DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

12/2016

Allergen-Screening II:

Crustacea, Egg, Fish, Milk, Molluscs, Mustard and Soya

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Inhalt / Content

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1. Introduction

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 2-20% of the allergenic ingredients concerned.
The respective raw materials were common in commerce egg powder, milk powder and soyflour and premixes produced by DLA from commercial mustard seeds and frozen shrimps, cod and squid (s. Tab. 2). The mustard seeds were crushed, ground with the addition carrier substances and sieved (mesh 400 µm). The frozen marine foods were crushed, dried and ground with addition of carriers and sieved by means of a centrifugal mill (mesh 500 µm).
The composition of the allergen-premixes is given in table 1. The premixes were used for spiking of the PT-samples 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: Potatos, E471, E304, E223, E100)	74 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (30% - 88%) - Sodium chloride (0,0% - 85%) - Sodium sulfate (0,0% - 7,7%) - Silicon dioxide (1,0% - 2,2%) - Allergens (2,4% - 20% each)	0,027 - 0,42 %

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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
Crustaceae: Shrimps *(Litopenaeus vannamei)*, getrocknet (Protein 63%)	positive (25 - 75)	negative	positive (50 - 150)	negative
Egg: Whole egg powder (Protein 47%)	positive (50 - 150)	positive (25 - 75)	negative	negative
Fish: Cod *(Gadus morhua)*, dried (Protein 56%)	negative	negative	positive (25 - 75)	positive (50 - 150)
Milk: Skimmed milk powder (Protein 37%)	positive (25 - 75)	negative	positive (50 - 150)	negative
Molluscs: Squid tubes *(Illex argentinus)*, dried (Protein 34%)	negative	positive (50 - 150)	negative	positive (25 - 75)
Mustard, yellow: Sinapis alba (Protein 31%)	negative	positive (50 - 150)	negative	negative
Mustard, brown: Brassica juncea (Protein 24%)	negative	negative	negative	positive (25 - 75)
Mustard, black: Brassica nigra (Protein 27%)	negative	negative	negative	positive (25 - 75)
Soya: Soyflour, not toasted (Protein 37%)	negative	negative	positive (50 - 150)	positive (25 - 75)

* Protein contents according to laboratory analysis (total nitrogen, Kjeldahl)
**Allergen contents of „food item" as indicated in the column of ingredients 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 98%, 99%, 91% and 82%, 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 0,5, 0,5, 0,7 and 0,9, respectively. The results of microtracer analysis are given in the documentation.

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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 29th week of 2016. The testing method was optional. The tests should be finished at September 16th 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 Crustaceae, Egg, Fish, Milk, Molluscs, Mustard (yellow/white, brown and black) and Soybean. The allergens are contained in a simple carrier matrix (75% potato powder / 25% 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.
All 17 participants submitted their results in time.

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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.

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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				

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4.1 Proficiency Test Crustaceae

4.1.1 ELISA-Results: Crustaceae (Shrimps)

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	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	BA	Lateral Flow
9	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	

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

Methods:

BA = Bioavid, R-Biopharm

ES = ELISA-Systems

IL = Immunolab

RS = Ridascreen® Fast, R-Biopharm

Comments:

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

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4.1.2 PCR-Results: Crustaceae (Shrimps)

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%)	IC	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
16	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	div	keine positiveprobe identifiziert

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

Methods:

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One participant could not detect any positive sample by means of an in-house PCR method.

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4.2 Proficiency Test Egg

4.2.1 ELISA-Results: Egg (Whole egg powder)

Qualitative valuation of results

Auswerte-nummer	Probe 1	Probe 2	Probe 3	Probe 4	Qualitative Bewertung	Qualitative Bewertung	Methode	Hinweis
	pos/neg	pos/neg	pos/neg	pos/neg	Übereinstimmungen mit Konsenswerten	Übereinstimmungen mit Dotierungen		
4a	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
16	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	MR	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
4b	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
9	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
17	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	VT	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	13	13	1	1
Number negative	0	0	12	12
Percent positive	100	100	8	8
Percent negative	0	0	92	92
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:
AQ = AgraQuant, RomerLabs
BA = Bioavid, R-Biopharm
MR = Morinaga ELISA
RS = Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

Comments:
The consensus values of results are in qualitative agreement with the spiking of samples. One participant indicated positive results for all samples by the ELISA method MR (not giving a plausible limit of detection).

