Evaluation Report
proficiency test

DLA ptALS3/2020

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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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)	0,06 - 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
Oat: Oat flakes (Protein 12%)	negative	negative	positive (25 - 75)	negative
Rye: Rye flour Type 1150 (Protein 9,1%)	negative	positive (25 - 75)	negative	negative
Wheat: Wheat flour mix-ture (Protein 11%)	positive (25 - 75)	negative	negative	negative
Peanut: commercial peanut butter (Protein 30%)	negative	positive (25 - 75)	positive (25 - 75)	negative
Lupine: Sweet lupine flour, (Protein 37%)	positive (50 - 150)	negative	positive (50 - 150)	negative
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 (25 - 75)	negative
Sesame: Seeds white, dried (Protein 22%)	negative	negative	positive (25 - 75)	negative
Sesame: Seeds black, dried (Protein 23%)	negative	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® Gluten	positive	positive	negative	negative
AgraStrip® Peanut	negative	positive	positive	negative
AgraStrip® Lupin	positive	negative	positive	negative
AgraStrip® Sesame	negative	negative	positive	positive

* Nachweisgrenze 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 71%, 94%, 83% and 100%, 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 1,0, 0,8, 0,9 and 0,3, 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. 0,39-0,41 (18,6°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 40th week of 2020. The testing method was optional. The tests should be finished at November 27th 2020 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*** *(Oat, Rye and Wheat),* ***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***
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. 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 24 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. No LC/MS results were submitted.

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 (wheat)	Sample 2 (rye)	Sample 3 (oat)	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		
23	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	AQ-G12	
5	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	AS	
24a	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	BF	
24b	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	BF-LF	
12	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	IL	
4	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
6	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
13	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
16	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
17	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
18	negative	positive	negative	negative	3/4 (75%)	2/4 (50%)	RS	
22	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	RS	
1	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	SP-Q	
4	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	SP-R5	

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

Methods:

AQ-G12 = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

BF-LF= AllerTrace (Lateral Flow), BioFront Technologies

IL = Immunolab

RS = Ridascreen®, R-Biopharm

SP-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins

SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins

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

Note concerning the qualitative assessment of sample 3 with oat: Non-contaminated oat do not contain gluten. This is in agreement with the consensus value of participants results. However, since oat is on the list of allergens to be labelled as "gluten-containing" cereals according to the EU Information Regulation (EU Regulation 1169/2011 Annex II), the qualitative evaluation of sample 3 was set with respect to the spiking to "positive". Analytically, the parameter gluten in the pure oat is "negative".

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 (wheat)	Sample 2 (rye)	Sample 3 (oat)	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	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
3	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	positive	positive		3/3 (100%)	3/3 (100%)	SFA	
19	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.1.2.2 PCR-Results: Oat

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (rye)	Sample 3 (Oat)	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	positive	positive	negative	2/4 (50%)	2/4 (50%)	SFA	
19	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
4	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. Low amounts of oat in samples 1 and 2 cannot be ruled out.

4.1.2.3 PCR-Results: Rye

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (rye)	Sample 3 (oat)	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	positive	negative	negative	4/4 (100%)	3/4 (75%)	SFA	
8	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	SFA-4p	
19	negative	positive	negative	negative	3/4 (75%)	4/4 (100%)	SFA-4p	sample 1 in traces
10	positive	positive	negative	negative	4/4 (100%)	3/4 (75%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the samples 2-4 are in qualitative agreement with the spiking of samples. Low amounts of rye in sample 1 containing wheat cannot be ruled out.

4.1.2.4 PCR-Results: Wheat

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (rye)	Sample 3 (oat)	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	positive	negativ	negativ	4/4 (100%)	3/4 (75%)	SFA	
8	positive	positive	negativ	negativ	4/4 (100%)	3/4 (75%)	SFA	
19	positive	negativ	negativ	negativ	3/4 (75%)	4/4 (100%)	SFA-4p	
4	positive	positive	negativ	negativ	4/4 (100%)	3/4 (75%)	div	
10	positive	positive	negativ	negativ	4/4 (100%)	3/4 (75%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results for samples 1, 3 and 4 are in qualitative agreement with the spiking of samples. For sample 2, spiked with rye, positive results were obtained. Low amounts of wheat in sample 2 cannot be ruled out.

