Evaluation Report

proficiency test

DLA ptALS3 (2021)

Allergen-Screening III:

Cereals containing Gluten, Peanut, Lupine, Celery and Sesame

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General Information on the proficiency test (PT)

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EP-Nummer *PT-Number*	DLA ptALS3 (2021)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (14. Februar 2022) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 14. Februar 2022
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficency test the following services were subcontracted: protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

The participation in proficiency testing schemes (PT) 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 0,9-10% of the allergenic ingredients concerned.
The respective raw materials for the allergens used were common in commerce cereal flakes, flours, mush, dried plant parts and seeds as well as fresh celery roots, of 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 the 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 (0,9% - 10% each)	0,04 - 0,5 %

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Barley: barley grain milled (Protein 7,7%)	negative	negative	positive (39)	negative
Rye: Rye grain milled (Protein 9,1%)	positive (46)	negative	negative	negative
Wheat: Wheat flour mixture (Protein 11%)	negative	negative	negative	positive (55)
Peanut: commercial peanut butter (Protein 30%)	negative	positive (83)	negative	positive (21)
Lupine: Sweet lupine flour (Protein 37%)	negative	positive (45)	positive (81)	negative
Celery: Leafs, dried (Protein 14%)	positive (83)	negative	negative	negative
Celery: Roots, dried (Protein 8,2%)	negative	negative	positive (85)	negative
Celery: Seeds, dried (Protein 20%)	negative	negative	negative	positive (75)
Sesame: Seeds white, dried (Protein 22%)	negative	positive (53)	negative	negative
Sesame: Seeds black, dried (Protein 23%)	positive (50)	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	positive	negative	positive	positive
AgraStrip® Peanut	negative	positive	negative	positive
AgraStrip® Lupin	negative	positive	positive	negative
AgraStrip® Sesame	positive	positive	negative	negative

* 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 96%, 90%, 35% and 80%, 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 0,71, 0,78, 1,2 and 0,89, 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. 0,29-0,39 (19-21°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 43rd week of 2021. The testing method was optional. The tests should be finished at December 24th 2021 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 ***Wheat, Rye, 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***
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 19 participants submitted at least one result.

3. Evaluation

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

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

3.1 Agreement with consensus values from participants

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

3.2 Agreement with spiking of samples

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

4. Results

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

The qualitative evaluation is carried out for each parameter for ELISA and PCR methods separately. Results of lateral flow methods are valuated together with ELISA methods, because they are usually based on antibody detection. Next generation sequencing methods are evaluated as DNA-based techniques together with the PCR methods.
No LC/MS method 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 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	AS	Lateral Flow
19	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	IL	
2	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
5	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	Samples 1, 3 and 4 rated "positive" by DLA, sample 2 as "negative".
7	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
17	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	RS	
18	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	RS	
6	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SP-R5	

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

Methods:

AS = AgraStrip (Lateral Flow), RomerLabs

IL = Immunolab

RS = Ridascreen®, R-Biopharm

SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins

Comment:

The consensus values of the results are in qualitative agreement with the spiking of samples 1 (46 mg/kg rye protein), 3 (39 mg/kg barley protein) and 4 (55 mg/kg wheat protein).

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 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
7	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	ASU	
11	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	GI	
4	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
13	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = not indicated / other method

Comment:

The consensus values of the results are in qualitative agreement with the spiking of samples 1 (46 mg/kg rye protein), 3 (39 mg/kg barley protein) and 4 (55 mg/kg wheat protein).

4.1.2.2 PCR-Results: Rye

Qualitative valuation of results

Evaluation number	Sample 1 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	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	negative	4/4 (100%)	4/4 (100%)	MS	
4	positive	negative	-	-	2/2 (100%	2/2 (100%	SFA-4p	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	6	0	0	1
Number negative	0	6	5	4
Percent positive	100	0	0	20
Percent negative	0	100	100	80
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

MS = Microsynth

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

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

div = not indicated / other method

Comment:

The consensus values of the results are in qualitative agreement with the spiking of sample 1 (46 mg/kg rye protein).

