Proficiency Tests

DLA

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Evaluation Report
proficiency test

DLA 13/2018

Allergen-Screening III:

Cereals containing Gluten, Peanut, Lupine, Celery and Sesame

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1st Correction 25/02/2019:
In the tables of the PCR results a before missing set of results (participant no. 12) was added. The qualitative valuation with respect to the consensus values was affected in some cases.

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

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EP-Nummer *PT-Number*	DLA 13/2018
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (25 February 2019) 1. Korrektur / 1st Correction Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 25 February 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Inhalt / Content

1. Introduction

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 5-10% of the allergenic ingredients concerned.
The respective raw materials were common in commerce cereal flakes, flours, nut butter, dried plant parts and seeds as well as fresh celery root, from which DLA produced allergen premixes (s. Tab. 2). If required the raw materials were crushed, dried, ground with the addition of carrier substances and sieved (mesh 400 µm) or sieved by means of a centrifugal mill (mesh 250 µm or 500 µm).
The composition of the basic matrix of PT samples 1-4 and of the allergen-premixes is given in table 1. The premixes were used for spiking of the PT-samples 1 to 4 (see Tab. 2).
After homogenisation the samples were portioned to approximately 20 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Samples 1 - 4**
Potato powder (Ingredients: Potatoes, E471, E304, E223, E100)	74 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (88% - 93%) - Sodium sulfate (0,0% - 5,5%) - Silicon dioxide (2,0% - 4,1%) - Allergens (5,0% - 10% each)	0,10 - 0,50 %

Table 2: Added amounts of allergenic ingredients positive in mg/kg ranges** given as food item

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Oat: Oat flakes, ground(Protein 12%)	positive (50 - 150)	negative	negative	negative
Rye: Rye flour Type 1150 (Protein 9,1%)	negative	negative	negative	positive (50 - 150)
Wheat: Wheat flour Type 550 (Protein 10,5%)	negative	positive (25 - 75)	negative	negative
Peanut: commercial peanut butter (Protein 30%)	negative	positive (50 - 150)	positive (25 - 75)	negative
Lupine: Sweet lupine flour, (Protein 37%)	positive (75 - 225)	negative	negative	positive (50 - 150)
Celery: Leafs, dried (Protein 14%)	negative	positive (75 - 225)	negative	negative
Celery: Roots, dried (Protein 8,2%)	negative	negative	positive (50 - 150)	negative
Celery: Seeds, dried (Protein 20%)	negative	negative	negative	positive (50 - 150)
Sesame: Seeds black, dried (Protein 22%)	positive (50 - 150)	negative	negative	negative
Sesame: Seeds white, dried (Protein 23%)	negative	negative	positive (50 - 150)	negative

* Protein contents according to laboratory analysis (total nitrogen, Kjeldahl general factor F=6,25)
**Allergen contents of „food item" as indicated in the column of ingredients according gravimetric mixing
Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

The detectability or absence of the allergens was tested by DLA using lateral flow assays. The results are in agreement with the spiking of the PT samples 1-4 (see Table 3).

Table 3: Verification of detectability of the added allergens by lateral flow assays (AgraStrip® LFD, Romer Labs®)

Lateral Flow Device (LFD)*	Sample 1	Sample 2	Sample 3	Sample 4
AgraStrip® Gluten G12	negative	positive	negative	positive
AgraStrip® Gluten	negative	positive	negative	positive
AgraStrip® Peanut	negative	positive	positive	negative
AgraStrip® Lupin	positive	negative	negative	positive
AgraStrip® Sesame	positive	negative	positiv	negative

* Nachweisgrenze (NWG) jeweils 1-10 mg/kg / Limit of detection (LOD) 1-10 mg/kg each

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 1-4 showed probabilities of 79%, 74%, 89% and 70%, 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 0,8, 0,9, 0,7 and 1,2 respectively. The HorRat value of sample 3 was slightly increased, while the probability was well > 25%. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,30 (23,3°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 4) were sent to every participating laboratory in the 44th week of 2018. The testing method was optional. The tests should be finished at December 14th 2018 the latest.

