DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

11/2016

Allergen-Screening I:

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

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Inhalt / Content

1. Introduction

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 1-2% of the allergenic ingredients concerned. The respective raw materials for the nuts used were commercial nut butters (containing 100% nuts) 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**
Maltodextrin	88,7 - 99,6 %
Allergen-Premixes Ingredients: - Maltodextrin (75% - 90%) - Sodium sulfate (6,1 - 14%) - Silicon dioxide (3,5 - 10%) - Nut butters (1,1% - 1,7% each)	0,43 - 1,3 %

Remarks to the sample matrix:
Because the carrier matrix consisted of maltodextrin and small amounts of inorganic substances, the expected protein and DNA amounts come exclusively from the allergenic ingredients. In PCR analysis only very small DNA amounts could be expected in the extracts by DNA amount estimation.

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Cashew (Protein 18,4%) - commercial nut butter	negative	positive (75 - 225)	negative	positive (50 - 150)
Hazelnut (Protein 15,9%) - commercial nut butter	positive (50 - 150)	negative	positive (25 - 75)	negative
Macadamia (Protein 9,4%) - Nuts, crushed	positive (50 - 150)	positive (25 - 75)	negative	negative
Almond (Protein 19,6%) - commercial nut butter	negative	positive (75 - 225)	positive (50 - 150)	negative
Brazil nut (Protein 14,8%) - Nuts, crushed	negative	negative	positive (75 - 225)	positive (50 - 150)
Pecan (Protein 12,2%) - Nuts, crushed	positive (75 - 225)	negative	negative	positive (50 - 150)
Pistachio (Protein 25,6%) - Nuts, crushed	negative	negative	negative	positive (50 - 150)
Walnut (Protein 13,9%) - Nuts, crushed	negative	positive (50 - 150)	negative	negative

* Protein contents according to laboratory analysis (total nitrogen, Kjeldahl)
**Allergen contents as „total nuts" according gravimetric mixing

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 40%, 87%, 96% and 20%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat values of 1,4, 0,9, 0,6 and 1,8, respectively. The results of microtracer analysis are given in the documentation.

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 2016. The testing method was optional. The tests should be finished at May 6th 2016 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 (>95% maltodextrin) in the range of 50 – 250 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***
In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

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 specifity, 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.
One participant submitted no results. All other participants submitted their results 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 [23, 24, 25, 26]. Furthermore matrix- and/or processing of samples can have strong impact on the detectability of allergens by ELISA and PCR methods.

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

3.1 Agreement with consensus values from participants

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**. A consensus value is determined unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples 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.

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		
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BC	Biocheck Cashew-Check Cross reactivity to Pistachio 4%
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IL	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	3	0	3
Number negative	3	0	3	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
BC = BioCheck
IL = Immunolab

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. The indicated cross-reactivity of method BC against pistachio has no impact, because pistachio is contained in sample 4 only.

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		
5	negative	positive	negative	negative	2/2 (100%)	3/4 (75%)	SFA ID	
7	negative	positive	negative	positive	2/2 (100%)	4/4 (100%)	SFA ID	
1	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
2	negative	positive	negative	positive	2/2 (100%)	4/4 (100%)	div	
3	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
8	negative	positive	negative	positive	2/2 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	4	0	3
Number negative	6	2	6	3
Percent positive	0	67	0	50
Percent negative	100	33	100	50
Consensus value	negative	none	negative	none
Spiking	negative	positive	negative	positive

Methods:

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

div = not indicated / other method

Comments:

Consensus values ≥75% were only obtained for the negative samples 1 and 3. For the spiked samples 2 and 4 results were varying with partly relatively high indications for the limits of detection of the respective methods (s. documentation).