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4.2.2 PCR-Results: Egg (Whole egg powder)

Qualitative valuation of results

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

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

Methods:

div = not indicated / other method

Comments:

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

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4.3 Proficiency Test Fish

4.3.1 ELISA-Results: Fish (Cod)

Qualitative valuation of results

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

	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:

AQ = AgraQuant, RomerLabs

BC = BioCheck

Comments:

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

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4.3.2 PCR-Results: Fish (Cod)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	div	no positive sample detected

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

Methods:

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. One participant could not detect any positive sample by means of an in-house PCR method.

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4.4 Proficiency Test Milk

4.4.1 ELISA-Results: Milk, Casein, beta-Lactoglobulin

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		
9a	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	Casein
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BA	Lateral Flow , Milk
9b	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	beta-Lactoglobulin
17a	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS1	Milk
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS2	Casein, beta-Lactoglobulin
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS2	Casein, beta-Lactoglobulin
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS2	Casein, beta-Lactoglobulin
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS2	Casein, beta-Lactoglobulin
17b	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS2	Casein, beta-Lactoglobulin
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS3	beta-Lactoglobulin
17c	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS3	beta-Lactoglobulin
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	VT	Milk
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	VT	Milk
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	VT	Milk
9c	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	Milk

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	15	0	15	0
Number negative	0	15	0	15
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, R-Biopharm
ES = ELISA-Systems
RS1 = Ridascreen® Fast R4612, R-Biopharm
RS2 = Ridascreen® Fast R4652, R-Biopharm
RS3 = Ridascreen® Fast R4902, R-Biopharm
VT = Veratox, Neogen
div = not indicated / other method

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

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4.4.2 PCR-Results: Milk (Skimmed milk powder)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
16	negative	negative	negative	negative	-	2/4 (50%)	div	no positive sample detected

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

Methods:

div = not indicated / other method

Comments:

One participant could not detect any positive sample by means of an in-house PCR method.

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4.5 Proficiency Test Molluscs

4.5.1 ELISA-Results: Molluscs (Squid)

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	-	4/4 (100%)	ET	

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

Methods:

ET = Elution Technologies

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. The participant noted a positive detection of sample 3 possibly due to a cross-reactivity to crustaceae in sample 3.

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4.5.2 PCR-Results: Molluscs (Squid)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	positive	negative	positive	3/3 (100%)	4/4 (100%)	IC	
14	negative	negative	negative	negative	2/3 (67%)	2/4 (50%)	IC	no positive sample detected
2	negative	positive	negative	positive	3/3 (100%)	4/4 (100%)	SFA-ID	
11	negative	positive	negative	positive	3/3 (100%)	4/4 (100%)	SFA-ID	
12	negative	positive	negative	negative	3/3 (100%)	3/4 (75%)	SFA-ID	sample with lower amount not detected

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	4	0	3
Number negative	5	1	5	2
Percent positive	0	80	0	60
Percent negative	100	20	100	40
Consensus value	negative	positive	negative	-
Spiking	negative	positive	negative	positive

Methods:
IC = Food Allergen Detection PCR Kit, real Time PCR, InCura
SFA-ID =Sure Food Allergen ID, Congen / r-Biopharm

Comments:
The consensus values of results are in qualitative agreement with the spiking of samples for samples 1, 2 and 3.
For the spiked sample with a lower amount of allergen the results were varying. One participant could not detect any positive sample by means of an in-house PCR method.

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4.6 Proficiency Test Mustard

4.6.1 ELISA-Results: Mustard

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	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ES	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS	
17	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

BA = Bioavid, R-Biopharm

ES = ELISA-Systems

RS = Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

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

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4.6.2 PCR-Results: Mustard

Qualitative valuation of results

4.6.2.1 Mustard, in general

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	4/4 (100%)	4/4 (100%)	SFA-ID	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

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

div = not indicated / other method

Comments:

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

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4.6.2.2 Mustard, yellow (Sinapis alba)

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	negative	positive	4/4 (100%)	3/4 (75%)	div	
16	negative	positive	negative	positive	4/4 (100%)	3/4 (75%)	div	

