Evaluation Report

proficiency test

DLA 13/2019

Allergen-Screening III:

Cereals containing Gluten, Peanut, Lupine, Celery and Sesame

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

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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: 12 February 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Keine As part of the present proficency test the following services were subcontracted: none
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 common in commerce cereal flakes, flours, nut butter, dried plant parts and seeds as well as fresh celery root, from which DLA produced allergen premixes (s. Tab. 2). If required the raw materials were crushed, dried, ground with the addition of carrier substances and sieved (mesh 400 µm) or sieved by means of a centrifugal mill (mesh 250 µm or 500 µm).

The composition of the allergen-premixes is given in table 1. The premixes were used for spiking of the PT-samples 1 to 4 (see Tab. 2).

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

Table 1: Composition of DLA-Samples

Ingredients	**Samples 1 - 4**
Potato powder (Ingredients: Potatoes, E471, E304, E223, E100)	74 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (88% - 93%) - Sodium sulfate (0,0% - 5,5%) - Silicon dioxide (2,0% - 4,1%) - Allergens (5,0% - 10% each)	0,10 - 0,60 %

Table 2: Added amounts of allergenic ingredients positive in mg/kg ranges** given as food item (for cereals as total protein)

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Barley: Barley grain, ground (Protein 7,3%)	negative	positive (25 - 75)	negative	negative
Rye: Rye flour Type 1150 (Protein 9,1%)	negative	negative	negative	positive (25 - 75)
Wheat: Wheat flour Type 550 (Protein 10,5%)	negative	negative	positive (25 - 75)	negative
Peanut: commercial peanut butter (Protein 30%)	positive (25 - 75)	positive (25 - 75)	negative	negative
Lupine: Sweet lupine flour, (Protein 37%)	positive (25 - 75)	negative	negative	positive (50 - 150)
Celery: Leafs, dried (Protein 14%)	negative	positive (50 - 150)	negative	negative
Celery: Roots, dried (Protein 8,2%)	negative	negative	negative	positive (50 - 150)
Celery: Seeds, dried (Protein 20%)	negative	negative	positive (50 - 150)	negative
Sesame: Seeds white, dried (Protein 22%)	negative	negative	positive (25 - 75)	negative
Sesame: Seeds black, dried (Protein 23%)	positive (25 - 75)	negative	negative	negative

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

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

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

Lateral Flow Device (LFD)*	Sample 1	Sample 2	Sample 3	Sample 4
AgraStrip® Gluten G12	negative	positive	positive	positive
AgraStrip® Peanut	positive	positive	negative	negative
AgraStrip® Lupin	positive	negative	negative	positive
AgraStrip® Sesame	positive	negative	positive	negative

* Nachweisgrenze jeweils 1-10 mg/kg / Limit of detection (LOD) 1-10 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 75%, 72%, 32% and 84%, 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 0,9, 1,0, 1,2 and 0,9, 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. 0,27 (17°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 44th week of 2019. The testing method was optional. The tests should be finished at December 13th 2019 the latest.

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

There are 4 different samples possibly containing the allergenic ingredients ***Gluten*** *(Wheat, Rye and Barley),* ***Peanut, Lupine, Celery*** *(Leaves / Stem, Root and Seed) and/or* ***Sesame*** *(white and black) 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***

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. 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 19 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 in case ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

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

4. Results

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

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

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Gluten Containing Cereals

4.1.1 ELISA-Results: Gluten, in general

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (barley)	Sample 3 (wheat)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
14	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
3	positive	positive	positive	positive	3/4 (75%)	3/4 (75%)	BF	
10	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	IL	
13	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	IL	
1a	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	MI	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
4	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
6a	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
9	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
11	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
15	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
19	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS	
6b	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SE-R5	
1b	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	VT-R5	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	15	15	15
Number negative	14	0	0	0
Percent positive	7	100	100	100
Percent negative	93	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

MI = Morinaga Institute ELISA

RS = Ridascreen®, R-Biopharm

SE-R5 = SensiSpec R5 ELISA Kit, Eurofins

VT-R5 = Veratox, Neogen

Comments:

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

4.1.2 PCR-Results: Cereals Containing Gluten

4.1.2.1 PCR-Results: Gluten, in general

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (barley)	Sample 3 (wheat)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
15	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
1	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, 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.

