Evaluation Report
proficiency test

DLA ptALS1 (2020)

Allergen-Screening I:

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

DLA - Proficiency Tests GmbH
Kalte Weide 21
24641 Sievershütten/Germany

proficiency-testing@dla-lvu.de *www.dla-lvu.de*

Coordinator of this PT:
Dr. Matthias Besler-Scharf

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptALS1 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (31 July 2020) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 31 July 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficency test the following services were subcontracted: protein determination
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.

Contents

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)	0,28 - 0,58 %

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

* 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	positive	positive	negative	negative
AgraStrip® Cashew/Pistachio	positive	positive	positive	weakly positive
AgraStrip® Hazelnut	negative	positive	negative	positive
AgraStrip® Macadamia	negative	negative	negative	positive
AgraStrip® Brazil Nut	positive	positive	negative	negative
AgraStrip® Walnut**	negative	weakly positive	weakly positive	positive

* Nachweisgrenze jeweils 1-10 mg/kg / Limit of detection (LOD) 1-10 mg/kg each
** Laut Herstellerangaben leichte Kreuzreaktivität zu Pecannuss / According to manufacturer's information slight cross-reactivity against pecan (Biofocus AgraStrips Allergens, www.romerlabs.com)

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 56%, 93%, 56% and 91%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 1,1, 0,7, 1,3 and 0,8, respectively. 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. 0,38 (18-20°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 12th week of 2020. The testing method was optional. The tests should be finished at May 29th 2020 the latest (extended).

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/or **Walnut** in a simple carrier matrix The evaluation of results is strictly qualitative (positive / negative).*

*The following **analysis methods** can be used:*

*a) **ELISA** and **Lateral Flow***
*b) **PCR***
*c) **LC/MS***

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. The results given as positive/negative were evaluated.
Queried and documented were the indicated results and details of the test methods like specificities, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 17 participants submitted at least one result in time.

3. Evaluation

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

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

3.1 Agreement with consensus values from participants

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined if ≥ 75% positive or negative results are available 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		
10	-	positive	-	negative	2/2 (100%)	2/2 (100%)	3M	Sample 1 and 3: cross-reactivity
8	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
13	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16a	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16b	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	Sample 1: cross-reactivity to pistachio
14	-	positive	-	negative	2/2 (100%)	2/2 (100%)	SP	Sample 1 and 3: cross-reactivity to pistachio

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

Several participants indicated cross-reactivity to pistachio for samples 1 and 3.

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		
1	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	MS	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
12	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
14	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
17	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-ID = Sure Food Allergen ID, 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.

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		
16a	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF	
16b	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IL	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	
7	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	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	
5	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	9	0	9
Number negative	9	0	9	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

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

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		
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
1	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample detected
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
17	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-ID = Sure Food Allergen ID, 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		
10	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	3M	
13	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
16a	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
16b	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
15	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	
14	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

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		
1	negative	negative	positive	negative	2/4 (50%)	2/4 (50%)	MS	
6	negative	positive	negative	positive	3/4 (75%)	3/4 (75%)	SFA	
12	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
3	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
11	negative	positive	negative	positive	3/4 (75%)	3/4 (75%)	SFA-ID	
15	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
17	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	2	1	6
Number negative	7	5	6	1
Percent positive	0	29	14	86
Percent negative	100	71	86	14
Consensus value	negative	none	negative	positive
Spiking	negative	negative	negative	positive

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 1, 3 and 4 are in qualitative agreement with the spiking of the samples.

For samples 2 (without addition of macadamia) inconsistent results were obtained so that no consensus value ≥75% could be established.

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		
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16a	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16b	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
13a	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ES	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
13b	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	12	12	0	0
Number negative	0	0	12	12
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

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

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		
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
1	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	MS	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
17	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	4	5	0	0
Number negative	1	0	5	5
Percent positive	80	100	0	0
Percent negative	20	0	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

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.

