Evaluation Report

proficiency test

DLA ptALS2 (2020)

Allergen-Screening II:

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

DLA - Proficiency Tests GmbH
Kalte Weide 21
24641 Sievershütten/Germany

proficiency-testing@dla-lvu.de *www.dla-lvu.de*

Coordinator of this PT:
Dr. Matthias Besler-Scharf

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptALS2 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (26 October 2020) 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: 26 October 2020
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.

Contents

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 amounts of allergenic ingredients positive in mg/kg ranges** given as food item

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Crustaceae: King Prawns *(Litopenaeus vannamei)*, *freeze*-dried (Protein 87%)	positive (75 - 150)	negative	positive (25 - 75)	negative
Egg: Whole egg powder (Protein 47%)	positive (75 - 150)	positive (25 - 75)	negative	negative
Fish: Cod *(Gadus morhua)*, freez-dried (Protein 88%)	negative	positive (75 - 150)	positive (25 - 75)	negative
Milk: Skimmed milk powder (Protein 37%)	positive (25 - 75)	negative	negative	positive (100 - 225)
Molluscs: Yesso Scallop *(Mizuhopecten yessoen-sis)*, freez-dried (Protein 76%)	negative	negative	positive (25 - 75)	positive (100 - 225)
Mustard, yellow: Sinapis alba (Protein 31%)	negative	positive (50 - 100)	negative	positive (50 - 100)
Mustard, brown: Brassica juncea (Protein 28%)	negative	negative	positive (50 - 100)	negative
Mustard, black: Brassica nigra (Protein 27%)	negative	positive (50 - 100)	negative	negative
Soya: Soyflour, not toasted (Protein 37%)	positive (75 - 150)	negative	negative	positive (25 - 75)

* 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	negative	positive	negative
AgraStrip® Egg	positive	positive	negative	negative
AgraStrip® Casein	positive	negative	negative	positive
AgraStrip® Soy	positive	negative	negative	positive
AgraStrip® Mustard	negative	positive	positive	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%, 89%, 98% 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 0,6, 0,8, 0,6 and 0,6, 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. 0,40 and 0,36 (21-22°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 25th week of 2020. The testing method was optional. The tests should be finished at August 28th 2020 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.

All 29 participants submitted at least one result in time.

3. Evaluation

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

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

3.1 Agreement with consensus values from participants

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

3.2 Agreement with spiking of samples

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

4. Results

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

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

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		
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
18	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
28	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
7	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	RS-F	
9	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	RS-F	
22	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
27	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	
19	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	
21	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	13	1	13	2
Number negative	0	12	0	11
Percent positive	100	8	100	15
Percent negative	0	92	0	85
Consensus value	positive	negative	positive	negative
Spiking	positive	negative	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

Participant 8 has pointed to a possible cross-reactivity to molluscs for the used ELISA method AQ (see documentation).

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		
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
27	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	SFA	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
20	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	negative	4/4 (100%)	4/4 (100%)	SFA	
25	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	

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

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

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		
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
28	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ES	
17	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MI-II	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MI-II	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MI-II	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MI-II	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
9	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
20	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
22	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
19	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
21	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

AS = AgraStrip (Lateral Flow), RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

4.2.2 PCR-Results: Egg (whole egg powder)

PCR methods were not applied by the participants.

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	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
26	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BC	
28	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BF	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
19	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
21	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	

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

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

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		
15	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	GS	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IM	
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MS	
1	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
5	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
16	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
20	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
21	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
23	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
24	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
25	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
26	negative	negative	positive	positive	2/4 (50%)	2/4 (50%)	SFA	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	17	18	1
Number negative	18	1	0	17
Percent positive	0	94	100	6
Percent negative	100	6	0	94
Consensus value	negative	positive	positive	negative
Spiking	negative	positive	positive	negative

Methods:

GI = GEN-IAL First Allergen

GS = Eurofins Genescan DNAnimal screen

IM = Imegen

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		
28	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
14a	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ES	Casein
14b	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ES	β-Lactoglobulin
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	Casein
8a	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	Casein
8b	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	β-Lactoglobulin
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS div	R-Biopharm Kit not specified
3	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	Casein
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
22	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	β-Lactoglobulin
20a	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	Casein
20b	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	β-Lactoglobulin
12	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	Casein
19	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	
21	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
RS div= R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins

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

4.4.2 PCR-Results: Milk (skimmed milk powder)

PCR methods were not applied by the participants.

