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Evaluation Report
proficiency test

DLA 13/2017

Allergen-Screening III:

Cereals containing Gluten, Peanut, Lupine, Celery and Sesame

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

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

Coordinator of this PT:
Dr. Matthias Besler-Scharf

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

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler-Scharf Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
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EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
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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.

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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 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 basic matrix of PT samples 1-4 and of the allergen-premixes is given in table 1. The premixes were used for spiking of the PT-samples 1 to 4 (see Tab. 2).
After homogenisation the samples were portioned to approximately 20 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

Ingredients	**Samples 1 - 4**
Potato powder (Ingredients: Potatoes, E471, E304, E223, E100)	74 - 76 %
Maltodextrin	24 - 26 %
Allergen-Premixes Ingredients: - Maltodextrin (88% - 93%) - Sodium sulfate (0,0% - 5,5%) - Silicon dioxide (2,0% - 4,1%) - Allergens (5,0% - 10% each)	0,10 - 0,50 %

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

Ingredients *	Sample 1	Sample 2	Sample 3	Sample 4
Barley: Barley seeds, ground(Protein 7,3%)	negative	positive (25 - 75)	negative	negative
Rye: Rye flour Type 1150 (Protein 9,1%)	negative	negative	positive (25 - 75)	negative
Wheat: Wheat flour Type 550 (Protein 10,5%)	positive (25 - 75)	negative	negative	negative
Peanut: commercial peanut butter (Protein 30%)	negative	positive (25 - 75)	negative	positive (25 - 75)
Lupine: Sweet lupine flour, (Protein 37%)	positive (25 - 75)	negative	positive (50 - 150)	negative
Celery: Leafs, dried (Protein 14%)	positive (25 - 75)	negative	negative	negative
Celery: Roots, dried (Protein 8,2%)	negative	negative	positive (75 - 225)	negative
Celery: Seeds, dried (Protein 20%)	negative	negative	negative	positive (50 - 150)
Sesame: Seeds black, dried (Protein 22%)	negative	negative	positive (25 - 75)	negative
Sesame: Seeds white, dried (Protein 23%)	negative	positive (25 - 75)	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 G12	positive	positive	positive	negative
AgraStrip® Peanut	negative	positive	negative	positive
AgraStrip® Lupin	positive	negative	positive	negative
AgraStrip® Sesame	negative	positive	positive	positive

* Nachweisgrenze (NWG) jeweils 1-10 mg/kg / Limit of detection (LOD) 1-10 mg/kg each

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1-4 showed probabilities of 95%, 84%, 77% and 92%, 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) [16, 17]. This gave HorRat values of 0,52, 0,68, 0,90 and 0,69, respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the samples (spoilage) and the content of EP-parameters (allergens) in a comparable matrix and water activity (a_w value <0.5). The stability of sample material is therefore given during the investigation period under consideration of given 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 2017. The testing method was optional. The tests should be finished at September 8th 2017 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 **Cereals containing Gluten** (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***

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

2.3 Submission of results

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

All 13 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 unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

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

4. Results

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

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

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Gluten Containing Cereals

4.1.1 ELISA-Results: Gluten

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (barley)	Sample 3 (rye)	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		
1	positivee	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
3	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
4	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
7	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
8	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
9	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
10	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
11	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
12	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
2	positive	positive	positive	-	3/3 (100%)	3/3 (100%)	RS-F	Sample 4: <LOQ

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

Methods:

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. For sample 4 one result was given as below the limit of quantification (and above the limit of detection).

4.1.2 PCR-Results: Gluten Containing Cereals

4.1.2.1 PCR-Results: Gluten, in general

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (barley)	Sample 3 (rye)	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		
13	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	2	2	2	0
Number negative	0	0	0	2
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-ID = Sure Food Allergen ID, R-Biopharm / Congen

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: Wheat

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (barley)	Sample 3 (rye)	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		
3	positive	negative	positive	negative	3/3 (100%)	3/4 (75%)	div	
4	positive	negative	positive	negative	3/3 (100%)	3/4 (75%)	div	
13	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	div	

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

Methods:

div = not indicated / other method

Comments:

The consensus value of the results for sample 1 is in qualitative agreement with the wheat spiking. For sample 3, spiked with rye, 2 positive results were obtained, so no consensus value can be given for this sample. For valuation of results, it is important to consider whether the methods used are specified as specific for wheat alone or both wheat and rye. Traces of wheat in sample 3 can not be excluded also.

