

Proficiency Tests

DLA

food
cosmetics
consumer goods
www.dla-lvu.de

Evaluation Report

proficiency test

DLA 11/2018

Allergen-Screening I:

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

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, 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)

<i>EP-Anbieter</i> <i>PT-Provider</i>	<p>DLA - Dienstleistung Lebensmittel Analytik GbR Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler-Scharf</p> <p>Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany</p> <p>Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de</p>
<i>EP-Nummer</i> <i>PT-Number</i>	DLA 11/2018
<i>EP-Koordinator</i> <i>PT-Coordinator</i>	Dr. Matthias Besler-Scharf
<i>Status des EP-Bericht</i> <i>Status of PT-Report</i>	<p>Abschlussbericht / Final report (20 July 2018)</p> <p>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.</p>
<i>EP-Bericht Freigabe</i> <i>PT-Report Authorization</i>	<p>Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) - <i>gezeichnet / signed M. Besler-Scharf</i></p> <p>Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) - <i>gezeichnet / signed G. Wichmann</i></p> <p>Datum / Date: 20 July 2018</p>
<i>Unteraufträge</i> <i>Subcontractors</i>	<p>Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben.</p> <p>In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.</p>
<i>Vertraulichkeit</i> <i>Confidentiality</i>	<p>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.</p> <p>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.</p>

Inhalt/Content

1. Introduction.....	5
2. Realisation.....	5
2.1 Test material.....	5
2.1.1 Homogeneity.....	7
2.1.2 Stability.....	7
2.2 Sample shipment and information to the test.....	8
2.3 Submission of results.....	8
3. Evaluation.....	9
3.1 Agreement with consensus values from participants.....	9
3.2 Agreement with spiking of samples.....	9
4. Results.....	10
4.1 Proficiency Test Cashew.....	11
4.1.1 ELISA-Results: Cashew.....	11
4.1.2 PCR-Results: Cashew.....	12
4.2 Proficiency Test Hazelnut.....	13
4.2.1 ELISA-Results: Hazelnut.....	13
4.2.2 PCR-Results: Hazelnut.....	14
4.3 Proficiency Test Macadamia.....	15
4.3.1 ELISA-Results: Macadamia.....	15
4.3.2 PCR-Results: Macadamia.....	16
4.4 Proficiency Test Almond.....	17
4.4.1 ELISA-Results: Almond.....	17
4.4.2 PCR-Results: Almond.....	18
4.5 Proficiency Test Brazil Nuts.....	19
4.5.1 ELISA-Results: Brazil Nuts.....	19
4.5.2 PCR-Results: Brazil Nuts.....	20
4.6 Proficiency Test Pecan.....	21
4.6.1 ELISA-Results: Pecan.....	21
4.6.2 PCR-Results: Pecan.....	22
4.7 Proficiency Test Pistachio.....	23
4.7.1 ELISA-Results: Pistachio.....	23
4.7.2 PCR-Results: Pistachio.....	24
4.8 Proficiency Test Walnut.....	25
4.8.1 ELISA-Results: Walnut.....	25
4.8.2 PCR-Results: Walnut.....	26
5. Documentation.....	27
5.1 Details by the participants.....	27
5.1.1 ELISA: Cashew.....	27
5.1.2 ELISA: Hazelnut.....	28
5.1.3 ELISA: Macadamia.....	29
5.1.4 ELISA: Almond.....	30
5.1.5 ELISA: Brazil Nuts.....	31
5.1.6 ELISA: Pecan.....	32
5.1.7 ELISA: Pistachio.....	33
5.1.8 ELISA: Walnut.....	34

5.1.9 PCR: Cashew.....35
5.1.10 PCR: Hazelnut.....36
5.1.11 PCR: Macadamia.....37
5.1.12 PCR: Almond.....38
5.1.13 PCR: Brazil Nuts.....39
5.1.14 PCR: Pecan.....40
5.1.15 PCR: Pistachio.....41
5.1.16 PCR: Walnut.....42
5.2 Homogeneity.....43
5.2.1 Mixture homogeneity before bottling.....43
5.3 Information on the Proficiency Test (PT).....45
6. Index of participant laboratories.....46
7. Index of references.....47

1. Introduction

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

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

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 1-2% of the allergenic ingredients concerned.

The respective raw materials for the nuts used were commercial nut butters and nut butters produced by DLA from commercial nuts (s. Tab. 2). The nuts were crushed, ground into nut butter and afterwards all butters were sieved (mesh 400 µm). From the nut butters thus obtained the allergen-premixes (see Tab. 1) were prepared with other additives and then used for spiking of the PT-sample 1 to 4 (see Tab. 2).