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		
23	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
24	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	BF	
6	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	IL	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	MI-II	
16	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
18	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
1	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
12	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	10	10	0
Number negative	10	0	0	10
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

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

MI-II = Morinaga Institute ELISA Kit II

RS = Ridascreen®, 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.

4.2.2 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		
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
20	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
2	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
3	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
17	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	Sample 3 in traces
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
16	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
21	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

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		
23	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AQ	
5	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	AS	
24	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	BF	
6	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	IL	
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	amounts > LOD evaluated as positive by DLA
19	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	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SP	

	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
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS = Ridascreen®, 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.

4.3.2 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		
4	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
11	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
14	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
17	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
19	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
15	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
21	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

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 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		
4	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
20	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
18	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	FP	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
3	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
6	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
17	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
19	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
10	negative	positive	positive	negative	3/4 (75%)	3/4 (75%)	div	
16	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
21	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

FP = foodproof Detection Kit, BIOTECON Diagnostics

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The 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 (without)	Sample 2 (without)	Sample 3 (white)	Sample 4 (black)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
23	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AQ	
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	AS	
19	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BC	
24	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	BF	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
18	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	amounts > LOD evaluated as positive by DLA
19	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	
17	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

RS = Ridascreen®, 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.

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 (without)	Sample 2 (without)	Sample 3 (white)	Sample 4 (black)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
17	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
19	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
21	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

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.

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
AQ-G12	23	10.09.20	pos	pos	neg	neg	4	Gluten	AQ-G12 = AgraQuant, RomerLabs
AS	5	29.10.20	positive	positive	negative	negative	2		AgraStrip Gluten/ Romer Labs
BF	24a	27/11	positive	positive	negative	negative	0,36	Gluten	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	24b		positive	positive	negative	negative			BF-LF = AllerTrace LFD (Lateral Flow), BioFront Technologies
IL	12	02.10.20	positive	positive	negative	negative	2 (LOQ)	Gliadin	IL = Immunolab
RS	4	09.10.20	positive	positive	negative	negative	3	Gluten	RS = Ridascreen®, R-Biopharm
RS	6		positive	positive	negative	negative			RS = Ridascreen®, R-Biopharm
RS	13	04.11.20	positive	positive	negative	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	16		positive	positive	negative	negative	3	Gliadin	RS = Ridascreen®, R-Biopharm
RS	17	02.11.20	positive	positive	negative	negative		Gliadin	RS = Ridascreen®, R-Biopharm
RS	18		negativ	positive	negative	negative	< 5		RS = Ridascreen®, R-Biopharm
RS	22	17.11.20	positive	positive	negative	negative	0,5 mg/kg	Gliadin	RS = Ridascreen®, R-Biopharm
SP-Q	1		positive	positive	negative	negative	2	Gluten	SP-Q = SensiSpec Ingezim Gluten R5 Quick, Eurofins
SP-R5	4	12.10.20	positive	positive	negative	negative	3,12	Gluten	SENSISpec Ingezim Gluten

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-G12	23	10001994			
AS	5				
BF	24a	GLU-EK	Monoclonal antibody-based assay	1:40 extraction performed at 60C for 1 hour	
BF-LF	24b				
IL	12				
RS	4	R7001	Recognizes prolamins (gliadins) from w heat, rye and barley, R5 from Mendez	acc. Manufacturer's instructions	Sample 1: >25; Sample 2: >25
RS	6				
RS	13	R7001			
RS	16	R7001			Sample 1 and Sample 2 are out of range
RS	17				
RS	18				
RS	22	R 7001	Monclonal antibody R5	cocktail solution (50°C 40 min), ethanol 80% (60 min)	
SP-Q	1	R.30.GLU.K2			
SP-R5	4	30.GLU.K2	Recognizes prolamins (gliadins) from w heat, rye and barley, R5 from Mendez	acc. Manufacturer's instructions	Sample 1: >50; Sample 2: >50

