DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

34/2016

GMO - Screening qualitative:

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

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1. Introduction

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

2. Realisation

2.1 Test material

The test materials are 5 different mixtures of common in commerce food mixtures from European and US-American suppliers (s. table 1). The raw materials were crushed, sieved, mixed and homogenized. The composition of the samples is given in table 1.
Before homogenization microtracer particles were added in order to check the accuracy of mixing. After homogenization during bottling aliquots were taken for microtracer analysis (s. 2.1.1).
The samples were portioned to approximately 10 g into metallised PET film bags and chronologically numbered.

Table 1: Composition of DLA-Samples

DLA-Sample	Ingredients (per 100 g)	GMO-Content Maize	GMO-Content Soya
1	Cake Mix Chocolate (100 g) Ingredients: sugar, wheat starch, low fat cocoa powder, raising agents: disodium diphosphate, sodium bicarbonate, starch, emulsifiers : E475, E471, E433, flavoring	-	-
2	Cake Mix Chocolate (90 g) Ingredients: sugar, wheat starch, low fat cocoa powder, raising agents: disodium diphosphate, sodium bicarbonate, starch, emulsifiers : E475, E471, E433, flavoring	-	-
	Soya Flour, European Supplier (7,4 g) Ingredients: **Soya flour toasted** Nutrients per 100 g: Protein 40 g, Carbohydrates 14 g, Fat 22 g	-	-
	Soya Chunks, USA-Supplier (2,7 g) Ingredients: **Soybean Flour** Nutrients per 100 g: Protein 47 g, Carbohydrates 17 g, Fat 0,8 g	-	positive (RR-Soya experimental)
3	Potato Flour (70 g) Ingredients: Potato flour Ingredients per 100 g: Protein 0,6 g, Carbohydrates 83 g, Fat 0,1 g	-	-
	Maize Flour, European-Supplier (20 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 77 g, Fat 1,0 g	-	-
	Soya Flour, European Supplier (10 g) Ingredients: **Soya flour toasted** Nutrients per 100 g: Protein 40 g, Carbohydrates 14 g, Fat 22 g	-	-
4	Bread Mix, gluten free (78,5 g) Ingredients: Maize starch, flax seed flour, buckwheat flour, pea bran, rice bran, apple fiber, salt sugar, thickener: guar gum Nutrients per 100 g: Protein 6,1 g, Carbohydrates 63 g, Fat 2,6 g	-	-
	Maize Semolina, European-Supplier (21,5 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 74 g, Fat 1 g	-	-
5	Bread Mix, gluten free (76 g) Ingredients: Maize starch, flax seed flour, buckwheat flour, pea bran, rice bran, apple fiber, salt sugar, thickener: guar gum Nutrients per 100 g: Protein 6,1 g, Carbohydrates 63 g, Fat 2,6 g	-	-
	Maize Semolina, European-Supplier (15 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 7,5 g, Carbohydrates 74 g, Fat 1 g	-	-
	Maize Flour, USA-Supplier (8,0 g) Ingredients: Maize Flour Nutrients per 100 g: Protein 9 g, Carbohydrates 79 g, Fat 0 g	positive (bt11-Maize experimental)	-

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1-5 showed probabilities from 77% to 98%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat values from 0,45 to 0,98. The results of microtracer analysis are given in the documentation.

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked for quantitative PTs and optionally the evaluation of the results of the participants will be done using the z'-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3].

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 18th week of 2016. The testing method was optional. The tests should be finished at June 17th 2016 the latest.

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

There are 5 different test samples (flours and baking mixtures) which possibly contain GMO ingredients of Bt11 maize and / or RR soy.
The evaluation will be done exclusively qualitative (positive/negative).
Results may be given as specific sequences, screening sequences (35S and NOS) and other events.
Every suitable method for determination of the analyte may be applied.

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 specifity, 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.
One of 18 participants submitted his results delayed after communication with DLA. All other 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 35S, NOS, GMO-Soya (RR), Lectin-DNA, GMO-Maize (bt11), Maize-DNA and other DNA results.