4.2 Proficiency Test Hazelnut

4.2.1 ELISA-Results: Hazelnut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	Detection depends on the degree of roasting

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

Methods:

ES = ELISA-Systems
RS = Ridascreen®, R-Biopharm
IL = Immunolab

Comments:

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

4.2.2 PCR-Results: Hazelnut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	positive	negative	2/2 (100%)	4/4 (100%)	ASU	
4	negative	negative	positive	negative	2/2 (100%)	3/4 (75%)	ASU	
5	positive	negative	positive	negative	2/2 (100%)	4/4 (100%)	SFA ID	
7	positive	negative	positive	negative	2/2 (100%)	4/4 (100%)	SFA ID	
1	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
3	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
8	positive	negative	negative	negative	2/2 (100%)	3/4 (75%)	div	

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

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID,
R-Biopharm / Congen

div = not indicated / other method

Comments:

Consensus values ≥75% were only obtained for the negative samples 2 and 4. For the spiked samples 1 and 3 results were varying with partly relatively high indications for the limits of detection of the respective methods (s. documentation).

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		
9	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	

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

Methods:

IL = Immunolab RS = Ridascreen®, R-Biopharm

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		
7	positive	positive	negative	negative	2/2 (100%)	4/4 (100%)	SFA ID	
1	negative	positive	negative	negative	2/2 (100%)	3/4 (75%)	div	
2	positive	positive	negative	-	1/2 (50%)	3/4 (75%)	div	
3	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
5	positive	positive	negative	positive	1/2 (50%)	3/4 (75%)	div	
8	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	4	0	1
Number negative	3	2	6	4
Percent positive	50	67	0	20
Percent negative	50	33	100	80
Consensus value	none	none	negative	negative
Spiking	positive	positive	negative	negative

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID,
R-Biopharm / Congen

div = not indicated / other method

Comments:

Consensus values ≥75% were only obtained for the negative samples 3 and 4. For the spiked samples 1 and 2 results were varying with partly relatively high indications for the limits of detection of the respective methods (s. documentation).

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		
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

IL = Immunolab
RS = Ridascreen®, R-Biopharm
div = not indicated / other method

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		
2	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	ASU	
4	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	ASU	
5	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	SFA ID	
6	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	SFA ID	
7	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	SFA ID	
1	negativ	negativ	negativ	negativ	2/4 (50%)	2/4 (50%)	div	
3	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	div	
8	negativ	positive	positive	negativ	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Method
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 nut

4.5.1 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		
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ET	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	

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

ET = Elution Technologies IL = Immunolab

Comments:

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

4.5.2 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		
2	negative	negative	positive	positive	2/2 (100%)	4/4 (100%)	ASU	
7	negative	negative	positive	positive	2/2 (100%)	4/4 (100%)	SFA ID	
1	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
3	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
5	negative	positive	positive	positive	1/2 (50%)	3/4 (75%)	div	
8	negative	negative	positive	negative	2/2 (100%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	1	4	3
Number negative	6	5	2	3
Percent positive	0	17	67	50
Percent negative	100	83	33	50
Consensus value	negative	negative	keiner	keiner
Spiking	negative	negative	positive	positive

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID,
R-Biopharm / Congen

div = not indicated / other method

Comments:

Consensus values ≥75% were only obtained for the negative samples 1 and 2. For the spiked samples 3 and 4 results were varying with partly relatively high indications for the limits of detection of the respective methods (s. documentation).

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		
6	positive	negative	negative	positive	4/4(100%)		ET	Sample 2 had a cross reaction with the Pecan Kit from the walnut content
9	positive	negative	negative	positive	4/4(100%)		IL	A weakly positive reaction at 4 ppm for sample 2 identified as cross-reactivity to walnut contamination

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

ET = Elution Technologies IL = Immunolab

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. The participants indicated for both ELISA-methods for the detection of pecan a cross-reactivity to walnut in sample 2.

4.6.2 PCR-Results: Pecan

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
7	positive	negative	negative	positive	2/2 (100%)	4/4 (100%)	SFA ID	
1	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
2	positive	-	negative	positive	1/2 (50%)	3/4 (75%)	div	
3	negative	negative	negative	negative	2/2 (100%)	2/4 (50%)	div	
5	positive	positive	negative	positive	1/2 (50%)	3/4 (75%)	div	
8	positive	negative	negative	positive	2/2 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	4	1	0	4
Number negative	2	4	6	2
Percent positive	67	20	0	67
Percent negative	33	80	100	33
Consensus value	none	negative	negative	none
Spiking	positive	negative	negative	positive

Methods:

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

div = not indicated / other method

Comments:

Consensus values ≥75% were only obtained for the negative samples 2 and 3. For the spiked samples 1 and 4 results were varying with partly relatively high indications for the limits of detection of the respective methods (s. documentation).