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 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	23	10.09.20	neg	pos	pos	neg	1	Food item, total	AQ = AgraQuant, RomerLabs
BF	24	27/11	negative	positive	positive	negative	0,24	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	6		negative	positive	positive	negative			IL = Immunolab
MI-II	4	09.10.20	negative	positive	positive	negative	0,2	peanut protein	MI-II = Morinaga Institute ELISA Kit II
RS	16		negative	positive	positive	negative	1,2	protein	RS = Ridascreen®, R-Biopharm
RS	18		negative	positive	positive	negative	< 2,5		RS = Ridascreen®, R-Biopharm
RS-F	13	27.10.20	negative	positive	positive	negative	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		negative	positive	positive	negative	1	peanut	SP = SensiSpec ELISA Kit, Eurofins
SP	12	02.10.20	negative	positive	positive	negative	1 (LOQ)	Food item, total	SP = SensiSpec ELISA Kit, Eurofins
SP	17	02.11.20	negative	positive	positive	negative		Food item, total	SP = SensiSpec ELISA Kit, Eurofins

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	23	10001990			
BF	24	PA3-EK	Monoclonal antibody-based assay	1:10 extraction performed at 60C for 10 minutes	Sample 2: >10; Probe 3: >10
IL	6				
MI-II	4	Morinaga Sensitive ELISA Kit II Peanut M2120	Recognizes peanut proteins	acc. Manufacturer's information	
RS	16	R6202			
RS	18				Sample 2 and Sample 3 are out of range
RS-F	13	R6202			
SP	1	HU0030019			
SP	12				
SP	17				

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
AQ	23	17/11	pos	neg	pos	neg	2	Food item, total	AQ = AgraQuant, RomerLabs
AS	5		positive	negative	positive	negative	2		AgraStrip Lupine/Romer Labs
BF	24	27/11	positive	negative	positive	negative	0,13	Food item, total	BF = MonoTrace ELISA, BioFront Technologies
IL	6		positive	negative	positive	negative			IL = Immunolab
RS	16		positive	negative	positive	negative	1	protein	RS = Ridascreen®, R-Biopharm
RS-F	13	02.11.20	positive	negative	positive	negative	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14		21,8	negative	>27	negative	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	19	30.10.20	positive	negative	positive	negative	0,5	See Further Remarks	RS-F= Ridascreen® Fast, R-Biopharm
SP	4	09.10.20	positive	negative	positive	negative	1,5	Lupine	SP = SensiSpec ELISA Kit, Eurofins
SP	12	02.10.20	positive	negative	positive	negative	2 (LOQ)	Food item, total	SP = SensiSpec ELISA Kit, Eurofins
SP	17	02.11.20	positive	negative	positive	negative		Food item, total	SP = SensiSpec ELISA Kit, Eurofins

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	23	10002049			
AS	5				
BF	24	LU2-EK	Monoclonal antibody-based assay	1:20 extraction performed at 60°C for 10 minutes	
IL	6				Sample 3 is out of range
RS	16	R6102			
RS-F	13	R6102			
RS-F	14				Reported as Lupin Protein
RS-F	19	R6102	As Per Kit Instructions	As per Kit Instructions	Sample 1: >25; Sample 3: >25
SP	4	HU0030011	Recognizes lupine proteins	As per Kit Instructions	
SP	12				
SP	17				

