Evaluation Report

proficiency test

DLA ptASW1 (2020)

Allergen Swab Test I:

Gluten, Peanut, Sesame and Soya

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General Information on the proficiency test (PT)

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Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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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

Eight test surfaces were provided for the qualitative detection of allergens in the range of 10 – 100 µg per test surface.
To prepare the test surfaces coated with allergens premixes were used at levels of about 5-10% of the allergenic ingredients concerned.
The allergen premixes were suspended in aqueous surfactant-containing solutions and defined aliquots were each spread out in petri dishes made of polystyrene. The test areas were then dried at 40°C overnight. A total of 4 petri dishes with halved partial areas were used, so that a total of 8 test areas were obtained.

The composition of the allergen suspensions is given in table 1. These premixes were used to spike the PT test areas A - D (see Table 2). The areas A and B should be tested for gluten and peanut and the areas C and D should be tested for sesame and soya.
Two sealed petri dishes were welded in into one metallized PET film bag.

Table 1: Composition of DLA-Samples

Ingredients	**Samples A - D**
surfactant containing aqueous solution	100 mL
Allergen-Premixes Ingredients: - Maltodextrin (30% - 88%) - Sodium chloride (0,0% - 85%) - Sodium sulfate (0,0% - 7,7%) - Silicon dioxide (1,0% - 2,2%) - allergens (5,0% - 10% each)	0,3 - 1,0 g

Table 2: Added amounts of allergenic ingredients, positive in brackets in µg/test surface (approx. 30 cm^2) ranges given as food item ** (cereals as total protein)

Ingredients *	Surface A	Surface B	Surface C	Surface D
Wheat: wheat flour type 550 (Protein 10,5%)	positive (80 - 110)	negative	-	-
Peanut: commercial nut butter (Protein 30%)	negative	positive (55 - 75)	-	-
Sesame: Seeds white, dried (Protein 22%)	-	-	positive (55 - 75)	negative
Soya: soya flour, untoasted (Protein 37%)	-	-	negative	positive (70 - 90)

* 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 A-D (see Table 3).

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

Lateral Flow Device (LFD)*	Surface A	Surface B	Surface C	Surface D
AgraStrip® Gluten	positive	negative	-	-
AgraStrip® Peanut	negative	positive	-	-
AgraStrip® Sesame	-	-	positive	negative
AgraStrip® Soy	-	-	negative	positive

* Nachweisgrenze jeweils 1-5 µg/25 cm^2 / Limit of detection (LOD) 1-5 µg/25 cm^2 each

2.1.1 Homogeneity

The homogeneity of the samples was ensured by applying equal amounts of suspended sample solution to each test area. The test areas were examined qualitatively for the relevant allergens using the allergen swab test. Quantitative tests were not carried out.

2.1.2 Stability

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 dry and dried products.
The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.
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].

2.2 Sample shipment and information to the test

The portions of the test materials (sample A to D) were sent to every participating laboratory in the 14th week of 2020. The testing method was optional. The tests should be finished at July 12th 2020 the latest (extended).

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

There are 4 plates (each with 2 test surfaces) possibly containing the allergenic parameters Gluten, Peanut, Sesame and Soya. Two areas are to be tested per allergen (one of them spiked with the relevant allergen). The amounts are in the range of 10 – 100 µg/test area. The analysis methods are optional.
The evaluation of results is strictly qualitative (positive / negative).

*Important note: The test areas are labeled with the **parameter to be tested** on the **backside of the plates**. A test field is only to be tested for this parameter.*

Please note the attached information on the proficiency test.
(see documentation, section 5.2 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.

14 of 15 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 on a test surface made of polystyrene 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 (or lateral flow) 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 (or lateral flow) and PCR methods separately. Results of lateral flow methods were valuated together with ELISA methods, because they are usually based on antibody detection.

The surfaces A and B should be tested for gluten (wheat) and peanut, and the surfaces C and D should be tested for sesame and soya as indicated on the 4 halfed petri dishes.