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

There are 4 different samples possibly containing the allergenic ingredients Gluten (Wheat, Rye and Oat), Peanut, Lupine, Celery (Leaves / Stem, Root and Seed) and/or Sesame (white and black) in a simple carrier matrix The evaluation of results is strictly qualitative (positive / negative).

The following analysis methods can be used:

a) ELISA and Lateral Flow
b) PCR

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 14 participants submitted at least one result in time.

3. Evaluation

Different ELISA- and PCR-methods for the determination of allergens in foods are eventually using different antibodies and target-DNA, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different valuation of the presence and/or content of the analyte [25, 26, 27, 28]. Furthermore matrix- and/or processing of samples can have strong impact on the detectability of allergens by ELISA and PCR methods.

Therefore in the present PT the allergenic ingredients were provided for analysis in a simple matrix without further processing.

3.1 Agreement with consensus values from participants

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

3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**.
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 qualitative evaluation is carried out for each parameter for ELISA and PCR methods separately. Results of lateral flow methods were valuated together with ELISA methods, because they are usually based on antibody detection.

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Gluten Containing Cereals

4.1.1 ELISA-Results: Gluten

Qualitative valuation of results

Evaluation number	Sample 1 (oat)	Sample 2 (wheat)	Sample 3 (without)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	positive	positive	negative	2/4 (50%)	1/4 (25%)	BF	
5	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	EF-R5	
4	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	IL	
14	positive	positive	negative	positive	3/4 (75%)	4/4 (100%)	IL	
2	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	
5	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	
6	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	
8	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	
9	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	
10	negative	positive	positive	positive	3/4 (75%)	2/4 (50%)	RS	
13	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	RS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	11	2	10
Number negative	10	0	9	1
Percent positive	9	100	18	91
Percent negative	91	0	82	9
Consensus value	negative	positive	negative	positive
Spiking	positive	positive	negative	positive

Methods:

BF = MonoTrace ELISA, BioFront Technologies

EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins

IL = Immunolab

RS = Ridascreen®, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples.

For sample 1 spiked with oat only one positive result was obtained. According to the test kit specifications or instructions, no (methods EF-R5, IL, RS) or only weak cross-reactivities (method BF) are indicated for certain varieties of oat. For the methods without (cross-) reactivity to oat, the qualitative valuation in relation to the consensus values (sample 1 "negative") is therefore to be preferred.

4.1.2 PCR-Results: Gluten Containing Cereals

4.1.2.1 PCR-Results: Gluten, in general

Qualitative valuation of results

Evaluation number	Sample 1 (oat)	Sample 2 (wheat)	Sample 3 (without)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
3	positive	positive	positive	positive	3/4 (75%)	3/4 (75%)	FP	Samples 1+3: < 10 mg/kg
7	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
4	negative	positive	negative	positive	3/4 (75%)	3/4 (75%)	div	
12	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

FP = foodproof Detection Kit, BIOTECON Diagnostics

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The positive results for sample 2 and 4 are in qualitative agreement with the spiking of samples. For sample 1 (with oat) two positive results and one negative result were obtained.

For the none spiked sample 3, a positive result was obtained.

4.1.2.2 PCR-Results: Oat

Qualitative valuation of results

Evaluation number	Sample 1 (oat)	Sample 2 (wheat)	Sample 3 (without)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

SFA = Sure Food Allergen, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The results are in qualitative agreement with the spiking of samples.

4.1.2.3 PCR-Results: Wheat

Qualitative valuation of results

Evaluation number	Sample 1 (oat)	Sample 2 (wheat)	Sample 3 (without)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	negative	negative	positive	positive	-	1/4 (25%)	div	
12	negative	positive	negative	positive	-	3/4 (75%)	div	specific for w heat and rye

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	1	1	2
Number negative	2	1	1	0
Percent positive	0	50	50	100
Percent negative	100	50	50	0
Consensus value	-	-	-	-
Spiking	negative	positive	negative	negative

Methods:

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The results are partly not in agreement with the spiking of samples. The specifity of the methods as indicated by the participant no. 12 has to be considered.

