Evaluation Report

proficiency test

DLA ptALS2 (2021)

Allergen-Screening II:

Crustaceae, Egg, Fish, Milk, Molluscs, Mustard and Soybean

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (3 November 2021) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 3 November 2021
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficency test the following services were subcontracted: protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Content

1. Introduction

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

2. Realisation

2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 5-10% of the allergenic ingredients concerned.
The respective raw materials for the allergens used were commercial egg powder, milk powder and soy flour and premixes produced by DLA from commercial mustard seeds as well as frozen king prawns, cod and mussels (s. Tab. 2). The mustard seeds were crushed, ground with addition of carrier substances and sieved (mesh 400 µm). The frozen products were crushed, freeze dried and ground with addition of carrier substances and sieved by means of a centrifugal mill (mesh 250 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: Potatoes, E471, E304, E223, E100)	74,6 – 74,8 %
Maltodextrin	24,8 – 25,0 %
Allergen-Premixes Ingredients: - Maltodextrin (30% - 88%) - Titanium dioxide (0,0% - 40%) - Sodium sulfate (0,0% - 7,7%) - Silicon dioxide (1,0% - 2,2%) - Allergens (5,0% - 10% each)	0,30 - 0,55 %

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Crustaceae: King Prawns *(Litopenaeus vannamei)*, *freeze*-dried (Protein 87%)	positive (120)	positive (53)	negative	negative
Egg: Whole egg powder (Protein 47%)	negative	positive (50)	positive (100)	negative
Fish: Cod *(Gadus morhua)*, freez-dried (Protein 88%)	negative	positive (99)	negative	positive (54)
Milk: Skimmed milk powder (Protein 37%)	negative	negative	positive (49)	positive (110)
Molluscs: Yesso Scallop *(Mizuhopecten yessoen-sis)*, freez-dried (Protein 76%)	positive (78)	negative	positive (180)	negative
Mustard, yellow: Sinapis alba (Protein 31%)	negative	positive (85)	negative	negative
Mustard, brown: Brassica juncea (Protein 28%)	negative	negative	negative	positive (85)
Mustard, black: Brassica nigra (Protein 27%)	positive (87)	negative	negative	negative
Soya: Soyflour, not toasted (Protein 37%)	negative	positive (110)	positive (53)	negative

* Protein contents according to laboratory analysis (total nitrogen, Kjeldahl general factor F=6,25
**Allergen contents of „food item" as indicated in the column of ingredients according gravimetric mixing
Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

The detectability or absence of the allergens was tested by DLA using lateral flow assays. The results are in agreement with the spiking of the PT samples 1-4 (see Table 3).

Table 3: Verification of detectability of the added allergens by lateral flow assays (AgraStrip® LFD, Romer Labs®)

Lateral Flow Device (LFD)*	Sample 1	Sample 2	Sample 3	Sample 4
AgraStrip® Crustaceae	positive	positive	negative	negative
AgraStrip® Egg	negative	positive	positive	negative
AgraStrip® Casein	negative	negative	positive	positive
AgraStrip® Soy	negative	positive	positive	negative
AgraStrip® Mustard	positive	positive	negative	positive

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **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 99%, 100%, 74% and 99%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 0,52, 0,49, 0,97 and 0,54, respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,35 (22°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 23rd week of 2021. The testing method was optional. The tests should be finished at August 6th 2021 the latest (extended).

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

*There are 4 different samples possibly containing the allergenic ingredients **Crustaceae, Egg, Fish, Milk, Molluscs, Mustard (yellow/white, brown and black)** and/or **Soybean** in a simple carrier matrix The evaluation of results is strictly qualitative (positive / negative).*

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

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

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

2.3 Submission of results

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

34 of 35 participants submitted at least one result. One participant submitted no results.

3. Evaluation

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

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

3.1 Agreement with consensus values from participants

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

3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**.
The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

4. Results

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

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

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Crustaceae

4.1.1 ELISA-Results: Crustaceae (King Prawns)

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%)	4/4 (100%)	AQ	
20	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
23	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
25	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
29	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
32	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
21	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
17	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	RS-F	
24	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	RS-F	
33	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
26	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
34	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	17	17	2	0
Number negative	0	0	15	17
Percent positive	100	100	12	0
Percent negative	0	0	88	100
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

AQ = AgraQuant, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

BF = MonoTrace ELISA, BioFront Technologies

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

The two positive results for sample 3 obtained by the ELISA method RS-F are possibly due to cross-reactivity to molluscs (see test kit instructions, Ridascreen Fast).

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

4.1.2 PCR-Results: Crustaceae (King Prawns)

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	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
21	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
30	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
31	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
33	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.2 Proficiency Test Egg

4.2.1 ELISA-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		
20	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
25	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
30	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
32	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
22	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
7	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
1	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
26	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
23	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
15	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
24	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
34	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
18	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

MI-II = Morinaga Institute ELISA Kit II

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.2.2 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		
30	negative	positive	positive	negative	-	4/4 (100%)	SFA	method indicated by participant

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

One participant submitted results for egg using PCR. The results are in qualitative agreement with the spiking of the samples and the consensus values of the ELISA results.