4.5 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		
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	3M	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16a	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
16b	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	DE	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	

	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

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

DE = Demeditec ELISA

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

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		
1	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	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
17	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	1	1	8	8
Percent positive	88	88	0	0
Percent negative	13	13	100	100
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.6 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		
10	negative	positive	positive	-	3/3 (100%)	3/3 (100%)	3M	Sample 4: cross-reactivity
8	negative	positive	positive	-	3/3 (100%)	3/3 (100%)	BF	
13	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	BF	
16a	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BF	
16b	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	Sample 4: cross-reactivity to walnut
14	negative	positive	positive	-	3/3 (100%)	3/3 (100%)	SP	Sample 4: cross-reactivity to walnut

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	8	8	1
Number negative	8	0	0	4
Percent positive	0	100	100	20
Percent negative	100	0	0	80
Consensus value	negative	positive	positive	negative
Spiking	negative	positive	positive	negative

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

Several participants indicated cross-reactivity to walnut for sample 4.

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		
1	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	MS	no positive sample detected
6	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	SFA	
12	negative	positive	negative	negative	2/2 (100%)	3/4 (75%)	SFA	
3	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	SFA-4p	
11	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	SFA-ID	
14a	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	div	
14b	negative	negative	positive	positive	1/2 (50%)	2/4 (50%)	div	Pecan and walnut not differentiated

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	5	5	1
Number negative	7	2	2	6
Percent positive	0	71	71	14
Percent negative	100	29	29	86
Consensus value	negative	none	none	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

SFA-ID = Sure Food Allergen ID, 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 the spiked samples 2 and 3 inconsistent results were obtained so that no consensus value ≥75% could be established.
A limit of detection of 100 mg/kg was indicated by participant 1 for the method MS. The spiked levels of pecan were below this detection limit. Without taking into account the results of participant 1, consensus values with 83% positive results for samples 2 and 3 are obtained.
Participant 14b indicated that no distinction is possible between pecan and walnut with the method used.

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		
10	positive	-	positive	negative	3/3 (100%)	3/3 (100%)	3M	Sample 2: cross-reactivity
15	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	BC	
8	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	BF	
13	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	BF	
16a	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	BF	
16b	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	IL	Sample 2: cross-reactivity to cashew
5	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	SP	Sample 2: cross-reactivity to cashew

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	8	2	8	0
Number negative	0	5	0	8
Percent positive	100	29	100	0
Percent negative	0	71	0	100
Consensus value	positive	none	positive	negative
Spiking	positive	negative	positive	negative

Methods:

3M = 3M Protein ELISA Kit

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of the results for samples 1, 3 and 4 are in qualitative agreement with the spiking of the samples.
For sample 2 (without addition of pistachio) inconsistent results were obtained so that no consensus value ≥75% could be established. Several participants indicated cross-reactivity to cashew for samples 2.

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	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	MS	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

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		
4	negative	positive	positive	positive	2/2 (100%)	2/4 (50%)	AQ	
10	negative	-	-	positive	2/2 (100%)	2/2 (100%)	AQ	Sample 2 and 3: cross-reactivity
15	negative	positive	positive	positive	2/2 (100%)	2/4 (50%)	BC	
8	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	BF	
13	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	BF	
16a	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	BF	
16b	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	BF-LF	Lateral Flow
12	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	IL	
2	negative	positive	positive	positive	2/2 (100%)		SP	
5	negative	negative	negative	positive	2/2 (100%)	4/4 (100%)	SP	Sample 2 and 3: cross-reactivity to pecan
14	negative	-	-	positive	2/2 (100%)	2/2 (100%)	SP	Sample 2 and 3: cross-reactivity to pecan

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	3	3	11
Number negative	11	6	6	0
Percent positive	0	33	33	100
Percent negative	100	67	67	0
Consensus value	negative	none	none	positive
Spiking	negative	negative	negative	positive

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of the results for samples 1 and 4 are in qualitative agreement with the spiking of the samples.
For sample 2 and 3 (both without addition of walnut) inconsistent results were obtained so that no consensus value ≥75% could be established.
Several participants indicated cross-reactivity to pecan for samples 2 and 3.
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		
1	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	MS	no positive sample detected
3	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
6	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
9	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
12	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
11	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
15	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
14a	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
14b	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	div	Walnut and pecan not differentiated
17	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

Participant 14b 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. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	10	12/05	*	positive	*	negative	0,9	Nut protein	3M
BF	8		negative	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13		negative	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	negative	positive	negative	negative	0,12	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	negative	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	negative	positive	negative	negative	2	Nut, total	IL = Immunolab
SP	5	09.04.20	negative*	positive	negative	negative	0.2	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	14	24.04.20	-	positive	-	negative	2	Nut, total	Selection of ELISA kits:

Other details to the Methods

Meth. Abbr.	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	10	E96CHW		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BF	8				
BF	13				
BF	16a		Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
IL	12				
SP	5				3 ppm identified as CR of pistachio
SP	14	HU0030004	recognizes cashew proteins	according to manufacturer's instructions	Sample 2: >50mg/kg; Sample 1 and 3: not feasible due to possible cross-reactivity with pistachio

5.1.2 ELISA: Hazelnut

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
BF	16a	29/5	negative	positive	negative	positive	0,04	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	negative	positive	negative	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	negative	positive	negative	positive	1	Nut, total	IL = Immunolab
MI-II	14	01.04.20	negative	positive	negative	positive	0,16	Nussprotein	MI-II = Morinaga Institute ELISA II
RS-F	7	17.04.20	negative	positive	negative	positive	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	10	13/05	negative	positive	negative	positive	2,5	Nut protein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13		negative	positive	negative	positive	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	2	30/03/2020	negative	positive	negative	positive	1	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	5	09.04.20	negative	positive	negative	positive	0.3	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	16a		Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
IL	12				
MI-II	14	M2119	recognizes hazelnut proteins	according to manufacturer's instructions	Sample 2: 2,3mg/kg; Sample 4: 4,2mg/kg
RS-F	7	R 6802			B(21,19 m/kg) D(31,24 mg/kg)
RS-F	10	R6802		R-Biopharm extraction buffer / 10 mins/ 60 degrees	
RS-F	13				
SP	2	HU0030010			
SP	5				

5.1.3 ELISA: Macadamia

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	10	12/05	negative	negative	negative	positive	0,3	Nut protein	3M
BF	13		negative	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	negative	negative	negative	positive	0,13	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	negative	negative	negative	positive	10	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	negative	negative	negative	positive	1	Nut, total	IL = Immunolab
IL	15	28.04.20	negative	negative	negative	positive	1	Nut, total	IL = Immunolab
SP	5	09.04.20	negative	negative	negative	positive	0.1	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	14	03.04.20	negative	negative	negative	positive	1	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	10	E96MAC		3M extraction buffer / 25 mins/ 50-60 degrees	
BF	13				
BF	16a		Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
IL	12				
IL	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	143.10ppm detected
SP	5				
SP	14	HU0030013:2	recognizes macada-mia nut protein	according to manufacturer's instructions	Sample 4: >30mg/kg

5.1.4 ELISA: Almond

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	4	01.04.20	positive	positive	negative	negative	0,102	food	AQ
BF	8		positive	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	positive	positive	negative	negative	0,15	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	positive	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ES	13a		positive	positive	negative	negative	0,5	Nut protein	ES = ELISA-Systems
IL	12	23.05.20	positive	positive	negative	negative	0,4	Nut, total	IL = Immunolab
RS-F	7	17.04.20	positive	positive	negative	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13b		positive	positive	negative	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	02.04.20	positive	positive	negative	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	2	30/03/2020	positive	positive	negative	negative	0,4	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	5	09.04.20	positive	positive	negative	negative	0.2	Nut, total	SP = SensiSpec, Eurofins Technologies
VT	10	13/05	positive	positive	negative	negative	2,5	Nut protein	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	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	4	COKAL0748			
BF	8				
BF	16a		Monoclonal antibody-based assay	1:20 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
ES	13a				
IL	12				
RS-F	7	R 6901			A (47,79 mg/kg) B (86,02 mg/kg)
RS-F	13b				
RS-F	14	R6901	recognizes almond proteins	according to manufacturer's instructions	Sample 1, 2: >18mg/kg
SP	2	HU0030001			
SP	5				
VT	10	8440		PBS / 15 mins/ 60 degrees	

5.1.5 ELISA: Brazil Nut

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	10	12/05	positive	positive	negative	negative	1.0	Nut protein	3M
BF	8		positive	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13		positive	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	positive	positive	negative	negative	0,14	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	positive	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
DE	15	28.04.20	positive	positive	negative	negative	1	Nut, total	Demeditec Diagnostics Gmbh
IL	12	23.05.20	positive	positive	negative	negative	1	Nut, total	IL = Immunolab
SP	5	09.04.20	positive	positive	negative	negative	0.2	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	14	06.04.20	positive	positive	negative	negative	4	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	10	E96BZL		3M extraction buffer / 25 mins/ 50-60 degrees	
BF	8				
BF	13				
BF	16a		Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
DE	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample A = 37.21ppm, Sample B = 71.13ppm
IL	12				
SP	5				
SP	14	HU0030018	recognizes Brazil nut proteins	according to manufacturer's instructions	Sample 1, 2: >30mg/kg