4.1.2.3 PCR-Results: Barley

Qualitative valuation of results

Evaluation number	Sample 1 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	MS	
4	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	SFA-4p	
3	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
13	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
14	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

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

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

div = not indicated / other method

Comment:

The consensus values of the results are in qualitative agreement with the spiking of sample 3 (39 mg/kg barley protein).

4.1.2.4 PCR-Results: Wheat

Qualitative valuation of results

Evaluation number	Sample 1 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	MS	
4	positive	negative	positive	positive	2/4 (50%)	2/4 (50%)	SFA-4p	
3	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
7	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
13	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
14	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

MS = Microsynth

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

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

div = not indicated / other method

Comments:

The consensus values of the results are in qualitative agreement with the spiking of sample 4 (55 mg/kg wheat protein).

One participant obtained positive results for samples 1 and 3 spiked with barley and rye by using method SFA-4p (Sure Food Allergen 4plex, R-Biopharm/Congen).

4.1.2.5 Further PCR-Results: Wheat and Rye

Qualitative valuation of results

Evaluation number	Sample 1 (rye)	Sample 2 (without)	Sample 3 (barley)	Sample 4 (wheat)	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%)	div	Duplex-PCR

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

Methods:

div = not indicated / other method

Comment:

The results are in qualitative agreement with the spiking of sample 1 (46 mg/kg rye protein) and sample 4 (55 mg/kg wheat protein) as well as with the consensus values obtained in the respective specific individual determinations (see 4.1.2.2 and 4.1.2.4).

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		
5	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	BA	Lateral Flow
18	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IL	
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	
19	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

BA = Bioavid (Lateral Flow), R-Biopharm

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of the results are in qualitative agreement with the spiking of samples 2 (38 mg/kg peanut) and 4 (21 mg/kg peanut).

One participant no positive result for any of the samples by Lateral Flow (Bioavid, R-Biopharm).

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		
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MS	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
10	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	-	-	-	1/1 (100%)	1/1 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	9	0	9
Number negative	10	0	9	0
Percent positive	0	100	0	100
Percent negative	100	0	100	0
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = not indicated / other method

Comment:

The consensus values of the results are in qualitative agreement with the spiking of samples 2 (38 mg/kg peanut) and 4 (21 mg/kg peanut).

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		
15	positive	positive	positive	positive	2/2 (100%)	2/4 (50%)	AQ	
18	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	IL	
19	negative	positive	positive	negative	2/2 (100%)	4/4 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	3	3	1
Number negative	2	0	0	2
Percent positive	33	100	100	33
Percent negative	67	0	0	67
Consensus value	none	positive	positive	none
Spiking	negative	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comments:

The consensus values of the results for samples 2 (45 mg/kg lupine) and 3 (81 mg/kg lupine) are in qualitative agreement with the spiking of the samples.

One participant obtained positive results for the unspiked samples 1 and 4, so that no consensus value of ≥75% could be determined.

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		
11	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	
2	negative	negative	negative	negative	2/4 (50%)	2/4 (50%)	MS	no positive sample identified
4	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
8	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
10	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA	
7	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
13	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
14	negative	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

GI = GEN-IAL First Allergen

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = not indicated / other method

Comment:

The consensus values of the results are in qualitative agreement with the spiking of samples 2 (45 mg/kg lupine) and 3 (81 mg/kg lupine).

4.4 Proficiency Test Celery

4.4.1 ELISA-Results: Celery

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

4.4.2 PCR-Results: Celery

Qualitative valuation of results

Evaluation number	Sample 1 (leafs)	Sample 2 (without)	Sample 3 (roots)	Sample 4 (seeds)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
7	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	ASU	
12	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	FP	Samples 1,3 and 4 rated "positive" by DLA, sample 2 as "negative".
11	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	GI	
1	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	GR	
2	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	MS	
4	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
8	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
10	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA	
3	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
13	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
16	positive	positive	positive	-	2/3 (67%)	2/3 (67%)	div	

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

Methods:

ASU = ASU §64 Methode/method

FP = foodproof Detection Kit, BIOTECON Diagnostics

GI = GEN-IAL First Allergen

GR = SPECIALfinder Assay, real time PCR, Generon

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = not indicated / other method

Comments:

The consensus values of the results are in qualitative agreement with the spiking of samples 1 (83 mg/kg celery leafs), 3 (85 mg/kg celery root) and 4 (75 mg/kg celery seed).

