Proficiency Tests
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Evaluation Report
proficiency test

DLA 11/2017

Allergen-Screening I:

Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio, Walnut

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

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EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (6 July 2017) 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 (Technischer Leiter / Technical Manager) - *gezeichnet / signed M. Besler* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - *gezeichnet / signed G. Wichmann* Datum / Date: 6 July 2017
Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
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 1-2% of the allergenic ingredients concerned.
The respective raw materials for the nuts used were commercial nut butters and nut butters produced by DLA from commercial nuts (s. Tab. 2). The nuts were crushed, ground into nut butter and afterwards all butters were sieved (mesh 400 µm). From the nut butters thus obtained the allergen-premixes (see Tab. 1) were prepared with other additives and then used for spiking of the PT-sample 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)	72 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (75% - 90%) - Sodium sulfate (6,1% - 14%) - Silicon dioxide (3,5% - 10%) - Nut butters (1,1% - 1,7% each)	0,25 - 0,80 %

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Cashew (Protein 18,4%) - commercial nut butter	positive (50 - 150)	negative	positive (25 - 75)	negative
Hazelnut (Protein 15,9%) - commercial nut butter	negative	positive (50 - 150)	negative	positive (25 - 75)
Macadamia (Protein 9,4%) - Nuts, crushed	negative	negative	positive (25 - 75)	positive (50 - 150)
Almond (Protein 19,6%) - commercial nut butter	positive (25 - 75)	positive (25 - 75)	negative	negative
Brazil nut (Protein 14,8%) - Nuts, crushed	positive (25 - 75)	negative	negative	positive (50 - 150)
Pecan (Protein 12,2%) - Nuts, crushed	negative	negative	positive (25 - 75)	positive (50 - 150)
Pistachio (Protein 25,6%) - Nuts, crushed	positive (25 - 75)	positive (50 - 150)	negative	negative
Walnut (Protein 13,9%) - Nuts, crushed	negative	positive (25 - 75)	negative	positive (50 - 150)

*Protein contents according to laboratory analysis (total nitrogen according to Kjeldahl)
**Allergen contents of „food item" in brackets 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® Almond	positive	positive	negative	negative
AgraStrip® Cashew/Pistachio	positive	positive	positive	slightly positive
AgraStrip® Hazelnut	negative	positive	slightly positive	positive
AgraStrip® Macadamia	negative	negative	positive	positive
AgraStrip® Brazil Nut	positive	slightly positive	slightly positive	positive
AgraStrip® Walnut	negative	positive	**slightly positive**	positive

* Nachweisgrenze jeweils 2-10 mg/kg / Limit of detection (LOD) 2-10 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 22%, 45%, 7% and 89%, respectively. 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 of 1,2, 1,2, 1,4 and 0,77, respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the samples (spoilage) and the content of EP-parameters (allergens) in a comparable matrix and water activity (a_w value <0.5). The stability of sample material is therefore given during the investigation period under consideration of given storage conditions.

2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 11th week of 2017. The testing method was optional. The tests should be finished at April 28th 2017 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 Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and Walnut. The allergens are contained in a simple carrier matrix in the range of mg/kg. 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.

13 out of 14 participants submitted at least one result. One participant submitted no results.

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 [23, 24, 25, 26]. 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 Cashew

4.1.1 ELISA-Results: Cashew

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		
11	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	AQ	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BA	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BC	
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	

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

Methods:

AQ = AgraQuant, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One positive result for sample 2 was submitted, which is probably because of a cross-reactivity of the test method against pistachio.

4.1.2 PCR-Results: Cashew

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		
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	GI	
7	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	SFA-ID	
9	-	-	positive	-	1/1 (100%)	1/1 (100%)	SFA-ID	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

GI = GEN-IAL First Allergen, Coring System Diagnostix

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One positive result each, for sample 2 and sample 4, was submitted. Cross-reactivities to other nuts are not described for the test method.

