



Evaluation Report

proficiency test

DLA 11/2019

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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<i>Vertraulichkeit</i> <i>Confidentiality</i>	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.

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1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].

The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.

Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

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	0,29 - 0,60 %
<u>Ingredients:</u> - Maltodextrin (75% - 90%) - Sodium sulfate (6,1% - 14%) - Silicon dioxide (3,5% - 10%) - Nut butters (1,1% - 1,7% each)	

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
Cashew (Protein 18,4%) - commercial nut butter	positive (25 - 75)	negative	negative	negative
Hazelnut (Protein 15,9%) - commercial nut butter	positive (25 - 75)	positive (50 - 150)	negative	negative
Macadamia (Protein 9,4%) - Nuts, crushed	positive (25 - 75)	positive (50 - 150)	negative	negative
Almond (Protein 19,6%) - commercial nut butter	negative	negative	positive (50 - 150)	positive (25 - 75)
Brazil nut (Protein 14,8%) - Nuts, crushed	positive (25 - 75)	negative	positive (50 - 150)	negative
Pecan (Protein 12,2%) - Nuts, crushed	negative	negative	negative	positive (50 - 150)
Pistachio (Protein 25,6%) - Nuts, crushed	negative	positive (25 - 75)	positive (50 - 150)	negative
Walnut (Protein 13,9%) - Nuts, crushed	negative	positive (25 - 75)	positive (25 - 75)	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® Almond	negative	negative	positive	positive
AgraStrip® Cashew/Pistachio	positive	positive	positive	negative
AgraStrip® Hazelnut	positive	positive	negative	negative
AgraStrip® Macadamia	positive	positive	negative	negative
AgraStrip® Brazil Nut	positive	negative	positive	negative
AgraStrip® Walnut	negative	positive	positive	schwach positive

* Nachweisgrenze jeweils 2-10 mg/kg / Limit of detection (LOD) 2-10 mg/kg each

** Laut Herstellerangaben Kreuzreaktivität zu Pecannuss / According to manufacturer's information cross-reactivity against pecan (Biofocus AgraStrips Allergens, www.romerlabs.com)

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **micro-tracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing dye coated iron particles of μm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of $\geq 5\%$ is equivalent to a good homogeneous mixture and of $\geq 25\%$ to an excellent mixture [14, 15].

The microtracer analysis of the present PT samples 1-4 showed probabilities of 29%, 88%, 48% and 86%, 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) [16, 17]. This gave HorRat values of 1,2, 0,8, 1,3 and 0,9, 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. 0,27 (24°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 10th week of 2019. The testing method was optional. The tests should be finished at April 18th 2019 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.

Out of 17 participants 15 submitted at least one result in time. One participant submitted no results and one participant submitted results in consultation with DLA delayed.

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 $\geq 75\%$ 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		
16	positive	positive	positive	negative	2/4 (50%)	2/4 (50%)	3M	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BA	
14	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BC	Sample 2 + 3: low cross-reactivity
1	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BF	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BF	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BF	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	EF	Sample 2 + 3: low cross-reactivity
12	positive	positive	positive	negative	2/4 (50%)	2/4 (50%)	IL	

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

Methods:

- 3M = 3M Protein ELISA Kit
- BA = Bioavid (Lateral Flow), R-Biopharm
- BC = BioCheck ELISA
- BF = MonoTrace ELISA, BioFront Technologies
- EF = SensiSpec ELISA Kit, Eurofins
- IL = Immunolab

Comments:

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

Two positive results were reported for samples 2 and 3, possibly due to cross-reactivity of the test methods to pistachio. Two other participants also indicated cross-reactivity for samples 2 and 3 (methods BC and EF).

Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
9	positive	positive	positive	negative	2/4 (50%)	2/4 (50%)	MS	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	SFA	
14	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
4	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	9	1	2	1
Number negative	0	8	7	8
Percent positive	100	11	22	11
Percent negative	0	89	78	89
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, 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.