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		
20	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
25	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	AQ	no positive sample detected
29	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
30	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	AQ	
32	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	AQ	
33	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BC	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	
34	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

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

4.3.2 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		
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IM	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MS	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
21	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
24	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
28	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
30	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
31	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
33	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
17	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
26	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

IM = IMEGEN, Spain

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.4 Proficiency Test Milk

4.4.1 ELISA-Results: Milk, Casein, β-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		
20	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AQ	
25	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AQ	
30	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AQ	
32	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AQ	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
11a	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MI-II	
11b	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MI-II	
26a	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MI-II	β-Lactoglobulin
26b	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	MI-II	Casein
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	Casein
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
15	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	β-Lactoglobulin
17	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
24	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	Casein
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	
34	negative	negative	positive	negative	3/4 (75%)	3/4 (75%)	SP	
26c	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	
18	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	22	21
Number negative	22	22	0	1
Percent positive	0	0	100	95
Percent negative	100	100	0	5
Consensus value	negative	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

AQ = AgraQuant, RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.4.2 PCR-Results: 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		
30	negative	negative	positive	positive	-	4/4 (100%)	SFA	method indicated by participant

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

One participant submitted results for milk using PCR. The results are in qualitative agreement with the spiking of the samples and the consensus values of the ELISA results.

4.5 Proficiency Test Molluscs

4.5.1 ELISA-Results: Molluscs (yesso scallop)

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	negative	positive	negative	3/3 (100%)	4/4 (100%)	3M	Sample 2: cross-reactivity to Crustaceae
20	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	AQ	
25	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	AQ	
32	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	AQ	
33	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	DE	
10	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	SP	
26	positive	negative	positive	negative	3/3 (100%)	4/4 (100%)	SP	
34	positive	positive	positive	negative	3/3 (100%)	3/4 (75%)	SP	Sample 2: w eak cross-reactivity to Crustaceae

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	8	4	8	0
Number negative	0	4	0	8
Percent positive	100	50	100	0
Percent negative	0	50	0	100
Consensus value	positive	none	positive	negative
Spiking	positive	negative	positive	negative

Methods:

3M = 3M Protein ELISA Kit

AQ = AgraQuant, RomerLabs

DE = Demeditec ELISA

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of sample 1, 2 and 4 are in qualitative agreement with the spiking of samples. For sample 2 (without addition of molluscs) no consensus value with ≥75% positive or negative results was obtained. Two participants pointed to a possible cross-reactivity to Crustaceae (methods 3M and SP). Samples 1 and 2 contain king prawns.
Possible cross-reactivities should be documented in the manufacturer's test kit information.

4.5.2 PCR-Results: Molluscs (yesso scallop)

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		
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	4L	
2	positive	negative	positive	-	3/3 (100%)	3/3 (100%)	SFA	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
21	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
23	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	SFA	
24	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
30	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
31	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
33	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	13	0	13	1
Number negative	0	13	0	11
Percent positive	100	0	100	8
Percent negative	0	100	0	92
Consensus value	positive	negative	positive	negative
Spiking	positive	negative	positive	negative

Methods:

ASU = ASU §64 Methode/method

IM = IMEGEN, Spain

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

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

4.6 Proficiency Test Mustard

4.6.1 ELISA-Results: Mustard, in general

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		
20	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	AQ	
25	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	AQ	
32	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	AQ	
22	positiv	positiv	negativ	negativ	3/4 (75%)	3/4 (75%)	AS	Lateral Flow
7	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
21	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	EZ	
6	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	RS-F	
14	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	RS-F	
23	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	RS-F	
24	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	RS-F	
10	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	SP	
26	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	SP	
34	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	SP	
18	positiv	positiv	negativ	positiv	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BA = Bioavid (Lateral Flow), R-Biopharm

EZ = Orsell EZPLATE

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples (sample 1 black mustard, sample 2 yellow mustard and sample 4 brown mustard).

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		
26	positive	positive	negative	positive	3/3 (100%)	4/4 (100%)	ASU	
2	positive	positive	positive	positive	3/3 (100%)	3/4 (75%)	SFA	
10	positive	positive	negative	positive	3/3 (100%)	4/4 (100%)	SFA	
21	positive	positive	-	positive	3/3 (100%)	3/3 (100%)	SFA	
12	positive	positive	positive	positive	3/3 (100%)	4/4 (100%)	SFA-4p	

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

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Some participants used PCR methods for the detection of mustard without differentiating the varieties.

The consensus values of results for sample 1, 2 and 4 are in qualitative agreement with the spiking of samples (sample 1 black mustard, sample 2 yellow mustard and sample 4 brown mustard).

For sample 3 (without the addition of mustard), no consensus value of ≥75% positive or negative results could be determined.

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		
16	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
28	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	CEN	
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	MS	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
13	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
17	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

CEN = European Committee for Standardization Method

MS = Microsynth

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

6 participants tested for mustard species by PCR. Yellow mustard (Sinapis alba) was detected in sample 2 by all of them.
The consensus values of results are in qualitative agreement with the spiking of samples.

4.6.2.3 Mustard, brown and black (Brassica juncea / 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		
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MS	
13	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
28	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

CEN = European Committee for Standardization Method

MS = Microsynth

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Moreover four participants detected Brassica species in sample 1 (containing black mustard, Brassica nigra) and sample 4 (containing brown mustard, Brassica juncea).
The consensus values of results are in qualitative agreement with the spiking of samples.