4.2 Proficiency Test Hazelnut

4.2.1 ELISA-Results: Hazelnut

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		
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ES	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

ES = ELISA-Systems

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

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

4.2.2 PCR-Results: Hazelnut

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	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IC	
9	-	positive	-	positive	2/2 (100%)	2/2 (100%)	SFA-ID	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

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

div = not indicated / other method

Comments:

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

4.3 Proficiency Test Macadamia

4.3.1 ELISA-Results: Macadamia

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	-	-	positive	positive	2/2 (100%)	2/2 (100%)	RS-F	Cross-reactivity to walnut, pecan, almond, hazelnut, cashew
4	negative	positive	positive	positive	4/4 (100%)	3/4 (75%)	RS-F	
10	negative	positive	positive	positive	4/4 (100%)	3/4 (75%)	RS-F	

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

Methods:

ES = ELISA-Systems

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 1, 3 and 4. For sample 2 in contrast to the spiking two positive results were obtained, probably because of cross-reactivities of the test method.

4.3.2 PCR-Results: Macadamia

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		
2	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	
11	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	div	
12	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	0	3	3
Number negative	2	3	0	0
Percent positive	33	0	100	100
Percent negative	67	100	0	0
Consensus value	none	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

div = not indicated / other method

Comments:

Consensus values ≥75% were obtained for sample 2, 3 and 4. In contrast to the spiking one positive result was obtained for sample 1.

4.4 Proficiency Test Almond

4.4.1 ELISA-Results: Almond

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	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

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

4.4.2 PCR-Results: Almond

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	positive	positive	negative	positive	3/4 (75%)	3/4 (75%)	ASU	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	GI	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IC	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	9	9	0	1
Number negative	0	0	9	8
Percent positive	100	100	0	11
Percent negative	0	0	100	89
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

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

div = not indicated / other method

Comments:

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

4.5 Proficiency Test Brazil Nuts

4.5.1 ELISA-Results: Brazil Nuts

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		
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BA	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
10	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	0	0	3
Number negative	0	3	3	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:

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

Comments:

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

4.5.2 PCR-Results: Brazil Nuts

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	positive	negative	negative	negative	3/4 (75%)	3/4 (75%)	ASU	
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
13	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	GI	
9	positive	-	-	positive	2/2 (100%)	2/2 (100%)	SFA-ID	
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
2	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	6
Number negative	0	6	6	1
Percent positive	100	0	0	86
Percent negative	0	100	100	14
Consensus value	positive	negative	negative	positive
Spiking	positive	negative	negative	positive

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen, Coring System Diagnostix

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One negative result was submitted for sample 4.

4.6 Proficiency Test Pecan

4.6.1 ELISA-Results: Pecan

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	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	BF	
10	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	BF	
5	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	ET	Sample 2 cross-reactivity to w alnut

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	2	3	3
Number negative	3	1	0	0
Percent positive	0	67	100	100
Percent negative	100	33	0	0
Consensus value	negative	none	positive	positive
Spiking	negative	negative	positive	positive

Methods:

BF = MonoTrace ELISA, BioFront Technologies

ET = Elution Technologies ELISA Kit

Comments:

Consensus values ≥75% were obtained for sample 1, 3 and 4. In contrast to the spiking two positive results and an indication for a cross-reactivity to walnut for sample 2 were submitted.

4.6.2 PCR-Results: Pecan

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	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	div	No positive sample identified
11	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	div	Walnut and Pecan

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

Methods:

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One positive result was submitted for sample 2, due to a cross-reactivity of the test-method to walnut. One participant identified no positive sample.

4.7 Proficiency Test Pistachio

4.7.1 ELISA-Results: Pistachio

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		
9	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	AQ-P	
8	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	BA	
4	positive	positive	negative	negative	3/3 (100%)	4/4 (100%)	BF	
10	positive	positive	negative	negative	3/3 (100%)	4/4 (100%)	BF	Cross-reactivity to cashew , hazelnut and w alnut

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	4	4	2	0
Number negative	0	0	2	4
Percent positive	100	100	50	0
Percent negative	0	0	50	100
Consensus value	positive	positive	none	negative
Spiking	positive	positive	negative	negative

Methods:

AQ-P = AgraQuant Plus, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 1, 2 and 4. In contrast to the spiking two positive results were obtained for sample 3, probably because of a cross-reactivity of the test methods, mainly to cashew.