5.1.6 ELISA: Pecan

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	10	12/05	negative	positive	positive	*	0,67	Nut protein	3M
BF	8		negative	positive	positive	-	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13		negative	positive	positive	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	negative	positive	positive	negative	0,17	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	negative	positive	positive	negative	5	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	negative	positive	positive	negative	2	Nut, total	IL = Immunolab
SP	5	09.04.20	negative	positive	positive	negative*	0.2	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	14	23.04.20	negative	positive	positive	-	2	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	10	E96PEC		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BF	8				
BF	13				
BF	16a		Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	PC1-EK used for evaluation
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
IL	12				
SP	5				4 ppm identified as CR of Walnut
SP	14	HU0030020	recognizes pecan proteins	according to manufacturer's instructions	Sample 2,3:>50mg/kg; Sample 4: not feasible due to possible cross-reactivity with walnut

5.1.7 ELISA: Pistachio

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	10	12/05	positive	*	positive	negative	1,89	Nut protein	3M
BC	15	13.05.20	positive	positive	positive	negative	1	Nut, total	BC = BioCheck ELISA
BF	8		positive	negative	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13		positive	negative	positive	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	positive	negative	positive	negative	0,12	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	positive	negative	positive	negative	5	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	positive	positive	positive	negative	1	Nut, total	IL = Immunolab
SP	5	09.04.20	positive	negative*	positive	negative	0.13	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	10	E96PST		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BC	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample A = 152.47ppm, Sample B = 13.20ppm, Sample C = 92.18ppm
BF	8				
BF	13				
BF	16a		Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at room temperature	
IL	12				Sample 002 cannot be clearly assessed due to cross-reaction to cashew (12%). Not sure of the result.
SP	5				20 ppm identified as CR of Cashew

5.1.8 ELISA: Walnut

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	4	02.04.20	negative	positive	positive	positive	0,81	food	AQ
AQ	10	21/05	negative	*	*	positive	2.0	Nut protein	Romer
BC	15	28.04.20	negative	positive	positive	positive	2	Nut, total	BC = BioCheck ELISA
BF	8		negative	negative	negative	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	13		negative	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	16a	29/5	negative	negative	negative	positive	0,22	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	16b	29/5	negative	negative	negative	positive	5	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	12	23.05.20	negative	negative	negative	positive	2	Nut, total	IL = Immunolab
SP	2	30/03/2020	negative	positive	positive	positive	2	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	5	09.04.20	negative	negative*	negative*	positive	0.35	Nut, total	SP = SensiSpec, Eurofins Technologies
SP	14	01.04.20	negative	-	-	positive	2	Nut, total	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	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	4	COKAL0948			
AQ	10	COKAL0948		Romer extraction buffer / 15 mins/ 60 degrees	*inconclusive due to cross-reactivity
BC	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample B = 2.89ppm, Sample C = 2.47ppm, Sample D = 126.04ppm
BF	8				
BF	13				
BF	16a		Monoclonal antibody-based assay	1:10 extraction ratio/10 minutes/60C	
BF-LF	16b		Monoclonal antibody-based assay	1:10 extraction ratio/1 minute at 60C	
IL	12				
SP	2	HU0030024			
SP	5				Background signal near LOQ identified as CR of Pecan nut
SP	14	HU0030024	recognizes walnut protein	according to manufacturer's instructions	Sample 4: >20mg/kg; Sample 2 and 3: not feasible due to possible cross-reactivity with pecan nut

5.1.9 PCR: Cashew

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		positive	positive	positive	negative	100	Nut-DNA	MS = Microsynth
SFA	6	01.04.20	negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		negative	positive	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	negative	positive	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	11		negative	positive	negative	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	14	01.04.20	negative	positive	negative	negative	8	Nut-DNA	Internal method
div	17		negative	positive	negative	negative	5	Nut, total	Internal method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	6				
SFA	8				
SFA	12				
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	Weight 2g
div	14			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
div	17		Ana 03	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.10 PCR: Hazelnut