One positive result for each of samples 2 and 4 and two positive results for sample 3 have been given.

Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BA	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	EF	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ES	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
16	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	

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

Methods:

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

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		
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
9	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample detected
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-4p = Sure Food Allergen 4plex, 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 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		
16	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	3M	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	EF	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	EF	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

Comments:

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

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		
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
9	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	7	7	0	0
Number negative	0	0	7	7
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 = Sure Food ALLERGEN, 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 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		
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BA	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BF	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BF	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	EF	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
15	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
16	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

BA = Bioavid (Lateral Flow), R-Biopharm
 BF = MonoTrace ELISA, BioFront Technologies
 EF = SensiSpec ELISA Kit, Eurofins
 IL = Immunolab
 RS-F= Ridascree® 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				
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MS	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
15	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	div	

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

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA = Sure Food ALLERGEN, 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.

One participant has indicated positive results for samples 1 and 2 (no almond added) (method not specified).

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		
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	3M	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	EF	
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	EF	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

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		
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, 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.

4.6 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		
16	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	3M	
2	negative	positive	positive	negative	1/2 (50%)	1/4 (25%)	BA	Pecan/walnut not differentiated, the positive sample not detected
1	negative	positive	positive	positive	2/2 (100%)	2/4 (50%)	BF	
6	negative	-	-	positive	2/2 (100%)	2/2 (100%)	BF	
13	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	BF	
14	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	DE	Sample 2 + 3: cross-reactivity
7	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	EF	Sample 2 + 3: low cross-reactivity to walnut
12	negative	positive	positive	positive	2/2 (100%)	2/4 (50%)	IL	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	3	3	7
Number negative	8	4	4	1
Percent positive	0	43	43	88
Percent negative	100	57	57	13
Consensus value	negative	none	none	positive
Spiking	negative	negative	negative	positive

Methods:

3M = 3M Protein ELISA Kit
 BA = Bioavid (Lateral Flow), R-Biopharm
 BF = MonoTrace ELISA, BioFront Technologies
 DE = Demeditec ELISA
 EF = SensiSpec ELISA Kit, Eurofins
 IL = Immunolab

Comments:

The consensus values of the results for samples 1 and 4 are in qualitative agreement with the spiking of the samples. For samples 2 and 3 (without addition of pecan nut) inconsistent results were obtained so that no consensus value $\geq 75\%$ could be established. Samples 2 and 3 each gave three positive results, possibly due to cross-reactivity against walnut. One participant indicated that it is not possible to distinguish between pecan and walnut with the method BA used. Two other participants indicated low cross-reactivity for samples 2 and 3 but reported negative results. Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
12	positive	positive	positive	positive	1 / 2 (50%)	1/4 (25%)	SFA	
14	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	SFA	
8	negative	positive	positive	positive	2/2 (100%)	2/4 (50%)	div	Pecan/walnut not differentiated
9	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	div	
11	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	div	
15	negative	negative	negative	negative	1 / 2 (50%)	3/4 (75%)	div	no positive sample detected

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	2	2	5
Number negative	5	4	4	1
Percent positive	17	33	33	83
Percent negative	83	67	67	17
Consensus value	negative	none	none	positive
Spiking	negative	negative	negative	positive

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen
 div = keine genaue Angabe / andere Methode
 div = not indicated / other method

Comments:

The consensus values of the results for samples 1 and 4 are in qualitative agreement with the spiking of the samples.
 For samples 2 and 3 (without addition of pecan) inconsistent results were obtained so that no consensus value $\geq 75\%$ could be established.
 Samples 2 and 3 each gave two positive results, possibly due to cross-reactivity against walnut. One participant indicated that it is not possible to distinguish between pecan and walnut with the method used.
 Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
16	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	3M	
2	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BA	
1	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
6	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
13	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
7	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	EF	Sample 1: cross-reactivity to cashew
12	positive	positive	positive	positive	2/3 (67%)	3/4 (75%)	IL	
15	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	IL	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	4	8	8	1
Number negative	4	0	0	7
Percent positive	50	100	100	13
Percent negative	50	0	0	88
Consensus value	none	positive	positive	negative
Spiking	negative	positive	positive	negative