4.1.2.4 PCR-Results: Rye

Qualitative valuation of results

Evaluation number	Sample 1 (oat)	Sample 2 (wheat)	Sample 3 (without)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	positive	positive	negative	negative	-	1/4 (25%)	div	
12	negative	negative	negative	negative	-	3/4 (75%)	div	specific for w heat and rye

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	1	0	0
Number negative	1	1	2	2
Percent positive	50	50	0	0
Percent negative	50	50	100	100
Consensus value	-	-	-	-
Spiking	negative	negative	negative	positive

Methods:

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The results are not in agreement with the spiking of samples.

4.2 Proficiency Test Peanut

4.2.1 ELISA-Results: Peanut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
1	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BF	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	

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

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of the results are in qualitative agreement with the spiking of samples.

4.2.2 PCR-Results: Peanut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	FP	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	
7	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	div	
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

FP = foodproof Detection Kit, BIOTECON Diagnostics

GI = GEN-IAL First Allergen

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples.

4.3 Proficiency Test Lupine

4.3.1 ELISA-Results: Lupine

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	EF	
3	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
14	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
2	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples.

4.3.2 PCR-Results: Lupine

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	div	no positive sample detected
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples.

4.4 Proficiency Test Celery

4.4.1 ELISA-Results: Celery

Comments:
None of the participants used the ELISA method for determination of celery.

4.4.2 PCR-Results: Celery

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (leaves)	Sample 3 (root)	Sample 4 (seed)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	negative	positive	negative	positive	3/3 (100%)	3/4 (75%)	ASU	
5	negative	positive	positive	positive	3/3 (100%)	4/4 (100%)	ASU	
12	negative	positive	negative	positive	3/3 (100%)	3/4 (75%)	ASU	
3	negative	positive	positive	positive	3/3 (100%)	4/4 (100%)	FP	
7	negative	positive	-	positive	3/3 (100%)	3/3 (100%)	SFA-ID	
2	negative	positive	positive	positive	3/3 (100%)	4/4 (100%)	SFA-Q	
8	negative	positive	negative	positive	3/3 (100%)	3/4 (75%)	SFA-Q	
9	negative	positive	positive	positive	3/3 (100%)	4/4 (100%)	div	
10	negative	negative	positive	positive	2/3 (75%)	3/4 (75%)	div	
11	negative	positive	positive	positive	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	9	6	10
Number negative	10	1	3	0
Percent positive	0	90	67	100
Percent negative	100	10	33	0
Consensus value	negative	positive	-	positive
Spiking	negative	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method
FP = foodproof Detection Kit, BIOTECON Diagnostics
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
For samples 1, 2 and 4 the consensus values of results are in qualitative agreement with the spiking of samples. For sample 3 (celery root) there are 6 positive and 3 negative results.

4.5 Proficiency Test Sesame

4.5.1 ELISA-Results: Sesame

Qualitative valuation of results

Evaluation number	Sample 1 (white)	Sample 2	Sample 3 (black)	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BC	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	EF	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	
3	negative	negative	positive	negative	3/4 (75%)	3/4 (75%)	IL	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	

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

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. None of the participants differentiated between black and white sesame.

4.5.2 PCR-Results: Sesame

Qualitative valuation of results

Evaluation number	Sample 1 (white)	Sample 2	Sample 3 (black)	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	positive	negative	2/4 (50%)	2/4 (50%)	div	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	no differentiation of w hite and black sesame

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

Methods:

ASU = ASU §64 Methode/method

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. None of the participants differentiated between black and white sesame.