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	14	01.04.20	negative	positive	negative	positive	10	Nut-DNA	ASU = ASU §64 Methode/method
MS	1		negative	negative	negative	negative	100	Nut-DNA	MS = Microsynth
SFA	3	25.03.20	negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	01.04.20	negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	negative	positive	negative	positive	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	11		negative	positive	negative	positive	5	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	17		negative	positive	negative	positive	range 5 to 10	Nut, total	CEN/TC 275/WG 12 N 317

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	14	L44.00-08:20120-01		CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
MS	1			Wizard Resin, qPCR	
SFA	3	S3602	Corylus	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA	6				
SFA	12				
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	weight 2g
div	17		Cor A1	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.11 PCR: Macadamia

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		negative	negative	positive	negative	100	Nut-DNA	MS = Microsynth
SFA	6	01.04.20	negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	negative	negative	negative	positive	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	3	25.03.20	negative	negative	negative	positive	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	11		negative	positive	negative	positive		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	15	07.05.20	negative	negative	negative	positive	1	Nut-DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	17		negative	negative	negative	positive	7 pg DNA	Nut-DNA	Internal method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	6				
SFA	12				
SFA-4p	3	S3403	Macadamia ternifolia	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	Weight 2g
SFA-Q	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample D = 14.33ppm
div	17		Vicilin gene	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.12 PCR: Almond

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	14	01.04.20	positive	positive	negative	negative	40	Nuss-DNA	ASU = ASU §64 Methode/method
MS	1		negative	positive	negative	negative	100	Nut-DNA	MS = Microsynth
SFA	6	01.04.20	positive	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	positive	positive	negative	negative	4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	17		positive	positive	negative	negative	range 5 to 10	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	14	L18.00-20:2014-08		CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
MS	1			Wizard Resin, qPCR	
SFA	6				
SFA	12				
div	17		ns LTP	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.13 PCR: Brazil Nuts

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		negative	negative	negative	negative	100	Nut-DNA	MS = Microsynth
SFA	6	01.04.20	positive	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	positive	positive	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	3	25.03.20	positive	positive	negative	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	11		positive	positive	negative	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	15	22.04.20	positive	positive	negative	negative	1	Nut-DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	14	01.04.20	positive	positive	negative	negative	10	Nut-DNA	Internal method
div	17		positive	positive	negative	negative	not determined	Nut-DNA	J. Verbr. Lebensm. (2014) 9:297-310

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	6				
SFA	12				
SFA-4p	3	S3403	Bertholletia excelsa	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	weight 2g
SFA-Q	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample A = 18.24ppm, Sample B = 14.98ppm
div	14			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
div	17		Albumin 2S	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.14 PCR: Pecan

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		negative	negative	negative	negative	100	Nut-DNA	MS = Microsynth
SFA	6	01.04.20	negative	positive	positive	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	negative	positive	negative	negative	4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	3	25.03.20	negative	positive	positive	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	11		negative	positive	positive	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	14a	01.04.20	negative	positive	positive	negative	5	Nuss-DNA	internal method
div	14b	01.04.20	negative	negative	positive	positive	4	Nut-DNA	internal method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	6				
SFA	12				
SFA-4p	3	S3403	Carya illinoinensis	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	Weight 2g
div	14a			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
div	14b			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / PCR / 45 cycles	Walnut/ Pecan

5.1.15 PCR: Pistachio

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		positive	negative	positive	negative	100	Nut-DNA	MS = Microsynth
SFA	3	25.03.20	positive	negative	positive	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	01.04.20	positive	negative	positive	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	02.04.20	positive	negative	positive	negative	4	Food/Food	SFA
SFA	12	29.05.20	positive	negative	positive	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	11		positive	negative	positive	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	15	07.05.20	positive	negative	positive	negative	1	Nut-DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	14	01.04.20	positive	negative	positive	negative	1	Nut-DNA	internal method
div	17		positive	negative	positive	negative	5	Nut, total	internal method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	3	S3614	Pistacia vera	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA	6				
SFA	7	S3614		CTAB / Kit / real time PCR	
SFA	12				
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	weight 2g
SFA-Q	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample A = 3.53ppm, Sample B = 4.36ppm
div	14			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
div	17		Vicilin gene	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.1.16 PCR: Walnut