Methods:

3M = 3M Protein ELISA Kit
 BA = Bioavid (Lateral Flow), R-Biopharm
 BF = MonoTrace ELISA, BioFront Technologies
 EF = SensiSpec ELISA Kit, Eurofins
 IL = Immunolab

Comments:

The consensus values of the results for samples 2, 3 and 4 are in qualitative agreement with the spiking of the samples. For sample 4 (without addition of pistachio) a positive result was obtained. For sample 1 (without addition of pistachio) inconsistent results were obtained so that no consensus value $\geq 75\%$ could be established. The positive results may be due to cross-reactivity against cashew, as reported by one participant. Possible cross-reactivities should be documented in the manufacturer's test kit information.

4.7.2 PCR-Results: Pistachio

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation Agreement with consensus value	Qualitative Valuation Agreement with spiking of samples	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg				
9	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	MS	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	SFA	
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
15	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, 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.

For samples 1 and 4 each (without addition of pistachio) a positive result was obtained.

Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
16	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	AQ	
2	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BA	
1	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
6	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
13	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	BF	
7	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	EF	Sample 4: cross-reactivity to pecan
12	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	IL	
15	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	IL	

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

Methods:

AQ = AgraQuant, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

IL = Immunolab

Comments:

The consensus values of the results for samples 1, 2 and 3 are in qualitative agreement with the spiking of the samples.

For sample 4 (without addition of walnut) inconsistent results were obtained so that no consensus value $\geq 75\%$ could be established. The positive results may be due to a cross-reactivity to pecan.

Another participant indicated a weak cross-reactivity but gave a negative result for sample 4.

Possible cross-reactivities should be documented in the manufacturer's test kit information.

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		
9	negative	negative	positive	negative	3/4 (75%)	3/4 (75%)	MS	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	SFA	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	div	Pecan/walnut not differentiated
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
15	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	7	8	2
Number negative	7	1	0	6
Percent positive	13	88	100	25
Percent negative	88	13	0	75
Consensus value	negative	positive	positive	negative
Spiking	negative	positive	positive	negative

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-4p = Sure Food Allergen 4plex, 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 the samples.

For samples 1 and 4 (without addition of walnut) one or two positive results were obtained. One participant indicated that no distinction is possible between pecan and walnut with the method used.

Possible cross-reactivities should be documented in the manufacturer's test kit information.

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
		Tag/Monat	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	z.B. Lebensmittel / Protein	Test-Kit + Anbieter
3M	16	10.04.19	positive	positive	positive	negative	0,9	Nut protein	3M = 3M Protein ELISA Kit
BA	2	11.04.19	positive	negative	negative	negative	1	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BC	14	06.04.19	positive	negative	negative	negative	2	Nut, total	BC = BioCheck ELISA
BF	1		positive	negative	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		positive	negative	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	positive	negative	negative	negative	0,12	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	7		positive	negative	negative	negative			EF = SensiSpec ELISA Kit, Eurofins
IL	12		positive	positive	positive	negative	2	Protein	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
3M	16	E96CHW			
BA	2	BL610-25			
BC	14	R6046	As Per Kit Instructions	As Per Kit Instructions	Samples B & C showed low cross reaction with ELISA - Confirmed as Negative with PCR
BF	1				
BF	6				
BF	13	CA2-EK	Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
EF	7				Samples 2+3 slightly positive below cut-off, possibly due to 4% cross-reactivity to pistachio
IL	12				