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		
26	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	DE	
8	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	ET	Sample 1 positive due to cross reactivity to crustacea suspected
17	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	IL	
4	-	negative	positive	positive	3/3 (100%)	3/3 (100%)	SP	Sample 1 traces at limit of detection
19	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	SP	Sample 1 and 3 positive due to cross reactivity to crustacea
21	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	0	6	6
Number negative	2	6	0	0
Percent positive	60	0	100	100
Percent negative	40	100	0	0
Consensus value	none	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

DE = Demeditec ELISA

ET = Elution Technologies ELISA Kit

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of sample 2, 3 and 4 are in qualitative agreement with the spiking of samples. For sample 1 (without addition of molluscs) no consensus value with ≥75% positive or negative results was obtained. Two participants have pointed to a possible cross-reactivity to Crustaceae (methods ET and SP). Samples 1 and 3 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		
22	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	4L	
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
18	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
20	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
21	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
25	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
26	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	13	13
Number negative	13	13	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:

4L = 4LAB Diagnostics

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.

4.6 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		
11	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
28	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	BF	
17	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	IL	
7	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
10	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
18	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
20	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
12	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SP	
19	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SP	
21	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SP	
4	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	VT	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

AS = AgraStrip (Lateral Flow), RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

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 2 and 4 yellow mustard, sample 2 black mustard and sample 3 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		
4	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
1	positive	positive	positive	positive	3/4 (75%)	3/4 (75%)	SFA	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
5	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
16	negative	-	-	-	1/1 (100%)	1/1 (100%)	SFA	
20	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
21	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
29	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	8	8	8
Number negative	8	0	0	0
Percent positive	11	100	100	100
Percent negative	89	0	0	0
Consensus value	negative	positive	positive	positive
Spiking	negative	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comments:

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

The consensus values of results are in qualitative agreement with the spiking of samples (sample 2 and 4 yellow mustard, sample 2 black mustard and sample 3 brown mustard).

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		
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
27	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
15	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MS	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Five participants tested for mustard species by PCR. Yellow mustard (Sinapis alba) was detected in sample 2 and 4 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		
27	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
15	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	
3a	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MS	
3b	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	MS	
9	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Moreover four participants detected Brassica species in sample 2 (containing black mustard, Brassica nigra) and sample 3 (containing brown mustard, Brassica juncea). One participant also obtained a positive result for sample 4.
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		
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
28	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
14	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ES	
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
19	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	MI-II	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
12	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
20	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
27	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
21	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	13	0	0	13
Number negative	0	13	13	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:

AS = AgraStrip (Lateral Flow), RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

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		
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
27	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
15	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	GI	
3	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	MS	
1	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	SFA	
2	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
16	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
20	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
21	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
9	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

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.

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	8	15.07.20	positive	negative	positive	negative	0,02	tropomyosin	AQ = AgraQuant, RomerLabs
AQ	18	Aug	pos	neg	pos	neg	0,1	Food item, total	AQ = AgraQuant, RomerLabs
BF	5		positive	negative	positive	negative	1	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
BF	28	28/8	positive	negative	positive	negative	0,07	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	17		positive	negative	positive	negative		Food item, total	IL = Immunolab
RS-F	7	30.07.20	positive	negative	positive	positive	20	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	9		positive	positive	positive	positive	2	Protein	R-BIOPHARM R7312
RS-F	22	08.07.20	positive	negative	positive	negative	20	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	27	29.06.20	positive	negative	positive	negative	2	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
SP	4	30.06.	positive	negative	positive	negative	0,02	Please select!	SP = SensiSpec, Eurofins Technologies
SP	12		positive	negative	positive	negative	0,02	Protein (tropomyosin)	SP = SensiSpec, Eurofins Technologies
SP	19	23.06.20	positive	negative	positive	negative	0,009	Shrimp Tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	positive	negative	positive	negative	0,01	Food item, total	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	8			as stipulated in kit insert	Kit present weak cross reactivity to molluscs
AQ	18	10002076 (COKAL2248)	Unknown		Reported as 'Crustacea'
BF	5				
BF	28		Monoclonal antibodies	1:10 extractraction ratio, 1 hour at 42C	no
IL	17				
RS-F	7	RIDASCREEN® FAST Crustacean (2nd generation) Art. No. R7312 / 14139	The antibody specifically detects crustacean proteins such as tropomyosin	As per kit instructions	no
RS-F	9				Sample 1 and Sample 3 are out of range
RS-F	22	R 7312	ANTI-TROPOMIOSIN	EXTRACTION: BUFFER 10 MINUTI / 60°C DETERMINATION 30 MINUTI / 20-25°C	
RS-F	27	R7312	Tropomyosin	As per kit instructions	reported as crustaceen
SP	4	HU0030006	recognizes the crustacean tropomyosin	According to manufacturer information	Tropomyosin crustaceans
SP	12	HU0030006			Reported as ug/Kg tropomyosin from crustaceans
SP	19				
SP	21				