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		
20	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
25	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
30	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
32	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
22	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	AS	Lateral Flow
26	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
7	negative	positive	negative	positive	2/4 (50%)	2/4 (50%)	RS-F	samples 3 and 4 interchanged?
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
24	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
34	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
18	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	17	15	1
Number negative	17	0	2	16
Percent positive	0	100	88	6
Percent negative	100	0	12	94
Consensus value	negative	positive	positive	negative
Spiking	negative	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.7.2 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		
16	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
1	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MS	
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
19	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
21	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
27	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
30	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
26	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
28	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA: Crustaceae

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	11	20.07.21	positive	positive	negative	negative	0,02	Troppomyosin	AQ = AgraQuant, RomerLabs
AQ	20		positive	positive	negative	negative	0,0045	Tropomyosin Protein	AQ = AgraQuant, RomerLabs
AQ	23	21.07.21	positive	positive	negative	negative	0,1	other: Crustacea Protein	AQ = AgraQuant, RomerLabs
AQ	25	21.07.21	positive	positive	negative	negative	0,0009	crustacean tropomyosin	AgraQuant, RomerLabs
AQ	29	22/07	positive	positive	negative	negative	0,1	Crustacae Protein	AQ = AgraQuant, RomerLabs
AQ	32	28.07.21	positive	positive	negative	negative	0,02	Crustacea Tropomyosin protein	AQ = AgraQuant, RomerLabs
BA	7		positive	positive	negative	negative	1 mg/kg	Food item, total	r-biopharm, Bioavid
BF	21		positive	positive	negative	negative	1	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
RS-F	15	29.06.21	positive	positive	negative	negative	2	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	17		positive	positive	positive	negative	2	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	24	02.07.21	1679,75	609,85	30,21	negative	2	Food item, total	RS = Ridascreen®, R-Biopharm
RS-F	33	04.08.21	positive	positive	negative	negative	20	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	8	28.06.21	positive	positive	negative	negative	0,02	Protein (tropomyosin)	SP = SensiSpec, Eurofins Technologies
SP	10		positive	positive	negative	negative		Please select!	SP = SensiSpec, Eurofins Technologies
SP	26	23.6.	positive	positive	negative	negative	0,02	crustacean tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	34	28.06.21	positive	positive	negative	negative	0,02	Tropomyosin	SP = SensiSpec, Eurofins Technologies
VT	5	07.06.21	positive	positive	negative	negative		Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	11			as stipulated in kit insert	limit of quantification reported in column I
AQ	20				
AQ	23	10002076			
AQ	25				
AQ	29	10002076		Romer Extraction Buffer / 15 min / Room Temp.	
AQ	32				
BA	7			aqueous extraction according to manufacturer	
BF	21				
RS-F	15	R 7312	ANTI-TROPOMIOSIN	EXTRACTION: BUFFER 10 MINUTI / 60°C DETERMINATION 30 MINUTI / 20-25°C	
RS-F	17				
RS-F	24	R7312			
RS-F	33	R7312	As Per Kit Instructions	As Per Kit Instructions	
SP	8	HU0030006			Reported as ug/Kg tropomyosin from crustaceans
SP	10				
SP	26	HU0030006/HU0030030	detects crustacean tropomyosin	As Per Kit Instructions	
SP	34				
VT	5	8520	Crustacea	PBS/15 minutes/30 C	

5.1.2 ELISA: Egg

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	20		negative	positive	positive	negative	0,5	Protein, total	AQ = AgraQuant, RomerLabs
AQ	25	20.07.21	negative	positive	positive	negative	0,05	egg protein	AgraQuant, RomerLabs
AQ	30	07.12.21	Negative	17.43 mg/kg	21.32 mg/kg	Negative	0,4	Egg white protein	AQ
AQ	32	06.08.21	negative	positive	positive	negative	0,4	Egg white protein	AQ = AgraQuant, RomerLabs
AS	22		negative	positive	positive	negative	2		AgraStrip Egg/Romer Labs
BA	7		negative	positive	positive	negative	1 mg/kg	Food item, total	r-biopharm, Bioavid
MI-II	1	22.06.21	negative	positive	positive	negative	10	Food item, total	MI-II = Morinaga Institute ELISA II
MI-II	8	25.06.21	negative	positive	positive	negative	0,31	Whole egg protein	MI-II = Morinaga Institute ELISA II
MI-II	11	14.07.21	negative	positive	positive	negative	0,31	egg protein	MI-II = Morinaga Institute ELISA II
MI-II	26	28.6.	negative	positive	positive	negative	0,31	whole egg protein	MI-II = Morinaga Institute ELISA II
RS	23	22.07.21	negative	positive	positive	negative	0,25	Whole egg powder	RS = Ridascreen®, R-Biopharm
RS-F	6	21.06.21	negative	positive	positive	negative	0,5	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13	07.07.21	negative	positive	positive	negative	0,1	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	25.06.21	<0,5	>13,5	>13,5	<0,5	0,5	egg	ridascreen FAST EGG PROTEIN R6402
RS-F	15	29.06.21	negative	positive	positive	negative	0,1	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	17		negative	positive	positive	negative	0,1	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	24	29.06.21	negative	50,42	107,03	negative	0,1	Food item, total	RS = Ridascreen®, R-Biopharm
SP	10		negative	positive	positive	negative		Please select!	SP = SensiSpec, Eurofins Technologies
SP	34	28.06.21	negative	positive	positive	negative	0,4	Egg white protein	SP = SensiSpec, Eurofins Technologies
VT	18	15.07.21	negative	positive (84.2ppm)	positive (176ppm)	negative	1	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	20				
AQ	25				
AQ	30	COKAL0848	Egg white residue	Extraction buffer	-
AQ	32				
AS	22				
BA	7			aqueous extraction according to manufacturer	
MI-II	1	M2111		extraction according to test kit instructions "Short Time Extraction Method"	
MI-II	8	M2111			Reported as whole egg protein mg/Kg
MI-II	11			as stipulated in kit insert // overnight extraction	limit of quantification reported in column I
MI-II	26	M2113	detects Ovalbumin	As per kit instructions	
RS	23	R6411			
RS-F	6	RIDASCREEN® FAST Ei / Egg Protein (ART. No R6402) / 15339	The antibodies specifically detect the antigens ovalbumin and ovomucoid of hen's egg	As per kit instructions	no
RS-F	13				
RS-F	14	R6402			
RS-F	15	R 6402	ANTI- OVOALBUMIN ANTI-OVOMUCOID	EXTRACTION: BUFFER 10 MINUTI / 60°C DETERMINATION 30 MINUTI / 20-25°C	
RS-F	17				
RS-F	24	R6402			
SP	10				
SP	34				
VT	18	8450		PBS/15min/60oC	