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
BA	2	11.04.19	positive	positive	negative	negative	1	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BF	6		positive	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	positive	positive	negative	negative	0,04	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	7		positive	positive	negative	negative	0.3/1	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
ES	3		positive	positive	negative	negative	0,25	hazelnut protein	ES = ELISA-Systems
IL	12		positive	positive	negative	negative	1	Protein	IL = Immunolab
RS-F	1		positive	positive	negative	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	15	08.04.19	pos	pos	neg	neg	1,2	Protein	R-BIOPHARM R6802
RS-F	16	16.04.19	positive	positive	negative	negative	2,5	Nut, total	RS = Ridascreen®, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	2	BL604-25			
BF	6				
BF	13	HC9-EK	Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
EF	7				
ES	3	§64 LFGB L 44.0.7:2006-06	detects hazelnut proteins	As Per Kit Instructions	
IL	12				
RS-F	1				
RS-F	15				
RS-F	16	R6802			

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
3M	16	10.04.19	positive	positive	negative	negative	2,5	Nut protein	3M = 3M Protein ELISA Kit
BF	1		positive	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		positive	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	positive	positive	negative	negative	0,13	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	3		positive	positive	negative	negative	1	Macadamia nut	Eurofins Technologies
EF	7		positive	positive	negative	negative	0.1/1	Nuss, gesamt	EF = SensiSpec ELISA Kit, Eurofins
IL	12		positive	positive	negative	negative	1	Protein	IL = Immunolab
IL	14	14.03.19	positive	positive	negative	negative		Nut, total	IL = Immunolab
IL	15	09.04.19	pos	pos	neg	neg	1	Protein	Immunolab MAC-E01

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
3M	16	E96MAC			
BF	1				
BF	6				
BF	13	MN1-EK	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
EF	3	HU0030013:2	detects macadamia nut protein	As Per Kit Instructions	
EF	7				
IL	12				
IL	14	MAC-E01	As Per Kit Instructions	As Per Kit Instructions	
IL	15				

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
BA	2	11.04.19	negative	negative	positive	positive	1	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BF	6		negative	negative	positive	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	negative	negative	positive	positive	0,15	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	7		negative	negative	positive	positive	0.2//0.4	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
IL	12		negative	negative	positive	positive	0,4	Protein	IL = Immunolab
RS-F	1		negative	negative	positive	positive	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	3		negative	negative	positive	positive	1,7	Almond	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	15	08.04.19	neg	neg	pos	pos	1.2	Protein	R-BIOPHARM R6901
VT	16	19.03.19	negative	negative	positive	positive	2,5	Nut, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	2	BL601-25			
BF	6				
BF	13	AP1-EK	Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
EF	7				
IL	12				
RS-F	1				
RS-F	3	R6901:2015-07	detects almond proteins	As per kit instructions	
RS-F	15				
VT	16	8440		Method #8440	

5.1.5 ELISA: Brazil Nut*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			
3M	16	09.04.19	positive	negative	positive	negative	1	Nut protein	3M = 3M Protein ELISA Kit
BF	1		positive	negative	positive	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		positive	negative	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	positive	negative	positive	negative	0,14	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	3		positive	negative	positive	negative	0,2	Brazil Nut	Eurofins Technologies
EF	7		positive	negative	positive	negative	0.2/1	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
IL	12		positive	negative	positive	negative	1	Protein	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
3M	16	E96BZL			
BF	1				
BF	6				
BF	13	BN-EK	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
EF	3	HU0030018	detects brazil nut proteins	As per kit instructions	
EF	7				
IL	12				

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
3M	16	11.04.19	negative	negative	negative	positive	0,7	Nut protein	3M = 3M Protein ELISA Kit
BA	2	11.04.19	negative	positive	positive	negative	10	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BF	1		negative	positive	positive	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		negative	-	-	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	negative	negative	negative	positive	0,17	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
DE	14	03.04.19	negative	negative	negative	positive		Nut, total	DE = Demeditec ELISA
EF	7		negative	negative	negative	positive	0.2/2	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
IL	12		negative	positive	positive	positive	2	Protein	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
3M	16	E96PEC			
BA	2	BL607-25			The Same Kit for Walnut and Pecan. Cross-reactivity Walnut: 100% Pecan
BF	1				
BF	6				
BF	13	PC4-EK	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
DE	14	DEPECE01	As Per Kit Instructions	As Per Kit Instructions	Samples B & C showed low cross reaction with ELISA - Confirmed as Negative with PCR
EF	7				the results of approx. 3 ppm for sample 2 and 3 were judged as cross-reactivity to walnut
IL	12				