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
AS	11	10.07.20	positive	positive	negative	negative	2		AgraStrip Egg/Romer Labs
BF	28	28/8	positive	positive	negative	negative	0,3	Whole egg powder	BF = MonoTrace ELISA, BioFront Technologies
ES	14		positive	positive	negative	negative	5 ppm	Egg white powder	ES = ELISA-Systems
IL	17		positive	positive	negative	negative		Please select!	IL = Immunolab
MI-II	3	07.07.20	positive	positive	negative	negative	10	Food item, total	MI-II = Morinaga Institute ELISA II
MI-II	4	29.06.	positive	positive	negative	negative	0,31	Please select!	MI-II = Morinaga Institute ELISA II
MI-II	8	20.07.20	positive	positive	negative	negative	0,312	egg protein	MI-II = Morinaga Institute ELISA II
MI-II	12		positive	positive	negative	negative	0,31	Whole Egg Protein	MI-II = Morinaga Institute ELISA II
RS-F	7	07.08.20	positive	positive	negative	negative	0,5	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	9		positive	positive	negative	negative	0,1	protein	R-BIOPHARM 6402
RS-F	20	14.08.20	positive	positive	negative	negative	0,5	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	22	07.07.20	positive	positive	negative	negative	0,5	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
SP	19	22.06.20	positive	positive	negative	negative	0.05	Egg white protein	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	positive	positive	negative	negative	0,05	Food item, total	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	
AS	11	1000003564			
BF	28		Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	no
ES	14	ES-6020, Transia	polyclonal, anti ovomucoid/ ovalbumin polyclonal, anti ovomucoid/ ovalbumin polyclonal, anti ovomucoid/ ovalbumin	Extractions buffer, 3x10min, room temperature	LOD 0,5 ppm
IL	17				
MI-II	3				
MI-II	4	M2111	recognizes the egg white protein ovalbumin	According to manufacturer information	whole egg protein
MI-II	8			as stipulated in kit insert	
MI-II	12	M2111			Reported as whole egg protein mg/Kg
RS-F	7	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	9				Sample 1 and Sample 2 are out of range
RS-F	20	RIDASCREEN® FAST Ei / Egg Protein (Art. Nr.: R6402)	The specific antibodies detect the egg white proteins ovalbumin and ovomucoid.	Preparation of the sample and test implementation following the instruction of RIDASCREEN® FAST Ei / Egg Protein (Art. Nr.: R6402) Lot 15339 - extraction with diluted Allergen Extraction buffer 10 min at 60°C	
RS-F	22	R 6402	ANTI- OVOALBUMIN ANTI-OVOMUCOID	EXTRACTION: BUFFER 10 MINUTI / 60°C DETERMINATION 30 MINUTI / 20-25°C	
SP	19				
SP	21				

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	8	06.07.20	negative	positive	positive	negative	4	Food item (cod)	AQ = AgraQuant, RomerLabs
BC	26	11.07.20	negative	positive	positive	negative	5	Food item, total	BC = BioCheck ELISA
BF	28	28/8	negative	positive	positive	negative	0,3	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	17		negative	positive	positive	negative		Food item, total	IL = Immunolab
SP	19	22.06.20	negative	positive	positive	negative	"1,4"	Food item, fresh	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	negative	positive	positive	negative	1,4	Food item, total	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	8			as stipulated in kit insert	
BC	26	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	
BF	28		Monoclonal antibodies	1:10 extractraction ratio, 1 hour boiling	no
IL	17				
SP	19				
SP	21				