After homogenisation the samples were portioned to approximately 20 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	Samples 1 - 4
Potato powder (Ingredients: Potatoes, E471, E304, E223, E100)	72 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes	0,25 - 0,80 %
<u>Ingredients:</u> - Maltodextrin (75% - 90%) - Sodium sulfate (6,1% - 14%) - Silicon dioxide (3,5% - 10%) - Nut butters (1,1% - 1,7% each)	

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

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

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

2.1.1 Homogeneity

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

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

The microtracer analysis of the present PT samples 1-4 showed probabilities of 62%, 59%, 47% and 93%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [16, 17]. This gave HorRat values of 1,1, 1,2, 1,4 and 0,92, respectively. The HorRat value of sample 3 was slightly increased, while the probability was well $> 25\%$. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_w value $< 0,5$).

The a_w value of the PT samples was approx. 0,23 (21-22°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 2018. The testing method was optional. The tests should be finished at May 4th 2018 the latest.

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

*There are 4 different samples possibly containing the allergenic ingredients Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and Walnut. The allergens are contained in a simple carrier matrix in the range of mg/kg. The evaluation of results is **strictly qualitative (positive / negative)**.*

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

- a) **ELISA and Lateral Flow**
- b) **PCR**

Please note the attached information on the proficiency test.

(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. The results given as positive/negative were evaluated.

Queried and documented were the indicated results and details of the test methods like specificities, test kit manufacturer and hints about the procedure.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

Out of 17 participants 16 submitted at least one result in time. One participant submitted no results.

3. Evaluation

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

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

3.1 Agreement with consensus values from participants

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined unless $\geq 75\%$ positive or negative results are present for a parameter.

The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**.

The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

The qualitative evaluation is carried out for each parameter for ELISA and PCR methods separately. Results of lateral flow methods were valuated together with ELISA methods, because they are usually based on antibody detection.

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Cashew

4.1.1 ELISA-Results: Cashew

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	positive	positive	negative	positive	2/3 (67%)	2/4 (50%)	BA	
10	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	BF	
12	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	BF	
1	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	ET	
15	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	IL	Sample 2: slightly positive reaction to cashew, due to cross-reactivity to pistachio

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	5	2	0	1
Number negative	0	3	5	4
Percent positive	100	40	0	20
Percent negative	0	60	100	80
Consensus value	positive	none	negative	negative
Spiking	positive	negative	negative	negative

Methods:

- BA = Bioavid (Lateral Flow), R-Biopharm
- BF = MonoTrace ELISA, BioFront Technologies
- ET = Elution Technologies ELISA Kit
- IL = Immunolab

Comments:

The consensus values of results for samples 1, 3 and 4 are in qualitative agreement with the spiking of samples. For the none-spiked sample 2 there were differing results, thus no consensus value $\geq 75\%$ could be determined.

Cross-reactivity to brazil nut, hazelnut and walnut is known for the method BA (validation report, R-Biopharm). For the method IL a weak cross-reactivity to pistachio is described, while for method ET no cross-reactivities to nuts (detection limits $<1\%$) are indicated (test kit instructions Immunolab and Elution Technologies).

Participant 15 (method IL) mentioned a weak positive detection of sample 2 and attributed it to the pistachio content in the sample (cross-reactivity).

4.1.2 PCR-Results: Cashew

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
4	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

SFA = Sure Food Allergen, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

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

4.2 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		
5	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	BA	
15	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
7	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	

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

BA = Bioavid (Lateral Flow), R-Biopharm

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. There was one positive result for sample 4 (method BA).

Cross-reactivity to walnut (and pumpkin seeds) is known for the method BA (validation report, R-Biopharm).

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		
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	multiplex together with peanut, walnut, cashew and pistachio
16	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4p	sample 4 slightly positive below judgement limit
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
13	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	SFA-ID	sample 3: traces
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method
 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 = 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	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ET	
15	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
12	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
16	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	possible cross-reactivity to hazelnut and walnut

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies

ET = Elution Technologies ELISA Kit

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

div = not indicated / other method

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		
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
2	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

SFA = Sure Food Allergen, R-Biopharm / Congen

div = not indicated / other method

Comments:

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

4.4 Proficiency Test Almond

4.4.1 ELISA-Results: Almond

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BA	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
7	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	

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

Methods:

AQ = AgraQuant, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

RS-F= Ridascreeen® Fast, R-Biopharm

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		
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food Allergen, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

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

4.5 Proficiency Test Brazil Nuts

4.5.1 ELISA-Results: Brazil Nuts

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BF	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ET	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ET	
15	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IL	