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Gluten

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	1	13/12	negative	positive	positive	negative	0,36	Gluten	BF = MonoTrace ELISA, BioFront Technologies
EF-R5	5		negative	positive	negative	po	3,12	Gluten	EF-R5 = SensiSpec Ingezim Gluten R5, Eurofins
IL	4	12.12.	negative	positive	negative	positive	4	Gluten	IL = Immunolab
IL	14	06.11.18	positive	positive	negative	positive	0.3	Gliadin	IL = Immunolab
RS	2	23.11.18	negative	positive	negative	positive	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	5	13.11.	negative	positive	negative	positive	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	6	01.11.18	negative	positive	negative	positive	1 PPM	Gliadin	Gliadin R-Biopharm
RS	8		negative	positive	negative	positive	3	Please select!	RS = Ridascreen®, R-Biopharm
RS	9	06.11.18	NEG	POS	NEG	POS	3	protein	R-Biopharm
RS	10	21.11.18	negative	positive	positive	positive	0,5	Protein	RIDASCREEN® Gliadin Test Kit
RS	13	09.11.	negative	positive	negative	positive	0,5	Gluten	RS = Ridascreen®, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
BF	1				
EF-R5	5	30.GLU.K2	R5 Mendez (detects prolamins from wheat, rye and barley)	As per kit instructions	
IL	4	GLU-E02			
IL	14				
RS	2	R7001	As per kit instructions	As per kit instructions	
RS	5	R7001	R5 Mendez (detects prolamins from wheat, rye and barley)	As per kit instructions	
RS	6				
RS	8				
RS	9	R7001			
RS	10	R7001			
RS	13	R7001	As per kit instructions	As per kit instructions	Sample 1: <5,0 mg/kg; Sample 2: 57,6 mg/kg; Sample 3: <5,0 mg/kg; Sample 4: >80 mg/kg

5.1.2 ELISA: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	6	01.11.18	negative	positive	positive	negative	0.1 PPM	Protein	AgraQuant Peanut Romer Labs
BF	1	13/12	negative	positive	positive	negative	0,24	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	4	12.12.	negative	positive	positive	negative	1	Peanut	IL = Immunolab
IL	14	06.11.18	negative	positive	positive	negative	0.1	Food item, total	IL = Immunolab
MI-II	5	9.11.	negative	positive	positive	negative	0,12	Peanut protein	MI-II = Morinaga Institute ELISA Kit II
RS	3	09.11.18	negative	positive	positive	negative	0,4	peanut	RS = Ridascreen®, R-Biopharm
RS-F	8		negative	positive	positive	negative	1,5	Please select!	RS-F= Ridascreen® Fast, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	6				
BF	1				
IL	4	PEA-E01			
IL	14				
MI-II	5	M2116	detects peanut proteins	As per kit instructions	
RS	3	R6202	anti-Arah1, anti-arah2		sample 3=360 ppm, sample 2=780 ppm
RS-F	8				

5.1.3 ELISA: Lupine

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	1	13/12	positive	negative	negative	positive	0,13	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
EF	5	14.11.	positive	negative	negative	poditive	1,5	Food item, total	EF = SensiSpec ELISA Kit, Eurofins
IL	3	11.12.18	positive	negative	negative	positive	0,4	lupine	IL = Immunolab
IL	14	06.11.18	positive	negative	negative	positive	0.2	Food item, total	IL = Immunolab
RS-F	2	23.11.18	positive	negative	negative	positive	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	8		positive	negative	negative	positive	0,7	Please select!	RS-F= Ridascreen® Fast, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
BF	1				
EF	5	HU0030011	detects lupin protein	As per kit instructions	
IL	3	LUP-E01		1g sample+20 ml extraction reagent/heating 60oC/15 min/ centrifuge 10 min/ incubate plate 20 min/ wash/ conjugate/incubate 20 min/ wash/ substrate/ 20 min/ stop/ read 450 nm	sample 1, 4 > 30 ppm
IL	14				
RS-F	2	R6102	As per kit instructions	As per kit instructions	
RS-F	8				