5.1.3 ELISA: Fish

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	20		negative	positive	negative	positive	1,4	Cod Parvalbumin Protein	AQ = AgraQuant, RomerLabs
AQ	25	04.08.21	negative	negative	negative	negative	1,4	parvalbumin	AgraQuant, RomerLabs
AQ	29	07/07	negative	positive	negative	positive	4.0	Fish Protein	AQ = AgraQuant, RomerLabs
AQ	30	07.12.21	Negative	73.77 mg/kg	Negative	44.51 mg/kg	4	Fish parvalbumin	AQ
AQ	32	06.08.21	negative	positive	positive	positive	4	Food item, total	AQ = AgraQuant, RomerLabs
BC	33	04.08.21	negative	positive	negative	positive	5	other: please fill in!	BC = BioCheck ELISA
SP	10		negative	positive	negative	positive		Please select!	SP = SensiSpec, Eurofins Technologies
SP	34	25.06.21	negative	positive	negative	positive	4	Food item, fresh	SP = SensiSpec, Eurofins Technologies

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	20				
AQ	25				
AQ	29	10002083		Romer Extraction Buffer / 15 min / 60°C	
AQ	30	COKAL2548	Fish parvalbumin	Extraction buffer	-
AQ	32				
BC	33	R6010	As Per Kit Instructions	As Per Kit Instructions	Cod, Fresh
SP	10				
SP	34				

5.1.4 ELISA: Milk

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	20		negative	negative	positive	positive	0,05	Total Milk Protein	AQ = AgraQuant, RomerLabs
AQ	25	28.07.21	negative	negative	positive	positive	0,05	milk protein	AgraQuant, RomerLabs
AQ	30	07.12.21	Negative	Negative	19.42 mg/kg	49.53 mg/kg	0,4	Milk protein	AQ
AQ	32	02.08.21	negative	negative	positive	positive	0,4	Protein, total	AQ = AgraQuant, RomerLabs
BA	7		negative	negative	positive	positive	1 mg/kg	Food item, total	r-biopharm, Bioavid
MI-II	11a	13.07.21	negative	negative	positive	positive	0,31	Milk protein	MI-II = Morinaga Institute ELISA II
MI-II	11b	13.07.21	negative	negative	positive	positive	0,31	Milk protein	MI-II = Morinaga Institute ELISA II
MI-II	26a	25.6.	negative	negative	positive	positive	0,031	ßLactoglobulin	MI-II = Morinaga Institute ELISA II
MI-II	26b	25.6.	negative	negative	positive	positive	0,25	Casein	MI-II = Morinaga Institute ELISA II
RS-F	1	22.06.21	negative	negative	positive	positive	10	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	2		negative	negative	positive	positive	1,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	4	12.07.21	negative	negative	positive	positive	0,7	other: Milkprotein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	6	21.06.21	negative	negative	positive	positive	0,5	Casein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13	06.07.21	negative	negative	positive	positive	0,7	Protein, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	23.06.21	<2,5	<2,5	16,1	24,1	2,5	milk	ridascreen FAST MILK R4652
RS-F	15	30.06.21	negative	negative	positive	positive	0,04	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	17		negative	negative	positive	positive	0,7	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	24	01.07.21	negative	negative	13,81	30,97	0,71	Food item, total	RS = Ridascreen®, R-Biopharm
SP	10		negative	negative	positive	positive		Please select!	SP = SensiSpec, Eurofins Technologies
SP	34	25.06.21	negative	negative	positive	negative	0,4	Casein+BLG	SP = SensiSpec, Eurofins Technologies
SP	26c	28.7.	negative	negative	positive	positive	0,4	Milkprotein	SP = SensiSpec, Eurofins Technologies
VT	18	15.07.21	negative	negative	positive (85.2ppm)	positive (142ppm)	1,3	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	20				
AQ	25				
AQ	30	COKAL2448	Milk protein	Extraction buffer	-
AQ	32				
BA	7			aqueous extraction according to manufacturer	
MI-II	11a		BLG	as stipulated in kit insert // overnight extraction	limit of quantification reported in column I
MI-II	11b		CASEIN	as stipulated in kit insert // overnight extraction	limit of quantification reported in column I
MI-II	26a	M2112	detects cow 's milk ßLac	As per kit instructions	*10 = total milk protein
MI-II	26b	M2113	detects cow 's milk Casein	As per kit instructions	*1,24 = total milk protein
RS-F	1	R4652		extraction according to test kit instructions "solid food"	
RS-F	2				
RS-F	4	R4652	see test kit instructions	As per kit instructions	
RS-F	6	RIDASCREEN® FAST casein Art. Nº R4612 / 22060	The antibodies specifically detect Casein	As per kit instructions	no
RS-F	13				
RS-F	14	R4652			
RS-F	15	R 4912	ANTI-COW BETALACTOGLOBULIN	EXTRACTION: BUFFER1 10 MIN/ 100°C BUFFER 2 10 MIN/60°C DETERMINATION 30 MINUTI / 20-25°C	
RS-F	17				
RS-F	24	R4612			
SP	10				
SP	34				
SP	26c	HU0030038		As per kit instructions	
VT	18	8470		PBS/15min/60oC	