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
BF	28	28/8	positive	negative	negative	positive	0,48	Milk powder	BF = MonoTrace ELISA, BioFront Technologies
ES	14a		positive	negative	negative	positive	10 ppm Casein	Skimmed milk powder equivalents	ES = ELISA-Systems
ES	14b		positive	negative	negative	positive	1 ppm ß-Lactglobulin	ß-Lactglobulin	ES = ELISA-Systems
IL	17		positive	negative	negative	positive		Milk powder	IL = Immunolab
MI-II	4	26.06.	positive	negative	negative	positive	0,31	Please select!	MI-II = Morinaga Institute ELISA II
MI-II	8a	29.07.20	positive	negative	negative	positive	0,312	Milk powder	MI-II = Morinaga Institute ELISA II
MI-II	8b	29.07.20	positive	negative	negative	positive	0,312	Milk powder	MI-II = Morinaga Institute ELISA II
RS div	1		positive	negative	negative	positive		Please select!	Selection ELISA-Kits:
RS-F	3	07.07.20	positive	negative	negative	positive	10	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	7	08.04.20	positive	negative	negative	positive	0,5	Casein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	9		positive	negative	negative	positive	0,7	protein	R-BIOPHARM 4652
RS-F	22	09.07.20	positive	negative	negative	positive	0,167	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	20a	04.08.20	positive	negative	negative	positive	2,5 mg/kg (ppm) casein	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	20b	04.08.20	positive	negative	negative	positive	0,167 mg/kg (ppm) β-lactoglobulin	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	12		positive	negative	negative	positive	0,2	Protein (casein)	SP = SensiSpec, Eurofins Technologies
SP	19	22.06.20	positive	negative	negative	positive	0.05	Casein+BLG	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	positive	negative	negative	positive	0,05	Food item, total	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	
BF	28		Monoclonal antibodies	1:10 extractraction ratio, 1 hour at 60C	no
ES	14a	ES-6030 Transia	polyclonal, anti bovine alpha-Casein	extraction buffer for both ELISAs, 2x15min and 1x10min, room temperature	we use 2 ELISAs: LOD 1 ppm skimmed milk powder equivalents or LOD 0.1 ppm ß-lactoglobulin
ES	14b	ES-6034 Transia	polyclonal, anti ß-Lactglobulin	extraction buffer for both ELISAs, 2x15min and 1x10min, room temperature	we use 2 ELISAs: LOD 1 ppm skimmed milk powder equivalents or LOD 0.1 ppm ß-lactoglobulin
IL	17				
MI-II	4	M2113 Casein	recognizes cow's milk casein	According to manufacturer information	milk protein
MI-II	8a		CASEIN	as stipulated in kit insert	
MI-II	8b		BLG	as stipulated in kit insert	
RS div	1			R biopharm	
RS-F	3				
RS-F	7	RIDASCREEN® FAST casein Art. N° R4612 / 22060	The antibodies specifically detect Casein	As per kit instructions	no
RS-F	9				
RS-F	22	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	20a	RIDASCREEN® FAST Casein (R4612)	The used antibodies specifically detect caseins of cow's milk.	Preparation of the sample and test implementation following the instruction of RIDASCREEN® FAST Casein (R4612), Lot 22060 casein - extraction with Extractor 2 cook it for 10 min at 100 °C in a water bath and then adding Allergen extraction buffer containing Additive 1 (A-AEP) and extract for 10 min at 60 °C in a water bath	
RS-F	20b	RIDASCREEN® FAST β-Lactoglobulin (Art. No. R4912)	The antibodies specifically detect β-lactoglobulin of cow's milk.	Preparation of the sample and test implementation following the instruction of RIDASCREEN® FAST β-Lactoglobulin (Art. No. R4912), Lot 24090 β-Lactoglobulin - extraction with Extractor 2 cook it for 10 min at 100 °C in a water bath and then adding Allergen extraction buffer containing Additive 1 (A-AEP) and extract for 10 min at 60 °C in a water bath	
SP	12	HU0030003			Milk detected as casein mg/Kg
SP	19				
SP	21				

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
DE	26	24.07.20	positive	negative	positive	positive	0,01	Food item, total	other: please fill in!
ET	8	06.07.20	positive	negative	positive	positive	1	molluscs protein	ET = Elution Technologies ELISA Kit
IL	17		negative	negative	positive	positive		Food item, total	IL = Immunolab
SP	4	30.06.	-	negative	positive	positive	0,03	Please select!	SP = SensiSpec, Eurofins Technologies
SP	19	23.06.20	positive*	negative	positive*	positive	0,017	Garden Snail Tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	negative	negative	positive	positive	0,0017	Food item, total	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	
DE	26	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	DeMediTec GmBH Test Kit
ET	8			as stipulated in kit insert	Use kit 3M with cross reactivity to crustacea. Result #1 = Cross reactivity to crustacea suspected.
IL	17				
SP	4	HU0030015/00300 39	recognizes the mollusc tropomyosin	According to manufacturer information	Tropomyosin molluscs; Sample 1: traces at the limit of detection (positive <0.03mg/kg)
SP	19				*positive due to cross-reaction of crustaceans
SP	21				