5.1.4 ELISA: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	6	01.11.18	positive	negative	positive	negative	0.2 PPM	Protein	AgraQuant Sesame Romer Labs
BC	2	23.11.18	positive	negative	positive	negative	2	Food item, total	BC = BioCheck ELISA
BF	1	14/12	positive	negative	positive	negative	0,3	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
EF	5	12.11.	positive	negative	positive	negative	1,5	Food item, total	EF = SensiSpec ELISA Kit, Eurofins
ES	8		positive	negative	positive	negative	0,125	Please select!	ES = ELISA-Systems
IL	3	10.12.18	negative	negative	positive	negative	0,4	sesame	IL = Immunolab
IL	14	06.11.18	positive	negative	positive	negative	0.2	Food item, total	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
AQ	6				
BC	2	R6029	As per kit instructions	As per kit instructions	
BF	1				
EF	5	HU0030022	detects sesame protein	As per kit instructions	
ES	8				
IL	3	SES -E01		1g sample+20 ml extraction reagent/heating 60oC/15 min/ centrifuge 10 min/ incubate plate 20 min/ wash/ conjugate/incubate 20 min/ wash/ substrate/ 20 min/ stop/ read 450 nm	sample 3 > 60 ppm
IL	14				

5.1.5 PCR: Gluten Cereals

5.1.5.1 PCR: Gluten, in general

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
FP	3	06.11.18	positive	positive	positive	positive	0,8	Allergen DNA	foodproof Detection Kit, BIOTECON Diagnostics
SFA-ID	7	16.11.18	positive	positive	negative	positive	0,4	Allergen-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	4	05.12.	negative	positive	negative	positive	10	Allergen-DNA	in house method
div	12		positive	positive	negative	positive			

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
FP	3	R302 64		extraction with foodproof Sample Preparation Kit III (S 400 06.1)/ 50 cycles	sample1 =4,05 ppm, sample 2= 760 ppm, sample3=7,0 ppm, sample 4= 1300 ppm
SFA-ID	7	S3606		Sure Food Prep Advanced Protokoll 1	Detection of gluten-containing cereals (Wheat, Spelt, Khorasan-Wheat, Rye, Barley, Oat)
div	4				
div	12				

5.1.5.2 PCR: Oat

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	7	16.11.18	positive	negative	negative	negative		Allergen-DNA	Sure Food Oat, R-Biopharm / Congen
div	5	8.11.	positive	negative	negative	negative	10	Allergen-DNA	internal method
div	12		positive	negative	negative	negative	50	Food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	7	S7004	Avena sativa	Sure Food Prep Advanced Protocol 1	LOD: 500 DNA copies
div	5		Oat-DNA	CTAB / Proteinase K / Promega Wizard DNA-CleanUp / PCR /45 Cycles	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	

5.1.5.3 PCR: Wheat

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	10	21.11.18	negative	negative	positive	positive		food item	in house
div	12		negative	positive	negative	positive		food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
div	10			Wizard	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	specific for wheat and rye

5.1.5.4 PCR: Rye

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	10	21.11.18	positive	positive	negative	negative		food item	in house
div	12		negative	negative	negative	negative	50	Food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
div	10			Wizard	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	specific for wheat and rye

5.1.6 PCR: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
FP	3	07.11.18	negative	positive	positive	negative	0,8	Allergen DNA	foodproof Detection Kit, BIOTECON Diagnostics
GI	4	06.12.	negative	positive	positive	negative	10	Allergen-DNA	GI = GEN-IAL First Allergen, Coring System Diagnostix
SFA-ID	7	16.11.18	negative	positive	positive	negative	0,4	Allergen-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	2	30.11.18	negative	positive	positive	negative	1	Food item, total	Sure Food Allergen Quant, R-Biopharm / Congen
SFA-Q	8		negative	positive	positive	negative	0,4	Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	5	8.11.	negative	positive	positive	negative	10	Please select!	internal method
div	9	08.11.18	NEG	POS	POS	NEG		Please select!	in-house method
div	10	21.11.18	negative	positive	positive	positive		food item	in house
div	11	20.11.18	negative	positive	positive	negative		Allergen-DNA	internal method pmPES
div	12		negative	positive	positive	negative	50	Food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
FP	3	R302 63		extraction with foodproof Sample Preparation Kit III (S 400 06.1)/ 50cycles	sample2=460 ppm, sample 3= 205 ppm
GI	4	PPEA 0050			
SFA-ID	7	S3603	Arachis hypogae	Sure Food Prep Advanced Protokoll 1	
SFA-Q	2	S3603	As per kit instructions	As per kit instructions	
SFA-Q	8				
div	5		Peanut DNA		
div	9				
div	10			Wizard	
div	11			in-house method	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	