5.1.5 ELISA: Molluscs

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
3M	11	21.07.21	positive	negative	positive	negative	1	mollusc protein	3M
AQ	20		positive	positive	positive	negative	0,0017	Protein, total	AQ = AgraQuant, RomerLabs
AQ	25	21.07.21	positive	positive	positive	negative	0,0009	mollusk tropomyosin	AgraQuant, RomerLabs
AQ	32	06.08.21	positive	positive	positive	negative	0,01	Mollusk Tropomyosin	Selection ELISA-Kits:
DE	33	04.08.21	positive	negative	positive	negative	10ug/kg	other: please fill in!	other: please fill in!
SP	10		positive	negative	positive	negative		Please select!	SP = SensiSpec, Eurofins Technologies
SP	26	22.7.	positive	negative	positive	negative	0,03	Mollusk Tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	34	25.06.21	positive	positive**	positive	negative	0,01	Tropomyosin	Selection ELISA-Kits:

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
3M	11			as stipulated in kit insert	limit of quantification reported in column I // Cross reactivity w ith crustaceae w as observed for sample 2
AQ	20				
AQ	25				
AQ	32				
DE	33	DEMOLE01	As Per Kit Instructions	As Per Kit Instructions	tropomyosin (ppb) - Demeditec Kit
SP	10				
SP	26	HU0030015/HU0030039	detects mollusk tropomyosin	As Per Kit Instructions	
SP	34				** Weakly positive due to cross-reactivity of crustatceans

5.1.6 ELISA: Mustard

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	20		positive	positive	negative	positive	1	Protein, total	AQ = AgraQuant, RomerLabs
AQ	25	23.07.21	positive	positive	negative	positive	1	wholw mustard	AgraQuant, RomerLabs
AQ	32	03.08.21	positive	positive	negative	positive	2	Food item, total	AQ = AgraQuant, RomerLabs
AS	22		positive	positive	negative	negative	2		AgraStrip Mustard/Romer Labs
BA	7		positive	positive	negative	positive	1 mg/kg	Food item, total	r-biopharm, Bioavid
EZ	21		positive	positive	negative	positive	2	Food item, total	ORSELL EZPLATE MUSTARD
RS-F	6	22.06.21	positive	positive	negative	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	21.07.21	>13,5	>13,5	<0,5	>13,5	0,5	mustard	ridascreen FAST MUSTARD R6152
RS-F	23	23.07.21	positive	positive	negative	positive	0,5	other: Mustard Powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	24	01.07.21	606,17	123,47	negative	683,17	0,1	Food item, total	RS = Ridascreen®, R-Biopharm
SP	10		positive	positive	negative	positive		Please select!	SP = SensiSpec, Eurofins Technologies
SP	26	25.6.	positive	positive	negative	positive		mustard	SP = SensiSpec, Eurofins Technologies
SP	34	28.06.21	positive	positive	negative	positive	2	Food item, total	SP = SensiSpec, Eurofins Technologies
VT	18	05.07.21	positive (143ppm)	positive (227ppm)	negative	positive (101ppm)	9,5	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	20				
AQ	25				
AQ	32				
AS	22				
BA	7			aqueous extraction according to instructions	
EZ	21			The kit that we used not distinguish the three varieties of mustard listed	
RS-F	6	Ridascreen® FAST Mustard R6152, R-Biopharm / 14489	The antibody specifically detects white, yellow, brown and black mustard.	As per kit instructions. Kit uses general mustard screening. Yellow, brown and black mustard cannot be differentiated	no
RS-F	14	R6152			
RS-F	23	R6152			
RS-F	24	R6152			
SP	10				
SP	26	HU0030016/HU0030040	detects mustard proteins (white, brown, black)	As per kit instructions	
SP	34				
VT	18	8400		tris-EDTA/15min/60oC	mustard in general