Two participants obtained one negative result each for sample 3 spiked with celery root by an ASU method and method GI (GEN-IAL First Allergen).

4.5 Proficiency Test Sesame

4.5.1 ELISA-Results: Sesame, in general

Qualitative valuation of results

Evaluation number	Sample 1 (black)	Sample 2 (white)	Sample 3 (without)	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		
15	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	AQ	
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	BA	Lateral Flow
18	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IL	
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	
19	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SP	

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

Methods:

BA = Bioavid (Lateral Flow), R-Biopharm

AQ = AgraQuant, RomerLabs

IL = Immunolab

SP = SensiSpec ELISA Kit, Eurofins

Comment:

The consensus values of the results are in qualitative agreement with the spiking of samples 1 (50 mg/kg sesame, black) and 2 (53 mg/kg sesame, white).

4.5.2 PCR-Results: Sesame, in general

Qualitative valuation of results

Evaluation number	Sample 1 (black)	Sample 2 (white)	Sample 3 (without)	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		
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	GI	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MS	
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
4	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
8	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	negative	-	2/3 (67%)	2/3 (67%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	9	8	0	0
Number negative	0	1	9	8
Percent positive	100	89	0	0
Percent negative	0	11	100	100
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

GI = GEN-IAL First Allergen

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = not indicated / other method

Comments:

The consensus values of the results are in qualitative agreement with the spiking of samples 1 (50 mg/kg sesame, black) and 2 (53 mg/kg sesame, white).

One participant obtained a negative result for sample 2 using an unspecified method.

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
IL	19		positive	negative	positive	positive	4	Gluten	IL = Immunolab
RS	2	04.11.21	positive	negative	positive	positive		Food item, total	RS = Ridascreen®, R-Biopharm
RS	5		106	<5	80	82	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	7	01.11.21	positive	negative	positive	positive	1	Gluten	RS = Ridascreen®, R-Biopharm
RS	17	18.11.21	positive	negative	positive	positive	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	18	25.11.	negative	negative	positive	positive			RS = Ridascreen®, R-Biopharm
SP-R5	6	22.11.21	positive	negative	positive	positive	2	Gluten	SP-R5 = SensiSpec Ingezim Gluten R5, 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	
IL	19				
RS	2	r7001		Sample extraction according to the "solid food" test kit instructions	
RS	5			cocktail extraction	
RS	7	R7001	R5		
RS	17	R7001	R5 antibodies against gliadins	according to kit instructions	
RS	18				
SP-R5	6	R.30.GLU.K2			

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
IL	18	25.11.	negative	positive	negative	positive			IL = Immunolab
SP	6	11.11.21	negative	positive	negative	positive	1	Peanut	SP = SensiSpec ELISA Kit, Eurofins
SP	19		negative	positive	negative	positive	1	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	
IL	18				
SP	6	HU0030019			
SP	19				

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	15	22.12.21	positive	positive	positive	positive	0,15	whole lupine	AgraQuant, RomerLabs
IL	18	25.11.	negative	positive	positive	negative			IL = Immunolab
SP	19		negative	positive	positive	negative	2	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	15				
IL	18				
SP	19				

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	15	23.12.21	positive	positive	negative	negative	0,31	whole sesame	AgraQuant, RomerLabs
IL	18	25.11.	positive	positive	negative	negative			IL = Immunolab
SP	6	09.11.21	positive	positive	negative	negative	2	Sesame	SP = SensiSpec ELISA Kit, Eurofins
SP	19		positive	positive	negative	negative	2	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	15				
IL	18				
SP	6	HU0030022			
SP	19				

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
ASU	7	11.11.21	positive	negative	negative	positive	80	Food item, total	ASU = ASU §64 Methode/method
GI	11	01.12.21	positive	negative	positive	positive			GI = GEN-IAL First Allergen
SFA	4		positive	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		positive	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	13		positive	negative	positive	positive		Allergen-DNA	In-house method
div	14	14.12.21	positive	negative	positive	positive		Wheat, spelt, kamut, rye, barley DNA	own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ASU	7	L 08.00-66	Gluten system of w heat and rye	Maxw ell RSC Pure Food GMO and Authentication Kit	1-plex
GI	11			extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
SFA	4	S3606	Specific DNA sequence of cereals containing gluten		Sample 2: very w eak pos.
SFA	8				
div	13				
div	14		Wheat, spelt, kamut, rye, barley DNA		Gluten and other prolamins from w heat, spelt, kamut, rye, barley DNA