5.1.7 PCR: Lupine

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	05.12.	positive	negative	negative	positive	10	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	5	8.11.	positive	negative	negative	positive	1	Allergen-DNA	ASU = ASU §64 Methode/method
SFA-ID	7	16.11.18	positive	negative	negative	positive	0,4	Allergen-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	8		positive	negative	negative	positive	0,4	Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	9	07.11.18	POS	NEG	NEG	POS		Please select!	in-house method
div	10	21.11.18	negative	negative	negative	negative		food item	in house
div	11	20.11.18	positive	negative	negative	positive		Allergen-DNA	internal method pmLupinelTS
div	12		positive	negative	negative	positive	50	Food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	ASU L 18.00-58 (V)			
ASU	5	L 08.00-58:2011-06	Lupin DNA	CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Real-time PCR /45 Cycles	
SFA-ID	7	S3611	Lupinus	Sure Food Prep Advanced Protocol 1	
SFA-Q	8				
div	9				
div	10			Wizard	
div	11			in-house method	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	

5.1.8 PCR: Celery

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	05.12.	negative	positive	negative	positive	4	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	5	8.11.	negative	positive	positive	positive	4	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	12		negative	positive	negative	positive	50	Food item	ASU = ASU §64 Methode/method
FP	3	09.11.18	negative	positive	positive	positive	0,1	Allergen DNA	foodproof Detection Kit, BIOTECON Diagnostics
SFA-ID	7	16.11.18	negative	positive	-	positive	0,4	Allergen-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	2	30.11.18	negative	positive	positive	positive	1	Food item, total	Sure Food Allergen Quant, R-Biopharm / Congen
SFA-Q	8		negative	positive	negative	positive	0,4	Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	9	07.11.18	NEG	POS	POS	POS		Please select!	in-house method
div	10	21.11.18	negative	negative	positive	positive		food item	in house
div	11	20.11.18	negative	positive	positive	positive		Allergen-DNA	in-house method pmApiumMat3

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	ASU L 08.00-56			
ASU	5	L 08.00-56:2014-08	celery DNA	CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Real-time PCR /45 Cycles	
ASU	12		part of mannitoldehydrogenase genes	2g sample, silica columns, RealTime-PCR, 45 Cycles	
FP	3	R302 60		extraction with foodproof Sample Preparation Kit III (S 400 06.1) / 50 cycles	sample 1=0,1, sample2 =65 ppm, sample 3 =0,4 ppm, sample 4=7,5 ppm
SFA-ID	7	S3605	Apium graveolens	Sure Food Prep Advanced Protokoll 1	
SFA-Q	2	S3605	As per kit instructions	As per kit instructions	
SFA-Q	8				
div	9				
div	10			Wizard	
div	11			in-house method	

5.1.9 PCR: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		day/moth	pos/neg	pos/neg	pos/neg	pos/neg	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	05.12.	positive	negative	positive	negative	10	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	5	8.11.	positive	negative	positive	negative	5	Allergen-DNA	ASU = ASU §64 Methode/method
SFA-Q	8		positive	negative	positive	negative	0,4	Please select!	Sure Food Allergen Quant, R-Biopharm / Congen
div	9	08.11.18	POS	NEG	POS	NEG		Please select!	in-house method
div	10	21.11.18	negative	positive	positive	negative		food item	in house
div	11	20.11.18	positive	negative	positive	negative		Allergen-DNA	in-house method pmCSN-Hex
div	12		positive	negative	positive	negative	50	Food item	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	ASU L 18.00-19			
ASU	5	L 18.00-19:2014-08	Sesame DNA	CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Real-time PCR /45 Cycles	
SFA-Q	8				
div	9				
div	10			Wizard	
div	11			in-house method	
div	12			2g sample, silica columns, RealTime-PCR, 45 Cycles	no differentiation between wihte and black sesame