5.1.7 ELISA: Soya

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
AQ	20		negative	positive	positive	negative	0,016	Soy Trypsin Inhibitor Protein	AQ = AgraQuant, RomerLabs
AQ	25	20.07.21	negative	positive	positive	negative	0,016	soy trypsin inhibitor	AgraQuant, RomerLabs
AQ	30	07.12.21	Negative	2.21 mg/kg	0.84 mg/kg	Negative	0,04	Soy trypsin inhibitor	AQ
AQ	32	06.08.21	negative	positive	positive	negative	0,3	Protein, total	AQ = AgraQuant, RomerLabs
AS	22		negative	positive	negative	negative	2		AgraStrip Soybean/Romer Labs
MI-II	26	24.6.	negative	positive	positive	negative	1,25	Soyprotein	MI-II = Morinaga Institute ELISA II
RS-F	6	22.06.21	negative	positive	positive	negative	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	7		negative	positive	negative	positive	5 mg/kg	Food item, total	RS = Ridascreen®, R-Biopharm
RS-F	8	28.06.21	negative	positive	positive	negative	2,5	Protein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12	27.07.21	negative	positive	positive	negative	2,5	Protein, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13	13.07.21	negative	positive	positive	negative	0,24	Protein, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14	01.07.21	<2,5	>20,0	>20,0	<2,5	2,5	soya protein	ridascreen FAST SOYA R7102
RS-F	17		negative	positive	positive	negative	0,24	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	24	01.07.21	negative	63,63	39,43	negative	0,24	Food item, total	RS = Ridascreen®, R-Biopharm
SP	10		negative	positive	positive	negative		Please select!	SP = SensiSpec, Eurofins Technologies
SP	34	28.06.21	negative	positive	positive	negative	0,04	Soy Trypsin Inhibitor	SP = SensiSpec, Eurofins Technologies
VT	18	08.07.21	negative	positive (103ppm)	positive (51ppm)	negative	2	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	20				
AQ	25				
AQ	30	COKAL0448	Soy trypsin inhibitor	Extraction buffer	-
AQ	32				
AS	22				
MI-II	26	M2117	detects the soyprotein beta-conglycinin	As per kit instructions	
RS-F	6	Ridascreen® FAST Soy R7102, R-Biopharm / 24180	Against Heat processed soya proteins. (Glycinin (408%, beta-conglycinin 7.3%, tripsin inhibitor 0.46%)	As per kit instructions	no
RS-F	7			r-biopharm in kit contained	
RS-F	8	R7102			Reported as soya protein mg/Kg
RS-F	12	R7102			
RS-F	13				
RS-F	14	R7102			
RS-F	17				
RS-F	24	R7102			
SP	10				
SP	34				
VT	18	8410		PBS/15min/60oC	

5.1.8 PCR: Crustaceae

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
SFA	2		positive	positive	negative	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	23.06.21	positive	positive	negative	negative	2,5	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		positive	positive	negative	negative		Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14	02.07.21	positive	positive	negative	negative	2	Crustaceans DNA	surefood allergen crustaceans s3612
SFA	21		positive	positive	negative	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	30	07.06.21	Positive	Positive	Negative	Negative	0,4	Allergen DNA	SFA
SFA	31		positive	positive	negative	negative	100	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	33	28.07.21	positive	positive	negative	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	13	02.07.21	positive	positive	negative	negative		Allergen-DNA	Accredited qPCR in-house method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	2				
SFA	6	SureFood® ALLERGEN Crustaceans Art. No. S3612 / 20150	Not specified in kit	As per kit instructions	no
SFA	10				
SFA	14	S3612			
SFA	21				
SFA	30	S3612	DNA	Spin column extraction	-
SFA	31	S3612	UNKNOWN	PCIA/ Qiagen cleanup kit/ qPCR 35 cycles	
SFA	33	S3612	As Per Kit Instructions	As Per Kit Instructions	
div	13				

5.1.9 PCR: Egg

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
SFA ?	30	07.06.21	Negative	Positive	Positive	Negative	2	Allergen DNA	SFA

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA ?	30	P0611	DNA	Spin column extraction	-

5.1.10 PCR: Fish

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	9	07.07.21	negative	positive	negative	positive	10	Food, dried	ASU = ASU §64 Methode/method
IM	3	21.06.21	negative	positive	negative	positive	4	Please select!	Other: IMEGEN
MS	1	22.06.21	negative	positive	negative	positive	10	Allergen-DNA	MS = Microsynth
SFA	2		negative	positive	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	01.07.21	negative	positive	negative	positive	2,5	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	positive	negative	positive		Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14	02.07.21	negative	positive	negative	positive	5	fish DNA	surefood allergen FISH s3610
SFA	21		negative	positive	negative	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	24	05.07.21	negative	positive	negative	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	28		negative	positive	negative	positive	2.5	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	30	07.06.21	Negative	Positive	Negative	Positive	1	Allergen DNA	SFA
SFA	31		negative	positive	negative	positive	100	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	33	28.07.21	negative	positive	negative	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7		negative	positive	negative	positive	1 mg/kg	Food item, total	selection PCR-Methods
div	13	02.07.21	negative	positive	negative	positive		Allergen-DNA	Accredited qPCR in-house method
div	17		negative	positive	negative	positive	0,008	Food item, total	Selection PCR-Methods
div	26	23.6.	negative	positive	negative	positive	20	Allergen-DNA	internal method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	9	L 00.00-167, 2019-03	Hoxc13-Gene	CTAB-Extraction with alpha-Amylase-, Proteinase K- and RNase Treatment	
IM	3			CTAB/ kit /PCR real time	
MS	1			Wizard Extraction, Real Time PCR	
SFA	2				
SFA	6	SureFood® ALLERGEN fish Art. No. S3610 / 20150	Not specified in kit	As per kit instructions	no
SFA	10				
SFA	14	S3610			
SFA	21				
SFA	24	S3610			
SFA	28	S3610		Extraction CTAB; real time PCR, 45 ciclos	
SFA	30	S3610	DNA	Spin column extraction	-
SFA	31	S3610	UNKNOWN	PCIA/ Qiagen cleanup kit/ qPCR 35 cycles	
SFA	33	S3610	As Per Kit Instructions	As Per Kit Instructions	
div	7			Mericon Food Kit, Quiagen	
div	13				
div	17				
div	26			CTAB / Proteinase K / Rnase A / Promega Maxwell / Realtime PCR / 45 Cycles	

5.1.11 PCR: Milk

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
SFA ?	30	07.06.21	Negative	Negative	Positive	Positive	2	Allergen DNA	SFA

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA ?	30	P0609	DNA	Spin column extraction	-