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

Methods:
div = not indicated / other method

4.6.2.2 Mustard, brown (Brassica juncea)

Auswerte-nummer	Probe 1	Probe 2	Probe 3	Probe 4	Qualitative Bewertung	Qualitative Bewertung	Methode	Hinweis
	pos/neg	pos/neg	pos/neg	pos/neg	Übereinstimmungen mit Konsenswerten	Übereinstimmungen mit Dotierungen		
4	negative	negative	negative	positive	-	4/4 (100%)	div	
16	negative	negative	negative	negative	-	3/4 (75%)	div	no positive sample detected

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	0	1
Number negative	2	2	2	1
Percent positive	0	0	0	50
Percent negative	100	100	100	50
Consensus value	negative	negative	negative	-
Spiking	negative	negative	negative	positive

Methods:
div = not indicated / other method

4.6.2.3 Mustard, black (Brassica nigra)

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	negative	negative	positive	-	4/4 (100%)	div	
16	negative	negative	negative	negative	-	3/4 (75%)	div	no positive sample detected

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	0	1
Number negative	2	2	2	1
Percent positive	0	0	0	50
Percent negative	100	100	100	50
Consensus value	negative	negative	negative	-
Spiking	negative	negative	negative	positive

Methods:
div = not indicated / other method

Comments:
Two participants tested for mustard species by PCR. Sinapis alba was detected in samples 2 and 4 by both of them. Only sample 2 was spiked with Sinapis alba. One participant detected Brassica species in sample 4 which is in agreement with the spiking of the samples.

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4.7 Proficiency Test Soya

4.7.1 ELISA-Results: Soya (Soyflour)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ES	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
6a	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
17	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
6b	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	VT	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	VT	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	VT	

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

ES = ELISA-Systems

IL = Immunolab

RS = Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

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

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4.7.2 PCR-Results: Soya (Soyflour)

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	negative	positive	positive	4/4 (100%)	4/4 (100%)	MS	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
7	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	SFA-Quant	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

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

SFA-Quant = Sure Food Allergen QUANT, Congen / r-Biopharm

div = not indicated / other method

Comments:

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

One participant detected all samples positive by means of PCR method SFA-Quant indicating a limit of quantification of 1 mg/kg.

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5. Documentation

5.1 Details by the participants

5.1.1 ELISA: Crustaceae

Primary data

Meth. Abr.	Evaluation number	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
ES	9	positive	negative	positive	negative	0,05	Tropomyosins crustaceae	ELISA-Systems, Residue Assay
ES	10	positive	negative	positive	negative	0,05	Protein, total	ELISA-Systems, Residue Assay
IL	13	positive	negative	positive	negative	0,001	Tropomyosin	Immunolab ELISA
RS	1	positive	negative	positive	negative	0,172	crustacean protein/food	Ridascreen Fast, r-Biopharm
RS	6	positive	negative	positive	negative	0,5	Food item, total	Ridascreen Fast, r-Biopharm
RS	17	positive	negative	positive	negative	2	Protein	r-Biopharm AG FAST Crustacean

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. Extraction Solution / Time / Temperature	
ES	9				
ES	10				
IL	13	CRU-E01	Tropomyosin		
RS	1	R 7302	CRUSTACEAN PROTEIN	One buffer extraction (60°C)	
RS	6	R7312	Crustacean Protein (Mainly tropomysin)	As Per Kit Instructions	
RS	17	R7312	specific	Allergen extraction buffer 10 minutes 60°C	

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5.1.2 ELISA: Egg

Primary data

Meth. Abr.	Evaluation number	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	4a	positive	positive	negative	negative	0,05	Egg white proteins	AgraQuant, RomerLabs
MR	16	positive	positive	positive	positive	0,01	Allergen-DNA	Morinage Egg Elisa
RS	1	positive	positive	negative	negative	0,1	whole egg powder/food	Ridascreen Fast, r-Biopharm
RS	3	positive	positive	negative	negative	0.5	Whole egg powder	Ridascreen Fast, r-Biopharm
RS	4b	positive	positive	negative	negative	0,1	Whole egg powder	Ridascreen Fast, r-Biopharm
RS	6	positive	positive	negative	negative	0,5	Food item, dry mass	Ridascreen Fast, r-Biopharm
RS	9	positive	positive	negative	negative	0,24	Whole egg powder	Ridascreen Fast, r-Biopharm
RS	12	positive	positive	negative	negative	0,5	Whole egg powder	Ridascreen, r-Biopharm
RS	13	positive	positive	negative	negative	0,1	Whole egg powder	Ridascreen Fast, r-Biopharm
RS	17	positive	positive	negative	negative	0,1	Whole egg powder	r-Biopharm AG FAST Egg/Ei
VT	8	positive	positive	negative	negative	2,5	Whole egg protein	Veratox Allergen, Neogen
div	5	positive	positive	negative	negative	0,1	Egg white proteins	