4.1.2.2 PCR-Results: Barley

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (barley)	Sample 3 (wheat)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
18	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	oat was also detected in sample 4
19	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

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.

4.1.2.3 PCR-Results: Rye

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (barley)	Sample 3 (wheat)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
1a	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	div	
1b	negative	negative	(positive)	positive	3/3 (100%)	3/3 (100%)	div	wheat and rye
18	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
19	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	1	5
Number negative	5	5	3	0
Percent positive	0	0	25	100
Percent negative	100	100	75	0
Consensus value	negative	negative	negative	positive
Spiking	negative	negative	negative	positive

Methods:

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. For sample 3 spiked with wheat two positive results were obtained. The result 1b was not considered for the evaluations because no differentiation was made between wheat and rye.

4.1.2.4 PCR-Results: Wheat

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (barley)	Sample 3 (wheat)	Sample 4 (rye)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	negative	positive	positive	4/4 (100%)	3/4 (75%)	SFA-4p	
1a	negative	negative	positive	(positive)	3/3 (100%)	3/3 (100%)	div	Wheat and other cereals with gliadin gene
1b	negative	negative	positive	(positive)	3/3 (100%)	3/3 (100%)	div	wheat and rye
6	negative	negative	positive	positive	4/4 (100%)	3/4 (75%)	div	
18	negative	negative	positive	positive	4/4 (100%)	3/4 (75%)	div	
19	negative	negative	positive	positive	4/4 (100%)	3/4 (75%)	div	

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

Methods:

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 for samples 1-3 are in qualitative agreement with the spiking of samples. For sample 4, spiked with rye, positive results were obtained. A slight contamination of the sample with wheat cannot be excluded.

The results 1 a+b for sample 4 were not considered for the evaluations because no differentiation was made between wheat and rye.

4.2 Proficiency Test Peanut

4.2.1 ELISA-Results: Peanut

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		
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BF	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BK	
10	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MI	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
19	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RS-F	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SE	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SE =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: Peanut

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%)	ASU	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
17	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, 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.

4.3 Proficiency Test Lupine

4.3.1 ELISA-Results: Lupine

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
14	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
3	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	BF	
2	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ES	
13	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	IL	
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
19	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SE	
15	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SE	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	11	0	0	11
Number negative	0	11	11	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

RS-F= Ridascreen® Fast, R-Biopharm

SE = SensiSpec ELISA Kit, Eurofins

Comments:

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

4.3.2 PCR-Results: Lupine

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
6	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
19	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
11	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
15	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
18	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, 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 Celery

4.4.1 ELISA-Results: Celery

None of the participants used the ELISA method for determination of celery.

4.4.2 PCR-Results: Celery

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (leaves)	Sample 3 (seed)	Sample 4 (root)	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	positive	positive	4/4 (100%)	4/4 (100%)	ASU	Sample 4: Traces at LOD
7	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
19	negative	positive	positive	negative	3/4 (75%)	3/4 (75%)	ASU	
5	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
15	negative	positive	positive	negative	3/4 (75%)	3/4 (75%)	SFA	
17	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
11	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
1	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
4	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
18	negative	positive	negative	negative	2/4 (50%)	2/4 (50%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	13	12	10
Number negative	13	0	1	3
Percent positive	0	100	92	77
Percent negative	100	0	8	23
Consensus value	negative	positive	positive	positive
Spiking	negative	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.5 Proficiency Test Sesame

4.5.1 ELISA-Results: Sesame, in general

Qualitative valuation of results

Evaluation number	Sample 1 (black)	Sample 2 (without)	Sample 3 (white)	Sample 4 (without)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1a	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BC	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
19	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SE	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SE	
1b	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SE = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

None of the participants differentiated between black and white sesame.

4.5.2 PCR-Results: Sesame, in general

Qualitative valuation of results

Evaluation number	Sample 1 (black)	Sample 2 (without)	Sample 3 (white)	Sample 4 (without)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
11	positive	negative	positive	positive	3/4 (75%)	3/4 (75%)	SFA-Q	
1	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
18	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	11	0	11	1
Number negative	0	11	0	10
Percent positive	100	0	100	9
Percent negative	0	100	0	91
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-Q = Sure Food Allergen Quant, 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.

None of the participants differentiated between black and white sesame.