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			
3M	16	11.04.19	positive	positive	positive	negative	1	Nut protein	3M = 3M Protein ELISA Kit
BA	2	11.04.19	negative	positive	positive	negative	1	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BF	1		negative	positive	positive	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		negative	positive	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	negative	positive	positive	negative	0,12	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	7		positive	positive	positive	negative	0.13/2	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
IL	12		positive	positive	positive	positive	1	Protein	IL = Immunolab
IL	15	09.04.19	pos	pos	pos	neg	1	Protein	Immunolab PIS-E01

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	
3M	16	E96PST			
BA	2	BL611-25			
BF	1				
BF	6				
BF	13	PV1-EK	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
EF	7				sample 1 - possible cross-reactivity to cashew
IL	12				
IL	15				

5.1.8 ELISA: Walnuss*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	16	17.04.19	negative	positive	positive	positive	2	Nut protein	AQ = AgraQuant, RomerLabs
BA	2	11.04.19	negative	positive	positive	negative	10	allergen/buffer	BA = Bioavid (Lateral Flow), R-Biopharm
BF	1		negative	positive	positive	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	6		negative	positive	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13	29.04.19	negative	positive	positive	negative	0,22	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
EF	7		negative	positive	positive	negative	0.35/2	Nut, total	EF = SensiSpec ELISA Kit, Eurofins
IL	12		negative	positive	positive	positive	2	Protein	IL = Immunolab
IL	15	12.04.19	neg	pos	pos	pos	1	Protein	Immunolab WAL-E01

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	16	COKAL0948			
BA	2	BL607-25			
BF	1				
BF	6				
BF	13	WJ4-EK	Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
EF	7				the result of 3 ppm for sample 4 was identified as cross-reactivity to pecan
IL	12				
IL	15				

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
MS	9	18.03.19	positive	positive	positive	negative		food item	Microsynth
SFA	6		positive	negative	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12		positive	negative	positive	negative	0,4	Protein	SureFood
SFA	14	04.04.19	positive	negative	negative	negative	1	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	3		positive	negative	negative	negative	10	Nut-DNA	internal method
div	4		positive	negative	negative	negative	5	Nut, total	Internal method
div	8		positive	negative	negative	negative	< 100	Nut, total	
div	11	12.3.199	positive	negative	negative	negative	21	Nut, total	biomers
div	15	08.04.19	pos	neg	neg	pos	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	
MS	9			Wizard Extraction and qPCR bys Rotorgene	
SFA	6				
SFA	12				
SFA	14	S3615	As Per Kit Instructions	As Per Kit Instructions	
div	3			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 Cycles	
div	4		Ana 03	Extraction: kit Food Macherey Nagel	
div	8		148bp - Product	/ Proteinase, RNase / Silica columns / Real Time PCR/ 45 Cycles	
div	11	Ehlert et al., Food Anal. Methods (2008) 1:136–143	cashew allergen (Ana o3) of the 2S albumin family	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	15				Limit of detection given as µg of DNA per kg of sample

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	3		positive	positive	negative	negative	10	Nuss-DNA	ASU = ASU §64 Methode/method
ASU	11	13.03.19	positive	positive	negative	negative	25	Nut, total	ASU = ASU §64 Methode/method
MS	9	18.03.19	negative	negative	negative	negative		food item	Microsynth
SFA	6		positive	positive	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12		positive	positive	negative	negative	0,4	Protein	SureFood
SFA-4p	10	18.03.19	positive	positive	negative	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	4		positive	positive	negative	negative	range 5 to 10	Nut, total	CEN/TC 275/WG 12 N 317
div	8		positive	positive	negative	negative	< 100	Nut, total	Auswahl PCR-Methoden
div	15	08.04.19	pos	pos	neg	neg	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	3	§64 LFGB L 44.00-08		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 Cycles	
ASU	11	L 44.00-8	corA1-Gen	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
MS	9			Wizard Extraction and qPCR by Rotorgene	
SFA	6				
SFA	12				
SFA-4p	10	S3402	Corylus	Sure Food Prep Advanced Protokoll 1	(K00 + K01)
div	4		Cor A1	Extraction: kit Food Macherey Nagel	
div	8		156bp - Product	/ Proteinase, RNase / Silika-Säulchen / Real Time PCR/ 45 Cycles	
div	15				Limit of detection given as µg of DNA per kg of sample