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. Extraction Solution / Time / Temperature	
AQ	4a	COKAL0848	Ovomucoid	Extraction and implementation according to testkit instructions	Sample 1: 48 mg/kg egg white proteins (mean 3 measurements), sample 2: 15,6 mg/kg egg white proteins (mean 3 measurements)
MR	16			Kit	
RS	1	R 6402	OVALBUMIN, OVOMUCOID	One buffer extraction (60°C)	
RS	3	14136		10min./60°C	
RS	4b	R6402	Egg white proteins	Extraction and implementation according to testkit instructions	Sample 1: 117,4 mg/kg whole egg powder, sample 2: 52,4 mg/kg whole egg powder
RS	6	R6402	Egg White Proteins - Ovalbumin and ovomucoid	As Per Kit Instructions	
RS	9				
RS	12	R6402		As Per Kit Instructions	
RS	13	R6402	Egg White Proteins - Ovalbumin and ovomucoid		
RS	17	R6402	specific	Allergen extraction buffer 10 minutes 60°C	
VT	8	Product 8450 / Lot 224016		Extraction:60C pre-heated PBS / 15 min @ 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	
div	5	in house method	Egg White Proteins		

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5.1.3 ELISA: Fish

Primary data

Meth. Abr.	Evaluation number	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	8	negative	negative	positive	positive	4	Fish protein	AgraQuant, RomerLabs
BC	6	negative	negative	positive	positive	5	other: please fill in!	BioCheck

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. Extraction Solution / Time / Temperature	
AQ	8	Lot FI1013-1601		Extraction:60C pre-heated PBS / shaking for 15 min @ room temp / centrifugation Determination: 4 parameter curve	
BC	6			As Per Kit Instructions	Reported as Fresh Cod

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5.1.4 ELISA: Milk

Primary data

Meth. Abr.	Evaluation number	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	9a	positive	negative	positive	negative	0,2	Protein, total	AgraQuant, RomerLabs
ES	9b	positive	negative	positive	negative	0,05	Protein, total	ELISA-Systems, Residue Assay
RS1	17a	positive	negative	positive	negative	0,7 ppm Milchprotein	FAST Milk: 0,7 ppm Milk protein	r-Biopharm AG FAST Milk
RS2	4	positive	negative	positive	negative	0,7	Protein, total	Ridascreen Fast, r-Biopharm
RS2	6	positive	negative	positive	negative	2,5	Protein, total	Ridascreen Fast, r-Biopharm
RS2	13	positive	negative	positive	negative	0,7	Protein, total	Ridascreen Fast, r-Biopharm
RS2	15	positive	negative	positive	negative	0,7	Milk protein	Ridascreen Fast, r-Biopharm
RS2	17b	positive	negative	positive	negative	0,71 ppm Casein	FAST Casein: 0,71 ppm Casein	FAST Casein
RS3	1	positive	negative	positive	negative	0,19	b-lactoglobulin/food	Ridascreen Fast, r-Biopharm
RS3	17c	positive	negative	positive	negative	0,19 ppm ß-Lactoglobulin	FAST ß-Lactoglobulin: 0,19 ppm ß-Lactoglobulin	FAST ß-Lactoglobulin
VT	3	positive	positive	positive	negative	2,5	Total Milk Allergen	Veratox Allergen, Neogen
VT	5	positive	negative	positive	negative	1	Skimmed milk powder	Veratox Allergen, Neogen
VT	12	positive	negative	positive	negative	2,2	Skimmed milk powder	Veratox Allergen, Neogen
div	9c	positive	negative	positive	negative			