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	23	17/11	neg	neg	pos	pos	2	Food item, total	AQ = AgraQuant, RomerLabs
AS	5		negative	negative	positive	positive	2		AgraStrip Sesame/Romer Labs
BC	19	30.10.20	negative	negative	positive	positive	2	Food item, total	BC = BioCheck ELISA
BF	24	27/11	negative	negative	positiv	positiv	0,3		BF = MonoTrace ELISA, BioFront Technologies
IL	6		negative	negative	positive	positive			IL = Immunolab
IL	9	05.11.2020	negative	negative	positive	positive	0,2 ppm	Food item, total	IL = Immunolab
RS	18		negative	negative	positive	positive	< 2,5		RS = Ridascreen®, R-Biopharm
RS-F	13	05.11.20	negative	negative	positive	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	14		negative	negative	>20	>20	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	19	30.10.20	negative	negative	positive	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		negative	negative	positive	positive	2	Sesame	SP = SensiSpec ELISA Kit, Eurofins
SP	4	12.10.20	negative	negative	positive	positive	1,5	Sesame	SP = SensiSpec ELISA Kit, Eurofins
SP	12	02.10.20	negative	negative	positive	positive	2 (LOQ)	Food item, total	SP = SensiSpec ELISA Kit, Eurofins
SP	17	02.11.20	negative	negative	positive	positive		Food item, total	SP = SensiSpec ELISA Kit, Eurofins

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	23	10002064			
AS	5				Sample 3: >20; Sample 4: >20
BC	19	R6028	As Per Kit Instructions	As per Kit Instructions	
BF	24	SE1-EK	Monoclonal antibody-based assay	1:20 extraction performed at 60C for 10 minutes	
IL	6				
IL	9	Sesame ELISA/Cat.: SES-E01/ Lot.: SES-138	Sesame protein	Extraction: 1 g of homogenized mixture suspended in 20 mL of pre-diluted extraction and sample dilution buffer/ 15 minutes of sample incubation in 60°C/10 minutes of 2000 x g centrifugation Determination: 100 µL of particle-free solution, ready-to-use standards applied per well/ 20 minutes incubation at room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL conjugate into each well/ 20 minutes incubation in room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL substrate solution into each well/ 20 minutes incubation in the dark, at room temperature/ add 100 µL Stop enzyme solution into each well/ Measure absorbance at 450 nm (reference 620 nm)	
RS	18				
RS-F	13	R7202			
RS-F	14				
RS-F	19	R7202	As Per Kit Instructions	As per Kit Instructions	
SP	1	HU0030022			
SP	4	HU0030022/00300	recognizes sesame proteins	As Per Kit Instructions	
SP	12				
SP	17				

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	2	06.10.20	positiv	positiv	positiv	negativ	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3		positiv	positiv	positiv	negativ	0,4	Gluten	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	26.10.20	positiv	positiv	positiv	-	<0,4	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	29.10.20	positiv	positiv	positiv	negativ	10	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	10		positiv	positiv	positiv	negativ		Allergen-DNA	house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	2	S3606	gluten-containing cereals	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	w heat like spelt and khorasan w heat, rye, barley, oats
SFA	3				
SFA	8		Gluten-specific DNA	CTAB + additional purification via column / real-time PCR	
SFA	19	S3606	As Per Kit Instructions	As per Kit Instructions	
div	10				

5.1.6 PCR: Oat

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	2	06.10.20	positive	positive	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	29.10.20	negative	negative	positive	negative	1	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	4	09.10.20	negative	negative	positive	negative	5	Food item, total	
div	10		negative	negative	positive	negative		Allergen-DNA	house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	2	S7004	Avena sativa	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	19	S7004	As Per Kit Instructions	As per Kit Instructions	
div	4	internal method		CTAB / Proteinase K / Rnase A / Promega Maxwell / Real-time PCR / 45 cycles	
div	10				

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	2	06.10.20	positive	positive	negative	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	8	19.10.20	positive	positive	negative	negative	< 0,1	Allergen-DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-4p	19	28.10.20	negative	positive	negative	negative	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	10		positive	positive	negative	negative		Allergen-DNA	house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	2	S7006	Secale cereale	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA-4p	8		rye -specific DNA	CTAB + additional purification via column / real-time PCR	
SFA-4p	19	S7006	As Per Kit Instructions	As per Kit Instructions	Sample 1 in traces
div	10				