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		
6	negative	positive	negative	positive	3/3 (100%)	3/3 (100%)	BC	Biocheck Pistachio-Check Cross reactivity to Cashew 12%
9	negative	negative	negative	positive	3/3 (100%)	3/3 (100%)	IL	A weakly positive reaction at 12 ppm for sample 2 identified as cross-reactivity to cashwe contamination

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

Methods:

BC = BioCheck IL = Immunolab

Comments:

The consensus values of results for samples 1, 3 and 4 are in qualitative agreement with the spiking of samples. The participants indicated for both ELISA-methods for the detection of pistachio a cross-reactivity to cashew. One participant valuated the result for sample 2 as weakly positive cross-reactivity to cashew, while the other participant indicated a positive result to pistachio in sample 2.

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		
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA ID	
7	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA ID	
1	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	
2	negative	-	negative	positive	3/4 (75%)	3/4 (75%)	div	
3	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	div	
8	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

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

div = not indicated / other method

Comments:

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

4.8 Proficiency Test Walnut

4.8.1 ELISA-Results: Walnut

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
6	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BC	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BK	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	

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

Methods:

BC = BioCheck
BK = BioKits, Neogen
IL = Immunolab

Comments:

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

4.8.2 PCR-Results: Walnut

Qualitative valuation of results

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

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

Methods:

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

div = not indicated / other method

Comments:

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

5. Documentation

5.1 Details by the participants

5.1.1 ELISA: Cashew

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
2	negative	positive	negative	positive		Nut, total	AQ	AgraQuant, RomerLabs
6	negative	positive	negative	positive	2	Nut, total	BC	
9	negative	positive	negative	positive	2	Nut, total	IL	Immunolab ELISA

Method:

AQ = AgraQuant, RomerLabs IL = Immunolab
BC = BioCheck

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
2	AQ	COKAL 3148			
6	BC		No data given	As per Kit Instructions	Biocheck Cashew-Check Cross reactivity to Pistachio 4%
9	IL	CAW-E01	polyclonal		

5.1.2 ELISA: Hazelnut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
2	positive	negative	positive	negative		Nut protein	ES	ELISA-Systems, Residue Assay
9	positive	negative	positive	negative	1	Nut, total	IL	Immunolab ELISA
5	positive	negative	positive	negative	1,5	Nut, total	RS	Ridascreen Fast, r-Biopharm
6	positive	negative	positive	negative	2,5	Nut, total	RS	Ridascreen Fast, r-Biopharm

Methods:

ES = ELISA-Systems
IL = Immunolab
RS = Ridascreen®, R-Biopharm

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
2	ES	L 44.00.7			
9	IL	HSN-E01	polyclonal		
5	RS	Ridascreen Fast, r-Biopharm R6802			
6	RS	Ridascreen Fast, r-Biopharm	No data given	As Per Kit Instructions	Detection depends on the degree of roasting

5.1.3 ELISA: Macadamia

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
9	positive	positive	negative	negative	1	Nut, total	IL	Immunolab ELISA
6	positive	positive	negative	negative	1	Nut, total	RS	Ridascreen Fast, r-Biopharm

Methods:

IL = Immunolab RS = Ridascreen®, R-Biopharm

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
9	IL	MAC-E01	polyclonal		
6	RS	Ridascreen Fast, r-Biopharm	All kinds of Macadamia	As Per Kit Instructions	

5.1.4 ELISA: Almond

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
9	negative	positive	positive	negative	0,4	Nut, total	IL	Immunolab ELISA
2	negative	positive	positive	negative		Nut, total	RS	Ridascreen Fast, r-Biopharm
5	negative	positive	positive	negative	1,7	Nut protein	RS	Ridascreen Fast, r-Biopharm
6	negative	positive	positive	negative	2,5	Nut, total	RS	Ridascreen Fast, r-Biopharm
4	negative	positive	positive	negative	10	Nut, total	div	in-house method

Methods:

IL = Immunolab
RS = Ridascreen®, R-Biopharm
div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
9	IL	ALM-E01	polyclonal		
2	RS	R6901			
5	RS	Ridascreen Fast, r-Biopharm R6901			
6	RS	Ridascreen Fast, r-Biopharm	Proteins from Almonds	As Per Kit Instructions	
4	div		Nut, total		

5.1.5 ELISA: Brazil nut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
6	negative	negative	positive	positive	1	Nut protein	ET	Elution Technologies Kit
9	negative	negative	positive	positive	1	Nut, total	IL	Immunolab ELISA

Methods:
ET = Elution Technologies IL = Immunolab

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
6	ET	Elution Technologies Kit	No data given	As Per Kit Instructions	
9	IL	PAR-E01	polyclonal		

5.1.6 ELISA: Pecan

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
6	positive	negative	negative	positive	0,67	Nut protein	ET	Elution Technologies Kit
9	positive	negative	negative	positive	2	Nut, total	IL	Immunolab ELISA

Methods:
ET = Elution Technologies IL = Immunolab

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
6	ET	Elution Technologies Kit	No data given	As Per Kit Instructions	Sample 2 had a cross reaction with the Pecan Kit from the walnut content
9	IL	PEC-E01	polyclonal		A weakly positive reaction at 4 ppm for sample 2 identified as cross-reactivity to walnut contamination

5.1.7 ELISA: Pistachio

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
6	negative	positive	negative	positive	1	Nut, total	BC	Biocheck
9	negative	negative	negative	positive	1	Nut, total	IL	Immunolab ELISA

Methods:

BC = BioCheck IL = Immunolab

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
6	BC		No data given	As Per Kit Instructions	Biocheck Pistachio-Check Cross reactivity to Cashew 12%
9	IL	PIS-E01	polyclonal		A weakly positive reaction at 12 ppm for sample 2 identified as cross-reactivity to cashwe contamination

5.1.8 ELISA: Walnut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
6	negative	positive	negative	negative	2	Nut, total	BC	BioCheck
2	negative	positive	negative	negative		Nut, total	BK	BioKits Assay Kit, Neogen
9	negative	positive	negative	negative	2	Nut, total	IL	Immunolab ELISA

Methods:

BC = BioCheck IL = Immunolab
BK = BioKits, Neogen

Other details to the Methods

Evaluation number	Meth. Abr.	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
6	BC		No data given	As Per Kit Instructions	Biocheck Walnut - Check
2	BK	902085J			
9	IL	WAL-E01	polyclonal		

5.1.9 PCR: Cashew

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
5	negative	positive	negative	negative	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
7	negative	positive	negative	positive	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	negative	negative	negative	25	pmCSN-Hex	div	in house method
2	negative	positive	negative	positive		Nut-DNA	div	in house method
3	neg	neg	neg	neg	100	ADN	div	House method
8	negative	positive	negative	positive	100	Nut-DNA	div	in house

Methods:

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

div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
5	SFA ID				
7	SFA ID				
1	div	-	nan o 3	CTAB; Magnetc Beads; Taqman real time PCR	
2	div			CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
3	div	House method	2s albumin	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
8	div		67bp	Wizard cleanup, Rotorgene6000	

5.1.10 PCR: Hazelnut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
2	positive	negative	positive	negative		Nut-DNA	ASU	ASU §64
4	negative	negative	positive	negative	10	Nut, total	ASU	ASU §64
5	positive	negative	positive	negative	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
7	positive	negative	positive	negative	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	negative	negative	negative	25	pmHZN-Cy5	div	in house method
3	neg	neg	neg	neg	100	ADN	div	House method
8	positive	negative	negative	negative	100	Nut-DNA	div	in house

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
2	ASU	L 44.00.8		CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
4	ASU	ASU §64	Nut, total	limit of detection: 10-25 mg/kg	
5	SFA ID				
7	SFA ID				
1	div	-	cor a 1	CTAB; Magnetc Beads; Taqman real time PCR	
3	div	Koppel y col., 2010	Cor	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
8	div		85bp	Wizard cleanup, Rotorgene6000	