4.1.2.3 PCR-Results: Barley

Qualitative valuation of results

Evaluation number	Sample 1 (wheat)	Sample 2 (barley)	Sample 3 (rye)	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	negative	positive	negative	negative	2/2 (100%)	4/4 (100%)	div	
13	positive	positive	positive	negative	2/2 (100%)	2/4 (50%)	div	

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

Methods:

div = not indicated / other method

Comments:

The results of the two participants for sample 2 and the unspiked sample 4 are in qualitative agreement with the spiking of barley. For sample 1 (addition of wheat) and sample 3 (addition of rye), a positive and a negative result were obtained. For valuation of results, it is important to consider whether the methods used are specified as specific for wheat alone or both wheat and rye.

4.1.2.4 PCR-Results: Rye

Comments:

No PCR-analyses were performed by the participants.

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		
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	BC	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	Mi	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
2	negative	positive	-	positive	3/3 (100%)	3/3 (100%)	RS-F	Sample 3: < LOQ
6	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RS-F	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	7	0	7
Number negative	7	0	6	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:

BC = BioCheck ELISA

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

The consensus values of the results are in qualitative agreement with the spiking of samples. For sample 3 one result was given as below the limit of quantification (and above the limit of detection).

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		
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

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

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		
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ES	
2	positive	-	positive	negative	3/3 (100%)	3/3 (100%)	RS-F	Sample 2: < LOQ
3	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	RS-F	

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

Methods:

ES = ELISA-Systems

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. For sample 2 one result was given as below the limit of quantification (and above the limit of detection).

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	
12	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
13	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
7	positive	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	5	0	5	0
Number negative	0	5	0	5
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-ID = Sure Food Allergen ID, R-Biopharm / Congen

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

Comments:
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 (leaves)	Sample 2 (without)	Sample 3 (root)	Sample 4 (seed)	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	positive	4/4 (100%)	4/4 (100%)	ASU	
12	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
13	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
3	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	negative	-	-	2/2 (100%)	2/2 (100%)	div	Sample 3 + 4: < LOQ
7	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:
ASU = ASU §64 Methode/method
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 results are in qualitative agreement with the spiking of samples. For samples 3 and 4 one result each was given as below the limit of quantification (and above the limit of detection).

4.5 Proficiency Test Sesame

4.5.1 ELISA-Results: Sesame

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (white)	Sample 3 (black)	Sample 4 (white)	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	BC	
5	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	BK	
1	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ES	
8	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ES	
11	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ES	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
3	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	RS-F	
12	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	VT	

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

Methods:

BC = BioCheck ELISA

BK = BioKits, Neogen

ES = ELISA-Systems

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples. None of the participants differentiated between black and white sesame.

4.5.2 PCR-Results: Sesame

Qualitative valuation of results

Evaluation number	Sample 1 (without)	Sample 2 (white)	Sample 3 (black)	Sample 4 (white)	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	
12	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
13	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
2	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
7	negative	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

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

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

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
RS	1	positive	positive	positive	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	3	positive	positive	positive	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	4	positive	positive	positive	negative			RS = Ridascreen®, R-Biopharm
RS	7	positive	positive	positive	negative	3	Gluten	RS = Ridascreen®, R-Biopharm
RS	8	positive	positive	positive	negative	1,0	Gluten	RIDASCREEN Gliadin, R-Biopharm
RS	9	positive	positive	positive	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	10	positive	positive	positive	negative	0,5	Gliadin	RS = Ridascreen®, R-Biopharm
RS	10	52,82	51,42	>80	<5,0	1	Gluten	RS = Ridascreen®, R-Biopharm
RS	11	positive	positive	positive	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	12	positive	positive	positive	negative	5	Gluten	RS = Ridascreen®, R-Biopharm
RS-F	2	63,06	62,41	108,73	<LOQ	1	Protein	Ridascreen Fast

