Evaluation Report
proficiency test

DLA ptALS1 (2021)

Allergen-Screening I:

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

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

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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: 4 August 2021
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.

Content

1. Introduction

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 1-10% of the allergenic ingredients concerned.
The respective raw materials for the nuts used were commercial nut butters or flours (coconut) 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)	74 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (75% - 90%) - Sodium chloride (0,0% - 85%) - Sodium sulfate (6,1% - 14%) - Silicon dioxide (1% - 10%) - Allergens (1,1% - 10% each)	0,05 - 0,6 %

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Cashew (Protein 18,4%) - commercial nut butter	negative	positive (47)	negative	negative
Coconut (Protein 18,8%) - commercial flour	positive (48)	negative	positive (150)	negative
Hazelnut (Protein 15,9%) - commercial nut butter	positive (36)	negative	positive (72)	negative
Macadamia (Protein 9,4%) - Nuts, crushed	positive (53)	negative	negative	positive (32)
Almond (Protein 19,6%) - commercial nut butter	negative	positive (53)	negative	positive (89)
Brazil nut (Protein 14,8%) - Nuts, crushed	negative	negative	positive (66)	positive (38)
Pecan (Protein 12,2%) - Nuts, crushed	positive (62)	negative	positive (38)	negative
Pistachio (Protein 25,6%) - Nuts, crushed	positive (54)	negative	negative	negative
Walnut (Protein 13,9%) - Nuts, crushed	negative	positive (52)	negative	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	positive	negative	positive
AgraStrip® Cashew/Pistachio	positive	positive	weakly positive	weakly positive
AgraStrip® Hazelnut	positive	negative	positive	negative
AgraStrip® Macadamia	positive	negative	negative	positive
AgraStrip® Brazil Nut	negative	negative	positive	positive
AgraStrip® Walnut**	positive	positive	weakly positive	negative

* 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 82%, 92%, 74% and 43%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 0,93, 0,78, 0,89 and 1,2, 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. 0,30 (15°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 2021. The testing method was optional. The tests should be finished at May 07th 2021 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, Coconut, 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 23 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. Next generation sequencing methods are evaluated as DNA-based techniques along with PCR methods.

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		
22	*	positive	negative	negative	3/3 (100%)	3/3 (100%)	3M	*inconclusive due to cross-reactivity
4	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
8	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
20	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
19	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	6	0	0
Number negative	5	0	6	6
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

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 sample 2 (47 mg/kg cashew).

One participant indicated cross-reactivity to pistachio for sample 1.

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		
13	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	GI	
3	positive	positive	positive	positive	1/4 (25%)	1/4 (25%)	MS	
17	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
14	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
16	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
6	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
20	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
21	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	15	1	1
Number negative	14	0	14	14
Percent positive	7	100	7	7
Percent negative	93	0	93	93
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 sample 2 (47 mg/kg cashew).

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		
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
20	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
22	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
18	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	
19	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	

	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:

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 1 (36 mg/kg hazelnut) and 3 (72 mg/kg hazelnut).

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		
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	positive	negative	3/4 (75%)	3/4 (75%)	MS	
17	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	NGS	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
16	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	SFA-4P	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
20	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	div	
21	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	13	1	14	1
Number negative	2	14	1	14
Percent positive	87	7	93	7
Percent negative	13	93	7	93
Consensus value	positive	negative	positive	negative
Spiking	positive	negative	positive	negative

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
MS = Microsynth
NGS = Next Generation Sequencing
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 1 (36 mg/kg hazelnut) and 3 (72 mg/kg hazelnut).

One participant has not detected a positive sample with the NGS method. Another participant identified only the higher-spiked sample 3 as positive using the MS method.

4.3 Proficiency Test Coconut

4.3.1 ELISA-Results: Coconut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
19	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The results of the two participants are in qualitative agreement with the spiking of samples 1 (48 mg/kg coconut) and 3 (150 mg/kg coconut).

4.3.2 PCR-Results: Coconut

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	3/3 (100%)	4/4 (100%)	MS	
17	negative	negative	positive	negative	3/3 (100%)	3/4 (75%)	NGS	sample with lower amount not detected
13	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

NGS = Next Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 2, 3 and 4 are in qualitative agreement with the spiking of sample 3 (150 mg/kg coconut). Inconsistent results were obtained for sample 1 (containing lower amounts of coconut), thus no consensus value ≥75% could be determined.

4.4 Proficiency Test Macadamia

4.4.1 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		
22	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	3M	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
11	-	negative	negative	positive	3/3 (100%)	3/3 (100%)	IL	sample 1 positive, possible cross-reaction w ith hazelnut
20	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
19	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples 1 (53 mg/kg macadamia) and 4 (32 mg/kg macadamia).
One participant indicated cross-reactivity to hazelnut for sample 1.