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

ET = Elution Technologies ELISA Kit

IL = Immunolab

Comments:

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

4.5.2 PCR-Results: Brazil Nuts

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg				
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
16	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

	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:

ASU = ASU §64 Methode/method

SFA = Sure Food Allergen, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

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

4.6 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		
5	positive	positive	positive	positive	2/2 (100%)	2/4 (50%)	BA	same kit for w walnut and pecan
10	-	positive	positive	-	2/2 (100%)	2/2 (100%)	BF	
12	positive	positive	positive	positive	2/2 (100%)	2/4 (50%)	BF	
1	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	ET	
15	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	IL	sample 1: slightly positive reaction to pecan, due to cross-reactivity to w walnut

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	2	5	5	2
Number negative	2	0	0	2
Percent positive	50	100	100	50
Percent negative	50	0	0	50
Consensus value	none	positive	positive	none
Spiking	negative	positive	positive	negative

Methods:

BA = Bioavid (Lateral Flow), R-Biopharm
 BF = MonoTrace ELISA, BioFront Technologies
 ET = Elution Technologies ELISA Kit
 IL = Immunolab

Comments:

Consensus values $\geq 75\%$ were obtained for samples 2 and 3. In contrast to the spiking two positive results were submitted for samples 1 and 4, which could be due to the reactivities / cross-reactivities described below.

For method BA reactivities of 100% are described for walnut and pecan and cross-reactivity to cashew and pistachio is known (validation report, R-Biopharm). For method BF a high cross-reactivity to walnut is indicated (test kit description BioFront Technologies).

4.6.2 PCR-Results: Pecan**Qualitative valuation of results**

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation Agreement with consensus value	Qualitative Valuation Agreement with spiking of samples	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg				
9	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	SFA	
6	negative	positive	negative	negative	3/3 (100%)	3/4 (75%)	SFA-ID	
2	positive	positive	negative	positive	1/3 (33%)	1/4 (25%)	div	
4	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	div	
8	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	5	3	1
Number negative	4	0	2	4
Percent positive	20	100	60	20
Percent negative	80	0	40	80
Consensus value	negative	positive	none	negative
Spiking	negative	positive	positive	negative

Methods:

SFA = Sure Food Allergen, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

Consensus values $\geq 75\%$ were obtained for samples 1, 2 and 4. For the sample 3 with a lower spiking level there were two negative results, thus no consensus value could be determined.

Participant no. 2 obtained positive results for the none-spiked samples 1 and 4. More details of the method or possible cross-reactivities were not provided.

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		
5	positiv	positiv	positiv	positiv	1/3 (33%)	1/4 (25%)	BA	
6	negativ	positiv	negativ	negativ	3/3 (100%)	4/4 (100%)	BC	
10	negativ	positiv	negativ	negativ	3/3 (100%)	4/4 (100%)	BF	
12	negativ	positiv	negativ	negativ	3/3 (100%)	4/4 (100%)	BF	
1	positiv	positiv	negativ	negativ	3/3 (100%)	3/4 (75%)	ET	
15	negativ	positiv	negativ	negativ	3/3 (100%)	4/4 (100%)	IL	sample 1: slightly positive reaction to pistachio, due to cross-reactivity to cashew

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	2	6	1	1
Number negative	4	0	5	5
Percent positive	33	100	17	17
Percent negative	67	0	83	83
Consensus value	none	positiv	negativ	negativ
Spiking	negativ	positiv	negativ	negativ

Methods:

- BA = Bioavid (Lateral Flow), R-Biopharm
- BC = BioCheck ELISA
- BF = MonoTrace ELISA, BioFront Technologies
- ET = Elution Technologies ELISA Kit
- IL = Immunolab

Comments:

Consensus values $\geq 75\%$ were obtained for samples 2, 3 and 4. For the none-spiked sample 1 there were two positive results, thus no consensus value could be determined.

In contrast to the spiking two positive results were submitted for samples 3 and 4, which could be due to the cross-reactivities described below.

Cross-reactivity to cashew, walnut, brazil nut and hazelnut is known for the method BA (validation report, R-Biopharm). For method ET no cross-reactivities to nuts (detection limits $< 1\%$) are indicated (test kit instruction Elution Technologies).

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		
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
3	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
6	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
10	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
13	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	SFA-ID	sample 2: traces
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
4	negative	positive	positive	negative	3/4 (75%)	3/4 (75%)	div	
8	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
16a	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	Real Time PCR
16b	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	PCR

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

Methods:

SFA = Sure Food Allergen, R-Biopharm / Congen
 SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
 div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples.
 Participant no. 4 obtained a positive result for the none-spiked sample 3. More details of the method or possible cross-reactivities were not provided.