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: Gluten, in general

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AS	14	07.11.19	negative	positive	positive	positive	5		AgraStrip Gluten G12 / Romer Labs
BF	3	13/12	positive	positive	positive	positive	0,36	Gluten	BF = MonoTrace ELISA, BioFront Technologies
IL	10	21.11.2019	negative	positive	positive	positive	0,3	Gluten	IL = Immunolab
IL	13		negative	positive	positive	positive		Gluten	IL = Immunolab
MI	1a	26.11.19	negative	positive	positive	positive	0,31	Gliadin	MI = Morinaga Institute ELISA
RS	2	02.12.19	negative	79,6	58,1	103	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	4	26.11.19	negative	positive	positive	positive	3	Gliadin	RS = Ridascreen®, R-Biopharm
RS	6a	12.11.	negative	positive	positive	positive	3	Gluten	RS = Ridascreen®, R-Biopharm
RS	8	26.11.19	negative	positive	positive	positive	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	9	04.12.19	negative	positive	positive	positive	1	Gluten	RS = Ridascreen®, R-Biopharm
RS	11	26.11.19	negative	positive	positive	positive	< 1,0 ppm	Food item, total	Ridascreen Gliadin
RS	15	14.11.19	NEG	POS	POS	POS	5	Please select!	RS = Ridascreen®, R-Biopharm
RS	19	06.11.19	negative	positive	positive	positive	1.0	Gluten	RS = Ridascreen®, R-Biopharm
SE-R5	6b	14.11.	negative	positive	positive	positive	3,12	Gluten	Eurofins Technologies
VT-R5	1b	27.11.19, 05.12.19	negative	positive	positive	positive	2,5	Gliadin	VT-R5 = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AS	14	COKAL0200AS			
BF	3	GLU-EK	Monoclonal	1:40 for 1 hour @ 60C	
IL	10	GLI-156	anti-gliadin	40% ethanol/ 5 min/ room temperature	-
IL	13				
MI	1a	M2114		according to kit instructions	
RS	2	R7001			
RS	4				
RS	6a	R7001	R5, detects prolamins from w heat, rye and barley	according to manufacturer's instructions	Sample 2: >50mg/kg; Sample 3: >50mg/kg; Sample 4: >50mg/kg
RS	8				
RS	9	R7001	monoclonal antibody R5	Kit instructions follow ed.	Sample 1: <5,0 mg/kg Sample 2: 56,27 mg/kg Sample 3: 61,09 mg/kg Sample 4: 104,9 mg/kg
RS	11	R7001	specific gliadin fraction from w heat	Cocktail solution, 50°C	
RS	15				
RS	19	R7001	R5	Cocktail solution/EtOH; 40 min 50°C / 1 h RT	
SE-R5	6b	SENSISpec Ingezim Test-Combination 30.GLU.K2	R5, detects prolamins from w heat, rye and barley	according to manufacturer's instructions	Sample 2: >50mg/kg; Sample 3: 57mg/kg; Sample 4: > 50mg/kg
VT-R5	1b	8510	R5	according to kit instructions	

5.1.2 ELISA: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detec-tion given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	3	13/12	positive	positive	negative	negative	0,24	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
BK	1	28.11.19	positive	positive	negative	negative	1	Peanut, total	BK = BioKits, Neogen
IL	10	14.11.2019	positive	positive	negative	negative	0,1	Food item, total	IL = Immunolab
IL	13		positive	positive	negative	negative		Peanut, total	IL = Immunolab
MI	6	12.11.	positive	positive	negative	negative	0,2	Erdnussprotein	MI = Morinaga Institute ELISA
RS-F	4	07.12.19	positive	positive	negative	negative	1,2	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	12	07.11.19	positive	positive	negative	negative	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	19	06.11.19	positive	positive	negative	negative	0.13	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SE	15	14.11.19	POS	POS	NEG	NEG		Please select!	SE = Eurofins SENSISpec
VT	2	28.11.19	positive	positive	negative	negative	2,5	Peanut	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
BF	3	PA3-EK	Monoclonal	1:10 for 10 minutes @ 60C	
BK	1	902048Q		according to kit instructions	
IL	10	ERN-154	anti-peanut	Extraction and sample dilution buffer (Tris)/ 15 min/ 60 degrees	-
IL	13				
MI	6	M2120	detects peanut proteins	according to manufacturer's instructions	Sample 1: >10mg/kg; Sample 2: >10mg/kg
RS-F	4				
RS-F	12	14498			
RS-F	19	R6202	Peanut protein	Extraction buffer from kit / 10 min 60°C	
SE	15				
VT	2	8430		only 1 g (instead of 5 g) sample extracted	