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. Extraction Solution / Time / Temperature	
RS	1	R7001	R5		
RS	3	R7001	AS Per Kit Instructions	AS Per Kit Instructions	
RS	4	R7001	Prolamine of wheat, rye, barley		
RS	7				
RS	8	R7001		Extraction: Cocktail solution (Mendez method) and incubation at 50C for 40 minutes; 80% ethanol addition and shaking for 1 hour; Centrifugation; samples are diluted with buffer. Determination: 4 parameter cure	
RS	9				
RS	10	R7001	AS Per Kit Instructions	Processing of the samples exactly according to kit instructions	qualitative result
RS	10	R7001	AS Per Kit Instructions	Processing of the samples exactly according to kit instructions	quanitative result
RS	11				
RS	12	R7001	R5 (Mendez), regonizes Prolamine (Gliadine) of wheat, rye, barley	According to the manufacturer	Sample 1: > 50; Sample 2: >50; Sample 3: >50
RS-F	2	R7002	R5	AS Per Kit Instructions	LOQ: 10 ppm

5.1.2 ELISA: Peanut

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BC	9	negative	positive	negative	positive	1	Food item, total	BC = BioCheck ELISA
Mi	12	negative	positive	negative	positive	0,31 (1,24 Peanut)	Peanutportein	MI = Morinaga Institute ELISA
RS-F	1	negative	positive	negative	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	2	negative	282,53	<LOQ	146,29	0,13	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	6	negative	positive	negative	positive	0,13 mg/kg peanut	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	13	negative	positive	negative	positive	0,13	Peanutportein	RS-F= Ridascreen® Fast, R-Biopharm
VT	11	negative	positive	negative	positive	2,5	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
BC	9				
Mi	12	M2116	Peanutprotein	According to the manufacturer	Sample 2: 5,1; Sample 4: 3,2 Peanutprotein (x4 = Peanut)
RS-F	1	R6202			
RS-F	2	R6202	Ara h 1 & Ara h 2	AS Per Kit Instructions	LOQ: 2.5 ppm
RS-F	6	R6202	The antibodies specifacally detect peanut proteins, including the peanut allergen Ara h 1 and Ara h 2		
RS-F	13	R6202			QE to green peas, lentils and fenugreek
VT	11				

5.1.3 ELISA: Lupine

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ES	12	positive	negative	positive	negative	0,5 (3,1 lupine flour)	lupine flour protein	ES = ELISA-Systems
RS-F	2	>27	<LOQ	69,38	negative	0,7	protein	Ridascreen Fast
RS-F	3	positive	negative	positive	negative	1	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm

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. Extraction Solution / Time / Temperature	
ES	12	ESLFP-48	lupine flour protein	AS Per Kit Instructions	Sample 1: >4; Sample 3: >4 Lupine flour protein (x6,2 = lupine flour)
RS-F	2	R6102	lupine protein	AS Per Kit Instructions	LOQ: 1.0 ppm quantitative results refer to lupine protein
RS-F	3	R6102	AS Per Kit Instructions	AS Per Kit Instructions	

5.1.4 ELISA: Sesame

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BC	9	negative	positive	positive	positive	2	Food item, total	BC = BioCheck ELISA
BK	5	negative	positive	positive	positive	6,3	Food item, total	BK = BioKits, Neogen
ES	1	negative	positive	positive	positive	0,5	Food item, total	ES = ELISA-Systems
ES	8	negative	positive	positive	positive	0,25	Sesame seed protein	Sesame Seed Protein Residues, ELISA Systems
ES	11	negative	positive	positive	positive	0,5	Food item, total	ES = ELISA-Systems
RS-F	2	negative	61,45	48,95	115,54	0,14	Food item, total	Ridascreen Fast
RS-F	3	negative	positive	positive	positive	2,5	Food item, total	RS-F= Ridascreen® Fast, R-Biopharm
VT	12	negative	positive	positive	positive	6	Food item, total	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction Solution / Time / Temperature	
BC	9				
BK	5	902070X	The polyclonal antibody specifically detects sesame proteins	Samples extracted in Biokits extraction buffer, room temp/shaking (150rpm)/15 minutes. Centrifuge at 2500rpm/10 mins.	
ES	1				
ES	8	ESSESRD-48	2S-albumin sesame seed protein	Extraction: Room temperature PBS extraction buffer / 15 min @ 60C in shaking w aterbath / centrifugation Determination: 4 parameter curve	
ES	11				
RS-F	2	R7202	sesame protein	As Per Kit Instructions	
RS-F	3	R7202	As Per Kit Instructions	As Per Kit Instructions	
VT	12	902070X	sesame protein	As Per Kit Instructions	Sample 2: >80; Sample 3: >80; Sesame 4: >80