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
AS	11	10.07.20	negative	positive	positive	positive	2		AgraStrip Mustard / Romer Labs
BF	28	28/8	negative	positive	positive	positive	0,13	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	17		negative	positive	positive	positive		Food item, total	IL = Immunolab
RS-F	7	06.08.20	negative	positive	positive	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	10		negative	positive	positive	positive	0,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	18	Aug	neg	pos	pos	pos	0,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	20	14.08.20	negative	positive	positive	positive	0,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	12		negative	positive	positive	positive	2	Food item, total	SP = SensiSpec, Eurofins Technologies
SP	19	22.06.20	negative	positive	positive	positive	1	food item, dried	SP = SensiSpec, Eurofins Technologies
SP	21	10.08.20	negative	positive	positive	positive	1	Food item, total	SP = SensiSpec, Eurofins Technologies
VT	4	26.06.	negative	positive	positive	positive	2,5	Mustard	Neogen Veratox Senf
VT	8	15.07.20	negative	positive	positive	positive	2,5	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	
AS	11	1000000977			
BF	28		Monoclonal antibodies	1:20 extractraction ratio, 1 hour at 60C	assay detects yellow/white, brown, black mustard
IL	17				Cross reactivity to brown mustard and black mustard
RS-F	7	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	10			mustard extraction buffer, 10 min, 60ºC	
RS-F	18	R6152	Unkown		yellow, brown and black reported in total as 'mustard'
RS-F	20	RIDASCREEN® FAST Senf/Mustard (Art. Nr.: R6152)	The antibodies used in the test specifically detect different kinds of mustard (yellow, white, brown, black mustard). The results are for mustard, in general.	Preparation of the sample and test implementation following the instruction of RIDASCREEN® FAST Senf/Mustard (Art. Nr.: R6152), Lot 14489 - extraction with diluted Allergen Extraction buffer 10 min at 60°C	The results are for mustard, in general.
SP	12	HU0030016			Test does NOT separate out species e.g. black, yellow ets. Reported as mg/Kg mustard.
SP	19				Cross-reactivty: Yellow 100%, brown 59%, black 50%
SP	21				
VT	4	8400	recognizes mustard protein from seeds of white mustard (Sinapis alba), black mustard (Brassica nigra) and brown mustard (Brassica juncea)	According to manufacturer information	
VT	8			as stipulated in kit insert	

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
AS	11	10.07.20	positive	negative	negative	positive	2		AgraStrip Soy / Romer Labs
BF	28	28/8	positive	negative	negative	positive	0,16	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
ES	14		positive	negative	negative	positive	25 ppm	Soya protein	ES = ELISA-Systems
IL	17		positive	negative	negative	positive		Please select!	IL = Immunolab
IL	19	23.06.20	positive	negative	negative	positive	0,2	Total protein	IL = Immunolab
MI-II	4	29.06.	positive	negative	negative	positive	0,31	Please select!	MI-II = Morinaga Institute ELISA II
MI-II	8	21.07.20	positive	negative	negative	positive	0,312	soya protein	MI-II = Morinaga Institute ELISA II
RS-F	7	03.08.20	positive	negative	negative	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	9		positive	negative	negative	positive	0,24	protein	R-BIOPHARM 7102
RS-F	12		positive	negative	negative	positive	2,5	Protein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	20	04.08.20	positive	negative	negative	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	27	23.06.20	positive	negative	negative	positive	0,24	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
SP	21	10.08.20	positive	negative	negative	positive	0,016	Please select!	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	
AS	11	1000002188			
BF	28		Monoclonal antibodies	1:20 extractraction ratio, 1 hour boiling	no
ES	14	ES-6012, Transia	polyclonal, anti-soy trypsin inhibitor and anti-soy flour protein	Extraction buffer, 2x30min and 1x15 min, room temperature	LOD 2,5 ppm
IL	17				
IL	19				
MI-II	4	M2117	recognizes the soy protein beta-conglycinin	According to manufacturer information	soy protein
MI-II	8			as stipulated in kit insert	
RS-F	7	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	9				Sample 1 and Sample 4 are out of range
RS-F	12	R7102			Reported as soya protein mg/Kg
RS-F	20	RIDASCREEN® FAST Soya (Art. No. R7102)	The antibodies specifically detect heated soya proteins	Preparation of the sample and test implementation following the instruction of RIDASCREEN® FAST Soya (Art. No. R7102), Lot 13339 - extraction with Extractor 3 and diluted Allergen Extraction Buffer for 10 min at 100 °C	
RS-F	27	R7102	heated soy proteins	according to test kit instructions	Reported as soy protein
SP	21				