3.1 Agreement with consensus values from participants

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

3.2 Agreement with spiking of samples

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

4. Results

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

The participant results and evaluation are tabulated as follows:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	

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

4.1 Test

4.1.1 Results: 35S-Screening-Sequence

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

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	15	0	1	16
Number negative	16	1	16	15	0
Percent positive	0	94	0	6	100
Percent negative	100	6	100	94	0
Consensus value	negative	positive	negative	negative	positive
Spiking	negative	positive	negative	negative	positive

Comments on results:

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

4.1.2 Results: NOS-Screening-Sequence

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
NOS	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
2	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
3	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
4	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
5	negative	positive	positive	negative	positive	4/5 (80%)	4/5 (80%)	
6	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
7	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
8	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
9	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
10	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
11	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
12	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
13	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
14	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
15	negative	positive	negative	negative	positive	5/5 (100%)	5/5 (100%)	
18		negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	15	1	0	16
Number negative	15	1	15	16	0
Percent positive	0	94	6	0	100
Percent negative	100	6	94	100	0
Consensus value	negative	positive	negative	negative	positive
Spiking	negative	positive	negative	negative	positive

Comments on results:

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

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

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
RR-Soja	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
4	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
6	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
7		positive				1/1 (100%)	1/1 (100%)	
10	negative	positive	negative	negative	positive	4/5 (80%)	4/5 (80%)	
11		positive			negative	2/2 (100%)	2/2 (100%)	
12	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
17	negative	positive	negative	negative	negative	5/5 (100%)	5/5 (100%)	
18	negative	negative	negative	negative	negative	4/5 (80%)	4/5 (80%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	8	0	0	1
Number negative	7	1	7	7	7
Percent positive	0	89	0	0	13
Percent negative	100	11	100	100	88
Consensus value	negative	positive	negative	negative	negative
Spiking	negative	positive	negative	negative	negative

Comments on results:

For all 5 samples consensus values with three times 100% and one time each 88% and 89% 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: Lectin-DNA (Soya-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Lectin	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
4	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
5	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
6	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
7	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	
12	positive	positive	positive	positive	positive	2/5 (40%)	2/5 (40%)	
18	negative	positive	positive	negative	negative	5/5 (100%)	5/5 (100%)	

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

Comments on results:

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

4.1.5 Results: GMO-Maize (bt11-Maize)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
bt11 Maize	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
6	negative	negative	negative	negative	positive	5/5 (100%)	5/5 (100%)	
7					positive	1/5 (20%)	1/5 (20%)	
10	negative	positive	negative	negative	positive	4/5 (80%)	4/5 (80%)	
11		negative			positive	2/2 (100%)	2/2 (100%)	
12	negative	negative	negative	negative	positive	5/5 (100%)	5/5 (100%)	
18	negative	negative	negative	negative	positive	5/5 (100%)	5/5 (100%)	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number positive	0	1	0	0	6
Number negative	4	4	4	4	0
Percent positive	0	20	0	0	100
Percent negative	100	80	100	100	0
Consensus value	negative	negative	negative	negative	positive
Spiking	negative	negative	negative	negative	positive

Comments on results:

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

4.1.6 Results: Maize-DNA (Maize-specific)

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Qualitative Valuation	Qualitative Valuation	Remarks
Mais	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples	
1	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	
4	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	
6	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	
7	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	
12	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	
18	negative	negative	positive	positive	positive	5/5 (100%)	5/5 (100%)	

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

Comments on results:

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

4.1.7 Results: Other Parameters (DNA)

Evaluation number	Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Remarks
	other DNA	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	
1	RR2 - Soya (MON89788)	negative	positive	negative	negative	negative	
3	FMV	negative	positive	negative	negative	negative	
4	FMV	negative	positive	negative	negative	negative	
5	Chloroplasts DNA	positive	positive	positive	positive	positive	
7	cry1Ab/Ac	negative	negative	negative	negative	positive	
8	FMV	negative	positive	negative	negative	negative	
9	CTP2-CP4 EPSPS	negative	positive	negative	negative	positive	
10a	FMV	negative	negative	negative	negative	negative	
10b	bar gene	negative	negative	negative	negative	negative	
11a	CTP2-CP4 EPSPS	negative	positive	negative	negative	positive	
11b	RR-Soya (GTS 40-3-2)		positive			negative	
11c	RR2 - Soya (MON89788)		positive			negative	
14	Plant-DNA	positive	positive	positive	positive	positive	
16	35S/NOS Screening	negative	positive	positive	positive	positive	