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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. Extraction Solution / Time / Temperature	
AQ	9a				
ES	9b				
RS1	17a	R 4612	specific	Extractor 2 10 Minutes 100°C; afterw ards Allergen extraction buffer 10 minutes 60°C	
RS2	4	R4652	Casein/ ß-Lactoglobulin	Extraction according to testkit instructions; additionally extraction protocol for strong gelling matrices (by R-Biopharm) applied	Sample 1: 13,63 mg/kg (mean 4 measurements), sample 3: 35,2 mg/kg (mean 4 measurements)
RS2	6	R4652	Caseins and b-lactoglobulins from Cow 's, Sheep, Goats and Buffalo's milk	As Per Kit Instructions	Reported as Milk Protein
RS2	13	R4652	Caseins and b-lactoglobulins (Cow 's, Sheep, Goats and Buffalo's milk)		
RS2	15	Art. Nr. 4652	Caseins and ß-Lactoglobulin	As Per Kit Instructions	
RS2	17b	R 4652	specific	Extractor 2 10 Minutes 100°C; afterw ards Allergen extraction buffer 10 Minutes 60°C	
RS3	1	R4902	COW'S, SHEEP'S, GOAT'S AND BUFFALO'S MILK	Tw o buffer extraction (100°C; 60°C)	Ridascreen Fast Casein, r-Biopharm R4612, LOD 0,24 mg protein/kg food, Tw o buffer extraction (100°C; 60°C), POSITIVE: sample n. 1 e n. 3
RS3	17c	R 4902	specific	Extractor 2 10 Minutes 100°C; afterw ards Allergen extraction buffer 10 minutes 60°C	
VT	3	228248		15min./60°C	
VT	5	8470	milk proteins	As Per Kit Instructions	
VT	12	8470		As Per Kit Instructions	
div	9c				

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5.1.5 ELISA: Molluscs

Primary data

Meth. Abr.	Evaluation number	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
ET	6	negative	positive	negative	positive	1	Protein, total	Elution Technologies Kit

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. Extraction Solution / Time / Temperature	
ET	6	E-75MSK	Mollusc Protein	As Per Kit Instructions	Note a positive result was noted on Sample 3 due to cross reaction with Crustacean in sample

5.1.6 ELISA: Mustard

Primary data

Meth. Abr.	Evaluation number	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
ES	12	negative	positive	negative	positive	0,5	Mustard powder	ELISA-Systems, Residue Assay
RS	6	negative	positive	negative	positive		Food item, total	Ridascreen Fast, r-Biopharm
RS	13	negative	positive	negative	positive	0,22	Mustard powder	Ridascreen Fast, r-Biopharm
RS	17	negative	positive	negative	positive	0,22	Mustard powder	r-Biopharm AG FAST Mustard/ Senf
VT	3	negative	positive	negative	positive	2,5	Total Mustard allergen	Veratox Allergen, Neogen
VT	8	negative	positive	negative	positive	2,5	Mustard	Veratox Allergen, Neogen
VT	9	negative	positive	negative	positive	1,5	Food item, total	Veratox Allergen, 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. Extraction Solution / Time / Temperature	
ES	12	ESMUS-48		As Per Kit Instructions	
RS	6	R6152	Yellow, White, Brown & Black Mustard	As Per Kit Instructions	
RS	13	R6152	mustard, in general		
RS	17	R 6152	specific	Allergen extraction buffer 10 Minutes 60°C	
VT	3	227775		15min./60°C	
VT	8	Product 8400 / Lot 232074		Extraction:60C pre-heated TRIS-EDTA / 15 min @ 60C in shaking waterbath / centrifugation Determination: 4 parameter curve	
VT	9				