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
ASU	15	18.08.20	positive	negative	positive	negative		Please select!	ASU = ASU §64 Methode/method
ASU	27	01.07.20	positive	negative	positive	negative		Please select!	ASU = ASU §64 Methode/method
SFA	1		positive	negative	positive	negative		Please select!	Selection PCR-Methods
SFA	2	23.06.20	positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		positive	negative	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	07.08.20	positive	negative	positive	positive	2,5	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		positive	negative	positive	negative	2	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	20	13.08.20	positive	negative	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	10.08.20	positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	23		positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	25	20.08.20	positive	negative	positive	negative	100	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	16		positive	negative	positive	negative	<0.4 mg/kg	Allergen DNA	SFA = Sure Food ALLERGEN, 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	15	L12.01-3 ; 07/2012		Simplex EasySpinFood DNA Kit / GEN-IAL, endpoint PCR with subsequent sequencing	
ASU	27	L12.01-3		according to ASU method	
SFA	1			CTAB extraction + PCR CONGEN	
SFA	2	S3612	Crustacea	Extraction with SureFood® Prep Advanced protocol 1 (S1053)	K01, QE to abalone (Haliotis) 100 %
SFA	5				
SFA	7	SureFood® ALLERGEN Crustaceans Art. No. S3612 / 20150	Not specified in kit	As per kit instructions	no
SFA	10			prep advance surefood/taq polymerase/ RT PCR/45 cycles	
SFA	20	SureFood® ALLERGEN Crustaceans - Art. No. S3612	The real-time PCR test detects DNA of crustaceans (Crustacea)	DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 11349	
SFA	21				
SFA	23	S3612/11349		Extraction= Sure Food PREP Advanced Determination = real tim	
SFA	25	S3612		qiagen dneasy kit/real time PCR/45 cycles	
SFA-ID	16	S3112		real time PCR	

5.1.9 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
GI	15	12.08.20	negative	positive	positive	negative	15	Allergen-DNA	First-Fish Kit / GEN-IAL
GS	12		negative	positive	positive	negative	0,001	Food item, total	Eurofins Genescan DNAnimal screen fish
IM	13	12.08.20	negative	positive	positive	negative	4	Please select!	other: IMEGEN
MS	3	13.07.20	negative	positive	positive	negative	10	Allergen-DNA	MS = Microsynth
SFA	1		negative	positive	positive	negative		Please select!	Selection PCR-Methods
SFA	2	23.06.20	negative	positive	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative	positive	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	31.07.20	negative	positive	positive	negative	2,5	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	positive	positive	negative	5	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		negative	positive	positive	negative	<1 mg/kg	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	20	13.08.20	negative	positive	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	10.08.20	negative	positive	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	23		negative	positive	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	24		negative	positive	positive	negative	1	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	25	20.08.20	negative	positive	positive	negative	100	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	26	22.08.20	negative	negative	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	4	13.07.	negative	positive	positive	negative	20	Allergen-DNA	Selection PCR methods
div	9		negative	positive	positive	negative	0,008		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	
GI	15	PHF0050, L10.00-12		Simplex EasySpinFood DNA Kit/GEN-IAL, RealTime PCR	
GS	12	5422211310			
IM	13			CTAB/ kit /PCR real time	
MS	3			Wizard Extraktion, Real Time PCR	
SFA	1			CTAB extraction + PCR CONGEN	
SFA	2	S3610	Osteichthyes (bony fish)	Extraction with SureFood® Prep Advanced protocol 1 (S1053)	K01, QE to muscovy duck (Cairina moschata) 100 %
SFA	5				
SFA	7	SureFood® ALLERGEN fish Art. No. S3610 / 20150	Not specified in kit	As per kit instructions	no
SFA	10			prep advance surefood/taq polymerase/ RT PCR/45 cycles	
SFA	16	S3610		real time PCR	
SFA	20	SureFood® ALLERGEN Fish - Art. No. S3610	The real-time PCR test detects DNA of fish	DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 14309	
SFA	21				
SFA	23	S3610/14309		Extraction= Sure Food PREP Advanced Determination = real time	
SFA	24	S3610	DNA fragment present solely in fish	CTAB DNA extraction/Real time PCR	Analyst: LP/AP
SFA	25	S3610			
SFA	26	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	
div	4	internal method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 cycles	
div	9				

5.1.10 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	22	23.07.20	negative	negative	positive	positive	A COPY OF HAPLOID GENOME	Allergen DNA	4L = 4LAB Diagnostics
SFA	1		negative	negative	positive	positive		Please select!	Selection PCR-Methods
SFA	2	23.06.20	negative	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative	negative	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	7	31.07.20	negative	negative	positive	positive		Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	negative	positive	positive	2	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	13	07.07.20	negative	negative	positive	positive	100	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18	Aug	neg	neg	pos	pos	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	20	13.08.20	negative	negative	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	10.08.20	negative	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	25	20.08.20	negative	negative	positive	positive	40	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	26	22.08.20	negative	negative	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	9		negative	negative	positive	positive	0,08		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	
4L	22	IC-02-1008	MOLLUSC DNA	EXTRACTION WITH GREES DAN FOOD KIT KIT IC-02-0095	
SFA	1			CTAB extraction + PCR CONGEN	
SFA	2	S3613	Gastropods, Decabrachia, Bivalvia	Extraction with SureFood® Prep Advanced protocol 1 (S1053)	K02
SFA	5				
SFA	7	SureFood® ALLERGEN mollusc Art. No. S3613 / 23040	Not specified in kit	As per kit instructions	no
SFA	10			prep advance surefood/taq polymerase/ RT PCR/45 cycles	
SFA	13			CTAB/ kit /PCR real time	
SFA	18	S3613	Uknown	Tris extraction with column clean-up, real-time PCR detection	
SFA	20	SureFood® ALLERGEN Molluscs - Art. No. S3613	The real-time PCR test detects DNA of molluscs	DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 13089	
SFA	21				
SFA	25	S3613			
SFA	26	As Per Kit Instructions	As Per Kit Instructions	As Per Kit Instructions	
div	9				