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	2	06.10.20	positive	positive	negative	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	19.10.20	positive	positive	negative	negative	< 0,1	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	19	28.10.20	positive	negative	negative	negative	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	4	09.10.20	positive	positive	negative	negative	10	Allergen-DNA	
div	10		positive	positive	negative	negative		Allergen-DNA	house method

other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
SFA	2	S7006	Triticum spp.	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	8		Wheat-specific DNA	CTAB + additional purification via column / real-time PCR	
SFA-4p	19	S7006	As Per Kit Instructions	As per Kit Instructions	
div	4	interne Methode		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real-time PCR / 45 Cycles	
div	10				

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	13	20.10.20	negativ	positiv	positiv	negativ	0,02	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	20	07.10.20	negativ	positiv	positiv	negativ	*	Food item, total	ASU = ASU §64 Methode/method
SFA	2	06.10.20	negativ	positiv	positiv	negativ	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3		negativ	positiv	positiv	negativ	0,4	Peanut	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	02.11.20	negativ	positiv	positiv	negativ		Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	8	19.10.20	negativ	positiv	positiv	negativ	< 0,4	Allergen-DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	4	09.10.20	negativ	positiv	positiv	negativ	5	Allergen-DNA	
div	10		negativ	positiv	positiv	negativ		Allergen-DNA	house method
div	16		negativ	positiv	positiv	negativ	8µg/kg		house method
div	21		negativ	positiv	positiv	negativ			house -internal 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	
ASU	13	ASU L 44.00-11 (2013-01)	Ara h2	Macherey und Nagel NucleoSpin Food Kit + Proteinase K	
ASU	20	L44.00.11	Ara h2	CTAB w ith/w ithout precipitation, Dneasy Mericon Food	
SFA	2	S3603	Arachis hypogae	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	3				
SFA	17				Sample 3 in traces
SFA-4p	8		Peanut-specific DNA	CTAB + additional purification via column / real-time PCR	LOD in ng/µl
div	4	internal method		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real-time PCR / 45 Cycles	
div	10				
div	16				validated in the laboratory for 0.1 %, as usually only used for adulterations
div	21			House-internal method	

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 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	4	09.10.20	positive	negative	positive	negative	1	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	13	20.10.20	positive	negative	positive	negative	5	Food item, total	ASU = ASU §64 Methode/method
SFA	2	06.10.20	positive	negative	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3		positive	negative	positive	negative	0,4	Lupine	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	20.10.20	positive	negative	positive	negative	< 0,4	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11	10.11.20	positive	negative	positive	negative	1	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14		positive	negative	positive	negative	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	02.11.20	positive	negative	positive	negative		Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	28.10.20	positive	negative	positive	negative	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	10		positive	negative	positive	negative		Allergen-DNA	house method
div	15	24.11.20	positive	negative	positive	negative	100	Allergen DNA	house method
div	16		positive	negative	positive	negative	8µg/kg		house method
div	21		positive	negative	positive	negative			house-internal 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	
ASU	4	§64 LFGB L08.00-58		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real-time PCR / 45 Cycles	
ASU	13	ASU L 08.00-58 (2011-06)	ITS-1	Macherey und Nagel NucleoSpin Food Kit + Proteinase K	
SFA	2	S3611	Lupinus	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	3				
SFA	8		Lupine-specific DNA	CTAB + additional purification via column / real-time PCR	
SFA	11	S3611	Not specified in kit	As per kit instructions	no
SFA	14				
SFA	17				
SFA	19	S3611	As Per Kit Instructions	As per Kit Instructions	
div	10				
div	15		L1PR10.1A (Ypro10.1a)	phenolchloroform exatrction, qiagen dneasy kit, end point PCR, 45 cycles, PAGE	
div	16				
div	21			House-internal method	