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5.1.7 ELISA: Soya

Primary data

Meth. Abr.	Evaluation number	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
ES	10	negative	negative	positive	positive	2,5	Protein, total	ELISA-Systems, Residue Assay
IL	12	negative	negative	positive	positive	16 ppb	Soy Protein	Immunolab ELISA
RS	3	negative	negative	positive	positive	42492	Soy Protein	Ridascreen Fast, r-Biopharm
RS	4	negative	negative	positive	positive	0,31	Protein, total	Ridascreen Fast, r-Biopharm
RS	5	negative	negative	positive	positive	0,3	Protein, total	Ridascreen Fast, r-Biopharm
RS	6a	negative	negative	positive	positive		Protein, total	Ridascreen Fast, r-Biopharm
RS	7	negative	negative	positive	positive	0,31	Soya protein	Ridascreen Fast, r-Biopharm
RS	13	negative	negative	positive	positive	0,31	Protein, total	Ridascreen Fast, r-Biopharm
RS	17	negative	negative	positive	positive	0,24	Protein	r-Biopharm AG FAST Soya
VT	6b	negative	negative	positive	positive		other: please fill in!	Veratox Allergen, Neogen
VT	8	negative	negative	positive	positive	2,5	soy flour	Veratox Allergen, Neogen
VT	9	negative	negative	positive	positive	0,96	soyflour	Veratox Allergen, 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. Extraction Solution / Time / Temperature	
ES	10				
IL	12	SOJ-E01		As Per Kit Instructions	
RS	3	16485		10min./100°C	
RS	4	R7102	soy protein	Extraction and implementation according to test kit instructions	Sample 3: 7,1 mg/kg (mean 2 measurements), sample 4: 4,6 mg/kg (mean 2 measurements)
RS	5	R7102	heated soy protein	As Per Kit Instructions	
RS	6a	R7102	Heat Treated Soya Proteins	As Per Kit Instructions	Reported as Soya Protein
RS	7				LOQ = 2,5
RS	13	R7102	heated soy protein		
RS	17	R 7102	spezifisch	Extractor 3 + AEP 10 Minutes 100°C	
VT	6b	8410	No Data	As Per Kit Instructions	Reported as Soy Flour
VT	8	Product 8410 / Lot 230354		Extraction:60C pre-heated PBS / 15 min @ 60C in shaking w aterbath / centrifugation Determination: 4 parameter curve	
VT	9				

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5.1.8 PCR: Crustacea

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
IC	14	positive	negative	positive	negative	5	Allergen DNA	Incura
SFA-ID	2	positive	negative	positive	negative	50	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	6	positive	negative	positive	negative	1	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	11	pos	neg	pos	neg	5	food/food	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	12	positiv	negative	positiv	negative	0,4	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
div	16	negative	negative	negative	negative	0,01	Allergen-DNA	in house method

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	
IC	14				
SFA-ID	2	R-Biopharm, S3112	unknown	Phenol Chloroform extraction/ Qiagen plant mini kit/ normalise to 10ng/uL using 260/280 ratio.	
SFA-ID	6	S3112		As Per Kit Instructions	
SFA-ID	11		unknown	Extraction: NucleoSpin Food (Macherey Na-gel)/ Real Time PCR/ 35 cycles	
SFA-ID	12	S3112		As per Kit Instructions	
div	16			Wizard Miniprep cleanup	

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5.1.9 PCR: Egg

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
div	11	pos	pos	neg	neg	0,001	ADN/ADN	in-house method

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	
div	11		cytochrome b/ ovalbumin/ Vitellogenin	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	

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5.1.10 PCR: Fish

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
SFA-ID	2	negative	negative	positive	positive	10	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	10	negative	negative	positive	positive	5 DNA copies	Allergen DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	11	neg	neg	pos	pos	5	food/food	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	12	negative	negative	positive	positive	0,4	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	14	negative	negative	positive	positive	0,4	Allergen DNA	Sure Food Allergen ID, Congen / r-Biopharm
div	3	negative	negative	positive	positive		Allergen DNA	in-house method
div	9	negative	negative	positive	positive	40	Allergen-DNA	in-house method
div	16	negative	negative	negative	negative	0,01	Allergen-DNA	in-house method

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-ID	2	R-Biopharm, S3110	unknown	Phenol Chloroform extraction/ Qiagen plant mini kit/ normalise to 10ng/uL using 260/280 ratio.	
SFA-ID	10				
SFA-ID	11		unknown	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 35 cycles	
SFA-ID	12	S3110		As Per Kit Instructions	
SFA-ID	14				
div	3	Herrero et al., 2014	18S RNA	real time PCR	
div	9				
div	16			Wizard Miniprep cleanup	

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5.1.11 PCR: Milk

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
div	16	negative	negative	negative	negative	0,01	Allergen-DNA	in-house method