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

4.4.2 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		
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample identified
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
10	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
13	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
20	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
21	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 1 (53 mg/kg macadamia) and 4 (32 mg/kg macadamia).

4.5 Proficiency Test Almond

4.5.1 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		
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ES	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
20	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
18	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	
19	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	
22	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	8	0	8
Number negative	8	0	8	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:

AQ = AgraQuant, RomerLabs

ES = ELISA-Systems

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 2 (53 mg/kg almond) and 4 (89 mg/kg almond).

4.5.2 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		
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample identified
17	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
5	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
16	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
15	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
20	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
21	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	13	0	13
Number negative	14	1	14	1
Percent positive	0	93	0	93
Percent negative	100	7	100	7
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 2 (53 mg/kg almond) and 4 (89 mg/kg almond).

4.6 Proficiency Test Brazil Nut

4.6.1 ELISA-Results: Brazil Nut

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		
22	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	3M	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BF	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BF	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
19	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	5	5
Number negative	5	5	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:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples 3 (66 mg/kg Brazil Nut) and 4 (38 mg/kg Brazil Nut).

4.6.2 PCR-Results: Brazil Nut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MS	
17	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	NGS	no positive sample identified
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
20	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
21	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	11	11
Number negative	12	12	1	1
Percent positive	0	0	92	92
Percent negative	100	100	8	8
Consensus value	negative	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 3 (66 mg/kg Brazil Nut) and 4 (38 mg/kg Brazil Nut).

4.7 Proficiency Test Pecan

4.7.1 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		
22	positive	*	positive	negative	3/3 (100%)	3/3 (100%)	3M	*inconclusive due to cross-reactivity
4	positive	positive	positive	negative	3/3 (100%)	3/3 (100%)	BF	** Pecan BioFront kit declares cross-reactivity with walnut. Sample 2 is positive for walnut and the result for pecan is positive
8	positive	positive	positive	negative	3/3 (100%)	3/3 (100%)	BF	
19	positive	positive*	positive	negative	3/3 (100%)	3/3 (100%)	SP	*Weakly positive, due to cross-reaction of walnut

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

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, BioFront Technologies

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 1 (62 mg/kg pecan) and 3 (38 mg/kg pecan).

Several participants obtained a positive result for sample 2 (without addition of pecan) and indicated cross-reactivity to walnut.
Possible cross-reactivities should be documented in the manufacturer's test kit information.

4.7.2 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		
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample identified
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	NGS	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
20	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
21	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	1	10	1	10
Percent positive	90	0	90	0
Percent negative	10	100	10	100
Consensus value	positive	negative	positive	negative
Spiking	positive	negative	positive	negative

Methods:

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 1 (62 mg/kg pecan) and 3 (38 mg/kg pecan).

4.8 Proficiency Test Pistachio

4.8.1 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		
22	positive	*	negative	negative	3/3 (100%)	3/3 (100%)	3M	*inconclusive due to cross-reactivity
4	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	BF	
8	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	BF	
11	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	IL	Sample 2 positive, assumed cross-reaction with cashew - confirmed as cross-contamination in PCR, therefore considered as negative.
20	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	IL	
19	positive	positive*	negative	negative	3/3 (100%)	3/4 (75%)	SP	*Weakly positive, due to cross-reaction of cashew

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	6	1	0	0
Number negative	0	3	6	6
Percent positive	100	25	0	0
Percent negative	0	75	100	100
Consensus value	positive	none	negative	negative
Spiking	positive	negative	negative	negative

Methods:

3M = 3M Protein ELISA Kit

BF = MonoTrace ELISA, 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 sample 1 (54 mg/kg pistachio).

Several participants obtained a positive result for sample 2 (without addition of pistachio) and indicated cross-reactivity to cashew.
Possible cross-reactivities should be documented in the manufacturer's test kit information.

4.8.2 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		
9	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	GI	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	MS	
17	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
16	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
1	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
10	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
20	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	15	0	0	0
Number negative	0	15	15	15
Percent positive	100	0	0	0
Percent negative	0	100	100	100
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 sample 1 (54 mg/kg pistachio).