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		
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
5	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	BA	same test kit for w walnut and pecan
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BC	
10	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
12	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
15	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

- AQ = AgraQuant, RomerLabs
- BA = Bioavid (Lateral Flow), R-Biopharm
- BC = BioCheck ELISA
- BF = MonoTrace ELISA, BioFront Technologies
- IL = Immunolab
- VT = Veratox, Neogen

Comments:

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

In contrast to the spiking two positive results were submitted for samples 2 and 3, which could be due to the reactivities / cross-reactivities described below.

For method BA reactivities of 100% are described for walnut and pecan and cross-reactivity to cashew and pistachio is known (validation report, R-Biopharm).

4.8.2 PCR-Results: Walnut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
10	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
13	positive	negative	negative	negative	3/4 (75%)	3/4 (75%)	SFA-ID	
2	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	div	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
16	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	div	

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

Methods:

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 = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. For the sample 4 with a lower spiking level there was a negative result.

Participant no. 2 and 16 obtained positive results for the none-spiked sample 3 and samples 2 and 3, respectively. More details of the methods or possible cross-reactivities were not provided.

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Cashew

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BA	5	27.04.18	positive	positive	negative	positive	1	allergen/buffer	BA = Bioavid
BF	10		positive	negative	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	12		positive	negative	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ET	1	19.04.18	positive	positive	negative	negative	0,3	Nut protein	ET = Elution Technologies ELISA Kit
IL	15	05.04.18	positive	negative	negative	negative	2 (LOQ)	Nut, total	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	5	BL610-25			
BF	10				
BF	12				
ET	1	E-75CSH		4.5mL extraction solution for 25mins at 60°C	
IL	15	CAW-E01			Definition: < LOQ = negative; Sample 2: slightly positive reaction to cashew, due to cross-reactivity to pistachio

5.1.2 ELISA: Hazelnut*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BA	5	27.04.18	negative	positive	positive	positive	1	allergen/buffer	BA = Bioavid
IL	15	05.04.18	negative	positive	positive	negative	1 (LOQ)	Nut, total	IL = Immunolab
RS-F	7		negative	positive	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12		negative	positive	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	12.04.18	negative	positive	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	5	BL604-25			
IL	15	HAZ-E01			Definition: < LOQ = negative
RS-F	7				
RS-F	12				
RS-F	14	R6802	hazelnut protein	As per kit instructions	

5.1.3 ELISA: Macadamia*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	10		positive	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ET	1	19.04.18	positive	negative	negative	positive	0,1	Nut protein	ET = Elution Technologies ELISA Kit
IL	15	05.04.18	positive	negative	negative	positive	1 (LOQ)	Nut, total	IL = Immunolab
RS-F	6	13.04.18	positive	negative	negative	positive	1ppm	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12		positive	negative	negative	positive	1	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
div	16	5.4.	positive	negative	negative	positive	1	Nut, total	Auswahl ELISA-Kits:

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BF	10				
ET	1	E-75MCD		4.5mL extraction solution for 25mins at 60°C	
IL	15	MAC-E01			Definition: < LOQ = negative
RS-F	6	R6852	As Per Kit Instructions	As Kit Instructions	
RS-F	12				
div	16	Eurofins Technologies Test-Combination HU0030013	detects macadamia protein	As Kit Instructions	Sample 1 >30mg/kg; Sample 2 <1mg/kg; Sample 3 <2mg/kg (LOQ modified due to possible cross-reactivity to hazelnut and w alnut); Sample 4 >30mg/kg

5.1.4 ELISA: Almond*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	14	13.04.18	positive	negative	positive	negative	0,4	Nuss, gesamt	AQ = AgraQuant, RomerLabs
BA	5	27.04.18	positive	negative	positive	negative	1	allergen/buffer	BA = Bioavid
BF	10		positive	negative	positive	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	15	05.04.18	positive	negative	positive	negative	0,4 (LOQ)	Nut, total	IL = Immunolab
RS-F	7		positive	negative	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12		positive	negative	positive	negative	2,5	Nut, total	RS-F= Ridascreen® Fast, R-Biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	14	COKAL 0748	almond protein / prunus protein	according to handbook	
BA	5	BL601-25			
BF	10				
IL	15	ALM-E01			Definition: < LOQ = negative
RS-F	7				
RS-F	12				