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	
div	16			Wizard Miniprep cleanup	

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5.1.12 PCR: Molluscs

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
IC	1	negative	positive	negative	positive	1 copia di genoma aploide = 1,6 pg	Allergen DNA	Incura
IC	14	negative	negative	negative	negative	5	Allergen DNA	Incura
SFA-ID	2	negative	positive	negative	positive	50	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	11	neg	pos	neg	pos	5	food/food	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	12	negative	positive	negative	negative		Food item, total	Sure Food Allergen ID, Congen / r-Biopharm

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	
IC	1	MOLLUSKIT REAL TIME PCR IC-02-1008	bivalvi 228 bp, cefalo-podi 150 bp, gastero-podi 157 bp	FOOD GREES DNA KIT INCURA IC-02-0095	
IC	14				
SFA-ID	2	R-Biopharm, S3113	unknown	Phenol Chloroform extraction/ Qiagen plant mini kit/ normalise to 10ng/uL using 260/280 ratio.	
SFA-ID	11		unknown	Extraction: NucleoSpin Food (Macherey Na-gel)/ Real Time PCR/ 35 cycles	
SFA-ID	12	S3113		As per kit instructions	

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5.1.13 PCR: Mustard, in general

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
SFA-ID	6	negative	positive	negative	positive	1	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	12	negative	positive	negative	positive	0,4		
SFA-ID	14	negative	positive	negative	positive	0,4	Allergen DNA	Sure Food Allergen ID, Congen / r-Biopharm
div	9	negative	positive	negative	positive	0,4	Allergen-DNA	in-house method
div	11	neg	pos	neg	pos	0,001	ADN/ADN	in-house method
div	16	negative	positive	negative	positive	0,01	Allergen-DNA	in-house method

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-ID	6	S3109		As Per Kit Instructions	
SFA-ID	12	S3109			
SFA-ID	14				
div	9				
div	11	Mustorp y col., 2008	sinA	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	
div	16			Wizard Miniprep cleanup	

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5.1.14 PCR: Senf, Sinapis alba

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
div	4	negative	positive	negative	positive	0,0001	Allergen-DNA	in-house method
div	16	negative	positive	negative	positive	0,01	Allergen-DNA	in-house method

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	
div	4		Sinapis alba	Macherey Nagel Nucleo Spin Food with optimizations: doubled sample weight, buffer changing (washing step with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination with UNG; in-house thermo profile	LOD in DNA-percent of referring plant
div	16			Wizard Miniprep cleanup	

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5.1.15 PCR: Mustard, Brassica juncea

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
div	4	negative	negative	negative	positive	0,0001	Allergen-DNA	in-house method
div	16	negative	negative	negative	negative	0,01	Allergen-DNA	in-house method

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	
div	4		Brassica juncea/nigra duplex	Macherey Nagel Nucleo Spin Food with optimizations: doubled sample weight, buffer changing (washing step with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination with UNG; in-house thermo profile	not distinguishing between Brassica juncea and nigra; LOD in DNA-percent of referring plant
div	16			Wizard Miniprep cleanup	

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5.1.16 PCR: Mustard, Brassica nigra

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
div	4	negative	negative	negative	positive	0,0001	Allergen-DNA	in-house method
div	16	negative	negative	negative	negative	0,01	Allergen-DNA	in-house method

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	
div	4		Brassica juncea/nigra duplex	Macherey Nagel Nucleo Spin Food with optimizations: doubled sample weight, buffer changing (washing step with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, Decontamination with UNG; in-house thermo profile	not distinguishing between Brassica juncea and nigra; LOD in DNA-percent of referring plant
div	16			Wizard Miniprep cleanup	

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5.1.17 PCR: Soya

Primary data

Meth. Abr.	Evaluation number	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 + Manufacturer
MS	4	negative	negative	positive	positive	0,00005	Allergen-DNA	Microsynth
SFA-ID	6	negative	negative	positive	positive	1	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	12	negative	negative	positive	positive	0,4	Food item, total	Sure Food Allergen ID, Congen / r-Biopharm
SFA-ID	14	negative	negative	positive	positive	0,4	Allergen DNA	Sure Food Allergen ID, Congen / r-Biopharm
SFA-Quant	7	positive	positive	positive	positive	0,4	Allergen DNA	Sure Food Allergen QUANT, Congen / r-Biopharm
div	9	negative	negative	positive	positive	40	Allergen-DNA	in-house method
div	11	neg	neg	pos	pos	0,001	ADN/ADN	in-house method
div	16	negative	negative	positive	positive	0,01	Allergen-DNA	in-house method