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1		negative	negative	negative	negative	100	Nut-DNA	MS = Microsynth
SFA	3	26.03.20	negative	negative	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	01.04.20	negative	negative	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9		negative	negative	negative	positive	2 mg/kg	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	29.05.20	negative	negative	negative	positive	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	11		negative	negative	negative	positive		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	15	07.05.20	negative	negative	negative	positive	1	Nut-DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	14a	01.04.20	negative	negative	negative	positive	5	Nuss-DNA	internal method
div	14b	01.04.20	negative	negative	positive	positive	4	Nut-DNA	internal method
div	17		negative	negative	negative	positive	5	Nut, total	Eur. Food Res. Technol. (2006) 223:373-377

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Resin, qPCR	
SFA	3	S3607	Juglans	Sure Food Prep Advanced Protocol 1 plus 300 µl LB	K01
SFA	6				
SFA	9			Sure Food Prep advanced extraction/PRC RT/45 cycles	
SFA	12				
SFA-ID	11			Maxwell RSC Pure Food GMO Kit	Weight 2g
SFA-Q	15	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	Sample D = 7.10ppm
div	14a			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45 cycles	
div	14b			CTAB / Amylase / Proteinase K / Promega Wizard DNA CleanUp / PCR / 45 cycles	Walnut/ Pecan
div	17		jug R2	Extraction: kit Food Macherey Nagel / 40 Cycles	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA -ptALS1 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	26,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	82	32,7
2	4,99	65	26,1
3	5,06	87	34,4
4	5,02	70	27,9
5	5,01	70	27,9
6	5,02	67	26,7
7	5,04	67	26,6
8	4,98	76	30,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	73,0	Particles
Standard deviation	7,78	Particles
χ^2 (CHI-Quadrat)	5,81	
Probability	**56**	%
Recovery rate	111	%

Normal distribution		
Number of samples	8	
Mean	29,1	mg/kg
Standard deviation	3,10	mg/kg
rel. Standard deviaton	10,7	%
Horwitz standard deviation	9,6	%
HorRat-value	**1,1**	
Recovery rate	111	%

Microtracer Homogeneity Test
DLA -ptALS1 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	22,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	61	24,5
2	5,01	64	25,5
3	4,99	62	24,8
4	5,00	65	26,0
5	5,05	67	26,5
6	5,06	66	26,1
7	4,98	56	22,5
8	5,02	73	29,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	64,2	Particles
Standard deviation	4,72	Particles
χ^2 (CHI-Quadrat)	2,43	
Probability	**93**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	25,6	mg/kg
Standard deviation	1,88	mg/kg
rel. Standard deviaton	7,3	%
Horwitz standard deviation	9,8	%
HorRat-value	**0,7**	
Recovery rate	113	%

Microtracer Homogeneity Test
DLA -ptALS1 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	20,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	59	23,5
2	4,97	53	21,3
3	5,00	41	16,4
4	5,01	51	20,4
5	5,01	48	19,2
6	4,97	50	20,1
7	4,99	47	18,8
8	4,99	39	15,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	48,5	Particles
Standard deviation	6,37	Particles
χ^2 (CHI-Quadrat)	5,86	
Probability	**56**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	19,4	mg/kg
Standard deviation	2,55	mg/kg
rel. Standard deviaton	13,1	%
Horwitz standard deviation	10,2	%
HorRat-value	**1,3**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA -ptALS1 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	22,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	61	24,4
2	5,02	56	22,3
3	5,01	71	28,3
4	5,02	63	25,1
5	5,03	67	26,6
6	4,96	57	23,0
7	5,05	61	24,2
8	4,99	64	25,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	62,5	Particles
Standard deviation	4,89	Particles
χ^2 (CHI-Quadrat)	2,68	
Probability	**91**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	24,9	mg/kg
Standard deviation	1,95	mg/kg
rel. Standard deviaton	7,8	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,8**	
Recovery rate	110	%

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 ptALS1 (2020)***
PT name	***Allergen-Screening I - 4 Samples qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and 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 (PT period), cooled 2 - 10°C (long term)*
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***
Last Deadline	***the latest June 19th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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