5.1.11 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	4	13.07.	negative	positive	positive	positive	5	Allergen-DNA	ASU
SFA	1		positive	positive	positive	positive		Please select!	Selection PCR-Methods
SFA	2	23.06.20	negative	positive	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		negative	positive	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	16.07.20	negative	positive	positive	positive		Allergen DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
SFA	16		negative	-	-	-	<0.4 mg/kg	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	20	13.08.20	negative	positive	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	10.08.20	negative	positive	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	29		negative	positive	positive	positive	0,4	other: In general	SFA = Sure Food ALLERGEN, 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	4	L 08.00-65:2017-10		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 cycles	
SFA	1			CTAB extraction + PCR CONGEN (mustard screening)	
SFA	2	S3609	yellow mustard (Sinapis alba), brown mustard (Brassica juncea), black mustard (Brassica nigra), ethiopian mustard (Brassica carinata), field mustard (Sinapis arvensis)	Extraction with SureFood® Prep Advanced protocol 1 (S1053)	K02, no differentiation between yellow, brown and black mustard
SFA	5				The kit used for mustard's determination detects all three species listed without distinction.
SFA	8	S3609		cleaning using SureFood Prep Advanced S1053, real time PCR, 45 cycles	
SFA	16	S3609		real time PCR	Test cannot distinguish between different types of mustard
SFA	20	SureFood® ALLERGEN Mustard - Art. No. S3609	The test detects DNA of white mustard (Sinapis alba), indian mustard (Brassica juncea) und black mustard (Brassica nigra). The results are for mustard, in general	DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 13059	The results are for mustard, in general.
SFA	21				
SFA	29			R-Biopharm Kit for extraction DNA. We used a real time PCR with 45 cycles.R-Biopharm Kit for extraction DNA. We used a real time PCR with 45 cycles.	

5.1.12 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	6	13.07.20	negative	positive	negative	positive	10	Food item, total	ASU = ASU §64 Methode/method
ASU	27	01.07.20	negative	positive	negative	positive		Please select!	ASU = ASU §64 Methode/method
GI	15	12.08.20	negative	positive	negative	positive	10	Allergen-DNA	GI = GEN-IAL First Allergen
MS	3	13.07.20	negative	positive	negative	positive	10	Allergen-DNA	MS = Microsynth
div	9		negative	positive	negative	positive	0,008		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	
ASU	6	L08.00-59	MADSD-F, MADSD-R	CTAB	
ASU	27	L08.00-65		according to ASU method	
GI	15	PMUS0050, L08.00-64		Simplex EasySpinFood DNA Kit/GEN-IAL, RealTime PCR	
MS	3			Wizard extraction, Real Time PCR	
div	9				

5.1.13 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
ASU	27	01.07.20	negative	positive	positive	negative		Please select!	ASU = ASU §64 Methode/method
GI	15	12.08.20	negative	positive	positive	negative	5	Allergen-DNA	GI = GEN-IAL First Allergen
MS	3a	13.07.20	negative	positive	positive	negative	10	Allergen-DNA	MS = Microsynth
MS	3b	13.07.20	negative	positive	positive	positive	10	Allergen-DNA	MS = Microsynth
div	9		negative	positive	positive	negative	0,008		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	
ASU	27	L08.00-65		according to ASU method	brown and black mustard
GI	15	PMUS0050, L08.00-64		Simplex EasySpinFood DNA Kit/GEN-IAL, RealTime PCR	brown mustard is detected together with black mustard
MS	3a			Wizard Extraktion, Real Time PCR	brown mustard
MS	3b			Wizard Extraktion, Real Time PCR	black mustard
div	9				black mustard