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	
MS	4	AllAllA Tetraplex	Lectin	Macherey Nagel Nucleo Spin Food w ith optimizations: doubled sample w eight, buffer changing (w ashing step w ith Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR w ith 45 cycles, Decontamination w ith UNG; in-house thermo profile	LOD in DNA-percent of referring plant
SFA-ID	6	S3101		As Per Kit Instructions	
SFA-ID	12	S3101		As Per Kit Instructions	
SFA-ID	14				
SFA-Quant	7				LOQ = 1 mg/kg
div	9				
div	11	Koppel y col., 2010	Le1	Extraction: NucleoSpin Food (Macherey Nagel)/ Real Time PCR/ 45 cycles	
div	16			Wizard Miniprep cleanup	

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5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 12-2016 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	40,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,40	126	46,7
2	5,12	115	44,9
3	5,10	123	48,2
4	5,03	119	47,3
5	5,04	121	48,0
6	5,06	111	43,9
7	5,05	121	47,9
8	5,19	112	43,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	119	Partikel
Standard deviation	5,13	Partikel
χ^2 (CHI-Quadrat)	1,56	
Probability	**98**	%
Recovery rate	115	%

Normal distribution		
Number of samples	8	
Mean	46,3	mg/kg
Standard deviation	2,00	mg/kg
rel. Standard deviaton	4,3	%
Horwitz standard deviation	9,0	%
HorRat-value	**0,5**	
Recovery rate	115	%

Microtracer Homogeneity Test
DLA 12-2016 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	24,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,08	73	28,7
2	5,04	69	27,4
3	5,08	65	25,6
4	5,15	70	27,2
5	4,97	69	27,8
6	5,12	72	28,1
7	5,09	66	25,9
8	5,06	74	29,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,8	Partikel
Standard deviation	3,22	Partikel
χ^2 (CHI-Quadrat)	1,04	
Probability	**99**	%
Recovery rate	111	%

Normal distribution		
Number of samples	8	
Mean	27,5	mg/kg
Standard deviation	1,27	mg/kg
rel. Standard deviaton	4,6	%
Horwitz standard deviation	9,7	%
HorRat-value	**0,5**	
Recovery rate	111	%

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Microtracer Homogeneity Test
DLA 12-2016 Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,21	105	40,3
2	5,00	101	40,4
3	5,18	104	40,2
4	5,07	112	44,2
5	5,02	105	41,8
6	5,11	107	41,9
7	5,07	96	37,9
8	5,09	92	36,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	102,8	Partikel
Standard deviation	6,32	Partikel
χ^2 (CHI-Quadrat)	2,72	
Probability	**91**	%
Recovery rate	120	%

Normal distribution		
Number of samples	8	
Mean	40,3	mg/kg
Standard deviation	2,48	mg/kg
rel. Standard deviaton	6,1	%
Horwitz standard deviation	9,2	%
HorRat-value	**0,7**	
Recovery rate	120	%

Microtracer Homogeneity Test
DLA 12-2016 Sample 4

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	23,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,11	65	25,4
2	5,06	75	29,6
3	5,15	61	23,7
4	5,02	65	25,9
5	5,04	59	23,4
6	5,05	66	26,1
7	5,05	61	24,2
8	5,01	56	22,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	63,5	Partikel
Standard deviation	5,72	Partikel
χ^2 (CHI-Quadrat)	3,60	
Probability	**82**	%
Recovery rate	108	%

Normal distribution		
Number of samples	8	
Mean	25,1	mg/kg
Standard deviation	2,26	mg/kg
rel. Standard deviaton	9,0	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,9**	
Recovery rate	108	%

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6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		SPAIN
		ITALY
		Germany
		Germany
		ITALY
		ITALY
		SWITZERLAND
		Germany
		Germany
		Germany
		AUSTRIA
		GREAT BRITAIN
		Germany
		GREAT BRITAIN
		SLOVAKIA
		CANADA

[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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