5.1.3 ELISA: Lupine

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AS	14	07.11.19	positive	negative	negative	positive	10		AgraStrip Lupin / Romer Labs
BF	3	13/12	positive	negative	negative	positive	0,13	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
ES	2	25.11.19	positive	negative	negative	positive	0,5	Lupine protein	ES = ELISA-Systems
IL	13		positive	negative	negative	positive		Lupine, total	IL = Immunolab
RS-F	1	02.12.2019 13.12.2019	positive	negative	negative	positive	1	Lupine protein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	4	07.12.19	positive	negative	negative	positive	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	5	13.12.19	positive	negative	negative	positive	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11	26.11.19	positive	negative	negative	positive	< 0,7 ppm	Food item, total	Ridascreen FAST Lupine
RS-F	19	12.11.19	positive	negative	negative	positive	0.7	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SE	6	12.11.	positive	negative	negative	positive	1,5	Lupine	Eurofins Technologies
SE	15	14.11.19	POS	NEG	NEG	POS		Please select!	SE = Eurofins SENSISpec

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AS	14	COKAL1510AS			
BF	3	LU2-EK	Monoclonal	1:20 for 10 minutes @ 60C	
ES	2	ESLFP-48			
IL	13				
RS-F	1	R6102		acording to kit instructions	Cross reactivity to chickpeas 0.31%
RS-F	4				
RS-F	5	R6102	As Per Kit Instructions	As Per Kit Instructions	
RS-F	11	R6102	specifically lupine proteins	Extraction buffer, 60°C	
RS-F	19	R6102	Lupine protein, polyclonal	Extraction buffer from kit / 10 min 60°C	
SE	6	HU0030011	detects lupine proteine	according to manufacturer's instructions	Sample 1: >25mg/kg; Sample 4: >25mg/kg
SE	15				

5.1.4 ELISA: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	1a	12.12.19	positive	negative	positive	negative	2	Sesame, total	AQ = AgraQuant, RomerLabs
AS	14	07.11.19	positive	negative	positive	negative	5		AgraStrip Sesame / Romer Labs
BC	5	13.12.19	positive	negative	positive	negative	2	Food item, total	BC = BioCheck ELISA
BF	3	13/12	positive	negative	positive	negative	0,3	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	10	19.11.2019	positive	negative	positive	negative	0,2	Food item, total	IL = Immunolab
IL	13		positive	negative	positive	negative		Sesame, total	IL = Immunolab
RS-F	12	07.11.19	positive	negative	positive	negative	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	19	13.11.19	positive	negative	positive	negative	0.14	Please select!	RS-F= Ridascreen® Fast, R-Biopharm
SE	6	13.11.	positive	negative	positive	negative	1,5	Sesame	Eurofins Technologies
SE	15	14.11.19	POS	NEG	POS	NEG		Please select!	SE = Eurofins SENSISpec
VT	1b	10.12.2019 16.12.2019	positive	negative	positive	negative	2,5	Sesame, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
AQ	1a	COKAL1948		according to kit instructions	
AS	14	COKAL1910AS			
BC	5	R6029	As Per Kit Instructions	As Per Kit Instructions	
BF	3	SE1-EK	Monoclonal	1:20 for 10 minutes @ 60C	
IL	10	SES-133	anti-sesame	Extraction and sample dilution buffer (Tris)/ 15 min/ 60 degrees	-
IL	13				
RS-F	12	13299			
RS-F	19	R7202	Sesame protein	Extraction buffer from kit / 10 min 60°C	
SE	6	HU0030022	recognizes sesame proteins	according to manufacturer's instructions	Sample 1: >20mg/kg; Sample 3: >20mg/kg
SE	15				
VT	1b	8530		according to kit instructions	