5.1.14 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	6	13.07.20	positive	negative	negative	positive	10	Food item, total	ASU = ASU §64 Methode/method
ASU	27	01.07.20	positive	negative	negative	positive		Please select!	ASU = ASU §64 Methode/method
GI	15	12.08.20	positive	negative	negative	positive	10	Allergen-DNA	GI = GEN-IAL First Allergen
MS	3	13.07.20	negative	negative	negative	positive	10	Allergen-DNA	MS = Microsynth
SFA	1		positive	positive	positive	positive		Please select!	Selection PCR-Methods
SFA	2	23.06.20	positive	negative	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5		positive	negative	negative	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16		positive	negative	negative	positive	<0.4 mg/kg	Allergen DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
SFA	20	13.08.20	positive	negative	negative	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	21	10.08.20	positive	negative	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	4	13.07.	positive	negative	negative	positive	5	Allergen-DNA	Selection PCR methods
div	9		positive	negative	negative	positive	0,02		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	
ASU	6	L08.00-59	Lectin-F; Lectin-R	CTAB	
ASU	27	L08.00-59 und L08.00-65		according to ASU methods	
GI	15	PSOY 0050, L08.00-65		Simplex EasySpinFood DNA Kit/GEN-IAL, RealTime PCR	
MS	3			Wizard extraction, Real Time PCR	
SFA	1			CTAB extraction + PCR CONGEN	
SFA	2	S3601	Glycine max	Extraction with SureFood® Prep Advanced protocol 1 (S1053)	K02
SFA	5				
SFA	16	S3601		real time PCR	
SFA	20	SureFood® ALLERGEN Soya - Art. No. S3601	The real-time PCR test detects soya DNA (Glycine max)	DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (45 cycles following kit setup instructions) with Bio-Rad CFX96, Lot 24060	
SFA	21				
div	4	internal method		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR 45 cycles	
div	9				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALS2 Sample 1

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	43	17,3
2	5,02	44	17,5
3	5,01	50	20,0
4	4,98	44	17,7
5	4,98	41	16,5
6	5,00	47	18,8
7	5,03	47	18,7
8	5,02	48	19,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,5	Particles
Standard deviation	2,85	Particles
χ^2 (CHI-Quadrat)	1,25	
Probability	**99**	%
Recovery rate	88	%

Normal distribution		
Number of samples	8	
Mean	18,2	mg/kg
Standard deviation	1,14	mg/kg
rel. Standard deviaton	6,3	%
Horwitz standard deviation	10,3	%
HorRat-value	**0,61**	
Recovery rate	88	%

Microtracer Homogeneity Test
DLA ptALS2 Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	67	26,6
2	4,97	75	30,2
3	5,03	72	28,6
4	5,02	74	29,5
5	5,02	77	30,7
6	5,03	65	25,8
7	4,99	78	31,3
8	4,96	64	25,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	71,5	Particles
Standard deviation	5,51	Particles
χ^2 (CHI-Quadrat)	2,97	
Probability	**89**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	28,6	mg/kg
Standard deviation	2,20	mg/kg
rel. Standard deviaton	7,7	%
Horwitz standard deviation	9,7	%
HorRat-value	**0,80**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA ptALS2 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	27,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	65	25,7
2	5,04	71	28,2
3	5,01	63	25,1
4	4,98	57	22,9
5	4,97	66	26,6
6	5,05	66	26,1
7	4,96	68	27,4
8	5,01	65	25,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,1	Particles
Standard deviation	3,96	Particles
χ^2 (CHI-Quadrat)	1,69	
Probability	**98**	%
Recovery rate	95	%

Normal distribution		
Number of samples	8	
Mean	26,0	mg/kg
Standard deviation	1,58	mg/kg
rel. Standard deviaton	6,1	%
Horwitz standard deviation	9,8	%
HorRat-value	**0,62**	
Recovery rate	95	%

Microtracer Homogeneity Test
DLA ptALS2 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	20,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	49	19,4
2	5,00	45	18,0
3	5,00	49	19,6
4	4,99	46	18,4
5	4,98	48	19,3
6	5,01	41	16,4
7	5,00	48	19,2
8	4,98	50	20,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	47,0	Particles
Standard deviation	2,95	Particles
χ^2 (CHI-Quadrat)	1,29	
Probability	**99**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	18,8	mg/kg
Standard deviation	1,18	mg/kg
rel. Standard deviaton	6,3	%
Horwitz standard deviation	10,3	%
HorRat-value	**0,61**	
Recovery rate	91	%

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 (2020)***
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 A + B:* *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), PCR and LC-MS can be applied for qualitative determinations.*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*One result each should be determined for Samples 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest August 28th 2020***
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
		SPAIN
		USA
		SPAIN
		CANADA
		ITALY
		SPAIN
		Germany
		Germany
		ITALY
		Germany
		FRANCE
		ITALY
		BRAZIL
		GREAT BRITAIN
		Germany
		Germany
		SWEDEN
		SPAIN
		SWITZERLAND
		ITALY
		Germany
		Germany
		Germany
		GREAT BRITAIN
		ITALY
		FRANCE
		GREAT BRITAIN
		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]