5.1.5 ELISA: Brazil Nuts*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	10		negative	positive	negative	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	12		negative	positive	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ET	1	19.04.18	negative	positive	negative	positive	0,1	Nut protein	ET = Elution Technologies ELISA Kit
ET	6	13.04.18	negative	positive	negative	positive	1ppm	Nut protein	ET = Elution Technologies ELISA Kit
IL	15	05.04.18	negative	positive	negative	positive	1 (LOQ)	Nut, total	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BF	10				
BF	12				
ET	1	E-75BZL		4.5mL extraction solution for 25mins at 60°C	
ET	6	E-75BZL	As Per Kit Instructions	As Kit Instructions	
IL	15	PAR-E01			Definition: < LOQ = negativ

5.1.6 ELISA: Pecan*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BA	5	27.04.18	positive	positive	positive	positive	1	allergen/buffer	BA = Bioavid
BF	10		-	positive	positive	-	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	12		positive	positive	positive	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ET	1	18.04.25	negative	positive	positive	negative	0,4	Nut protein	ET = Elution Technologies ELISA Kit
IL	15	05.04.18	negative	positive	positive	negative	2 (LOQ)	Nut, total	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	5	BL607-25			same kit for walnut
BF	10				
BF	12				
ET	1	E-75PCN		4.5mL extraction solution for 25mins at 60°C	
IL	15	PEC-E01			Definition: < LOQ = negative; sample 1: slightly positive reaction to pecan, due to cross-reactivity to walnut

5.1.7 ELISA: Pistachio*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BA	5	27.04.18	positive	positive	positive	positive	1	allergen/buffer	BA = Bioavid
BC	6	13.04.18	negative	positive	negative	negative	1ppm	Nut, total	BC = BioCheck ELISA
BF	10		negative	positive	negative	negative	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	12		negative	positive	negative	negative	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
ET	1	19.04.18	positive	positive	negative	negative	0,3	Nut protein	ET = Elution Technologies ELISA Kit
IL	15	05.04.18	negative	positive	negative	negative	1 (LOQ)	Nut, total	IL = Immunolab

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BA	5	BL611-25			
BC	6	R6042	As Per Kit Instructions	As Kit Instructions	Sample 1 showed cross reaction with Pistachio ELISA
BF	10				
BF	12				
ET	1	E-75PST		4.5mL extraction solution for 25mins at 60°C	
IL	15	PIS-E01			Definition: < LOQ = negative; sample 1: slightly positive reaction to pistachio, due to cross-reactivity to cashew

5.1.8 ELISA: Walnut*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	1	18.04.18	positive	negative	negative	positive	0,35	Nut protein	AQ = AgraQuant, RomerLabs
BA	5	27.04.18	positive	positive	positive	positive	1	allergen/buffer	BA = Bioavid
BC	6	13.04.18	positive	negative	negative	positive	2ppm	Nut, total	BC = BioCheck ELISA
BF	10		positive	negative	negative	positive	1	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
BF	12		positive	negative	negative	positive	2	Nut, total	BF = MonoTrace ELISA, BioFront Technologies
IL	15	05.04.18	positive	negative	negative	positive	2 (LOQ)	Nut, total	IL = Immunolab
VT	7		positive	negative	negative	positive	2,5	Nut, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	1	COKAL0948		20mL extraction solution for 15mins at 60°C	
BA	5	BL607-25			same kit for pecan
BC	6	R6016	As Per Kit Instructions	As Kit Instructions	
BF	10				
BF	12				
IL	15	WAL-E01			Definition: < LOQ = negative
VT	7				

5.1.9 PCR: Cashew*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	9		positive	negative	negative	negative	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	3	25.04.	positive	negative	negative	negative	10	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	28.04.18	positive	negative	negative	negative	8	other: µg nut DNA / kg of sample	other: intern method
div	4		positive	negative	negative	negative		Nut-DNA	in-house method
div	8		positive	negative	negative	negative		Nut-DNA	in-house method pmCSN-Hex
div	11		positive	negative	negative	negative	5	Nut, total	in-house method
div	14	18.04.18	positive	negative	negative	negative	0,01	ng/µl DNA	Ehler et al, 2008
div	16	27.3.	positive	negative	negative	negative	8	Nut-DNA	

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	9	S3615			
SFA-ID	3	S3115		CTAB / Prot.K / QIAquick / Real-Time PCR	
div	2				
div	4			Real Time PCR	
div	8	-	-	in-house method	
div	11		Ana 03	Extraction: kit Food Macherey Nagel	
div	14		Ana o 3 AY081853	DNeasy mericon food kit Qiagen	
div	16	in-house method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45	