5.1.5 PCR: Gluten, in general

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA	5	04.12.19	negative	positive	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	14.11.19	NEG	POS	POS	POS	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q	8	07.11.19	negative	positive	positive	positive	0,4	Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	1	17.12.19	negative	positive	positive	positive		Wheat, spelt, kamut, rye, barley, oats	own PCR method
div	16		negative	positive	positive	positive		Please select!	Selection PCR-Methods

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	5	S3606	As Per Kit Instructions	As Per Kit Instructions	
SFA	15				
SFA-Q	8				
div	1				
div	16				

5.1.6 PCR: Barley

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-4p	5	13.12.19	negative	positive	negative	negative	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	17.12.19	negative	positive	negative	negative		Barley	own PCR method
div	18		negative	positive	negative	negative		Allergen-DNA	NGS
div	19		negative	positive	negative	negative	1.0	Allergen-DNA	Literature method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA-4p	5	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	1				
div	18			FFS Promega	oat w as also detected in sample 4
div	19			CTAB /Chloroform extraction/ Clean up: FFS Kit Promega (Maxw ell)	

5.1.7 PCR: Rye

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-4p	5	13.12.19	negative	negative	negative	positive	1	Please select!	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1a	17.12.19	negative	negative	positive	positive		Rye	own PCR method
div	1b	17.12.19	negative	negative	positive	positive		Wheat and rye	own PCR method
div	18		negative	negative	negative	positive		Allergen-DNA	NGS
div	19		negative	negative	negative	positive	1.0	Allergen-DNA	Literature method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA-4p	5	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	1a				
div	1b				
div	18			FFS Promega	
div	19			CTAB /Chloroform extraction/ Clean up: FFS Kit Promega (Maxw ell)	

5.1.8 PCR: Wheat

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-4p	5	13.12.19	negative	negative	positive	positive	1	Please select!	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	1a	17.12.19	negative	negative	positive	positive		Wheat and other cereals with gliadin gene	own PCR method
div	1b	17.12.19	negative	negative	positive	positive		Wheat and rye	own PCR method
div	6	15.11.	negative	negative	positive	positive	10	Wheat DNA	Internal method
div	18		negative	negative	positive	positive		Allergen-DNA	NGS
div	19		negative	negative	positive	positive	1.0	Allergen-DNA	Literature method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA-4p	5	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	1a				
div	1b				
div	6			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime-PCR / 45 cycles	
div	18			FFS Promega	
div	19			CTAB /Chloroform extraction/ Clean up: FFS Kit Promega (Maxw ell)	

5.1.9 PCR: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	2	10.12.19	positive	positive	negative	negative	5 pg	Allergen-DNA	Selection PCR-Methods
ASU	7	05.11.19	positive	positive	-	-	*	Food item, total	ASU
SFA	11	19.11.19	positive	positive	negative	negative	≤ 0,4 mg/kg	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	14.11.19	POS	POS	NEG	NEG	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	05.11.19	positive	positive	negative	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q	8	07.11.19	positive	positive	negative	negative	0,4	Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	1	17.12.19	positive	positive	negative	negative		Peanut	own PCR method
div	4	07.12.19	positive	positive	negative	negative	8	Allergen DNA	other: please fill in!
div	6	15.11.	positive	positive	negative	negative	10	Peanut DNA_	internal method
div	16		positive	positive	negative	negative		Please select!	Selection PCR-Methods

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	2	L 44.00-11: 2013-01		Qiagen Mericon Food Kit	
ASU	7	L44.00.11	Ara h2	CTAB with / without precipitation, Dneasy Mericon Food	Validated in the laboratory for 0.1%, as it is usually only used for adulteration
SFA	11	S3603		SureFood® PREP Advanced	
SFA	15				
SFA	17	REF. KIT S3603 / LOT14098		EXTRACTION/ REAL TIME PCR	
SFA-Q	8				
div	1				
div	4				
div	6			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime-PCR / 45 cycles	
div	16				