4.7.2 PCR-Results: Pistachio

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		
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

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

div = not indicated / other method

Comments:

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

4.8 Proficiency Test Walnut

4.8.1 ELISA-Results: Walnut

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	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BK	
3	negative	positive	-	positive	3/3 (100%)	3/3 (100%)	NL	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	NL	

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

NL = nutriLinia® Allergen-ELISA

Comments:

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

4.8.2 PCR-Results: Walnut

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		
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
9	-	positive	-	positive	2/2 (100%)	2/2 (100%)	SFA-ID	
5	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	div	Walnut and Pecan
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

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

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One positive result was submitted for sample 3, which is because of a cross-reactivity of the test method against pecan.

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: Cashew

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	11	22.03.17	positive (>50)	positive (28)	positive (>50)	negative	2	Nut, total	AgraQuant, RomerLabs
BA	8	21.04.17	positive	negative	positive	negative	1	food	BioAvid, LTF/ r-biopharm
BC	5	18.04.17	positive	negative	positive	negative	2	Nut, total	BioCheck ELISA
BF	4	07.04.17	positive	negative	positive	negative	2	Nut, total	BioFront Technologies
BF	10		positive	negative	positive	negative	2	Nut, total	BioFront MonoTrace Cashew
RS-F	3	31.03.17	positive	negative	positive	negative	0,09	Nut, total	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. Extractionbuffer / Time / Temperature	
AQ	11	COKAL3148	Cashew	As Per Kit Instructions	
BA	8	BL-610	Cashew Nutprotein	Extractionbuffer r-biopharm, Almond-Kit/10 min/60°C	
BC	5	R6046	As Per Kit Instructions	As Per Kit Instructions	
BF	4	CA2-EK			
BF	10	CA2-EK- 96			
RS-F	3	R6872	Cashewprotein	As Per Kit Instructions	Cross-reactivity to pistachio

5.1.2 ELISA: Hazelnut

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ES	11	20.03.17	negative	positive (>4)	negative	positive (2,2)	0,5	Nutprotein	ELISA-Systems, Residue Assay
RS-F	3	30.03.17	negative	positive	negative	positive	1,5	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	6	24.03.	negative	positive	negative	positive	2,5	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	8	21.04.17	negative	positive	negative	positive	1,5	Nut, total	Ridascreen, r-Biopharm
RS-F	10		negative	positive	negative	positive	2,5	Nut, total	Ridascreen Fast, r-Biopharm
VT	4	20.04.17	negative	positive	negative	positive	2,5	Nut, total	Veratox Allergen, Neogen

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. Extractionbuffer / Time / Temperature	
ES	11	ESHRD-48	Hazelnutprotein	As Per Kit Instructions	
RS-F	3	R 6802	Hazelnutprotein	As Per Kit Instructions	
RS-F	6				
RS-F	8	R6802	Hazelnutprotein	Extractionbuffer r-biopharm, Hazelnut/10 min/60°C	
RS-F	10	R6802			
VT	4	8420			

5.1.3 ELISA: Macadamia

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
RS-F	3	23.03.17	-	-	positive	positive	0,38	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	4	25.04.17	negative	positive	positive	positive	1	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	10		negative	positive	positive	positive	1	Nut, total	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. Extractionbuffer / Time / Temperature	
RS-F	3	R6852	Macadamiaproteine	As Per Kit Instructions	Cross-reactivity to walnut, pecan, almond, hazelnut, cashew
RS-F	4	r6852			
RS-F	10	R6852			