5.1.10 PCR: Hazelnut*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4		negative	positive	positive	negative		Nut-DNA	ASU = ASU §64 Methode/method
ASU	14	18.04.18	negative	positive	positive	negative	0,01	ng/µl DNA	ASU
ASU	16	27.3.	negative	positive	positive	negative	8	Nut-DNA	ASU = ASU §64 Methode/method
SFA	9		negative	positive	positive	negative	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-4p	3	12.04.	negative	positive	positive	negative	1	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	10		negative	positive	positive	negative	0,4	Nut-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13		negative	negative	negative	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	28.04.18	negative	positive	positive	negative	8	other: µg nut DNA / kg of sample	other: intern method
div	8		negative	positive	positive	negative		Nut-DNA	in-house method pmHZN-Cy5
div	11		negative	positive	positive	negative	range 5 to 10	Nut, total	CEN/TC 275/WG 12 N 317

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	ASU L44.00-8		Real Time PCR	
ASU	14	44.00-8	Cor a 1 AF136945	DNeasy mericon food kit Qiagen	as multiplex together with peanut, walnut, cashew and pistachio
ASU	16	L 44.00-08, mod.		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45	
SFA	9	S3602			
SFA-4p	3	S3402		CTAB / Prot.K / QIAquick / Real-Time PCR	Sample 4 slightly positive below judgement limit
SFA-ID	10				
SFA-ID	13				Sample 3: traces
div	2				
div	8	-	-	in-house method	
div	11		Cor A1	Extraction: kit Food Macherey Nagel	

5.1.11 PCR: Macadamia*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	9		positive	negative	negative	positive	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
div	2	27.04.18	positive	negative	negative	positive	8	other: µg nut DNA / kg of sample	other: intern method
div	8		positive	negative	negative	positive		Nut-DNA	in-house method pmMAS-TxRed
div	11		positive	negative	negative	positive	7 pg DNA	Nut-DNA	Internal method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	9	S3616			
div	2				
div	8	-	-	in-house method	
div	11		Vicilin gene	Extraction: kit Food Macherey Nagel	

5.1.12 PCR: Almond*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4		positive	negative	positive	negative		Nut-DNA	ASU = ASU §64 Methode/method
ASU	14	24.04.18	positive	negative	positive	negative	0,004	ng/µl DNA	ASU
ASU	16	27.3.	positive	negative	positive	negative	40	Nut-DNA	ASU = ASU §64 Methode/method
SFA	9		positive	negative	positive	negative	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	3	13.04.	positive	negative	positive	negative	10	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	10		positive	negative	positive	negative	4	Nut-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13		positive	negative	positive	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	28.04.18	positive	negative	positive	negative	8	other: µg nut DNA / kg of sample	other: intern method
div	8		positive	negative	positive	negative		Nut-DNA	in-house method pmMAD-Hex
div	11		positive	negative	positive	negative	range 5 to 10	Nut, total	J. Verbr. Lebensm. (2014) 9:297-310

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	ASU L18.00-20		Real Time PCR	
ASU	14	18.00-22	PRU AV1 Genes (BQ641046)	DNeasy mericon food kit Qiagen	
ASU	16	L 18.00-20, mod.		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45	
SFA	9	S3604			
SFA-ID	3	S3104		CTAB / Prot.K / QIAquick / Real-Time PCR	
SFA-ID	10				
SFA-ID	13				
div	2				
div	8	-	-	in-house method	
div	11		ns LTP	Extraction: kit Food Macherey Nagel	

5.1.13 PCR: Brazil Nuts*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	14	24.04.18	negative	positive	negative	positive	0,004	ng/µl DNA	ASU
ASU	16	27.3.	negative	positive	negative	positive		Nut-DNA	ASU = ASU §64 Methode/method
SFA	9		negative	positive	negative	positive	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	3	26.04.	negative	positive	negative	positive	10	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	6	13.04.18	negative	positive	negative	positive	1ppm	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	27.04.18	negative	positive	negative	positive	8	other: µg nut DNA / kg of sample	other: intern method
div	4		negative	positive	negative	positive		Nut-DNA	in-house method
div	8		negative	positive	negative	positive		Nut-DNA	in-house method pmPRS-TxRed
div	11		negative	positive	negative	positive	not determined	Nut-DNA	J. Verbr. Lebensm. (2014) 9:297-310

