SureFood PREP Plant X, according to manual
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food
18		Testkit Fa. Congen, ASU L00.00-119	Testkit Fa. Congen
20	Terminator of Agrobacterium tumefaciens (nos)	ASU L 00.00-116	Machery & Nagel Nucleo Spin Food Kit, 200 mg sample weight

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5.1.3 GMO-Soya (RR-Round-Up-Ready-Soya)

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
4		SureFood GMO Quant RoudUp Ready Soya	Sure Food Prep Allergen; 150mg sample weight
5	Target - DNA	Biotecon Diagnostics	foodproof GMO Sample Preparation Kit
6			
9		Gen-Ial	Maxwell FFS Kit
11	target-Sequence	manufacturer	prepman ultra applyed biosystem-no clean up- taqman universal master mix applyed biosystem
12		ASU § 35 LMBG	QIAamp® DNA Stool Mini Kit (Fa. Qiagen)
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	CTP-Gen - P-35S	L 00.00-105	In house (CTAB and Clean Up by innuPrep Micro Kit)
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food
19			
20		ASU L 00.00-105	Machery & Nagel Nucleo Spin Food Kit, 200 mg sample weight

5.1.4 Lectin-DNA (Soya-specific)

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
4		SureFood GMO Quant RoudUp Ready Soya	Sure Food Prep Allergen; 150mg sample weight
9		Gen-Ial	Maxwell FFS Kit
11	target-Sequence	manufacturer	prepman ultra applyed biosystem-no clean up- taqman universal master mix applyed biosystem
12		Vodkin et al., 1983; Cell: 34, 1023-1031	QIAamp® DNA Stool Mini Kit (Fa. Qiagen)
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	Lectin (Le1) Gen	RC 2008 Event specific Method - QT/GM/009	In house (CTAB and Clean Up by innuPrep Micro Kit)
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food
20	Lektin Gen	ASU L 00.00-105	Machery & Nagel Nucleo Spin Food Kit, 200 mg sample weight

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5.1.5 GMO-Maize (bt11-Maize)

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
6		GenControl BT11Mais-Kit, GENIAL (EN ISO 21570 und §64 LFGB L00.00-105:2006 (Methode C7 konform)	Genomic DNA from food, NucleoSpin Kit, Macherey und Nagel
9		Gen-Ial	Maxwell FFS Kit
11	target-Sequence	manufacturer	prepman ultra applyed biosystem-no clean up- taqman universal master mix applyed biosystem
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	5' -host Genom - Bt11-ev-p1	JRC 2008 Event specific Method - QT/ZM/015	In house (CTAB and Clean Up with innuPrep Micro Kit)
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food

5.1.6 Maize-DNA (Maize-specific)

Evaluation number	Result given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequenz / -DNA	Supplier / Method	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
3	DNA	In-house method STM No. 01-076 (based on journal Eur Food Res Technol(2008) 226 : 1221- 1228	High Pure PCR Template Preparation Kit (Column Extraction)
9		Gen-Ial	Maxwell FFS Kit
13		In-house method	Magnetic Bead Extraction, no clean-up
14			Quiagen
15	Adh1 Gen	RC 2008 Event specific Method - QT/ZM/015	In house (CTAB and Clean Up with innuPrep Micro Kit)
17		§64 LFGB	CTAB-Lysis with Macherey Nagel NucleoSpin® Food
20	Invertase Gen	Brodmann (2002)	Machery & Nagel Nucleo Spin Food Kit, 200 mg sample weight

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5.1.7 Other Parameters (DNA)

Evaluation number	Results given as	Test-Kit or Literature	Remarks to DNA-Extraction
	Target-Sequence	Supplier / Methdd	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
4			Surefood GMO Plant
6a		GenControl RT Triplex III-Kit, GEN-IAL	SureFood Prep Plant X
6b		GenControl RT RR Soja -Kit, GEN-IAL (EN ISO 21570 and §64 LFGB L00.00-105:2006 (Methode C2 konform)	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
6c		GenControl RT RR Soja -2 Kit, GEN-IAL (DIN EN ISO 21570 konform)	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
7		Screening Pflanzen-DNA / Hausverfahren	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
8			Mericon Food Kit (Qiagen)
9		Congen	Mericon Food Kit (Qiagen)
12		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.	Mericon Food Kit (Qiagen)
15a	ctp2-cp4-epsps	Huber et al. 2013	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15b	PFMV	Mano et al. 2009	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15c			according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15d			according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15e			according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15f			according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
15g			according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
16	FMV	R-Biopharm	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17a		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17b		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17c		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17d		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17e		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
17f		§64 LFGB	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
18		Testkit Fa. Congen, ASU L00.00-119	according to Schweizer Lebensmittelbuch, Chapter 52B, Mai 1998: Extraction with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
20	transition from CTP2 to CP4-EPSPS-Gen	ASU L 00.00-125	SureFood® PREP Plant (Congen) for Extraction and clean up of plant DNA from food

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5.2 Details by participants to PCR-reaction