5.1.4 ELISA: Almond

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	6	31.03.	positive	positive	negative	negative	0,4	Nut, total	AgraQuant, RomerLabs
RS-F	3	28.03.17	positive	positive	negative	negative	1,2	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	8	21.04.17	positive	positive	negative	negative	1,2	Nut, total	Ridascreen, r-Biopharm
RS-F	10		positive	positive	negative	negative	2,5	Nut, total	Ridascreen Fast, r-Biopharm
RS-F	11	22.03.17	positive (>18)	positive (>18)	negative	negative	2,5	Nut, total	Ridascreen Fast, r-Biopharm
VT	4	21/04/17	positive	positive	negative	negative	2,5	Nut, total	Veratox Allergen, Neogen

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. Extractionbuffer / Time / Temperature	
AQ	6				
RS-F	3	R 6901	Almondprotein	As Per Kit Instructions	
RS-F	8	R6901	Almondprotein	Extractionbuffer r-biopharm, Almond-Kit/10 min/60°C	
RS-F	10	R6901			
RS-F	11	R6901	Almondprotein	As Per Kit Instructions	
VT	4	8440			

5.1.5 ELISA: Brazil Nuts

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BA	8	21.04.17	positive	negative	negative	positive	1	food	BioAvid, LTF/ r-biopharm
BF	4	27/04/17	positive	negative	negative	positive	2	Nut, total	BioFront Technologies
BF	10		positive	negative	negative	positive	2	Nut, total	BioFront Mono Trace Brasil

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. Extractionbuffer / Time / Temperature	
BA	8	BL-602	Brazil Nut protein	Extractionbuffer r-biopharm, Almond-Kit/10 min/60°C	
BF	4	BN-EK			
BF	10	BN-EK- 96			

5.1.6 ELISA: Pecan

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	4	07.04.17	negative	positive	positive	positive	2	Nut, total	BioFront Technologies
BF	10		negative	positive	positive	positive	2	Nut, total	BioFront Mono Trace Pecan
ET	5	18.04.17	negative	negative	positive	positive	0,67	Nut protein	Elution Technologies Kit

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. Extractionbuffer / Time / Temperature	
BF	4	PC4-EK			
BF	10	PC4-EK- 96			
ET	5	E-75PCN	As Per Kit Instructions	As Per Kit Instructions	Sample 2 Cross reacted with Kit due to presence of walnut

5.1.7 ELISA: Pistachio

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ-P	9		positive	positive	positive	negative	1	Nut protein	AgraQuant F.A.S.T., RomerLabs
BA	8	21.04.17	positive	positive	positive	negative	1	food	BioAvid, LTF/ r-biopharm
BF	4	25/04/17	positive	positive	negative	negative	2	Nut, total	BioFront Technologies
BF	10		positive	positive	negative	negative	2	Nut, total	BioFront Mono Trace Pistachio
NL	3	17.03.17	positive	positive	positive	-	0,13	Nut, total	nutriLinia, Transia

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. Extractionbuffer / Time / Temperature	
AQ-P	9				
BA	8	BL-611	Pistachio proteine	Extractionbuffer r-biopharm, Almond-Kit/10 min/60°C	
BF	4	PV1-EK			
BF	10	PV1-EK- 96			
NL	3	NC-6019	Pistachioprotein	As Per Kit Instructions	Cross-reactivity to Cashew 12%, Hazelnut 0,17%,Walnut 0,0008%

5.1.8 ELISA: Walnut

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	4	27/04/17	negative	positive	negative	positive	2	Nut, total	BioFront Technologies
BF	10		negative	positive	negative	positive	2	Nut, total	BioFront Mono Trace Walnut
BK	11	20.03.17	negative	positive	negative	positive (>60)	3	Nut, total	BioKits Assay Kit, Neogen
NL	3	31.03.17	negative	positive	-	positive	0,6	Nut, total	nutriLinia, Transia
NL	8	21.04.17	negative	positive	negative	positive	0,6	Nut, total	nutriLinia E ELISA, Transia