4.9 Proficiency Test Walnut

4.9.1 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		
22	*	positive	*	negative	2/2 (100%)	2/2 (100%)	AQ	*inconclusive due to cross-reactivity
4	negative	positive	negative	negative	2/2 (100%)	4/4 (100%)	BF	
12	positive	positive	positive	negative	2/2 (100%)	2/4 (50%)	BS	in samples 1 and 3 possible cross-reaction w ith cashew and pecan.
11	negative	positive	negative	negative	2/2 (100%)	4/4 (100%)	IL	
20	positive	positive	positive	negative	2/2 (100%)	2/4 (50%)	IL	
19	positive*	positive	positive*	negative	2/2 (100%)	2/4 (50%)	SP	*Weakly positive, due to cross-reaction of pecan

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	2	6	2	0
Number negative	2	0	2	6
Percent positive	50	100	50	0
Percent negative	50	0	50	100
Consensus value	none	positive	none	negative
Spiking	negative	positive	negative	negative

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

BS = BioSystems

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of the results for samples 2 and 4 are in qualitative agreement with the spiking of the sample 2 (52 mg/kg walnut).

Several participants obtained positive results for samples 1 and 3 (without addition of walnut) and indicated cross-reactivity to pecan. Possible cross-reactivities should be documented in the manufacturer's test kit information.

4.9.2 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		
13	negative	positive	negative	positive	3/4 (75%)	3/4 (75%)	ASU	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	MS	no positive sample identified
17	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
12	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
16	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
6	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
10	positive	positive	positive	negative	2/4 (50%)	2/4 (50%)	div	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
20	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
21	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	14	1	1
Number negative	14	1	14	14
Percent positive	7	93	7	7
Percent negative	93	7	93	93
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next Generation Sequencing

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 the results are in qualitative agreement with the spiking of the sample 2 (52 mg/kg walnut).

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	22	27/04	*	positive	negative	negative	0,9	Nut protein	3M
BF	4	19.03.21	negative	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	8		negative	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
RS-F	11	29.03.21	negative	positive	negative	negative	5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	20		negative	positive	negative	negative	1,2		RS = Ridascreen®, R-Biopharm
SP	19	15.03.21	negative	positive	negative	negative	2	Nut, total	SENSISpec

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	22	E96CHW		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BF	4				
BF	8				
RS-F	11	R6872			
RS-F	20	R6872			Sample 2 is outside the Measuring range.
SP	19				

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
RS-F	4	17.03.21	positive	negative	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11	23.03.21	positive	negative	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12	22.03.21	positive	negative	positive	negative	2,5	hazelnut, total	RS = Ridascreen®, R-Biopharm
RS-F	20		positive	negative	positive	negative	1,2		RS-F= Ridascreen® Fast, R-Biopharm
RS-F	22	08/04	positive	negative	positive	negative	2,5	Nut protein	RS-F= Ridascreen® Fast, R-Biopharm
SP	18		positive	negative	positive	negative			
SP	19	15.03.21	positive	negative	positive	negative	1	Nut, total	SENSISpec

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	
RS-F	4				
RS-F	11	R6802			
RS-F	12	R6802	recognizes hazelnut antigens		Sample 1: Count >10 (>20); Sample 3: >10 (> 20)
RS-F	20	R6802			Sample 1 and sample 3 are outside the measuring range.
RS-F	22	R6802		R-Biopharm extraction buffer / 10 mins/ 60 degrees	
SP	18				SENSISpec kit
SP	19				

5.1.3 ELISA: Coconut

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	8		positive	negative	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
SP	19	15.03.21	positive	negative	positive	negative	2	Nut, total	SENSISpec

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	8				
SP	19				

5.1.4 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	22	26/04	positive	negative	negative	positive	0,3	nut protein	3M
BF	4	17.03.21	positive	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	8		positive	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	11	25.03.21	-	negative	negative	positive	2		IL = Immunolab
IL	20		positive	negative	negative	positive	1		IL = Immunolab
SP	19	15.03.21	positive	negative	negative	positive	1	Nut, total	SENSISpec

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	22	E96MAC		3M extraction buffer / 25 mins/ 50-60 degrees	
BF	4				
BF	8				
IL	11	MAC-E01/E04			Sample 1 positive, possible cross-reaction with hazelnut
IL	20	MAC-E01			Sample 1 and 4 are outside the measuring range
SP	19				

5.1.5 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	11	25.03.21	negative	positive	negative	positive	0,4	Nut, total	AQ = AgraQuant, RomerLabs
ES	4	19.03.21	negative	positive	negative	positive	0,5	Nut protein	ES = ELISA-Systems
RS-F	4	12.04.21	negative	positive	negative	positive	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12	23.03.21	negative	positive	negative	positive	2,5	Almond, total	RS = Ridascreen®, R-Biopharm
RS-F	20		negative	positive	negative	positive	1,2		RS = Ridascreen®, R-Biopharm
SP	18		negative	positive	negative	positive			
SP	19	15.03.21	negative	positive	negative	positive	0,4	Nut, total	SENSISpec
VT	22	07/05	negative	positive	negative	positive	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	11	COKAL0748			
ES	4				
RS-F	4				
RS-F	12	R6901	detects antigens of the almond	-	Sample 2: Number > 10 (>20); Sample 4: Number > 10 (>20)
RS-F	20	R6901			Sample 2 and sample 4 are outside the measuring range.
SP	18				SENSISpec kit
SP	19				
VT	22	8440		PBS/15 min/60 Degrees	