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	14	18.00-22	Brazil nut 2S albumin Gen M17146, M80400, X57027, X57028, X54490, AB044391	DNeasy mericon food kit Qiagen	
ASU	16	§64LFGB L14.02-4, mod.		CTAB / Proteinase K / Promega Wizard DNA CleanUp / PCR / 45	
SFA	9	S3617			
SFA-ID	3	S3117		CTAB / Prot.K / QIAquick / Real-Time PCR	
SFA-ID	6	S3117	As Per Kit Instructions	As Kit Instructions	
div	2				
div	4			Real Time PCR	
div	8	-	-	in-house method	
div	11		Albumin 2S	Extraction: kit Food Macherey Nagel	

5.1.14 PCR: Pecan*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	9		negative	positive	positive	negative	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	6	13.04.18	negative	positive	negative	negative	1ppm	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	27.04.18	positive	positive	negative	positive	80	other: µg nut DNA / kg of sample	other: intern method
div	4		negative	positive	positive	negative		Nut-DNA	in-house method
div	8		negative	positive	positive	negative		Nut-DNA	in-house method pmPAS-Atto

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	9	S3618		QE to Carya Ovata 100 %	
SFA-ID	6	S3118	As Per Kit Instructions	As Kit Instructions	
div	2				
div	4			Real Time PCR	
div	8	-	-	in-house method	

5.1.15 PCR: Pistachio*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	9		negative	positive	negative	negative	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	3	20.04.	negative	positive	negative	negative	10	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	6	16.04.18	negative	positive	negative	negative	1ppm	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	10		negative	positive	negative	negative	0,4	Nut-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13		negative	negative	negative	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	27.04.18	negative	positive	negative	negative	80	other: µg nut DNA / kg of sample	other: intern method
div	4		negative	positive	positive	negative		Nut-DNA	in-house method
div	8		negative	positive	negative	negative		Nut-DNA	in-house method pmPIST-Fam
div	11		negative	positive	negative	negative	5	Nut, total	Internal method
div	14	18.08.18	negative	positive	negative	negative	0,01	ng/µl DNA	Köppel et al, 2012
div	16a	27.3.	negative	positive	negative	negative	0,4	Nut-DNA	
div	16b	27.3.	negative	positive	negative	negative	0,4	Nut-DNA	

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	9	S3614			
SFA-ID	3	S3114		CTAB / Prot.K / QIAquick / Real-Time PCR	
SFA-ID	6	S3114	As Per Kit Instructions	As Kit Instructions	
SFA-ID	10				
SFA-ID	13				Sample 2: traces
div	2				
div	4			Real Time PCR	
div	8	-	-	in-house method	
div	11		Vicilin gene	Extraction: kit Food Macherey Nagel	
div	14		Dehydrin Y07600	DNeasy mericon food kit Qiagen	
div	16a	in-house method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45	
div	16b	in-house method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / PCR / 45	

5.1.16 PCR: Walnut*Primary data*

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	9		positive	negative	negative	positive	0,4	Nut-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-4p	3	12.04.	positive	negative	negative	positive	1	Nut, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	6	16.04.18	positive	negative	negative	positive	1ppm	Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	10		positive	negative	negative	positive	0,4	Nut-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-ID	13		positive	negative	negative	negative		Nut, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	28.04.18	positive	positive	positive	positive	8	other: µg nut DNA / kg of sample	other: intern method
div	4		positive	negative	negative	positive		Nut-DNA	in-house method
div	8		positive	negative	negative	positive		Nut-DNA	in-house method pmWLZ-Atto
div	11		positive	negative	negative	positive	5	Nut, total	Eur. Food Res. Technol. (2006) 223:373-377
div	14	18.08.14	positive	negative	negative	positive	0,025	ng/µl DNA	Brezna et al, 2006
div	16	27.3.	positive	positive	positive	positive	2	Nut-DNA	

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specificity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	9	S3607			
SFA-4p	3	S3402		CTAB / Prot.K / QIAquick / Real-Time PCR	
SFA-ID	6	S3107	As Per Kit Instructions	As Kit Instructions	
SFA-ID	10				
SFA-ID	13				
div	2				
div	4			Real Time PCR	
div	8	-	-	in-house method	
div	11		jug R2	Extraction: kit Food Macherey Nagel	
div	14		Jug r 2 AF066055	DNeasy mericon food kit Qiagen	
div	16	in-house method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real Time PCR / 45	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test

DLA 11-2018 Sample 1

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	76	30,2
2	5,05	60	23,8
3	5,02	55	21,9
4	5,02	61	24,3
5	5,00	53	21,2
6	4,95	59	23,8
7	5,04	64	25,4
8	5,00	62	24,8

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	61,2	Particles
Standard deviation	6,84	Particles
χ^2 (CHI-Quadrat)	5,35	
Probability	62	%
Recovery rate	105	%