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. Extractionbuffer / Time / Temperature	
BF	4	WJ4-EK			
BF	10	WJ4-EK- 96			
BK	11	902085J	Walnut protein	As Per Kit Instructions	
NL	3	NC-6013	Walnut protein	As Per Kit Instructions	Cross-reacitivity to Pecan 0,85%, Hazelnut 0,022%, Pistachio: 0,0013%, Brazil Nuts 0,0005%
NL	8	NC-613	Jug r1; Jug r2	Extractionbuffer NutriLinia, Walnut-E/15 min/60°C	

5.1.9 PCR: Cashew

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
GI	13	22.03.	positive	negative	positive	negative	2mg/kg	food	First-Cashew/GEN-IAL
SFA-ID	7		positive	positive	positive	positive		Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	9		-	-	positive	-	5 DNA copies	Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		positive	negative	positive	negative		Nut-DNA	Choice PCR-Methods
div	6	27.4.	positive	negative	positive	negative	0,01 ng/µl	Nut-DNA	
div	12		positive	negative	positive	negative	5	Nut, total	Internal 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	
GI	13			First-DNA all tissue Kit/ GEN-IAL	
SFA-ID	7				
SFA-ID	9				
div	1			Real Time PCR	
div	6	A. Ehlert et al. (2008)	ana o 3	as Multiplex together with Pistachio, Peanut, Walnut und Cashew	
div	12		Ana 03	Extraction: kit Food Macherey Nagel	

5.1.10 PCR: Hazelnut

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	6	25.04.	negative	positive	negative	positive	0,01 ng/µl	Nut-DNA	ASU §64 Methode/method
ASU	11	20.03.17	negative	positive	negative	positive	8	Nut-DNA	ASU §64 Methode/method
GI	13	22.03.	negative	positive	negative	positive	10mg/kg	Please choose!	First-Hazelnut/GEN-IAL
IC	3		negative	positive	negative	positive		Please choose!	Food Allergen Detection PCR Kit, real Time PCR, InCura
SFA-ID	9		-	positive	-	positive	5 DNA copies	Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		negative	positive	negative	positive		Nut-DNA	Choice PCR-Methods
div	2		negative	positive	negative	positive		Please choose!	realtime PCR
div	12		negative	positive	negative	positive	5	Nut, total	CEN/TC 275/WG 12 N 317

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	6	ASU L 44.00-8 (mod.)	cor a 1	as Multiplex together with Pistachio, Peanut, Walnut und Cashew	
ASU	11	§64 LFGB L44.00-08, mod.	Hazelnut DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/45 Cyklen	
GI	13			First-DNA all tissue Kit/ GEN-IAL	
IC	3				
SFA-ID	9				
div	1			Real Time PCR	
div	2				
div	12		Cor A1	Extraction: kit Food Macherey Nagel	

5.1.11 PCR: Macadamia

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	2		negative	negative	positive	positive		Please choose!	realtime PCR
div	11	20.03.17	positive	negative	positive	positive	0,5	Nut-DNA	other: please insert!
div	12		negative	negative	positive	positive	5	Nut, total	Internal 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	2				
div	11	interal method	Macadamia DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real Time PCR/45 Cycles	
div	12		Vicilin gene	Extraction: kit Food Macherey Nagel	

5.1.12 PCR: Almond

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	6	26.04.	positive	positive	negative	positive	0,004 ng/µl	Nut-DNA	ASU §64 Methode/method
GI	13	22.03.	positive	positive	negative	negative	5mg/kg	Please choose!	First-Almond/GEN-IAL
IC	3		positive	positive	negative	negative		Please choose!	Food Allergen Detection PCR Kit, real Time PCR, InCura
SFA-ID	5	27.04.17	positive	positive	negative	negative	1	Nut, total	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	7		positive	positive	negative	negative		Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		positive	positive	negative	negative		Nut-DNA	Auswahl PCR-Methoden
div	2		positive	positive	negative	negative		Please choose!	realtime PCR
div	11	20.03.17	positive	positive	negative	negative	40	Nut-DNA	other: please insert!
div	12		positive	positive	negative	negative	5	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310