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
SFA	12		positive	positive	negative	negative	0,4	Protein	SureFood
SFA	14	05.04.19	positive	positive	negative	negative	1	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	4		positive	positive	negative	negative	7 pg DNA	Nut-DNA	Internal method
div	8		positive	positive	negative	negative	< 100	Nut, total	
div	9	18.03.19	positive	positive	negative	negative		food item	in house
div	11	12.03.19	positive	positive	negative	negative	21	Nut, total	biomers
div	15	08.04.19	pos	pos	neg	neg	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	12				
SFA	14	S3616	As Per Kit Instructions	As Per Kit Instructions	
div	4		Vicilin gene	Extraction: kit Food Macherey Nagel	
div	8		93bp - Product	/ Proteinase, RNase / Silika-Säulchen / Real Time PCR/ 45 Cycles	
div	9			Wizard Extraktion and qPCR by Rotorgene	
div	11	Brezna et al. 2010	vicilin precursor gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	15				Limit of detection given as µg of DNA per kg of sample

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	3		negative	negative	positive	positive	40	Nut-DNA	ASU = ASU §64 Methode/method
ASU	11	12.03.19	negative	negative	positive	positive	20	Nut, total	ASU = ASU §64 Methode/method
MS	9	18.03.19	negative	negative	positive	positive		food item	Microsynth
SFA	6		negative	negative	positive	positive	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12		negative	negative	positive	positive	4	Protein	SureFood
div	4		negative	negative	positive	positive	range 5 to 10	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310
div	8		negative	negative	positive	positive	< 100	Nut, total	
div	15	08.04.19	pos	pos	pos	pos	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	3	§64 LFGB L 18.00-20		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 Cycles	
ASU	11	L 18.00-20	non specific lipid transfer protein	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
MS	9			Wizard Extraktion and qPCR by Rotorgene	
SFA	6				
SFA	12				
div	4		ns LTP	Extraction: kit Food Macherey Nagel	
div	8		82bp - Produkt	/ Proteinase, RNAse / Silica columns / Real Time PCR/ 45 Cycles	
div	15				Limit of detection given as µg of DNA per kg of sample

5.1.13 PCR: Brazli 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	3		positive	negative	positive	negative	20	Nut-DNA	ASU = ASU §64 Methode/method
ASU	11	12.03.19	positive	negative	positive	negative	20	Nut, total	ASU = ASU §64 Methode/method
SFA	12		positive	negative	positive	negative	0,4	Protein	SureFood
div	4		positive	negative	positive	negative	not determined	Nut-DNA	J. Verbr. Lebensm. (2014) 9:297-310
div	8		positive	negative	positive	negative	< 100	Nut, total	
div	9	18.03.19	positive	negative	positive	negative		food item	in house
div	15	04.04.19	pos	neg	pos	neg	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	3	§64 LFGB L 18.00-21		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 Cycles	
ASU	11	L 18.00-21	Ber e 1 Gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
SFA	12				
div	4		Albumin 2S	Extraction: kit Food Macherey Nagel	
div	8		102bp - Product	/ Proteinase, RNase / Silica columns / Real Time PCR/ 45 Cycles	
div	9			Wizard Extraction and qPCR by Rotorgene	
div	15				Limit of detection given as µg of DNA per kg of sample