5.1.10 PCR: Lupine

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detec-tion given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	2	27.11.19	positive	negative	negative	positve	1 pg	Allergen-DNA	Selection PCR-Methods
ASU	6	15.11.	positive	negative	negative	positive	0,5	Lupine DNA_	ASU
ASU	19	13.11.19	positive	negative	negative	positive	1.0	Allergen-DNA	RT-PCR according to ASU
SFA	5	04.12.19	positive	negative	negative	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11	18.11.19	positive	negative	negative	positive	≤ 0,4 mg/kg	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	14.11.19	POS	NEG	NEG	POS	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	05.11.19	positive	negative	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q	8	07.11.19	positive	negative	negative	positive	0,4	Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	1	17.12.19	positive	negative	negative	positive		Lupine	own PCR method
div	4	10.12.19	positive	negative	negative	positive	80	Allergen DNA	other: please fill in!
div	16		positive	negative	negative	positive		Please select!	Selection PCR-Methods
div	18		positive	negative	negative	positive		Allergen-DNA	NGS

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	2	L 08.00-58(V):2011-06		Qiagen Mericon Food Kit	
ASU	6	§64 LFGB L 08.00-58 (V): 2011-06		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime-PCR / 45 cycles	
ASU	19	ASU L 08.00-58(V)_2011-06	ITS-Sequence	CTAB /Chloroform extraction/ Clean up: FFS Kit Promega (Maxw ell)	
SFA	5	S3602	As Per Kit Instructions	As Per Kit Instructions	
SFA	11	S3611		SureFood® PREP Advanced	PC is negative
SFA	15				
SFA	17	REF. KIT S3611 / LOT 12248		EXTRACTION/ REAL TIME PCR	
SFA-Q	8				
div	1				
div	4				
div	16				
div	18			FFS Promega	

5.1.11 PCR: Celery

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	6	15.11.	negative	positive	positive	positive	10	Celery DNA_	ASU
ASU	7	05.11.19	-	positive	positive	positive	80'	Food item, total	ASU
ASU	19	11.11.19	negative	positive	positive	negative	1.0	Allergen-DNA	RT-PCR according to ASU
SFA	5	04.12.19	negative	positive	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	14.11.19	NEG	POS	POS	NEG	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	05.11.19	negative	positive	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	11	19.11.19	negative	positive	positive	positive	≤ 0,4 mg/kg	Allergen-DNA	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	8	07.11.19	negative	positive	positive	positive	0,4	Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	1	17.12.19	negative	positive	positive	positive		Celery	own PCR method
div	2	26.11.19	negative	positive	positive	positive	5 pg	Allergen-DNA	Selection PCR-Methods
div	4	15.11.19	negative	positive	positive	positive	8	Allergen DNA	other: please fill in!
div	16		negative	positive	positive	positive		Please select!	Selection PCR-Methods
div	18		negative	positive	negative	negative		Allergen-DNA	NGS

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	6	§64 LFGB L 08.00-56: 2014-08		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime-PCR / 45 cycles	Probe 4: Spuren an der NWG
ASU	7	L08.00.56	mannitol dehydrogenase	CTAB with / without precipitation, Dneasy Mericon Food	limit of detection previously determined in the laboratory
ASU	19	ASU L 08.00-56_2014-08	mannitol dehydrogenase	CTAB /Chloroform extraction/ Clean up: FFS Kit Promega (Maxwell)	
SFA	5	S3605	As Per Kit Instructions	As Per Kit Instructions	
SFA	15				
SFA	17	REF. KIT S3605 / LOT 13059		EXTRACTION/ REAL TIME PCR	
SFA-ID	11	S3105		SureFood® PREP Advanced	NC is positive
SFA-Q	8				
div	1				
div	2	DIN CEN/TS 15634-2, edition April 2012		Qiagen Mericon Food Kit	
div	4				
div	16				
div	18			FFS Promega	

5.1.12 PCR: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	2	27.11.19	positive	negative	positive	negative	5 pg	Allergen-DNA	Selection PCR-Methods
ASU	6	15.11.	positive	negative	positive	negative	10	Sesame DNA_	ASU
SFA	5	04.12.19	positive	negative	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	15	14.11.19	POS	NEG	POS	NEG	0,4	Please select!	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	05.11.19	positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-Q	8	07.11.19	positive	negative	positive	negative	0,4	Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
SFA-Q	11	18.11.2019 & 21.11.19	positive	negative	positive	positive	≤ 0,4 ppm	Allergen-DNA	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	1	17.12.19	positive	negative	positive	negative		Sesame	own PCR method
div	4	07.12.19	positive	negative	positive	negative	8	Allergen DNA	other: please fill in!
div	16		positive	negative	positive	negative		Please select!	Selection PCR-Methods
div	18		positive	negative	positive	negative		Allergen-DNA	NGS