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	6	ASU L 18.00-22	PRU AV1 Gens		
GI	13			First-DNA all tissue Kit/ GEN-IAL	
IC	3				
SFA-ID	5	S3104	As Per Kit Instructions	As Per Kit Instructions	
SFA-ID	7				
div	1			Real Time PCR	
div	2				
div	11	internal Method	Almond DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real Time PCR/45 Cyklen	
div	12		ns LTP	Extraction: kit Food Macherey Nagel	

5.1.13 PCR: Brazil Nuts

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	6	26.04.	positive	negative	negative	negative	0,004 ng/µl	Nut-DNA	ASU §64 Methode/method
ASU	11	20.03.17	positive	negative	negative	positive		Nut-DNA	ASU §64 Methode/method
GI	13	24.03.	positive	negative	negative	positive	20mg/kg	Please choose!	First-Allergen Tetra II /GEN-IAL
SFA-ID	9		positive	-	-	positive	5 DNA copies	Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		positive	negative	negative	positive		Nut-DNA	Choice PCR-Methods
div	2		positive	negative	negative	positive		Please choose!	realtime PCR
div	12		positive	negative	negative	positive	5	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310

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	6	ASU L 18.00-22	Brazil nut 2S albumin Gen		
ASU	11	§64LFGB L14.02-4, mod.	Paranuss DANN	CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real Time PCR/45 Cyklen	
GI	13			First-DNA all tissue Kit/ GEN-IAL	
SFA-ID	9				
div	1			Real Time PCR	
div	2				
div	12		Albumin 2S	Extraction: kit Food Macherey Nagel	

5.1.14 PCR: Pecan

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	1		negative	negative	positive	positive		Nut-DNA	Choice PCR-Methods
div	2		negative	negative	positive	positive		Please choose!	realtime PCR
div	8	21.04.17	negative	negative	negative	negative	10	Please choose!	interal method
div	11	20.03.17	negative	positive	positive	positive	2	Nut-DNA	other: please insert!

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	1			Real Time PCR	
div	2				
div	8		jug r1		
div	11	internal Method	Walnut/Pecan DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/45 Cycles	Walnut and Pecan

5.1.15 PCR: Pistachio

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-ID	7		positive	positive	negative	negative		Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
div	1		positive	positive	negative	negative		Nut-DNA	Choice PCR-Methods
div	2		positive	positive	negative	negative		Please choose!	realtime PCR
div	6	27.4.	positive	positive	negative	negative	0,01 ng/µl	Nut-DNA	
div	11	20.03.17	positive	positive	negative	negative	8	Nut-DNA	other: please insert!
div	12		positive	positive	negative	negative	5	Nut, total	Internal 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-ID	7				
div	1			Real Time PCR	
div	2				
div	6	R. Köppel et al. (2012)	dehydrin	as Multiplex together with Pistachio, Peanut, Walnut und Cashew	
div	11	internal Method	Pistachio DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/45 Cycles	
div	12		Vicilin gene	Extraction: kit Food Macherey Nagel	

5.1.16 PCR: Walnut

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
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-ID	7		negative	positive	negative	positive		Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	9		-	positive	-	positive	5 DNA copies	Nut-DNA	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	5	27.04.17	negative	positive	negative	positive	1	Nut, total	Sure Food Allergen Quant, R-Biopharm / Congen
div	1		negative	positive	negative	positive		Nut-DNA	Choice PCR-Methods
div	2		negative	positive	negative	positive		Please choose!	realtime PCR
div	6	25.04.	negative	positive	negative	positive	0,025 ng/µl	Nut-DNA	
div	8	21.04.17	negative	positive	negative	positive	10	Nut, total	interal Method
div	11	20.03.17	negative	positive	positive	positive	2	Nut-DNA	other: please insert!
div	12		negative	positive	negative	positive	5	Nut, total	Eur. Food Res. Technol. (2006) 223:373-377