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
SFA	12		positive	positive	positive	positive	4	Protein	SureFood
SFA	14	05.04.19	negative	negative	negative	positive	1	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	8		negative	positive	positive	positive	< 100	Nut, total	
div	9	18.03.19	negative	negative	negative	positive		food item	in house
div	11	13.03.19	negative	negative	negative	positive	25	Nut, total	biomers
div	15	08.04.19	neg	neg	neg	neg	80		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	12				
SFA	14	S3618	As Per Kit Instructions	As Per Kit Instructions	
div	8		189bp - Product	/ Proteinase, RNase / Silica columns / Real Time PCR/ 45 Cycles	Pecanuss/Walnut is not differentiated
div	9			Wizard Extraktion und qPCR mittels Rotorgene	
div	11	Brezna et al., Eur Food Res Technol 2007 DOI 10.1007/s00217-007-0639-3	pecan putative vicilin-like seed storage protein gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	15				Limit of detection given as µg of DNA per kg of sample

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
MS	9	18.03.19	negative	positive	positive	positive		food item	Microsynth
SFA	5	22.03.19	negative	positive	positive	negative	4	Food/Food	SFA
SFA	6		negative	positive	positive	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12		positive	positive	positive	negative	0,4	Protein	SureFood
div	3		negative	positive	positive	negative	1	Nut-DNA	interne Methode
div	4		negative	positive	positive	negative	5	Nut, total	Internal method
div	8		negative	positive	positive	negative	< 100	Nut, total	
div	11	13.03.19	negative	positive	positive	negative	20	Nut, total	biomers
div	15	08.04.19	neg	pos	pos	neg	80		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	
MS	9			Wizard Extraction and qPCR by Rotorgene	
SFA	5	S3614		CTAB / Kit / real time PCR	
SFA	6				
SFA	12				
div	3			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 Cycles	
div	4		Vicilin gene	Extraction: kit Food Macherey Nagel	
div	8		88bp - Product	/ Proteinase, RNAse / Silica columns / Real Time PCR/ 45 Cycles	
div	11	Brezna et al., Eur Food Res Technol (2008) 228:197–203	Pistacia vera, internal transcribed spacer	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	15				Limit of detection given as µg of DNA per kg of sample

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
MS	9	18.03.19	negative	negative	positive	negative		food item	Microsynth
SFA	6		negative	positive	positive	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12		positive	positive	positive	negative	0,4	Protein	SureFood
SFA-4p	10	18.03.19	negative	positive	positive	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	4		negative	positive	positive	negative	5	Nut, total	Eur. Food Res. Technol. (2006) 223:373-377
div	8		negative	positive	positive	positive	< 100	Nut, total	
div	11	13.03.19	negative	positive	positive	negative	20	Nut, total	biomers
div	15	08.04.19	neg	pos	pos	pos	8		in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	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	
MS	9			Wizard Extraction and qPCR by Rotorgene	
SFA	6				
SFA	12				
SFA-4p	10	S3402	Juglans	Sure Food Prep Advanced Protokoll 1	(K00 + K01)
div	4		jug R2	Extraction: kit Food Macherey Nagel	
div	8		189bp - Product	/ Proteinase, RNase / Silica columns / Real Time PCR/ 45 Cycles	Pecan/Walnut is not differentiated
div	11	Brezna et al., Eur. Food Res Technol (2006)223: 373-377	jug r2 Gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	15				Limit of detection given as µg of DNA per kg of sample

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test

DLA 11-2019 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	33,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	98	39,0
2	5,04	73	29,0
3	4,99	97	38,9
4	5,05	96	38,0
5	4,99	95	38,1
6	5,04	111	44,0
7	5,05	89	35,2
8	5,03	91	36,2

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	93,8	Particles
Standard deviation	10,71	Particles
χ^2 (CHI-Quadrat)	8,56	
Probability	29	%
Recovery rate	112	%

Normal distribution

Number of samples	8	
Mean	37,3	mg/kg
Standard deviation	4,26	mg/kg
rel. Standard deviaton	11,4	%
Horwitz standard deviation	9,3	%
HorRat-value	1,2	
Recovery rate	112	%