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	4	09.10.20	negative	positive	positive	positive	5	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	13	19.10.20	negative	positive	positive	positive	100	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	20	07.10.20	negative	positive	positive	positive	80	Food item, total	ASU = ASU §64 Methode/method
FP	18	29.10.20	negative	positive	positive	positive	1 ppm	Allergen DNA	FP = foodproof Detection Kit, BIOTECON Diagnostics
SFA	2	06.10.20	negative	positive	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3		negative	positive	positive	positive	0,4	Celery	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6		negative	positive	positive	positive			SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	02.11.20	negative	positive	positive	positive		Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	28.10.20	negative	positive	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	8	19.10.20	negative	positiv	positiv	positiv	< 0,4	Allergen-DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	10		negative	positive	positive	negative		Allergen-DNA	house method
div	16		negative	positive	positive	positive	8 µg/kg		in-house method
div	21		negative	positive	positive	positive			in-house method

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	4	§64 LFGB L08.00-56		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real-time PCR / 45 Cycles	
ASU	13	ASU L 08.00-56 (2020-08)	Mannitolde-hydrogenase	Macherey und Nagel NucleoSpin Food Kit + Proteinase K	
ASU	20	L08.00.56	Mannitoldehydrogenase	CTAB w ith/w ithout precipitation, Dneasy Mericon Food	
FP	18	PB-22/LM w yd. 1 z dn. 15.11.2016			
SFA	2	S3605	Apium graveolens	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	3				Detection limit in haploid genome copies
SFA	6				
SFA	17				
SFA	19	S3605	As Per Kit Instructions	As per Kit Instructions	
SFA-4p	8		Celery-specific DNA	CTAB + additional purification via column / real-time PCR	
div	10				
div	16				Limit of detection so far determined in the laboratory
div	21			house internal method	

Other details to the Methods

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	4	09.10.20	negative	negative	positive	positive	10	Allergen-DNA	ASU = ASU §64 Methode/method
ASU	13	19.10.20	negative	negative	positive	positive	10	Food item, total	ASU = ASU §64 Methode/method
SFA	2	06.10.20	negative	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	3		negative	negative	positive	positive	0,4	Sesame	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	20.10.20	negative	negative	positive	positive	< 0,4	Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	14		negative	negative	positive	positive	0,4	Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	17	02.11.20	negative	negative	positive	positive		Allergen DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	19	28.10.20	negative	negative	positive	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7		negative	negative	positive	positive		Allergen DNA	Hans-Ulrich Waiblinger et al., 2014.other: Journal fu¨r Verbraucherschutz und Lebensmittelsicherheit Ring trial validation of single and multiplex real-time PCR methods for the detection and quantification of the allergenic food ingredients sesame, almond, lupine and Brazil nut
div	10		negative	negative	positive	positive		Allergen-DNA	house method
div	16		negative	negative	positive	positive	8 µg/kg		house method
div	21		negative	negative	positive	positive			House-internal 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	
ASU	4	§64 LFGB L18.00-19		CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real-time PCR / 45 Cycles	
ASU	13	ASU L 18.00-19 (2014-08)	ITS-1	Macherey und Nagel NucleoSpin Food Kit + Proteinase K	
SFA	2	S3608	Sesamum indicum	Extraction using SureFood® Prep Advanced Protocol 1 (S1053)	
SFA	3				
SFA	8		sesame-specific DNA	CTAB + additional purification via column / real-time PCR	
SFA	14				
SFA	17				
SFA	19	S3608	As Per Kit Instructions	As Per Kit Instructions	
div	7		Sesam:e 2S Albumine-Gene, 66 bp (Brzezinski 2007)	1. DNA extraction w ith CTAB 2. DNA extraction according to Wizard Magnetic DNA Purification for Food/Promega /FF 3750, 3. Aria Mx Real time PCR System, Agilent Technologies, 45 cycles	
div	10				
div	16				
div	21			House-internal method	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALS3 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	29,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	78	31,2
2	5,02	83	33,1
3	4,98	79	31,7
4	5,01	84	33,5
6	5,04	65	25,8
8	4,97	88	35,4
9	5,04	80	31,7
10	4,96	74	29,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	78,9	Particles
Standard deviation	7,16	Particles
χ^2 (CHI-Quadrat)	4,55	
Probability	**71**	%
Recovery rate	108	%