5.1.6 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
MS	2		positive	negative	negative	negative		Allergen-DNA	MS = Microsynth
SFA-4p	4		positive	negative	-	-	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	17.11.21	positive	negative	negative	negative	10	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	7	11.11.21	positive	negative	negative	negative	50	Food item, total	Dolch et al.; Food Control 101 (2019) 180-188
div	13		positive	negative	negative	negative		Allergen-DNA	In-house method
div	14	14.12.21	positive	negative	negative	positive		Allergen-DNA	own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MS	2			Wizard extraction, Real Time PCR	
SFA-4p	4	S7006			Sample 2: w eak pos.
SFA-ID	3	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	7	--	O-methyl transferase gene	Maxw ell RSC Pure Food GMO and Authentication Kit	3-plex
div	13				
div	14		secalin gene		rye DNA

5.1.7 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
MS	2		negative	negative	positive	negative		Allergen-DNA	MS = Microsynth
SFA-4p	4		negative	negative	positive	positive	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	17.11.21	negative	negative	positive	negative	10	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	7	11.11.21	negative	negative	positive	negative	50	Food item, total	Dolch et al.; Food Control 101 (2019) 180-188
div	13		negative	negative	positive	negative		Allergen-DNA	In-house method
div	14	10.12.21	negative	negative	positive	negative		Allergen-DNA	own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MS	2			Wizard extraction, Real Time PCR	
SFA-4p	4	S7006			
SFA-ID	3	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	7	--	γ-hordein gene	Maxw ell RSC Pure Food GMO and Authentication Kit	3-plex
div	13				
div	14		hordein gene		barley DNA

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
MS	2		negative	negative	negative	positive		Allergen-DNA	MS = Microsynth
SFA-4p	4		positive	negative	positive	positive	1	Food item, total	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	17.11.21	negative	negative	negative	positive	10	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	7	07.12.21	negative	negative	negative	positive	80	Food item, total	Dolch et al.; Food Control 101 (2019) 180-188
div	10		negative	negative	negative	positive		Allergen-DNA	
div	13		negative	negative	negative	positive		Allergen-DNA	In-house method
div	14	10.12.21	negative	negative	negative	positive		Allergen-DNA	own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MS	2			Wizard extraction, Real Time PCR	
SFA-4p	4	S7006			
SFA-ID	3	S7006	As Per Kit Instructions	As Per Kit Instructions	
div	7	--	Wheat Proline-rich protein	Maxw ell RSC Pure Food GMO and Authentication Kit	3-plex
div	10	Dolch et al. 2019 Imai et al. 2012Dolch et al. 2019 Imai et al. 2012	Proline-rich protein (prp)	real-time PCR	
div	13				
div	14		gamma gliadin gene		w heat sp. DNA

5.1.9 PCR: Rye and 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
div	14	10.12.21	positive	negative	negative	positive		Allergen-DNA	Own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
div	14		wheat and rye DNA		Duplex wheat and rye DNA

5.1.10 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	7	11.11.21	negative	positive	negative	positive	20	Food item, total	ASU = ASU §64 Methode/method
ASU	12	05.11.21	negative	positive	negative	positive	*	Food item, total	ASU = ASU §64 Methode/method
GI	11	30.11.21	negative	positive	negative	positive			GI = GEN-IAL First Allergen
MS	2		negative	positive	negative	positive		Allergen-DNA	MS = Microsynth
SFA	4		negative	positive	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		negative	positive	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	positive	negative	positive		Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	13		negative	positive	negative	positive		Allergen-DNA	In-house method
div	14	17.11.21	negative	positive	negative	positive		Allergen-DNA	own PCR-method
div	16	09.12.21	negative	-	-	-	0,4		