5.1.6 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	22	27/04	negative	negative	positive	positive	1,0	Nut protein	3M
BF	4	22.03.21	negative	negative	positive	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	8		negative	negative	positive	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	11	08.04.21	negative	negative	positive	positive	4	Nut, total	IL = Immunolab
SP	19	15.03.21	negative	negative	positive	positive	1	Nut, total	SENSISpec

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	22	E96BZL		3M extraction buffer / 25 mins/ 50-60 degrees	
BF	4				
BF	8				
IL	11	PAR-E01/E04			
SP	19				

5.1.7 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	22	27/04	positive	*	positive	negative	0,7	Nut protein	3M
BF	4	17.03.21	positive	positive	positive	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	8		positive	positive	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
SP	19	15.03.21	positive	positive*	positive	negative	2	Nut, total	SENSISpec

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	22	E96PEC		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BF	4				** Pecan BioFront kit declares cross-reactivity to walnut. Sample 2 is positive for walnut and the result for pecan is positive
BF	8		The kit declares cross-reactivity to walnut		
SP	19			*Weakly positive, due to cross-reaction of walnut	

5.1.8 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	22	07/04	positive	*	negative	negative	1,0	Nut protein	3M
BF	4	30.03.21	positive	negative	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	8		positive	negative	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	11	22.03.21	positive	negative	negative	negative	2,8	Nut, total	IL = Immunolab
IL	20		positive	positive	negative	negative	1		RS = Ridascreen®, R-Biopharm
SP	19	15.03.21	positive	positive*	negative	negative	1	Nut, total	SENSISpec

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	22	E96PST		3M extraction buffer / 25 mins/ 50-60 degrees	*inconclusive due to cross-reactivity
BF	4				
BF	8				
IL	11	PIS-E01/E04			Sample 2 positive, assumed cross-reaction with cashew - confirmed as cross-contamination in PCR, therefore considered as negative.
IL	20	PIS-E01			Sample 2 out of measuring range
SP	19			*Weakly positive, due to cross-reaction of cashew	

5.1.9 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	22	06/05	*	positive	*	negative	2	Nut protein	Romer
BF	4	19.03.21	negative	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BS	12	23.03.21	positive	positive	positive	negative	2	Nut, total	Biosystem
IL	11	24.03.21	negative	positive	negative	negative	5	Nut, total	IL = Immunolab
IL	20		positive	positive	positive	negative	1		RS = Ridascreen®, R-Biopharm
SP	19	15.03.21	positive*	positive	positive*	negative	2	Nut, total	SENSISpec

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	
AQ	22	COKAL0948		Romer extraction buffer/ 15 min/ 60 degrees	*inconclusive due to cross-reactivity
BF	4				
BS	12	14130	-	-	Sample 1: Counts 2,7; Sample 2: >6,0 (> 60,0);Sample 3: counts 2,3. For Sample 1 and 3 possible cross-reactions w ith cashew and pecan
IL	11	WAL-E01/E04			
IL	20	WAL-E01			Sample 2 out of measuring range
SP	19			*Weakly positive, due to cross-reaction of pecan	

5.1.10 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
ASU	13	22.3.+15.4	negative	positive	negative	negative	10	Nut, total	ASU = ASU §64 Methode/method
GI	9		negative	positive	negative	negative	0,4	protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	positive	positive	positive	positive	10	Nut-DNA	MS = Microsynth
NGS	17		negative	positive	negative	negative	NA	DNA portion	NGS
SFA	5		negative	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	16.03.21	negative	positive	negative	negative	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	14	16.06.21	negative	positive	negative	negative	1	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		negative	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	1	25.03.21	negative	positive	negative	negative	ca. 100	Nut-DNA	
div	6		negative	positive	negative	negative	5	Nut, total	house method
div	10		negative	positive	negative	negative		Allergen-DNA	house method
div	11	19.03.21	negative	positive	negative	negative	n.a	Nut-DNA	Köppel et al, „Two quantitative hexaplex real-time PCR systems for the detec-tion and quantification of DNA from twelve allergens in food“, Eur Food Res Technol, 2012
div	20		negative	positive	negative	negative	8µg/kg		
div	21		negative	positive	negative	negative			house 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	
ASU	13	Lifeprint	ITS2	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	4-plex
GI	9	PCAS 0050		Extraction with Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA extraction: Nucleo Spin Food kit – MN	
SFA	5				
SFA	7	S3615	Anacardium occidentale	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K02
SFA	8				
SFA	14	S3615	according Testkit manufactor	As Per Kit Instructions	
SFA	16		Cashew -specific DNA	CTAB + post-cleaning with colum / Realtime PCR	
div	1		F: 5'-cca tga agt gaa gca gta g-3', R: 5'-gac tct gtg ctg att cta cta ctc-3'	house method (Triplex)	Limit of detection: 0.02 ng/ul Nut-DNA
div	6		Ana 03	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				
div	11			Macherey & Nagel NucleoSpin Food Kit	
div	20				
div	21			house method	