5.1.5 PCR: Gluten Cereals

5.1.5.1 PCR: Gluten, in general

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
SFA-ID	13	positive	positive	positive	negative	0,4	Gluten	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	positive	positive	positive	negative	5	Protein	in house, Zeltner et al. 2009

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. Extraction Solution / Time / Temperature	
SFA-ID	13	S3106			
div	2	-	HMW Glutenin B1-1	ReliaPrep, Promega	

5.1.5.2 PCR: Wheat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	3	positive	negative	positive	negative	10	Food item, total	
div	4	positive	negative	positive	negative			in-house method
div	13	positive	negative	negative	negative	5 DNA-copies	Allergen-DNA	in-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.	Antibody	e.g. Extraction Solution / Time / Temperature	
div	3	PGE29A	As Per Kit Instructions	As Per Kit Instructions	Generon Wheat
div	4				
div	13				

5.1.5.3 PCR: Barley

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	2	negative	positive	negative	negative	100	Food item, total	
div	13	positive	positive	positive	negative	5 DNA copies	Allergen-DNA	in-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.	Antibody	e.g. Extraction Solution / Time / Temperature	
div	2	-	Hor3		
div	13				

5.1.5.4 PCR: Rye

None of the participants used the PCR method for determination of rye.

5.1.6 PCR: Peanut

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	negative	positive	negative	positive			ASU = ASU §64 Methode/method
SFA-ID	13	negative	positive	negative	positive	1	Peanut	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	negative	positive	negative	positive	1	Food item	in house, Köppel et al. 2012
div	7	negative	positive	negative	positive	0,008	Allergen DNA	
div	12	negative	positive	negative	positive	40	Allergen-DNA	

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. Extraction Solution / Time / Temperature	
ASU	4				
SFA-ID	13	S3103			
div	2	-	Ara d 2	ReliaPrep, Promega	
div	7				
div	12	internal method		Proteinase K, CTAB, Promega Wizard DNA CleanUp, Real-time PCR	

5.1.7 PCR: Lupine

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	positive	negative	positive	negative			ASU = ASU §64 Methode/method
ASU	12	positive	negative	positive	negative	0,4	Allergen-DNA	ASU = ASU §64 Methode/method
SFA-ID	13	positive	negative	positive	negative	0,4	Lupine	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	positive	negative	positive	negative	1	food item	in house,§64
div	7	positive	negative	positive	negative	0,08	Allergen DNA	

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. Extraction Solution / Time / Temperature	
ASU	4				
ASU	12	L 08.00-58		Proteinase K, CTAB, Promega Wizard DNA CleanUp, Real-time PCR	
SFA-ID	13	S3111			
div	2	-	IST-1	ReliaPrep, Promega	
div	7				

5.1.8 PCR: Celery

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	positive	negative	positive	positive			ASU = ASU §64 Methode/method
ASU	12	positive	negative	positive	positive	4	Allergen-DNA	ASU = ASU §64 Methode/method
SFA-4p	13	positive	negative	positive	positive	0,4	Celery	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-ID	3	positive	negative	positive	positive	1	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	14	negative	<LOQ	<LOQ	1	food item	in house, Köppel et al. 2012
div	7	positive	negative	positive	positive	0,008	Allergen DNA	

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. Extraction Solution / Time / Temperature	
ASU	4				
ASU	12	L 08.00-56		Proteinase K, CTAB, Promega Wizard DNA CleanUp, Real-time PCR	
SFA-4p	13	S3401			
SFA-ID	3	S3105	As Per Kit Instructions	As Per Kit Instructions	
div	2	-	Mannitoldehy.	ReliaPrep, Promega	LOQ: 10 ppm
div	7				