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-ID	7				
SFA-ID	9				
SFA-Q	5	S3107 & S3207	As Per Kit Instructions	As Per Kit Instructions	
div	1			Real Time PCR	
div	2				
div	6	B. Brezna et al (2006)	jug r 2	as Multiplex together with Pistachio, Peanut, Walnut und Cashew	
div	8		jug r1		
div	11	internal Method	Walnut/Pecan DNA	CTAB/Proteinase K/Promega Wizard DNA CleanUp/45 Cycles	Walnut and Pecan
div	12		jug R2	Extraction: kit Food Macherey Nagel	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 11-2017 Sample 1

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	44,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	101	39,9
2	5,03	88	35,0
3	5,10	123	48,2
4	5,02	105	41,8
5	5,09	96	37,7
6	5,02	98	39,0
7	5,00	96	38,4
8	5,02	119	47,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	103,2	Partikel
Standard deviation	11,8	Partikel
χ^2 (CHI-Quadrat)	9,40	
Probability	**22**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	40,9	mg/kg
Standard deviation	4,67	mg/kg
rel. Standard deviaton	11,4	%
Horwitz standard deviation	9,2	%
HorRat-value	**1,2**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA 11-2017 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	31,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	75	29,9
2	5,00	72	28,8
3	5,10	58	22,7
4	5,04	76	30,2
5	5,05	56	22,2
6	5,04	67	26,6
7	5,03	60	23,9
8	5,06	64	25,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	66,0	Partikel
Standard deviation	8,00	Partikel
χ^2 (CHI-Quadrat)	6,79	
Probability	**45**	%
Recovery rate	84	%

Normal distribution		
Number of samples	8	
Mean	26,2	mg/kg
Standard deviation	3,17	mg/kg
rel. Standard deviaton	12,1	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,2**	
Recovery rate	84	%

Microtracer Homogeneity Test
DLA 11-2017 Sample 3

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	48,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,07	121	47,7
2	5,00	97	38,8
3	5,25	134	51,0
4	5,05	126	49,9
5	5,07	103	40,6
6	5,06	88	34,8
7	5,13	110	42,9
8	5,17	115	44,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	112	Partikel
Standard deviation	14,4	Partikel
χ^2 (CHI-Quadrat)	13,0	
Probability	**7**	%
Recovery rate	90	%

Normal distribution		
Number of samples	8	
Mean	43,8	mg/kg
Standard deviation	5,64	mg/kg
rel. Standard deviaton	12,9	%
Horwitz standard deviation	9,1	%
HorRat-value	**1,4**	
Recovery rate	90	%

Microtracer Homogeneity Test
DLA 11-2017 Sample 4

Weight whole sample	1,03	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	38,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,08	73	28,7
2	5,06	83	32,8
3	5,08	77	30,3
4	5,00	71	28,4
5	5,13	78	30,4
6	5,02	84	33,5
7	5,21	70	26,9
8	5,05	74	29,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	76,3	Partikel
Standard deviation	5,64	Partikel
χ^2 (CHI-Quadrat)	2,92	
Probability	**89**	%
Recovery rate	79	%

Normal distribution		
Number of samples	8	
Mean	30,0	mg/kg
Standard deviation	2,22	mg/kg
rel. Standard deviaton	7,4	%
Horwitz standard deviation	9,6	%
HorRat-value	**0,77**	
Recovery rate	79	%

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 11-2017***
PT name	***Allergen-Screening I - 4 Samples qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio, Walnut***
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:* *Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and Walnut* *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 April 28th 2017***
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, 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
		GREAT BRITAN
		SWITZERLAND
		ITALY
		Germany
		Germany
		ITALY
		Germany
		Germany
		FRANCE
		Germany
		Germany
		GREAT BRITAN
		CANADA
		SPAIN

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

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

7. Index of references

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