5.1.11 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	13	22.3.+15.4	positive	negative	positive	negative	5	Nut, total	ASU = ASU §64 Methode/method
GI	9		positive	negative	positive	negative	0,4	protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	positive	negative	10	Nut-DNA	MS = Microsynth
NGS	17		negative	negative	negative	negative	NA	DNA portion	NGS
SFA	5		positive	negative	positive	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	24.03.21	positive	negative	positive	negative	0,4	hazelnut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4P	7	17.03.21	positive	negative	positive	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-4P	16		positive	positive	positive	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	24.03.21	positive	negative	positive	negative	ca. 100	Nut-DNA	
div	6		positive	negative	positive	negative	range 5 to 10	Nut, total	CEN/TC 275/WG 12 N 317
div	10		positive	negative	positive	negative		Allergen-DNA	house method
div	11	19.03.21	positive	negative	positive	negative	n.a	Nut-DNA	Köppel et al, „Two quantitative hexaplex real-time PCR systems for the detec-tion and quantification of DNA from twelve allergens in food“, Eur Food Res Technol, 2012
div	15	01.04.21	positive	negative	positive	negative	0,4	hazelnut-DNA	LifePrint: Detection of hazelnut-DNA
div	20		positive	negative	positive	positive	8µg/kg		
div	21		positive	negative	positive	negative			house 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	
ASU	13	Lifeprint	ITS2	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	4-plex
GI	9	PHAZ 0050		Extraction mit Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA extraction :Nucleo Spin Food kit - MN	
SFA	5				
SFA	12	S3602			
SFA-4P	7	S3402	Corylus	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K01
SFA-4P	16		Hazenut-specific DNA	CTAB + post-cleaning with colum / Multiplex Realtime PCR	
div	1		F: 5'-ggc aag ttc gtg agc agg ttc -3', R: 5'-ctt tcg gaa tag tca cag tga g -3'	house method (Triplex)	Limit of Detection:. 0.02 ng/ul Nut-DNA
div	6		Cor A1	Extraktion: kit Food Macherey Nagel / 40 Cycles	
div	10				traces for sample 1
div	11			Macherey & Nagel NucleoSpin Food Kit	
div	15	MI763 Rev.0		Extraction> Nucleo Spin Food/TANBeaad Nukleinsäureextraktor; Real Time PCR> QuantStudio5/7500 Fast/CFX-96 deep well	
div	20				
div	21			house method	

5.1.12 PCR: Coconut

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	3	30.03.21	positive	negative	positive	negative	10	Nut-DNA	MS = Microsynth
NGS	17		negative	negative	positive	negative	NA	DNA portion	NGS
div	13	25.3.+28.4	positive	negative	positive	negative	250	Nut, total	biomers

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	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplast genes	DNA Extraction: Nucleo Spin Food kit – MN	
div	13	Impetus	Actin-Gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	

5.1.13 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
GI	9		positive	negative	negative	positive	0,4	protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	negative	negative	10	Nut-DNA	MS = Microsynth
NGS	17		positive	negative	negative	positive	NA	DNA portion	NGS
SFA	5		positive	negative	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4P	7	16.03.21	positive	negative	negative	positive	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	23.03.21	positive	negative	negative	positive	Approx. 100	Nut-DNA	
div	6		positive	negative	negative	positive	7 pg DNA	Nut-DNA	house method
div	10		positive	negative	negative	positive		Allergen-DNA	house method
div	13	22.3.+19.4	positive	negative	negative	positive	40	Nut, total	biomers
div	20		positive	negative	negative	positive	8µg/kg		
div	21		positive	negative	negative	positive			house 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	
GI	9	PMAC 0050		Extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA Extraction: Nucleo Spin Food kit - MN	
SFA	5				
SFA-4P	7	S3403	Macadamia ternifolia	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K02
div	1		F: 5'-acg aga acc tgc tgc ttt ttg -3', R: 5'-tct ccc cgc gag gaa gtt -3'	house method (Triplex)	Limit of Detection approx. 0.02 ng/ul Nut DNA
div	6		Vicilin gene	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				
div	13	Brezna et al. 2010	vicilin precursor Gene	Maxw ell® RSC PureFood GMO and Authentication Kit, Promega	
div	20				
div	21			house method	