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	
ASU	7	L 00.00-169	Peanut: mitoch. ATPase 6 gene	Maxwell RSC Pure Food GMO and Authentication Kit	4-plex
ASU	12	L44.00.11	Ara h2	CTAB with/without precipitation, Dneasy Mericon Food	*validated in the lab for 0.1% as commonly used only for falsification
GI	11			Extraction with Simplex Easy Spin Food Kit/GEN-IAL	
MS	2			Wizard extraction, Real Time PCR	
SFA	4	S3603	Specific DNA sequence (Arachis hypogaea)		
SFA	8				
SFA	10				
div	13				
div	14		Peanut DNA		Peanut DNA
div	16				

5.1.11 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
GI	11	30.11.21	negative	positive	positive	negative			GI = GEN-IAL First Allergen
MS	2		negative	negative	negative	negative		Allergen-DNA	MS = Microsynth
SFA	4		negative	positive	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		negative	positive	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		negative	positive	positive	negative		Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7	10.11.21	negative	positive	positive	negative	10	Food item, total	Galan et al.; Food Chem. 2011, 127, 834–841
div	13		negative	positive	positive	negative		Allergen-DNA	In-house method
div	14	23.11.21	negative	positive	positive	negative		Allergen-DNA	own PCR-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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
GI	11			Extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
MS	2			Wizard extraction, Real Time PCR	
SFA	4	S3611	Specific DNA sequence (Lupinus spp.)		
SFA	8				
SFA	10				
div	7	--	mitoch. initiator tRNA-MET-Gene	Maxw ell RSC Pure Food GMO and Authentication Kit	1-plex
div	13				
div	14		Lupine DNA		Lupine DNA

5.1.12 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	7	11.11.+7.12.21	positive	negative	negative	positive	10	Food item, total	ASU = ASU §64 Methode/method
ASU	12	05.11.21	positive	negative	positive	positive	80	Food item, total	ASU = ASU §64 Methode/method
FP	5		32,04	< 0,1	0,35	2,08		Allergen-DNA	FP = foodproof Detection Kit, BIOTECON Diagnostics
GI	11	30.11.21	positive	negative	negative	positiv			GI = GEN-IAL First Allergen
GR	1	10.11.2021	positive	negative	positive	positive		Food item, total	GR = SPECIALfinder Assay, real time PCR, Generon
MS	2		positive	negative	positive	positive		Allergen-DNA	MS = Microsynth
SFA	4		positive	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		positive	negative	positive	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	10		positive	negative	positive	positive		Allergen-DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	3	17.11.21	positive	negative	positive	positive	1	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	13		positive	negative	positive	positive		Allergen-DNA	In-house method
div	14	18.11.21	positive	negative	positive	positive		Allergen-DNA	own PCR-method
div	16	09.12.21	positive	positive	positive	-	0,4		

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	
ASU	7	L 08.00-65	Mannitol dehydrogenase gene	Maxwell RSC Pure Food GMO and Authentication Kit	4-plex; Sample 3: Traces of celery on the LOD
ASU	12	L08.00.56	Mannitoldehydrogenase	CTAB with/without precipitation, Dneasy Mericon Food	
FP	5			Mericon Food Kit	
GI	11			Extraction with Simplex Easy Spin Food Kit/GEN-IAL	
GR	1	PVA15M-50	specific DNA sequence of celery	Extraction Kit Genomic DNA from food Macherey-Nagel / real time PCR / 40 cycles	
MS	2			Wizard extraction, Real Time PCR	
SFA	4	S3605	Specific DNA sequence (Apium graveolens)		
SFA	8				
SFA	10				
SFA-ID	3	S3605	As Per Kit Instructions	As Per Kit Instructions	
div	13			Sample 3: Traces	
div	14		Celery DNA		Celery DNA
div	16				

5.1.13 PCR: Sesame, 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
GI	11	30.11.21	positive	positive	negative	negative			GI = GEN-IAL First Allergen
MS	2		positive	positive	negative	negative		Allergen-DNA	MS = Microsynth
SFA	1	10.11.2021	positive	positive	negative	negative		Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	4		positive	positive	negative	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8		positive	positive	negative	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7	11.11.21	positive	positive	negative	negative	20	Food item, total	I. Laube; Sesamum indicum
div	13		positive	positive	negative	negative		Allergen-DNA	In-house method
div	14	23.11.21	positive	positive	negative	negative		Allergen-DNA	own PCR-method
div	16	09.12.21	positive	negative	negative	-	0,4		