Normal distribution

Number of samples	8	
Mean	24,4	mg/kg
Standard deviation	2,73	mg/kg
rel. Standard deviaton	11,2	%
Horwitz standard deviation	9,89	%
HorRat-value	1,1	
Recovery rate	105	%

Microtracer Homogeneity Test

DLA 11-2018 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	18,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	53	21,1
2	4,99	62	24,8
3	5,04	61	24,2
4	4,96	48	19,4
5	5,05	47	18,6
6	5,01	62	24,8
7	4,96	58	23,4
8	4,98	64	25,7

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	56,9	Particles
Standard deviation	6,75	Particles
χ^2 (CHI-Quadrat)	5,60	
Probability	59	%
Recovery rate	121	%

Normal distribution

Number of samples	8	
Mean	22,7	mg/kg
Standard deviation	2,70	mg/kg
rel. Standard deviaton	11,9	%
Horwitz standard deviation	10,0	%
HorRat-value	1,2	
Recovery rate	121	%

Microtracer Homogeneity Test

DLA 11-2018 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	18,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	52	20,6
2	5,02	49	19,5
3	4,96	54	21,8
4	5,02	52	20,7
5	4,99	41	16,4
6	5,03	51	20,3
7	4,99	39	15,6
8	4,96	37	14,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	46,9	Particles
Standard deviation	6,63	Particles
χ^2 (CHI-Quadrat)	6,57	
Probability	47	%
Recovery rate	100	%

Normal distribution		
Number of samples	8	
Mean	18,7	mg/kg
Standard deviation	2,65	mg/kg
rel. Standard deviaton	14,2	%
Horwitz standard deviation	10,3	%
HorRat-value	1,4	
Recovery rate	100	%

Microtracer Homogeneity Test

DLA 11-2018 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	14,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	36	14,5
2	4,99	40	16,0
3	5,02	35	13,9
4	5,00	39	15,6
5	5,05	38	15,0
6	4,96	38	15,3
7	4,99	29	11,6
8	5,07	34	13,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	36,1	Particles
Standard deviation	3,57	Particles
χ^2 (CHI-Quadrat)	2,47	
Probability	93	%
Recovery rate	99	%

Normal distribution		
Number of samples	8	
Mean	14,4	mg/kg
Standard deviation	1,43	mg/kg
rel. Standard deviaton	9,89	%
Horwitz standard deviation	10,7	%
HorRat-value	0,92	
Recovery rate	99	%

5.3 Information on the Proficiency Test (PT)

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

<i>PT number</i>	DLA 11-2018
<i>PT name</i>	Allergen-Screening I - 4 Samples qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and Walnut
<i>Sample matrix</i>	Samples 1-4: Carrier matrix / ingredients: potato powder (appr. 75%), maltodextrin (appr. 25%), other food additives and allergenic foods
<i>Number of samples and sample amount</i>	4 different Samples 1-4: 20 g each
<i>Storage</i>	Samples A + B: room temperature (long term cooled 2 - 10°C)
<i>Intentional use</i>	Laboratory use only (quality control samples)
<i>Parameter</i>	Qualitative: Cashew, Hazelnut, Macadamia, Almond, Brazil Nuts, Pecan, Pistachio and Walnut Samples 1-4: appr. 25 - 250 mg/kg
<i>Methods of analysis</i>	The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.
<i>Notes to analysis</i>	The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.
<i>Result sheet</i>	One result each should be determined for Samples 1-4. The results should be filled in the result submission file.
<i>Units</i>	positiv / negativ (limit of detection mg/kg)
<i>Number of digits</i>	at least 2
<i>Result submission</i>	The result submission file should be sent by e-mail to: pt@dla-lvu.de
<i>Deadline</i>	the latest 04th May 2018
<i>Evaluation report</i>	The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.
<i>Coordinator and contact person of PT</i>	Matthias Besler-Scharf, PhD

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		CANADA
		ITALY
		Germany
		Germany
		Germany
		Germany
		ITALY
		Germany
		ITALY
		FRANCE
		Germany
		Germany
		GREAT BRITAIN
		FRANCE
		Germany
		SPAIN
		SPAIN

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment - General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Analytical Laboratories ; J.AOAC Int., 76(4), 926 - 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 - 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Horwitz Equation as Quality Benchmark in ISO/IEC 17025 Testing Laboratory, Rivera & Rodriguez, Bufete de ingenieros industriales, S.C. (Corrigendum 2014)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel - Nachweis von Lebensmittelallergenen - Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006

23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes¹, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contaminations in foodstuffs by ELISA in microtiterplates]
31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contaminations in chocolate and chocolate products by ELISA in microtiterplates]