5.1.14 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	13	22.3.+19.4	negative	positive	negative	positive	40	Nut, total	ASU = ASU §64 Methode/method
GI	9		negative	positive	negative	positive	0,4	protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	negative	negative	10	Nut-DNA	MS = Microsynth
NGS	17		negative	positive	negative	positive	NA	DNA	NGS
SFA	5		negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	17.03.21	negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11	17.03.21	negative	positive	negative	positive	1	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		negative	positive	negative	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	1	24.03.21	negative	positive	negative	positive	ca. 100	Nut-DNA	
div	6		negative	positive	negative	positive	Range 5 to 10	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310
div	10		negative	positive	negative	positive		Allergen-DNA	house method
div	15	01.04.21	negative	positive	negative	positive	0,4	Almond-DNA	LifePrint: detection of Almond DNA
div	20		negative	positive	negative	positive	8µg/kg		
div	21		negative	positive	negative	positive			house 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	
ASU	13	L 18.00-20	non specific lipid transfer protein	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
GI	9	PALM0050		Extraction with Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA Extraction: Nucleo Spin Food kit - MN	
SFA	5				
SFA	7	S3604	Prunus dulcis	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K01
SFA	11			Macherey & Nagel NucleoSpin Food Kit	
SFA	16		Almond-specific DNA	CTAB + Post-cleaning via column / Realtime PCR	
div	1		F: 5'-cct agc gga gga tcc atc atc -3', R: 5'-gta ggt ctc aat gag ctt gaa gag -3'	house method (Triplex)	Limit of Detection: approx. 0.02 ng/ul Nut DNA
div	6		PRU AV1	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				
div	15	MI762 Rev.1		Extraction> Nucleo Spin Food/TANBeaad Nucleic acid extraction Real Time PCR> QuantStudio5/7500 Fast/CFX-96 deep well	
div	20				
div	21			house method	

5.1.15 PCR: 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
ASU	11	18.03.21	negative	negative	positive	positive	10	Nut-DNA	ASU L 18.00-21 (2014-08)
ASU	13	23.3.+20.4	negative	negative	positive	positive	40	Nut, total	ASU = ASU §64 Methode/method
GI	9		negative	negative	positive	positive	0,4	Protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	positive	positive	10	Nut-DNA	MS = Microsynth
NGS	17		negative	negative	negative	negative	NA	DNA portion	NGS
SFA	5		negative	negative	positive	positive	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4P	7	16.03.21	negative	negative	positive	positive	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	23.03.21	negative	negative	positive	positive	approx. 100	Nut-DNA	
div	6		negative	negative	positive	positive	4,5pg DNA	Nut-DNA	J. Verbr. Lebensm. (2014) 9:297-310
div	10		negative	negative	positive	positive		Allergen-DNA	house method
div	20		negative	negative	positive	positive	8µg/kg		
div	21		negative	negative	positive	positive			house 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	
ASU	11	ASU L 18.00-21 (2014-08)		Macherey & Nagel NucleoSpin Food Kit	
ASU	13	L 18.00-21	Ber e 1 Gene	Maxw ell® RSC PureFood GMO and Authentication Kit, Promega	
GI	9	PBRAZ 0050		Extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA Extraction: Nucleo Spin Food kit – MN	
SFA	5				
SFA-4P	7	S3403	Bertholletia excelsa	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K02
div	1		F: 5'-tgc aac ctc agt ccc atg ag-3', R: 5'-tgg cta gtg gca gat tca gaa c-3'	house method (Triplex)	Limit of Detection: approx. 0.02 ng/ul Nut DNA
div	6		Albumin 2S	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				
div	20				
div	21			house method	

5.1.16 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
GI	9		positive	negative	positive	negative	0,4	Protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	negative	negative	10	Nut-DNA	MS = Microsynth
NGS	17		positive	negative	positive	negative	NA	DNA portion	NGS
SFA	5		positive	negative	positive	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14	16.03.21	positive	negative	positive	negative	1	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4P	7	16.03.21	positive	negative	positive	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	23.03.21	positive	negative	positive	negative	ca. 100	Nut-DNA	
div	13	25.3.+19.4	positive	negative	positive	negative	40	Nut, total	biomers
div	20		positive	negative	positive	negative	8µg/kg		
div	21		positive	negative	positive	negative			house 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	
GI	9	PPEC 0050		Extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA Extraction: Nucleo Spin Food kit – MN	
SFA	5				
SFA	14	S3618	As Per Kit Instructions	As Per Kit Instructions	
SFA-4P	7	S3403	Carya illinoinensis	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K02, QE to scale bark hickory (Carya ovata) 100 %.
div	1		F: 5'-ccg cga aga gaa agc aga g -3', R: 5'-tca tgt ctc gac ctg agt cc-3'	house method (Triplex)	Limit of detection: approx. 0.02 ng/ul Nut DNA
div	13	Brezna et al., Eur Food Res Technol 2007 DOI 10.1007/s00217-007-0639-3	pecan putative vicilin-like seed storage protein gene	Maxw ell® RSC PureFood GMO and Authentication Kit, Promega	
div	20				
div	21			house method	