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	Limit of Detection	Remarks to DNA-Extraction
35S	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	84 bp	according to ASU § 64 L00.00-118 (Primersequence, PCR-Preparation, PCR-Program)		Wizard-Kit from Promega
2		food proof GMO Screening kit / Biotecon	10 genome equivalents / RU	food proof-sample prepkit III
3	35S	R-Biopharm		R-Biopharm, SureFood PREP Advanced, according to manual
4	Target-sequence	Sure Food GMO Screen 4-plex	5 copies according to manual	SureFood® PREP Basic
5		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.		QIAamp® DNA Stool Mini Kit (Qiagen)
6		Internal method	5 copies	Macherey Nagel - nucleobond
7	82 bp-Fragment from P35S-sequence	L00.00-122, 2008-06, modified	10 copies/PCR	according to Swiss Food Methods SLMB, Chapter 52B, May 1998: Extraction w with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
8		r-biopharm / SureFood GMO Screen 4plex, S 2126	< 5 copies	r-biopharm / SureFood PREP Advanced, S 1053, Protocol 2
9		in-house method		CTAB
10	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
11		Gen-ial GmbH	10 copies	Genomic DNA from food (M+N)
12		GEN-IAL		FFS-Kit, Promega
13		GEN-IAL		
14		ASU L00.00.31		modified CTAB-method with clean-up
15		Biotecon		Biotecon foodproof Sample Preparation Kit S 400 061; Biotecon foodproof GMO Screening Kit R 302 17
18				

5.1.2 NOS-Screening Sequence

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
NOS	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	82 bp	according to ASU § 64 L00.00-118 (Primersequence, PCR-Preparation, PCR-Program)		Wizard-Kit from Promega
2				
3	NOS	R-Biopharm		R-Biopharm, SureFood PREP Advanced, according to manual
4	Target-sequence	Sure Food GMO Screen 4-plex	5 copies according to manual	SureFood® PREP Basic
5		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.		QIAamp® DNA Stool Mini Kit (Qiagen)
6		Internal method	5 copies	Macherey Nagel - nucleobond
7	84 bp-Fragment from T-nos-sequence	L00.00-122, 2008-06, modified	10 copies/PCR	according to Swiss Food Methods SLMB, Chapter 52B, May 1998: Extraction w with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
8		r-biopharm / SureFood GMO Screen 4plex, S 2126	< 5 copies	r-biopharm / SureFood PREP Advanced, S 1053, Protocol 2
9		in-house method		CTAB
10	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
11		Gen-ial GmbH	10 copies	Genomic DNA from food (M+N)
12		GEN-IAL		FFS-Kit, Promega
13		GEN-IAL		
14		ASU L00.00.31		modified CTAB-method with clean-up
15				
18				

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

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
RR-Soja	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	172 bp	according to ASU § 64 L00.00-118 (Primersequence, PCR-Preparation, PCR-Program)		Wizard-Kit from Promega
4	Target-sequence	SureFoodGMO QUANT RoundUp Ready Soya	5 copies according to manual	SureFood® PREP Basic
6		Internal method	5 copies	Macherey Nagel - nucleobond
7	84 bp Fragment of transition from construct to 5'-flanking region of plant genome	EURL-GMFF Soybean Line 40-3-2, CRLVL08/05VP, 2009-01, modified	10 copies/PCR	Mericon Food Kit (Qiagen)
10	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
11				
12		GEN-IAL		FFS-Kit, Promega
17	Target DNA		0	
18				

5.1.4 Lectin-DNA (Soya-specific)

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
Lectin	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	118 bp	according to ASU § 64 L00.00-118 (Primersequence, PCR-Preparation, PCR-Program)		Wizard-Kit from Promega
4	Target-sequence	SureFoodGMO QUANT RoundUp Ready Soy	5 copies according to manual	SureFood® PREP Basic
5		Vodkin et al., 1983; Cell: 34, 1023-1031		QIAamp® DNA Stool Mini Kit (Qiagen)
6		in-house method	5 copies	Macherey Nagel - nucleobond
7	74 bp-Fragment from soya specific lectin 1-sequence	EURL-GMFF Soybean Line 40-3-2, CRLVL08/05VP, 2009-01, modified	10 copies/PCR	Mericon Food Kit (Qiagen)
12		GEN-IAL		FFS-Kit, Promega
18				