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	2	L.18.00-19:2014-08		Qiagen Mericon Food Kit	
ASU	6	§64 LFGB L 08.00-19: 2014-08		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime-PCR / 45 cycles	
SFA	5	S3608	As Per Kit Instructions	As Per Kit Instructions	
SFA	15				
SFA	17	REF. KIT S3608 / LOT 12178		EXTRACTION/ REAL TIME PCR	
SFA-Q	8				
SFA-Q	11	S3208		SureFood® PREP Advanced	Sample 2 w as repeated
div	1				
div	4				
div	16				
div	18			FFS Promega	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 13-2019 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	33,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	81	32,1
2	5,05	68	26,9
5	5,03	78	31,0
6	5,05	69	27,3
7	5,06	72	28,5
8	5,10	82	32,2
9	5,08	64	25,2
10	5,00	68	27,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	72,7	Particles
Standard deviation	6,66	Particles
χ^2 (CHI-Quadrat)	4,27	
Probability	**75**	%
Recovery rate	85	%

Normal distribution		
Number of samples	8	
Mean	28,8	mg/kg
Standard deviation	2,64	mg/kg
rel. Standard deviaton	9,15	%
Horwitz standard deviation	9,65	%
HorRat-value	**0,95**	
Recovery rate	85	%

Microtracer Homogeneity Test
DLA 13-2019 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	25,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	68	27,1
4	5,07	76	30,0
5	5,08	59	23,2
6	5,01	69	27,5
7	5,00	61	24,4
8	5,02	58	23,1
9	5,13	70	27,3
10	5,12	60	23,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,1	Particles
Standard deviation	6,48	Particles
χ^2 (CHI-Quadrat)	4,51	
Probability	**72**	%
Recovery rate	99	%

Normal distribution		
Number of samples	8	
Mean	25,8	mg/kg
Standard deviation	2,56	mg/kg
rel. Standard deviaton	9,94	%
Horwitz standard deviation	9,81	%
HorRat-value	**1,0**	
Recovery rate	99	%

Microtracer Homogeneity Test
DLA 13-2019 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	35,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,08	89	35,0
3	5,17	87	33,7
4	5,06	108	42,7
6	5,01	109	43,5
7	5,14	98	38,1
8	5,13	99	38,6
9	5,02	105	41,8
10	5,14	84	32,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	97,5	Particles
Standard deviation	10,67	Particles
χ^2 (CHI-Quadrat)	8,17	
Probability	**32**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	38,3	mg/kg
Standard deviation	4,19	mg/kg
rel. Standard deviaton	10,9	%
Horwitz standard deviation	9,24	%
HorRat-value	**1,2**	
Recovery rate	107	%

Microtracer Homogeneity Test
DLA 13-2019 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	26,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,13	58	22,6
2	5,01	55	22,0
3	5,10	66	25,9
4	5,02	67	26,7
6	5,04	58	23,0
8	5,07	63	24,9
9	5,08	70	27,6
10	5,05	56	22,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	61,6	Particles
Standard deviation	5,55	Particles
χ^2 (CHI-Quadrat)	3,50	
Probability	**84**	%
Recovery rate	93	%

Normal distribution		
Number of samples	8	
Mean	24,3	mg/kg
Standard deviation	2,19	mg/kg
rel. Standard deviaton	9,00	%
Horwitz standard deviation	9,90	%
HorRat-value	**0,91**	
Recovery rate	93	%

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 13-2019***
PT name	***Allergen-Screening III - 4 Samples qualitative: Cereals containing Gluten** (Wheat, Rye and Barley)**, Peanut, Lupine, Celery** (Leaves / Stem, Root and Seed)**, Sesame** (white and black)*
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: **Cereals containing Gluten** (Wheat, Rye and Barley)**, Peanut, Lupine, Celery** (Leaves / Stem, Root and Seed)**, Sesame** (white and black)* *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 December 13th 2019***
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
		USA
		SWITZERLAND
		SPAIN
		Germany
		Germany
		FRANCE
		Germany
		ITALY
		SPAIN
		POLAND
		Germany
		SWITZERLAND
		SPAIN
		Germany
		Germany
		GREAT BRITAIN
		Germany
		FRANCE
		Germany

[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]