5.1.17 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	positivee / negative	positivee / negative	positivee / negative	positivee / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
GI	9		positive	negative	negative	negative	0,4	Protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	positive	negative	negative	negative	10	Nut-DNA	MS = Microsynth
NGS	17		positive	negative	negative	negative	NA	DNA portion	NGS
SFA	2	22.03.21	positive	negative	negative	negative	4		SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		positive	negative	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	17.03.21	positive	negative	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		positive	negative	negative	negative	0,4	Nut-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		positive	negative	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	1	25.03.21	positive	negative	negative	negative	ca. 100	Nut-DNA	
div	6		positive	negative	negative	negative	5	Nut, total	house method
div	10		positive	negative	negative	negative		Allergen-DNA	house method
div	11	19.03.21	positive	negative	negative	negative	n.a	Nut-DNA	Köppel et al, „Two quantitative hexaplex real-time PCR systems for the detection and quantification of DNA from twelve allergens in food“, Eur Food Res Technol, 2012
div	13	25.3.+19.4	positive	negative	negative	negative	5	Nut, total	biomers
div	15	01.04.21	positive	negative	negative	negative	0,4	Pistachio-DNA	LifePrint: Detection of pistachio DNA
div	20		positive	negative	negative	negative	80µg/kg		

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	
GI	9	PPIST 0050		Extraction with Simplex Easy Spin Food Kit/GEN-IAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplast genes	DNA extraction :Nucleo Spin Food kit - MN	
SFA	2	S3614		CTAB / Kit / real time PCR	
SFA	5				
SFA	7	S3614	Pistacia vera	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K01
SFA	8				
SFA	16		Pistachio specific DNA	CTAB + Post cleaning via column / Realtime PCR	
div	1		F: 5'-cga gta tca gaa ccg att cag tgt-3', R: 5'-cca gaa gca acg gtg aca aa-3'	house method (Triplex)	Detection limit: approx. 0.02 ng/ul nut DNA
div	6		Vicilin-gene	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				
div	11			Macherey & Nagel NucleoSpin Food Kit	
div	13	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	MI417 Rev.2		Extraction> Nucleo Spin Food/TANBeaad Nucleic acid extractor Real Time PCR> QuantStudio5/7500 Fast/CFX-96 deep well	
div	20				

5.1.18 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	positivee / negativee	positivee / negativee	positivee / negativee	positivee / negativee	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	13	22.3.+15.4	negative	positive	negative	positive	5	Nut, total	ASU = ASU §64 Methode/method
GI	9		negative	positive	negative	negative	0,4	Protein	GI = GEN-IAL First Allergen
MS	3	30.03.21	negative	negative	negative	negative	10	Nut-DNA	MS = Microsynth
NGS	17		negative	positive	negative	negative	NA	DNA portion	NGS
SFA	2	22.03.21	negative	positive	negative	negative	4		SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	24.03.21	negative	positive	negative	negative	0,4	Nut, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4P	7	17.03.21	negative	positive	negative	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-4P	16		negative	positive	negative	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	24.03.21	negative	positive	negative	negative	approx. 100	Nut-DNA	
div	6		negative	positive	negative	negative	5	Nut, total	Eur. Food Res. Technol. (2006) 223:373-377
div	10		positive	positive	positive	negative		Allergen-DNA	house method
div	11	18.03.21	negative	positive	negative	negative	n.a	Nut, total	Köppel et al, „Two quantitative hexaplex real-time PCR systems for the detec-tion and quantification of DNA from twelve allergens in food“, Eur Food Res Technol, 2012
div	20		negative	positive	negative	negative	8µg/kg		
div	21		negative	positive	negative	negative			house 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	
ASU	13	AGES	Chloroplast DNA	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	4-plex
GI	9	PWAL 0050		Extraction with Simplex Easy Spin Food Kit/GENIAL	
MS	3			Wizard/ Realtime	
NGS	17		Nuclear and chloroplat genes	DNA Extraction: Nucleo Spin Food kit - MN	
SFA	2	S3607		CTAB / Kit / real time PCR	
SFA	5				
SFA	12	S3607	-		
SFA-4P	7	S3402	Juglans	Sure Food Prep Advanced Protokoll 1 zzgl. 200 µl LB	K01
SFA-4P	16		Walnut-specific DNA	CTAB + Post-cleaning via column / Multiplex Realtime PCR	
div	1		F: 5'-gcg cag aga aag cag ag -3', R: 5'-ctc atg tct cga cct aat gct -3'	house method (Triplex)	Detection limit: approx. 0.02 ng/ul nut DNA
div	6		jug R2	Extraction: kit Food Macherey Nagel / 40 Cycles	
div	10				Traces in sample 3
div	11			Macherey & Nagel NucleoSpin Food Kit	
div	20				
div	21			house method	