5.2.1 35S-Screening Sequence

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
1		
2	Real Time PCR	
3	Real-time PCR	
4	real time PCR, 45 cycles, Kit Amplification control, LOD=5copies	For sample 5 small amounts of DNA detectable
5	foodproof GMO Screenig Kit	
6	Real-time PCR, Amplificate length GenContol-Kit: p35S – 82Bp, Tnos – 84Bp, EPSPS – 105Bp	
7	Gel-PCR	
8		
9	Real Time PCR	
10	Biotecon real time pcr kit	
11	real time PCR	
12	Gelelektrophorese/45 Cycles/ 123 bp/ Bt11-Mais, RR-Soja	test in dupletes
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	
16	Real Time PCR, SureFood GVO 4plex, R-Biopharm, according to manual	
17	Real Time PCR, 45 Cycles, positive tested routine sample	questionable: Detection of traces, in parts of the sample
18	Real Time PCR	
20	Real-Time PCR	

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5.2.2 NOS-Screening Sequence

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
1		
2	Real Time PCR	
3	Real-time PCR	
4	real time PCR, 45 cycles, Kit Amplification control, LOD=5copies	
5	foodproof GMO Screenig Kit	
6	Real-time PCR, Amplifikatlängen GenContol-Kit: p35S – 82Bp, Tnos – 84Bp, EPSPS – 105Bp	
7	Gel-PCR	
8		
9	Real Time PCR	
10		
11	real time PCR	
12	Gelelektrophorese/45 Cycles/ 180 bp/ Bt11-Mais, RR-Soja	test in dupletes
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	
16	Real Time PCR, SureFood GVO 4plex, R-Biopharm, nach Anleitung	
17	Real Time PCR, 45 Cycles, positive tested routine sample	questionable: Detection of traces, in parts of the sample
18	Real Time PCR	
20		

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5.2.3 GMO-Soya (RR-Round-Up-Ready-Soya)

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
3	Real-time PCR	
4	real time PCR, 45 cycles, Kit Amplification control, LOD=5copies	positive results are valuated detectable in the laboratory
5	foodproof GMO Screenig Kit	
6		
9	Real Time PCR	
11	real time PCR	
12	Gelelektrophorese/45 Cycles/ 169 bp/ RR-Soja	
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	
17	Real Time PCR, 45 Cycles, positive tested routine sample	questionable: Detection of traces, in parts of the sample
19		
20		

5.2.4 Lectin-DNA (Soya-specific)

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
3	Real-time PCR	
4	real time PCR, 45 cycles, Kit Amplification control, LOD=5copies	negative results are valuated not detectable in the laboratory
9	Real Time PCR	
11	real time PCR	
12	Gel electrophoresis/45 Cycles/ 438 bp/ RR-Soja	
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	
17	Real Time PCR, 45 Cycles, positive tested routine sample	
20		Sample 1 and 2 very weak positive and therefore not suitable for analysis of gm-soya

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5.2.5 GMO-Maize (bt11-Maize)

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
3	Real-time PCR	
6	Real-time PCR, Amplificate length 127 bp	
9	Real Time PCR	
11	real time PCR	
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	
17	Real Time PCR, 45 Cycles, certified reference material	

5.2.6 Maize-DNA (Maize-specific)

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
3	Real-time PCR	
9	Real Time PCR	
13	gel electrophoresis	
14	Internal method	
15	Real Time PCR / 50 Cycles /	only when high DNA-yields of sample 3 were extracted traces of maize-specific DNA could be detected
17	Real Time PCR, 45 Cycles, positive tested routine sample	
20		

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5.2.7 Other Parameters (DNA)

Evaluation number	Notes to PCR-Reaction	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	
4		
6a	Real-time PCR, Amplificate length GenContol-Kit: p35S – 82Bp, Tnos – 84Bp, EPSPS – 105Bp	
6b	Real-time PCR, Amplifikatlänge 74 bp	
6c	Real-time PCR, Amplificate length 139 bp	
7	Gel-PCR	
8		
9	Real Time PCR	
12	Gel electrophoresis/45 Cycles/ 200-600 bp/ Bt11-Maize, Bt176, RR-Soya	
15a	Real Time PCR / 50 Cycles /	
15b	Real Time PCR / 50 Cycles /	
15c		
15d		
15e		
15f		
15g		
16	Real Time PCR, SureFood GVO 4plex, R-Biopharm, according to manual	
17a	Real Time PCR, 45 Cycles, positive tested routine sample	
17b	Real Time PCR, 45 Cycles, certified reference material	
17c	Real Time PCR, 45 Cycles, certified reference material	
17d	Real Time PCR, 45 Cycles, certified reference material	
17e	Real Time PCR, 45 Cycles, positive tested routine sample	
17f	Real Time PCR, 45 Cycles, positive tested routine sample	
18	Real Time PCR	
20		

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6. Index of participant laboratories

Teilnehmer / Participants	Ort / Town
	Germany
	Thailand
	Germany
	Germany
	France
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Germany
	Netherlands
	Italy
	United Kingdom

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

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7. Index of references

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