Normal distribution		
Number of samples	8	
Mean	31,5	mg/kg
Standard deviation	2,86	mg/kg
rel. Standard deviaton	9,1	%
Horwitz standard deviation	9,5	%
HorRat-value	**0,95**	
Recovery rate	108	%

Microtracer Homogeneity Test
Microtracer Homogeneity Test
DLA ptALS3 Sample 2
DLA ptALS3 Sample 3

Weight whole sample	1,01	kg
Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Weight per particle	2,0	µg
Addition of tracer	24,8	mg/kg
Addition of tracer	23,4	mg/kg

Result of analysis
Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
[illegible]	[illegible]	[illegible]	[illegible]
1	5,02	60	23,9
1	5,02	61	24,3
2	5,03	64	25,4
2	5,03	54	21,5
5	4,99	50	20,0
5	5,00	63	25,2
6	5,01	56	22,4
6	5,00	68	27,2
7	5,00	63	25,2
7	5,02	54	21,5
8	4,96	58	23,4
8	5,00	61	24,4
9	5,01	56	22,4
9	5,00	59	23,6
10	5,03	61	24,3
10	4,98	68	27,3

Poisson distribution		
Number of samples	8	
Number of samples	8	
Degree of freedom	7	
Degree of freedom	7	
Mean	58,5	Particles
Mean	61,0	Particles
Standard deviation	4,43	Particles
Standard deviation	5,56	Particles
χ^2 (CHI-Quadrat)	2,34	
χ^2 (CHI-Quadrat)	3,55	
Probability	**94**	%
Probability	**83**	%
Recovery rate	94	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Number of samples	8	
Mean	23,4	mg/kg
Mean	24,4	mg/kg
Standard deviation	1,77	mg/kg
Standard deviation	2,22	mg/kg
rel. Standard deviaton	7,6	%
rel. Standard deviaton	9,1	%
Horwitz standard deviation	10,0	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,76**	
HorRat-value	**0,92**	
Recovery rate	94	%
Recovery rate	104	%

Microtracer Homogeneity Test
DLA ptALS3 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	21,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	64	25,4
2	5,02	59	23,5
3	5,01	60	24,0
4	5,01	59	23,6
6	4,98	59	23,7
8	4,98	60	24,1
9	4,98	63	25,3
10	5,02	60	23,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	60,5	Particles
Standard deviation	1,91	Particles
χ^2 (CHI-Quadrat)	0,42	
Probability	**100**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	24,2	mg/kg
Standard deviation	0,76	mg/kg
rel. Standard deviaton	3,2	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,32**	
Recovery rate	113	%

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 ptALS3 (2020)***
PT name	**Allergen-Screening III- 4 Samples qualitative: *Cereals containing Gluten (Wheat, Rye and Oat), 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 1 - 4:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Wheat, Rye, Oat, Peanut, Lupine, Celery (Leaves / Stem, Root and Seed) and 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 November 27th 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
		USA
		SPANIEN
		ITALIEN
		Deutschland
		Deutschland
		SPANIEN
		Deutschland
		Deutschland
		Deutschland
		FRANKREICH
		ITALIEN
		GROSSBRITANNIEN
		POLEN
		ÖSTERREICH
		Deutschland
		SERBIEN
		POLEN
		ITALIEN
		Deutschland
		GROSSBRITANNIEN
		FRANKREICH
		Deutschland
		GROSSBRITANNIEN
		USA

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

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

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