5.1.12 PCR: Molluscs

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
4L	15	05.07.21	positive	negative	positive	negative	A COPY OF APLOID GENOME	Allergen DNA	4L = 4LAB Diagnostics
SFA	2		positive	negative	positive	-	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3	21.06.21	positive	negative	positive	negative	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	23.06.21	positive	negative	positive	negative	2,5	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		positive	negative	positive	negative		Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14	02.07.21	positive	negative	positive	negative	0,4	MOLLUSCS DNA	surefood allergen MOLLUCS s3613
SFA	21		positive	negative	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	23	02.07.21	positive	negative	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	24	05.07.21	positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	30	07.06.21	Positive	Negative	Positive	Negative	0,4	Allergen DNA	SFA
SFA	31		positive	negative	positive	negative	10	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	33	28.07.21	positive	negative	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	17		positive	negative	positive	negative	0,08	Food item, total	Selection PCR-Methods

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
4L	15	IC-02-1008	MOLLUSC DNA	EXTRACTION WITH GREES DAN FOOD KIT KIT IC-02-0095	
SFA	2				On sample 4, for the molluscs, we noticed a strong inhibition even though we repeated the analysis several times. We did not manage to get a result.
SFA	3			CTAB/ kit /PCR real time	
SFA	6	SureFood® ALLERGEN mollusc Art. No. S3613 / 23040	Not specified in kit	As per kit instructions	no
SFA	10				
SFA	14	S3613			
SFA	21				
SFA	23	S3613			
SFA	24	S3613			
SFA	30	S3613	DNA	Spin column extraction	-
SFA	31	S3613	UNKNOWN	PCIA/ Qiagen cleanup kit/ qPCR 35 cycles	
SFA	33	S3613	As Per Kit Instructions	As Per Kit Instructions	
div	17				

5.1.13 PCR: Mustard, in general

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	26	23.6.	positive	positive	negative	positive	4	Allergen-DNA	ASU = ASU §64 Methode/method
SFA	2		positive	positive	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		positive	positive	negative	positive		Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21		positive	positive	-	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	12	27.07.21	positive	positive	positive	positive	0,4	Allergen DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	26	§ 64 LFGB L 08.00-65:2017-10		CTAB / Proteinase K / Rnase A / Promega Maxwell / Realtime PCR / 45 Cycles	
SFA	2				Our test does not allow to make a difference between the different type of mustard, therefore the result for mustard in general is added in thie line.
SFA	10				
SFA	21			The kit that we used not distinguish the three varieties of mustard listed	
SFA-4p	12	S3401		Extraction with S1053 Surefood prep advanced (R-Biopharm)/ 35 cycles- 4 plex program in RidaCycler	Our test cannot diferenciate these 3 different kinds of mustard

5.1.14 PCR: Mustard, Sinapis alba

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	16	20.07.21	negative	positive	negative	negative	10	Food item, total	ASU = ASU §64 Methode/method
CEN	28		negative	positive	negative	negative	5	Food item, total	UNE CEN/TS 15634-5
MS	1	22.06.21	negative	positive	negative	negative	10	Allergen-DNA	MS = Microsynth
div	9	13.07.21	negative	positive	negative	negative	10	Food item, total	in-house method according to ASU L 08.00-59, 2013-01
div	13	22.07.21	negative	positive	negative	negative		please select	Accredited qPCR in-house method
div	17		negative	positive	negative	negative	0,008	Food item, total	Selection PCR-Methods

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	16	L08.00-59	MADSD-F; MADSD-R	CTAB	
CEN	28	UNE CEN/TS 15634-5	74 pb	Extraction CTAB; real time PCR multiplex, 50 ciclos	Sonda and primers for detection white Sinapis alba, and sonda y primers for detection brown/black Brassica nigra/Brassica juncea
MS	1			Wizard Extraction, Real Time PCR	
div	9		c-DNA for MADS-D-Protein from Sinapis alba	CTAB-Extraction with alpha-Amylase-, Proteinase K- and RNase Treatment	
div	13				
div	17				

5.1.15 PCR: Mustard, Brassica juncea/ Brassica nigra

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
MS	1	22.06.21	positive	negative	negative	positive	10	Allergen-DNA	MS = Microsynth
div	13	21.07.21	positive	negative	negative	positive		Allergen-DNA	Accredited qPCR in-house method
div	17		positive	negative	negative	positive	0,008	Food item, total	Selection PCR-Methods
div	28		positive	negative	negative	positive	5	Food item, total	Palle Reich et al. (2013). Food Chemistry

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
MS	1			Wizard Extraction, Real Time PCR	
div	13				the method does not distinguish betw een brow n and black mustard
div	17				
div	28	Palle Reich et al. (2013). Food Chemistry	76 pb	Extraction CTAB; real time PCR multiplex, 50 ciclos	Sonda and primers for detection w hite Sinapis alba, and sonda y primers for detection brow n/black Brassica nigra/Brassica juncea

5.1.16 PCR: Soya

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	16	20.07.21	negative	positive	positive	negative	10	Food item, total	ASU = ASU §64 Methode/method
MS	1	22.06.21	negative	positive	positive	negative	10	Allergen-DNA	MS = Microsynth
SFA	2		negative	positive	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	positive	positive	negative		Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	02.08.21	negative	positive	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21		negative	positive	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	27	15.07.21	negative	positive	positive	negative	0,4	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	30	07.06.21	Negative	Positive	Positive	Negative	0,4	Allergen DNA	SFA
SFA-4p	12	27.07.21	negative	positive	positive	negative	0,4	Allergen DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	9	12.07.21	negative	positive	positive	negative	50	Food item, total	in-house method according to ASU L 08.00-59, 2013-01
div	13	05.07.21	negative	positive	positive	negative		Allergen-DNA	Accredited qPCR in-house method
div	17		negative	positive	positive	negative	0,002	Food item, total	Selection PCR-Methods
div	26	23.6.	negative	positive	positive	negative	10	Allergen-DNA	internal method
div	28		negative	positive	positive	negative	5	Food item, total	ISO 21570