5.1.5 GMO-Maize (bt11-Maize)

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
bt11 Maize	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
6		in-house method	5 copies	Macherey Nagel - nucleobond
7	68 bp-Fragment Fragment of transition from construct to 5'-flanking region of plant genome	EURL-GMFF Bt11 Mais, CRLVL10/07VP, 2008-06, modified	10 copies/PCR	according to Swiss Food Methods SLMB, Chapter 52B, May 1998: Extraction w with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
10	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
11		Gen-ial GmbH	20 copies	Genomic DNA from food (M+N)
12		GEN-IAL		FFS-Kit, Promega
18				

5.1.6 Maize-DNA (Maize-specific)

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
Maize	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	226 bp	according to ASU § 64 L00.00-118 (Primersequence, PCR-Preparation, PCR-Program)		Wizard-Kit der Firma Promega
4	Target-Sequence / -DNA	ASU 00.00-105	6 copies	SureFood® PREP Basic
6		in-house method	5 copies	Macherey Nagel - nucleobond
7	79 bp-Fragment from maize-specific high-mobility-group-Protein-Gene (hmg)	L 00.00-105, 2014-02	20 copies/PCR	according to Swiss Food Methods SLMB, Chapter 52B, May 1998: Extraction w with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
12		GEN-IAL		FFS-Kit, Promega
18	Invertase			

5.1.7 Other Parameters (DNA)

Evaluation number	Result given as	Test-Kit or Literature	Limit of Detection	Remarks to DNA-Extraction
	Target-sequence / -DNA	Supplier / Method	Copies / ct-value	e.g. Extraction / Enzymes / Clean-Up / DNA-Quality
1	139 bp	according to Charles Delobel C. Et al. (2013): Event-specific Method for the Quantification of Soybean Line MON 89788 Using Real-time PCR v 1.01 - Validation Report and Validated Method		Wizard-Kit from Promega
3	FMV	R-Biopharm		R-Biopharm, SureFood PREP Advanced, according to manual
4	Target-sequence	Sure Food GMO Screen 4-plex	5 copies according to manual	SureFood® PREP Basic
5		§64 LFGB, 00.00-31, mod./ 15.05-1, mod./ 23.01.22-1, mod.		QIAamp® DNA Stool Mini Kit (Qiagen)
7	74 bp-Fragment from cry1Ab/Ac-DNA-Sequenz	L15.06-3, 2013-08	10 copies/PCR	according to Swiss Food Methods SLMB, Chapter 52B, May 1998: Extraction w with SDS, Guanidine HCl, Proteinase K; Wizard DNA clean-up (Promega)
8		r-biopharm / SureFood GMO Screen 4plex, S 2126	< 5 copies	r-biopharm / SureFood PREP Advanced, S 1053, Protocol 2
9		in-house method		CTAB
10a	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
10b	Target - DNA	Biotecon Diagnostics	50	foodproof GMO Sample Preparation Kit
11a		Gen-ial GmbH	10 copies	Genomic DNA from food (M+N)
11b		Gen-ial GmbH	8 copies	Genomic DNA from food (M+N)
11c		Gen-ial GmbH	2 copies	Genomic DNA from food (M+N)
14		Screening Plant-DNA / in-house method		modified CTAB-method with clean-up
16	35S/NOS Screening	Biotecon	ct35	DNA Extraction Biotecon

5.2 Details by participants to PCR-reaction

5.2.1 35S-Screening Sequence

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
35S	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	Duplex Method; PCR 84 and 82 bp + Gel electrophoresis	01.06.16	
2	5´Nucllease	23.05.16	
3	Real Time PCR, SureFood GVO 4plex, R-Biopharm, according to manual	16.06.16	
4	Real Time PCR, 45 cycles		
5	Gel electrophoresis/45 Cycles/ 123 bp/ Bt11-Maize, RR-Soya		testing in double determination, sample 1 and 2 initally undiluted show ed inhibition, successful amplification of Spike-DNA at sample dilution 1:100
6	real time PCR	June 8, 2016	
7	Real-time PCR	11.05 / 10.06	
8	Real time PCR	10.05.	
9	Real-Time PCR	27.05.16	
10	foodproof GMO Screenig Kit	20.05.2016	
11	real-time PCR, 45 Zyklen	May	
12	Real Time PCR	25.5.	
13	Real Time PCR	10.05.16	
14	Gel electrophoresis		
15	Real Time PCR	17.05.16	
18			