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 13-2018 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	39,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	111	44,7
2	5,00	112	44,8
3	5,02	114	45,4
4	5,06	105	41,5
5	5,09	115	45,2
6	5,01	101	40,3
7	5,00	121	48,4
8	5,03	98	39,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	109,6	Particles
Standard deviation	7,83	Particles
χ^2 (CHI-Quadrat)	3,91	
Probability	**79**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	43,7	mg/kg
Standard deviation	3,12	mg/kg
rel. Standard deviaton	7,14	%
Horwitz standard deviation	9,06	%
HorRat-value	**0,79**	
Recovery rate	112	%

Microtracer Homogeneity Test
DLA 13-2018 Sample 2

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	101	40,1
2	5,02	97	38,6
3	5,05	92	36,4
4	5,03	84	33,4
5	5,00	93	37,2
6	5,06	102	40,3
7	5,06	90	35,6
8	5,00	80	32,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	92,4	Particles
Standard deviation	7,54	Particles
χ^2 (CHI-Quadrat)	4,31	
Probability	**74**	%
Recovery rate	171	%

Normal distribution		
Number of samples	8	
Mean	36,7	mg/kg
Standard deviation	3,00	mg/kg
rel. Standard deviaton	8,16	%
Horwitz standard deviation	9,30	%
HorRat-value	**0,88**	
Recovery rate	171	%

Microtracer Homogeneity Test
DLA 13-2018 Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	95	38,2
2	5,04	81	32,1
3	4,97	86	34,6
4	5,02	99	39,4
5	5,05	94	37,2
6	5,06	87	34,4
7	5,00	84	33,6
8	5,03	89	35,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	89,4	Particles
Standard deviation	6,17	Particles
χ^2 (CHI-Quadrat)	2,99	
Probability	**89**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	35,6	mg/kg
Standard deviation	2,46	mg/kg
rel. Standard deviaton	6,9	%
Horwitz standard deviation	9,3	%
HorRat-value	**0,7**	
Recovery rate	107	%

Microtracer Homogeneity Test
DLA 13-2018 Sample 4

Weight whole sample	1,010	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	42	16,8
2	5,06	41	16,2
3	4,95	44	17,8
4	4,97	48	19,3
5	5,02	40	15,9
6	5,10	51	20,0
7	5,00	52	20,8
8	5,05	37	14,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	44,4	Particles
Standard deviation	5,45	Particles
χ^2 (CHI-Quadrat)	4,69	
Probability	**70**	%
Recovery rate	77	%

Normal distribution		
Number of samples	8	
Mean	17,7	mg/kg
Standard deviation	2,17	mg/kg
rel. Standard deviaton	12,3	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,2**	
Recovery rate	77	%

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 13-2018***
PT name	**Allergen-Screening III - 4 Samples qualitative: Cereals containing Gluten (Wheat, Rye, Barley and Oat), Peanut, Lupine, Celery (Leaves / Stem, Root and Seed), Sesame (white and black)**
Sample matrix	*Samples 1-4:* *Carrier matrix / ingredients: potato powder (appr. 75%), maltodextrin (appr. 25%), other food additives and allergenic foods*
Number of samples and sample amount	*4 different Samples 1-4: 20 g each*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: **Gluten (Wheat, Rye and Oat), Peanut, Lupine, Celery (Leaves / Stem, Root and Seed) and Sesame (white and black)*** *Samples 1-4: appr. 25 - 250 mg/kg*
Methods of analysis	*The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.*
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 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 14th December 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

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

[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.]

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