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence / -DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	16	L08.00-59	Lectin F; Lectin R	CTAB	
MS	1			Wizard Extraction, Real Time PCR	
SFA	2				
SFA	10				
SFA	19	S3601 / batch 21021		Extraction DNA with Sure Food prepadvanced (Biopharm/Congen)	
SFA	21				
SFA	27	S3601	Target-Sequence located within ITS-part (*internal transcribed spacer*) of the soybean genome	Real Time PCR	
SFA	30	S3601	DNA	Spin column extraction	-
SFA-4p	12	S3401		Extraction with S1053 Surefood prep advanced (R-Biopharm)/ 35 cycles- 4 plex program in RidaCycler	
div	9		Soja-Lectin-Gen	CTAB-Extraction with alpha-Amylase-, Proteinase K- and RNase Treatment	
div	13				
div	17				
div	26			CTAB / Proteinase K / Rnase A / Promega Maxwell / Realtime PCR / 45 Cycles	
div	28	ISO 21570	81 pb	Extraction CTAB; real time PCR, 45 ciclos	lectin

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALS2 (2021) 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	25,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	66	26,5
2	5,02	62	24,7
3	4,96	63	25,4
4	4,98	65	26,1
5	5,01	60	24,0
6	5,03	58	23,1
7	5,02	59	23,5
8	5,02	60	23,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	61,6	Particles
Standard deviation	3,14	Particles
χ^2 (CHI-Quadrat)	1,12	
Probability	**99**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	24,6	mg/kg
Standard deviation	1,25	mg/kg
rel. Standard deviaton	5,09	%
Horwitz standard deviation	9,88	%
HorRat-value	**0,52**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA ptALS2 (2021) 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	24,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	61	24,3
2	5,02	56	22,3
3	4,97	62	24,9
4	5,01	61	24,4
5	4,99	61	24,4
6	5,02	59	23,5
7	5,00	54	21,6
8	4,97	59	23,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,1	Particles
Standard deviation	2,87	Particles
χ^2 (CHI-Quadrat)	0,97	
Probability	**100**	%
Recovery rate	95	%

Normal distribution		
Number of samples	8	
Mean	23,6	mg/kg
Standard deviation	1,15	mg/kg
rel. Standard deviaton	4,85	%
Horwitz standard deviation	9,94	%
HorRat-value	**0,49**	
Recovery rate	95	%

Microtracer Homogeneity Test
DLA ptALS2 (2021) 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	28,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	72	28,9
2	5,02	71	28,3
3	4,99	79	31,7
4	5,03	61	24,3
5	5,04	66	26,2
6	5,02	65	25,9
7	4,98	67	26,9
8	4,98	78	31,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,9	Particles
Standard deviation	6,57	Particles
χ^2 (CHI-Quadrat)	4,33	
Probability	**74**	%
Recovery rate	100	%

Normal distribution		
Number of samples	8	
Mean	27,9	mg/kg
Standard deviation	2,63	mg/kg
rel. Standard deviaton	9,40	%
Horwitz standard deviation	9,69	%
HorRat-value	**0,97**	
Recovery rate	100	%

Microtracer Homogeneity Test
DLA ptALS2 (2021) Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	60	24,1
2	5,01	57	22,8
3	5,03	64	25,4
4	4,99	56	22,4
5	5,02	57	22,7
6	5,03	57	22,7
7	4,97	53	21,3
8	4,98	56	22,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	57,5	Particles
Standard deviation	3,11	Particles
χ^2 (CHI-Quadrat)	1,18	
Probability	**99**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	23,0	mg/kg
Standard deviation	1,24	mg/kg
rel. Standard deviaton	5,41	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,54**	
Recovery rate	103	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptALS2 (2021)***
PT name	***Allergen-Screening II - 4 Samples qualitative: Crustaceae, Egg, Fish, Milk, Molluscs, Mustard*** *(yellow/white, brown and black),* ***Soybean***
Sample matrix	*Samples 1-4:* *Carrier matrix / ingredients: potato powder (appr. 75%), maltodextrin (appr. 25%), other food additives and allergenic foods*
Number of samples and sample amount	*4 different Samples 1-4: 20 g each*
Storage	*Samples 1 - 4:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative:* ***Crustaceae, Egg, Fish, Milk, Molluscs, Mustard*** *(yellow/white, brown and black) and* ***Soybean*** *Samples 1-4: appr. 25 - 250 mg/kg*
Methods of analysis	*The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*One result each should be determined for Samples 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest August 06th 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		MALAYSIA
		GREECE
		Germany
		SPAIN
		CANADA
		CANADA
		ITALY
		SPAIN
		Germany
		Germany
		Germany
		FRANCE
		ITALY
		Germany
		GREAT BRITAIN
		GREECE
		USA
		SPAIN
		Germany
		SPAIN
		SWITZERLAND
		ITALY
		SPAIN
		ITALY
		Germany
		Germany
		GREAT BRITAIN
		ITALY
		GREAT BRITAIN
		FRANCE
		GREAT BRITAIN
		GREAT BRITAIN
		USA
		GREAT BRITAIN

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Hor-witz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Ana-lyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estim-ates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messun-gen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Check-ing procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Re-quirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)

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