5.2.2 NOS-Screening Sequence

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
NOS	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	Duplex Method; PCR 84 and 82 bp + Gel electrophoresis	01.06.16	
2			
3	Real Time PCR, SureFood GVO 4plex, R-Biopharm, according to manual	16.06.16	
4	Real Time PCR, 45 cycles		
5	Gel electrophoresis/45 Cycles/ 180 bp/ Bt11-Maize, RR-Soya		testing in double determination, sample 1 and 2 initally undiluted show ed inhibition, successful amplification of Spike-DNA at sample dilution 1:100
6	real time PCR	June 8, 2016	
7	Real-time PCR	11.05 / 10.06	
8	Real time PCR	10.05.	
9	Real-Time PCR	27.05.16	
10	foodproof GMO Screenig Kit	20.05.2016	
11	real-time PCR, 45 cycles	May	
12	Real Time PCR	25.5.	
13	Real Time PCR	10.05.16	
14	Gel electrophoresis		
15			
18			

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

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
RR-Soya	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	PCR 172 bp + Gel elektrophoresis; MON-4032	16.06.16	
4	Real Time PCR, 45 cycles		
6	real time PCR	June 8, 2016	
7	Real-time PCR	14,06	
10	foodproof GMO Screenig Kit	20.05.2016	
11		May	
12	Real Time PCR	25.5.	
17	real time PCR	18.05.16	
18			

5.2.4 Lectin-DNA (Soya-specific)

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
Lectin	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	PCR 118 bp + Gel electrophoresis; Soya-Lectin Gene	16.06.16	
4	Real Time PCR, 45 cycles		
5	Gel electrophoresis/45 Cycles/ 438 bp/ RR-Soya		testing in double determination, sample 1 and 2 initally undiluted show ed inhibition, successful amplification of Spike-DNA at sample dilution 1:100
6	real time PCR	June 8, 2016	
7	Real-time PCR	10.06.	
12	Real Time PCR	25.5.	
18			

5.2.5 GMO-Maize (bt11-Maize)

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
bt11 Maize	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
6	real time PCR	June 13, 2016	
7	Real-time PCR	12.05.2016, 18.05.2016	
10	foodproof GMO Screenig Kit	20.05.2016	
11	real-time PCR, 45 cycles	May	
12	Real Time PCR	25.5.	
18			

5.2.6 Maize-DNA (Maize-specific)

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
Maize	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	PCR 226 bp + Gel electrophoresis; Maize-Invertase-Gene	16.06.16	
4	Real Time PCR, 50 cycles		
6	real time PCR	June 13, 2016	
7	Real-time PCR	11.05.2016, 18.05.2016	
12	Real Time PCR	25.5.	
18			

5.2.7 Other Parameters (DNA)

Evaluation number	Notes to PCR-Reaction	Date of Analysis	Further Remarks
	e.g. Real Time PCR / Gel electrophoresis / Cycles / Lenght of Amplificates / Reference material	day / month	
1	PCR 139 bp + Gel electrophoresis; MON-89788 (GMO-Soya)	16.06.16	
3	Real Time PCR, SureFood GVO 4plex, R-Biopharm, according to manual	16.06.2016	
4	Real Time PCR, 45 cycles		
5	Gel elektrophoresis/45 Cycles/ 200-600 bp/ Bt11-Maize, Bt176, RR-Soya		testing in double determination, sample 1 and 2 initally undiluted showed inhibition, successful amplification of Spike-DNA at sample dilution 1:100
7	Real-time PCR	12.05.2016, 18.05.2016	
8	Real time PCR	10.05.	
9	Real-Time PCR	27.05.2016	
10a	foodproof GMO Screenig Kit	20.05.2016	FMV
10b	foodproof GMO Screenig Kit	20.05.2016	bar gene
11a	real-time PCR, 45 cycles	May	
11b	real-time PCR, 45 cycles	May	
11c	real-time PCR, 45 cycles	May	
14	Gel electrophoresis		
16	Real Time Screening Kit Biotecon 35 S/NOS	24.05.2016	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 34-2016 Sample 1