5.1.11 PCR: Macadamia

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
7	positive	positive	negative	negative	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	positive	negative	negative	25	pmMAS-TxRed	div	in house method
2	positive	positive	negative	-		Nut-DNA	div	in house method
3	neg	neg	neg	neg	100	ADN	div	House method
5	positive	positive	negative	positive	0,4*	Nut-DNA	div	in house method CONGEN
8	negative	negative	negative	negative	100	Nut-DNA	div	in house

Methods:

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

div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
7	SFA ID				
1	div	-	amp2	CTAB; Magnetc Beads; Taqman real time PCR	
2	div			CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
3	div	House method	vicilin precursor (AMP2)	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
5	div				* < = 0.4 mg allergenic substance/kg in non-processed corn flour
8	div		73bp	Wizard cleanup, Rotorgene6000	

5.1.12 PCR: Almond

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
2	negative	positive	positive	negative		Nut-DNA	ASU	ASU §64
4	negative	positive	positive	negative	10	Nut, total	ASU	ASU §64
5	negative	positive	positive	negative	4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
6	negative	positive	positive	negative	1	Nut, total	SFA ID	SureFood ID, Congen
7	negative	positive	positive	negative	4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	negative	negative	negative	25	pmMAD-Hex	div	in house method
3	neg	pos	pos	neg	100	ADN	div	House method
8	negative	positive	positive	negative	100	Nut-DNA	div	in house

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID,
R-Biopharm / Congen
div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
2	ASU	L 18.00-20		CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
4	ASU	ASU §64	Nut, total	limit of detection: 10-25 mg/kg	Sample 2 weakly positive, reason: no clear DNA-pellet during extraction, therefore probably less DNA extracted from sample 2
5	SFA ID				
6	SFA ID			As Per Kit Instructions	
7	SFA ID				
1	div	-	prudu1.01	CTAB; Magnetic Beads; Taqman real time PCR	
3	div	Koppel y col., 2010	Cor I	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
8	div		129bp	Wizard cleanup, Rotorgene6000	

5.1.13 PCR: Brazil nut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
2	negative	negative	positive	positive		Nut-DNA	ASU	ASU §64
7	negative	negative	positive	positive	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	negative	negative	negative	25	pmPRS-TxRed	div	in house method
3	neg	neg	neg	neg	100	ADN	div	House method
5	negative	positive	positive	positive	0,4*	Nut-DNA	div	in house method CONGEN
8	negative	negative	positive	negative	100	Nut-DNA	div	in house

Methods:

ASU = ASU §64 Method
SFA ID= Sure Food Allergen ID, R-Biopharm / Congen
div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
2	ASU	L 18.00-21		CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
7	SFA ID				
1	div	-	2s strorage protein	CTAB; Magnetc Beads; Taqman real time PCR	
3	div	House method	Sulfur rich water soluble	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
5	div				* < = 0,4 mg allergenic substance/kg in non-processed corn flour
8	div		50-80bp	Wizard cleanup, Rotorgene6000	

5.1.14 PCR: Pecan

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
7	positive	negative	negative	positive	4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	negative	negative	negative	25	pmPAS-Atto680	div	in house method
2	positive	-	negative	positive		Nut-DNA	div	in house method
3	neg	neg	neg	neg	1000	ADN	div	House method
5	positive	positive	negative	positive	4*	Nut-DNA	div	in house method CONGEN
8	positive	negative	negative	positive	100	Nut-DNA	div	in house

Methods:

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

div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
7	SFA ID				
1	div	-	pec1a1a1	CTAB; Magnetc Beads; Taqman real time PCR	
2	div			CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
3	div	House method	Vicilin like	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
5	div				*< = 4 mg allergenic substance/kg in non-processed corn flour
8	div		141bp	Wizard cleanup, Rotorgene6000	

5.1.15 PCR: Pistachio

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
5	negative	negative	negative	positive	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
7	negative	negative	negative	positive	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	positive	negative	negative	positive	25	pmPist-Fam	div	in house method
2	negative	-	negative	positive		Nut-DNA	div	in house method
3	neg	neg	neg	neg	1000	ADN	div	House method
8	negative	negative	negative	positive	100	Nut-DNA	div	in house

Methods:

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

div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
5	SFA ID				
7	SFA ID				
1	div	-	18s rRNA	CTAB; Magnetc Beads; Taqman real time PCR	
2	div			CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
3	div	Engel y col., 2008	Dehidrin (Cor)	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
8	div		77bp	Wizard cleanup, Rotorgene6000	

5.1.16 PCR: Walnut

Primary data

Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Meth. Abr.	Method
	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein		Test-Kit + Manufacturer
5	negative	positive	negative	negative	0.4mg/kg	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
7	negative	positive	negative	negative	0,4	Nut-DNA	SFA ID	Sure Food Allergen ID, Congen / r-Biopharm
1	negative	positive	negative	negative	25	pmWLZ-Atto	div	in house method
2	negative	positive	negative	negative		Nut-DNA	div	in house method
3	neg	neg	neg	neg	1000	ADN	div	House method
8	negative	positive	negative	negative	100	Nut-DNA	div	in house

Methods:

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

div = not indicated / other method

Other details to the Methods

Evaluation number	Meth. Abr.	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	
5	SFA ID				
7	SFA ID				
1	div	-	vicilin like protein	CTAB; Magnetc Beads; Taqman real time PCR	
2	div			CTAB / Protease K / Chloroform + Promega Wizard/ End Point PCR/ 4% Agarose Gel / 45 Cycles	
3	div	Wang y col., 2009	Vicilin like protein	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	DNA extraction of the sample was insufficient to implement the LOD established in the assay
8	div		88bp	Wizard cleanup, Rotorgene6000	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 11-2016 Sample 1

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,13	43	16,8
2	5,29	50	18,9
3	5,34	49	18,4
4	5,21	43	16,5
5	5,14	35	13,6
6	5,38	50	18,6
7	5,3	58	21,9
8	5,61	41	14,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	46,1	Particles
Standard deviation	6,94	Particles
χ^2 (CHI-Quadrat)	7,30	
Probability	**40**	%
Recovery rate	87	%

Normal distribution		
Number of samples	8	
Mean	17,4	mg/kg
Standard deviation	2,62	mg/kg
rel. Standard deviaton	15,0	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,4**	
Recovery rate	87	%

Microtracer Homogeneity Test
DLA 11-2016 Sample 2

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	17,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,26	45	17,1
2	5,34	49	18,4
3	5,34	41	15,4
4	5,16	48	18,6
5	5,1	48	18,8
6	5,45	46	16,9
7	5,24	47	17,9
8	5,24	56	21,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	47,5	Particles
Standard deviation	4,62	Particles
χ^2 (CHI-Quadrat)	3,15	
Probability	**87**	%
Recovery rate	106	%

Normal distribution		
Number of samples	8	
Mean	18,1	mg/kg
Standard deviation	1,76	mg/kg
rel. Standard deviaton	9,7	%
Horwitz standard deviation	10,4	%
HorRat-value	**0,9**	
Recovery rate	106	%

Microtracer Homogeneity Test
DLA 11-2016 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	37,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,12	92	35,9
2	5,22	90	34,5
3	5,21	80	30,7
4	5,33	97	36,4
5	5,7	93	32,6
6	5,2	88	33,8
7	5,51	91	33,0
8	5,46	88	32,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	89,9	Particles
Standard deviation	5,11	Particles
χ^2 (CHI-Quadrat)	2,03	
Probability	**96**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	33,7	mg/kg
Standard deviation	1,91	mg/kg
rel. Standard deviaton	5,7	%
Horwitz standard deviation	9,4	%
HorRat-value	**0,6**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA 11-2016 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	11,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,33	37	13,9
2	5,55	28	10,1
3	5,1	32	12,5
4	5,13	44	17,2
5	5,62	34	12,1
6	5,21	45	17,3
7	5,93	33	11,1
8	5,33	39	14,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	36,7	Particles
Standard deviation	7,15	Particles
χ^2 (CHI-Quadrat)	9,74	
Probability	**20**	%
Recovery rate	124	%

Normal distribution		
Number of samples	8	
Mean	13,6	mg/kg
Standard deviation	2,65	mg/kg
rel. Standard deviaton	19,5	%
Horwitz standard deviation	10,8	%
HorRat-value	**1,8**	
Recovery rate	124	%

6. Index of participant laboratories

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

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

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

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