5.1.9 PCR: Sesame

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	negative	positive	positive	positive			ASU = ASU §64 Methode/method
ASU	12	negative	positive	positive	positive	40	Allergen-DNA	ASU = ASU §64 Methode/method
SFA-ID	13	negative	positive	positive	positive	0,4	Sesame	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	2	negative	positive	positive	positive	1	food item	in house,§64
div	7	negative	positive	positive	positive	0,008	Allergen DNA	

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. Extraction Solution / Time / Temperature	
ASU	4				
ASU	12	L 18.00-19		Proteinase K, CTAB, Promega Wizard DNA CleanUp, Real-time PCR	
SFA-ID	13	S3108			
div	2	-	2S albumin gene	ReliaPrep, Promega	
div	7				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 13-2017 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	51,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,94	149	60,3
2	5,00	157	62,8
3	5,18	148	57,1
4	4,94	152	61,5
5	5,10	160	62,7
6	5,06	147	58,1
7	5,13	152	59,3
8	5,05	140	55,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	150,7	Particles
Standard deviation	6,75	Particles
χ^2 (CHI-Quadrat)	2,12	
Probability	**95**	%
Recovery rate	117	%

Normal distribution		
Number of samples	8	
Mean	59,7	mg/kg
Standard deviation	2,67	mg/kg
rel. Standard deviaton	4,48	%
Horwitz standard deviation	8,65	%
HorRat-value	**0,52**	
Recovery rate	117	%

Microtracer Homogeneity Test
DLA 13-2017 Sample 2

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	52,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	126	49,8
2	4,94	136	55,1
3	5,00	138	55,2
4	4,99	152	60,9
5	5,02	143	57,0
6	5,08	139	54,7
7	5,00	140	56,0
8	5,09	151	59,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	140,6	Particles
Standard deviation	8,37	Particles
χ^2 (CHI-Quadrat)	3,49	
Probability	**84**	%
Recovery rate	108	%

Normal distribution		
Number of samples	8	
Mean	56,0	mg/kg
Standard deviation	3,33	mg/kg
rel. Standard deviaton	5,95	%
Horwitz standard deviation	8,73	%
HorRat-value	**0,68**	
Recovery rate	108	%

Microtracer Homogeneity Test
DLA 13-2017 Sample 3

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	82	32,4
2	5,02	85	33,9
3	5,09	79	31,0
4	5,03	69	27,4
5	5,12	75	29,3
6	4,96	74	29,8
7	5,08	74	29,1
8	5,09	90	35,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	78,5	Particles
Standard deviation	6,73	Particles
χ^2 (CHI-Quadrat)	4,04	
Probability	**77**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	31,0	mg/kg
Standard deviation	2,66	mg/kg
rel. Standard deviaton	8,58	%
Horwitz standard deviation	9,54	%
HorRat-value	**0,90**	
Recovery rate	104	%

Microtracer Homogenitätstest
DLA 13-2017 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	33,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	86	34,3
2	5,06	91	36,0
3	4,98	93	37,3
4	5,00	82	32,8
5	5,09	96	37,7
6	5,08	101	39,8
7	4,97	87	35,0
8	5,00	85	34,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	90,1	Particles
Standard deviation	5,78	Particles
χ^2 (CHI-Quadrat)	2,59	
Probability	**92**	%
Recovery rate	109	%

Normal distribution		
Number of samples	8	
Mean	35,9	mg/kg
Standard deviation	2,30	mg/kg
rel. Standard deviaton	6,41	%
Horwitz standard deviation	9,34	%
HorRat-value	**0,69**	
Recovery rate	109	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 13-2017***
PT name	***Allergen-Screening III - 4 Samples qualitative: Cereals containing Gluten*** *(Wheat, Rye, Barley 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 A + B: room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative:* ***Cereals containing Gluten*** *(Wheat, Rye, Barley)****, Peanut, Lupine, Celery*** *(Leaves / Stem, Root and Seed),* ***Sesame*** *(white and black)* *Samples 1-4: appr. 25 - 250 mg/kg*
Methods of analysis	*The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*One result each should be determined for Samples 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection mg/kg)*
Number of digits	*possibly at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest December 08th 2017***
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, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. 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

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

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

7. Index of references

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