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	24,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,11	112	24,6
2	8,99	114	25,4
3	9,89	129	26,1
4	9,71	121	24,9
5	9,35	108	23,1
6	9,58	120	25,1
7	9,65	118	24,5
8	9,39	127	27,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	119	Particles
Standard deviation	5,52	Particles
χ^2 (CHI-Quadrat)	1,80	
Probability	**97**	%
Recovery rate	102	%

Normal distribution		
Number of samples	8	
Mean	25,1	mg/kg
Standard deviation	1,17	mg/kg
rel. Standard deviaton	4,7	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,47**	
Recovery rate	102	%

Microtracer Homogeneity Test
DLA 34-2016 Sample 2

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	12,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,33	52	11,1
2	8,60	57	13,3
3	9,47	59	12,5
4	9,48	66	13,9
5	9,15	52	11,4
6	9,03	56	12,4
7	9,45	68	14,4
8	8,86	52	11,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	57,7	Particles
Standard deviation	5,43	Particles
χ^2 (CHI-Quadrat)	3,58	
Probability	**83**	%
Recovery rate	101	%

Normal distribution		
Number of samples	8	
Mean	12,6	mg/kg
Standard deviation	1,18	mg/kg
rel. Standard deviaton	9,41	%
Horwitz standard deviation	10,9	%
HorRat-value	**0,86**	
Recovery rate	101	%

Microtracer Homogeneity Test
DLA 34-2016 Sample 3

Weight whole sample	1,03	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	16,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	10,09	65	12,9
2	9,03	59	13,1
3	9,28	65	14,0
4	9,00	64	14,2
5	8,50	65	15,3
6	9,47	72	15,2
7	10,25	74	14,4
8	10,21	70	13,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	66,8	Particles
Standard deviation	4,19	Particles
χ^2 (CHI-Quadrat)	1,84	
Probability	**97**	%
Recovery rate	86	%

Normal distribution		
Number of samples	8	
Mean	14,1	mg/kg
Standard deviation	0,88	mg/kg
rel. Standard deviaton	6,27	%
Horwitz standard deviation	10,7	%
HorRat-value	**0,58**	
Recovery rate	86	%

Microtracer Homogeneity Test
DLA 34-2016 Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,63	101	21,0
2	9,11	91	20,0
3	8,55	83	19,4
4	9,10	101	22,2
5	9,05	96	21,2
6	9,49	104	21,9
7	9,09	97	21,3
8	9,08	99	21,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	96,4	Particles
Standard deviation	4,42	Particles
χ^2 (CHI-Quadrat)	1,42	
Probability	**98**	%
Recovery rate	95	%

Normal distribution		
Number of samples	8	
Mean	21,1	mg/kg
Standard deviation	0,97	mg/kg
rel. Standard deviaton	4,59	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,45**	
Recovery rate	95	%

Microtracer Homogeneity Test
DLA 34-2016 Sample 5

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	10,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,49	51	10,7
2	9,43	48	10,2
3	8,93	47	10,5
4	9,79	41	8,4
5	9,86	47	9,5
6	9,20	53	11,5
7	10,03	57	11,4
8	9,36	42	9,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	48,3	Particles
Standard deviation	5,32	Particles
χ^2 (CHI-Quadrat)	4,11	
Probability	**77**	%
Recovery rate	93	%

Normal distribution		
Number of samples	8	
Mean	10,2	mg/kg
Standard deviation	1,12	mg/kg
rel. Standard deviaton	11,02	%
Horwitz standard deviation	11,3	%
HorRat-value	**0,98**	
Recovery rate	93	%

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		Germany
		Germany
		FRANCE
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		NETHERLANDS
		Germany
		GREAT BRITAIN

[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

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Anan-lytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. European Network of GMO Laboratories, Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing, Version 25-01-2005
17. Powell J, Owen L, Reliability of food measurements: the application of proficiency testing to GMO analysis, Accred Qual. Assur. 7, 392-402 (2002)
18. Thompson M, GMO Proficiency testing: Interpreting z-scores derived from log-transformed data, amc technical brief, No. 18 Dec 2004
19. Thompson M et al., Scoring in Genetically Modified Organism Proficiency Tests Based on Log-Transformed Results, J. AOAC Int., 89(1), 232-239 (2006)
20. Žel J et al., Calculation of Measurement Uncertainty in Quantitative Analysis of Genetically Modified Organisms Using Intermediate Precision - A Practical Approach, J. AOAC Int., 90(2), 582-586 (2007)