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	
GI	11			Extraction w ith Simplex Easy Spin Food Kit/GEN-IAL	
MS	2			Wizard extraction, Real Time PCR	
SFA	1	S3608	Specific DNA sequence of sesame	Extraction Kit Genomic DNA from food by Macherey-Nagel / real time PCR / 45 cycles	
SFA	4	S3608	Specific DNA sequence (Sesamum indicum)		
SFA	8				
div	7	--	omega-6 fatty acid desaturase gene; 247 bps	Maxw ell RSC Pure Food GMO and Authentication Kit	1-plex
div	13				
div	14		sesame DNA		sesame DNA
div	16				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALS3 (2021) Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	31,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	50	19,8
2	5,01	56	22,4
3	5,00	48	19,2
4	5,07	60	23,7
5	5,06	56	22,1
6	5,05	56	22,2
7	5,07	54	21,3
8	5,01	50	20,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	53,7	Particles
Standard deviation	3,87	Particles
χ^2 (CHI-Quadrat)	1,95	
Probability	**96**	%
Recovery rate	69	%

Normal distribution		
Number of samples	8	
Mean	21,3	mg/kg
Standard deviation	1,54	mg/kg
rel. Standard deviaton	7,2	%
Horwitz standard deviation	10,1	%
HorRat-value	**0,71**	
Recovery rate	69	%

Microtracer Homogeneity Test
DLA ptALS3 (2021) Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	27,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,08	72	28,3
2	5,00	74	29,6
3	5,00	64	25,6
4	5,01	67	26,7
5	5,09	83	32,6
6	5,08	69	27,2
7	5,05	70	27,7
8	5,09	73	28,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	71,5	Particles
Standard deviation	5,38	Particles
χ^2 (CHI-Quadrat)	2,83	
Probability	**90**	%
Recovery rate	101	%

Normal distribution		
Number of samples	8	
Mean	28,3	mg/kg
Standard deviation	2,13	mg/kg
rel. Standard deviaton	7,5	%
Horwitz standard deviation	9,7	%
HorRat-value	**0,78**	
Recovery rate	101	%

Microtracer Homogeneity Test
DLA ptALS3 (2021) Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	36,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	100	39,5
2	5,04	96	38,1
3	5,01	88	35,1
4	5,01	109	43,5
5	5,11	84	32,9
6	5,04	108	42,9
7	5,02	104	41,4
8	5,03	85	33,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	96,8	Particles
Standard deviation	10,39	Particles
χ^2 (CHI-Quadrat)	7,81	
Probability	**35**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	38,4	mg/kg
Standard deviation	4,12	mg/kg
rel. Standard deviaton	10,7	%
Horwitz standard deviation	9,2	%
HorRat-value	**1,2**	
Recovery rate	105	%

Microtracer Homogeneity Test
DLA ptALS3 (2021) Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,09	78	30,6
2	5,04	74	29,4
3	5,06	79	31,2
4	5,01	70	27,9
5	5,09	68	26,7
6	5,03	84	33,4
7	4,99	64	25,7
8	5,05	76	30,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	74,1	Particles
Standard deviation	6,38	Particles
χ^2 (CHI-Quadrat)	3,84	
Probability	**80**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	29,4	mg/kg
Standard deviation	2,53	mg/kg
rel. Standard deviaton	8,6	%
Horwitz standard deviation	9,6	%
HorRat-value	**0,89**	
Recovery rate	105	%

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 (2021)***
PT name	***Allergen-Screening III - 4 Samples qualitative: Cereals containing Gluten (Wheat, Rye, 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 1 - 4:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Gluten / Wheat, Rye, Barley, 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), PCR or LC/MS can be applied for qualitative determinations.*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*One result each should be determined for Samples 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest December 24th 2021***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		GREECE
		SWITZERLAND
		Germany
		SPAIN
		Germany
		Germany
		FRANCE
		BRAZIL
		Germany
		GREAT BRITAIN
		GREECE
		Germany
		AUSTRIA
		SWITZERLAND
		Germany
		Germany
		Germany
		Germany
		GREAT BRITAIN

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

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

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