Microtracer Homogeneity Test

DLA 11-2019 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	34,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	57	22,7
2	5,00	63	25,2
3	5,03	60	23,9
4	5,02	67	26,7
5	5,03	71	28,2
6	5,10	73	28,6
7	5,03	69	27,4
8	4,99	62	24,8

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	65,2	Particles
Standard deviation	5,36	Particles
χ^2 (CHI-Quadrat)	3,08	
Probability	88	%
Recovery rate	76	%

Normal distribution

Number of samples	8	
Mean	26,0	mg/kg
Standard deviation	2,13	mg/kg
rel. Standard deviaton	8,2	%
Horwitz standard deviation	9,8	%
HorRat-value	0,8	
Recovery rate	76	%

Microtracer Homogeneity Test**DLA 11-2019 Sample 3**

Weight whole sample	1,03	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	18,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	43	17,3
2	5,02	54	21,5
3	4,99	46	18,4
4	5,02	63	25,1
5	4,99	47	18,8
6	4,99	54	21,6
7	5,09	43	16,9
8	5,03	51	20,3

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	50,1	Particles
Standard deviation	6,83	Particles
χ^2 (CHI-Quadrat)	6,51	
Probability	48	%
Recovery rate	111	%

Normal distribution

Number of samples	8	
Mean	20,0	mg/kg
Standard deviation	2,72	mg/kg
rel. Standard deviation	13,6	%
Horwitz standard deviation	10,2	%
HorRat-value	1,3	
Recovery rate	111	%

Microtracer Homogeneity Test**DLA 11-2019 Sample 4**

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	52	20,7
2	4,98	64	25,7
3	5,00	56	22,4
4	5,03	57	22,7
5	4,98	57	22,9
6	5,07	66	26,0
7	5,02	54	21,5
8	5,03	52	20,7

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	57,2	Particles
Standard deviation	5,16	Particles
χ^2 (CHI-Quadrat)	3,25	
Probability	86	%
Recovery rate	98	%

Normal distribution

Number of samples	8	
Mean	22,8	mg/kg
Standard deviation	2,06	mg/kg
rel. Standard deviation	9,01	%
Horwitz standard deviation	10,0	%
HorRat-value	0,90	
Recovery rate	98	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

<i>PT number</i>	DLA 11-2019
<i>PT name</i>	Allergen-Screening I - 4 Samples qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio, Walnut
<i>Sample matrix</i>	Samples 1-4: Carrier matrix / ingredients: potato powder (appr. 75%), maltodextrin (appr. 25%), other food additives and allergenic foods
<i>Number of samples and sample amount</i>	4 different Samples 1-4: 20 g each
<i>Storage</i>	Samples A + B: room temperature (long term cooled 2 - 10°C)
<i>Intentional use</i>	Laboratory use only (quality control samples)
<i>Parameter</i>	Qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio, Walnut Samples 1-4: appr. 25 - 250 mg/kg
<i>Methods of analysis</i>	The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.
<i>Notes to analysis</i>	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.
<i>Result sheet</i>	One result each should be determined for Samples 1-4. The results should be filled in the result submission file.
<i>Units</i>	positiv / negativ (limit of detection mg/kg)
<i>Number of digits</i>	at least 2
<i>Result submission</i>	The result submission file should be sent by e-mail to: pt@dla-lvu.de
<i>Deadline</i>	the latest <u>April 18th 2019</u>
<i>Evaluation report</i>	The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.
<i>Coordinator and contact person of PT</i>	Matthias Besler-Scharf PhD / Alexandra Scharf M.Sc.

* 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
		SPAIN
		USA
		CANADA
		ITALY
		Germany
		Germany
		AUSTRIA
		Germany
		SWITZERLAND
		FRANCE
		Germany
		Germany
		GREAT BRITAIN
		FRANCE
		GREAT BRITAIN
		SPAIN
		ITALY

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswertebereichs nicht angegeben.]

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

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