5.1.19 PCR: 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
	15	01.04.21	positive	positive	positive	positive	0,4	Nut-DNA	SPECIALfinder MC Tree Nuts and Peanut MultiSCREEN Kit - Generon

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	
	15	MI776 Rev.1		Extraction> Nucleo Spin Food/TANBeaad Nucleic acid extractor Real Time PCR> QuantStudio5/7500 Fast/CFX-96 deep w ell	

5.1.20 PCR: Peanut

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
	16		negative	negative	negative	negative	0,4	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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	
	16		Peanut-specific DNA	CTAB + post-purification via column / multiplex real-time PCR	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA -ptALS1 Sample 1

Weight whole sample	1,03	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	25,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	60	24,0
2	5,04	61	24,2
3	4,98	63	25,3
4	5,02	60	23,9
5	4,99	64	25,7
6	4,99	57	22,8
7	5,05	57	22,6
8	4,97	74	29,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	62,0	Particles
Standard deviation	5,70	Particles
χ^2 (CHI-Quadrat)	3,67	
Probability	**82**	%
Recovery rate	98	%

Normal distribution		
Number of samples	8	
Mean	24,8	mg/kg
Standard deviation	2,28	mg/kg
rel. Standard deviaton	9,2	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,93**	
Recovery rate	98	%

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	28,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	69	27,7
2	5,04	67	26,6
3	4,98	64	25,7
4	4,95	70	28,3
5	4,99	62	24,8
6	4,96	66	26,6
7	4,98	72	28,9
8	5,02	57	22,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,9	Particles
Standard deviation	4,99	Particles
χ^2 (CHI-Quadrat)	2,65	
Probability	**92**	%
Recovery rate	92	%

Normal distribution		
Number of samples	8	
Mean	26,4	mg/kg
Standard deviation	2,00	mg/kg
rel. Standard deviaton	7,6	%
Horwitz standard deviation	9,8	%
HorRat-value	**0,78**	
Recovery rate	92	%

Microtracer Homogeneity Test
DLA -ptALS1 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	35,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	95	37,7
2	4,99	86	34,5
3	5,00	97	38,8
4	5,01	98	39,1
5	5,03	86	34,2
6	4,99	83	33,3
7	4,99	81	32,5
8	5,03	101	40,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	90,9	Particles
Standard deviation	7,50	Particles
χ^2 (CHI-Quadrat)	4,33	
Probability	**74**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	36,3	mg/kg
Standard deviation	2,99	mg/kg
rel. Standard deviaton	8,3	%
Horwitz standard deviation	9,3	%
HorRat-value	**0,89**	
Recovery rate	103	%

Microtracer Homogeneity Test
DLA -ptALS1 Sample 4

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	91	36,3
2	5,02	73	29,1
3	4,99	81	32,5
4	4,98	92	36,9
5	4,98	80	32,1
6	4,97	70	28,2
7	5,01	93	37,1
8	5,01	92	36,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	84,0	Particles
Standard deviation	9,16	Particles
χ^2 (CHI-Quadrat)	7,00	
Probability	**43**	%
Recovery rate	127	%

Normal distribution		
Number of samples	8	
Mean	33,6	mg/kg
Standard deviation	3,67	mg/kg
rel. Standard deviaton	10,9	%
Horwitz standard deviation	9,4	%
HorRat-value	**1,2**	
Recovery rate	127	%

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 (2021)***
PT name	***Allergen-Screening I - 4 Samples qualitative: Cashew, Hazelnut, Coconut, 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 1 - 4:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Cashew, Hazelnut, Coconut, 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), PCR and LC-MS 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 May 07th 2021***
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
		CANADA
		ITALY
		Germany
		Germany
		Germany
		FRANCE
		ITALY
		SPAIN
		Germany
		AUSTRIA
		Germany
		SWITZERLAND
		SWITZERLAND
		FRANCE
		ITALY
		ITALY
		Germany
		GREAT BRITAIN
		FRANCE
		Germany
		SPAIN
		PORTUGAL

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

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

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