The participant results and evaluation are tabulated as follows:

Evaluation number	Surface A	Surface B	Surface C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		

	Surface A	Surface B	Surface C	Surface D
Number positive				
Number negative				
Percent positive				
Percent negative				
Consensus value				
Spiking				

4.1 Proficiency Test Gluten containing Cereals

4.1.1 ELISA and Lateral Flow Results: Gluten

Qualitative valuation of results

Evaluation number	Surface A	Surface B	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
13a	positive	negative	2/2 (100%)	2/2 (100%)	BF	
13b	positive	negative	2/2 (100%)	2/2 (100%)	BF-LF	Lateral Flow
7a	positive	negative	2/2 (100%)	2/2 (100%)	IL	
14	positive	negative	2/2 (100%)	2/2 (100%)	IL	
1	positive	negative	2/2 (100%)	2/2 (100%)	RS	
2	positive	negative	2/2 (100%)	2/2 (100%)	RS	
3	positive	negative	2/2 (100%)	2/2 (100%)	RS	
7b	positive	negative	2/2 (100%)	2/2 (100%)	RS	
9	positive	negative	2/2 (100%)	2/2 (100%)	RS	
11	positive	negative	2/2 (100%)	2/2 (100%)	RS	
12	positive	negative	2/2 (100%)	2/2 (100%)	RS	
10	positive	negative	2/2 (100%)	2/2 (100%)	RV-3D	Lateral Flow
9	positive	negative	2/2 (100%)	2/2 (100%)	SP-R5	
5	positive	negative	2/2 (100%)	2/2 (100%)	VT-R5	
8	positive	negative	2/2 (100%)	2/2 (100%)	VT-R5	

	Surface A	Surface B
Number positive	15	0
Number negative	0	15
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

BF = MonoTrace ELISA, BioFront Technologies

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

IL = Immunolab

RS = Ridascreen®, R-Biopharm

RV-3D = Reveal 3D (Lateral Flow), Neogen

SP-R5 = SensiSpec Ingezim Gluten R5, Eurofins

VT-R5 = Veratox, Neogen

Comments:

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

4.1.2 PCR-Results: Wheat

Qualitative valuation of results

Evaluation number	Surface A	Surface B	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	positive	negative	2/2 (100%)	2/2 (100%)	4L	
4	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
6	positive	negative	2/2 (100%)	2/2 (100%)	SFA	

	Surface A	Surface B
Number positive	3	0
Number negative	0	3
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

4L = 4LAB Diagnostics

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comments:

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

4.2 Proficiency Test Peanut

4.2.1 ELISA and Lateral Flow Results: Peanut

Qualitative valuation of results

Evaluation number	Surface A	Surface B	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
13a	negative	positive	2/2 (100%)	2/2 (100%)	BF	
13b	negative	positive	2/2 (100%)	2/2 (100%)	BF-LF	Lateral Flow
5	negative	positive	2/2 (100%)	2/2 (100%)	IL	
9	negative	positive	2/2 (100%)	2/2 (100%)	MI	
2	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
11	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
12	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
14	negative	positive	2/2 (100%)	2/2 (100%)	SP	
8	negative	positive	2/2 (100%)	2/2 (100%)	VT	

	Surface A	Surface B
Number positive	0	9
Number negative	9	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

BF = MonoTrace ELISA, BioFront Technologies

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

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.2.2 PCR-Results: Peanut

Qualitative valuation of results

Evaluation number	Surface A	Surface B	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	positive	2/2 (100%)	2/2 (100%)	GI	
3	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
4	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
6	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
7	negative	positive	2/2 (100%)	2/2 (100%)	div	
9	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface A	Surface B
Number positive	0	6
Number negative	6	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

4.3 Proficiency Test Sesame

4.3.1 ELISA and Lateral Flow Results: Sesame

Qualitative valuation of results

Evaluation number	Surface C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
13a	positive	negative	2/2 (100%)	2/2 (100%)	BF	
13b	positive	negative	2/2 (100%)	2/2 (100%)	BF-LF	Lateral Flow
7	positive	negative	2/2 (100%)	2/2 (100%)	IL	
2	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
11	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
9	positive	negative	2/2 (100%)	2/2 (100%)	SP	
14	positive	negative	2/2 (100%)	2/2 (100%)	SP	
8	positive	negative	2/2 (100%)	2/2 (100%)	VT	

	Surface C	Surface D
Number positive	9	0
Number negative	0	9
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

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

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

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

4.3.2 PCR-Results: Sesame

Qualitative valuation of results

Evaluation number	Surface C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
9	positive	negative	2/2 (100%)	2/2 (100%)	ASU	
5	positive	negative	2/2 (100%)	2/2 (100%)	GI	
3	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
4	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
6	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
12	positive	negative	2/2 (100%)	2/2 (100%)	SFA	

	Surface C	Surface D
Number positive	6	0
Number negative	0	6
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Comments:

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

4.4 Proficiency Test Soya

4.4.1 ELISA and Lateral Flow Results: Soya

Qualitative valuation of results

Evaluation number	Surface C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	positive	2/2 (100%)	2/2 (100%)	AQ	
13a	negative	positive	2/2 (100%)	2/2 (100%)	BF	
13b	negative	positive	2/2 (100%)	2/2 (100%)	BF-LF	Lateral Flow
9	negative	positive	2/2 (100%)	2/2 (100%)	MI	
1	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
2	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
3	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
8	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
11	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
12	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
14	negative	positive	2/2 (100%)	2/2 (100%)	SP	

	Surface C	Surface D
Number positive	0	11
Number negative	11	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

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

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

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

4.4.2 PCR-Results: Soya

Qualitative valuation of results

Evaluation number	Surface C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
5	negative	positive	2/2 (100%)	2/2 (100%)	GI	
3	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
4	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
6	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
7	negative	positive	2/2 (100%)	2/2 (100%)	div	
9	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface C	Surface D
Number positive	0	6
Number negative	6	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

GI = GEN-IAL First Allergen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

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	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	13a		positive	negative	X	X	0,36	Gluten	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	13b		positive	negative	X	X	5	Gluten	BF = AllerTrace LFD, BioFront Technologies
IL	7a	13.05.20	positive	negative	X	X	0,4	Gluten	IL = Immunolab
IL	14	11.04.20	positive	negative	X	X	0.3	Gliadin	IL = Immunolab
RS	1		positive	negative	X	X	5	Gluten	RS = Ridascreen®, R-Biopharm
RS	2	08.04.	positive	negative	X	X	0,5 - 1,0	Gluten	RS = Ridascreen®, R-Biopharm
RS	3		positive	negative	X	X	1,0ppm	Gluten	Ridascreen Gliadin
RS	7b	13.05.20	positive	negative	x	x	0,5	Gluten	RS = Ridascreen®, R-Biopharm
RS	9a	28.05.20	positive	negative	X	X	0,125	Gluten	RS = Ridascreen®, R-Biopharm
RS	11	07.05.20	positive	negative	X	X	1	Gluten	AOAC 2012.01
RS	12	05.06.20	positive	negative	X	X	<0,125µg/ Fläche	Gluten	RS = Ridascreen®, R-Biopharm
RV	10		positive	negative	X	X		Gluten	RV = Reveal 3D (Lateral Flow), Neogen
SP	9b	29.05.20	positive	negative	-	-	0,078	Gluten	SP = SensiSpec, Eurofins Technologies
VT-R5	5	21.05.20	positive	negative	X	X	3	Gluten	VT-R5 = Veratox, Neogen
VT-R5	8	29.05.20	positive	negative	X	X	5 (**)	Gluten	VT-R5 = Veratox, Neogen

Continuation ELISA: Gluten

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	
BF	13a		Anti-gliadin monoclonal antibody	Swab collection performed and swab tip broken off into 1 ml swab solution. 4 ml of extraction buffer added and incubated for 1 hour at 60C.	
BF-LF	13b		Anti-gliadin monoclonal antibody	Swab collection performed and swab tip broken off into 2 ml of extraction buffer. Shake for 20 seconds, incubate for 1 minute at room temp.	
IL	7a	MEI10,01 / GLU-E02	ND	Short Application Protocol for Swab Test in Combination with the Immunolab Gliadin ELISA, GLU-E02 Version: 2013-07-19	
IL	14			Application of Eurofins Technologies SENSISwab kit	
RS	1				
RS	2	R7001	R5: Anti -Gliadin	Coctail solution/60% EtOH; loq:0,5 µg/swab	
RS	3	R7001	specific gliadin fraction of wheat	Cocktail solution, 50°C	
RS	7b	MEI10,01 / R7001	R5	Application note: Allergen swabbing using ELISA tests – RIDASCREEN®FAST Allergen (4. Swab method for gluten) 2017-06-28	
RS	9a	R7001	R5 antibody of Mendez detects Prolamins (Gliadins) of wheat, rye and barley	Area swabbed according to instructions of SENSISwab Swab Test Kit, Swab extracted and solution used for testing	
RS	11	AOAC 2012.01 / RIDASCREEN - GLIADIN Art.No. R7001			
RS	12	R7001	R5	Petri dish sampled with swabs soaked in ethanol, then rinsed petri dish with 2 ml of 80% ethanol. Continue according to the manufacturer's instructions!	Area A = 27,5µg/area
RV	10	901031P	Gluten	5 min	
SP	9b	SENSISpec Ingezim Test Combination 30.GLUK2:2015	R5 antibody of Mendez detects Prolamins (Gliadins) of wheat, rye and barley	Area swabbed according to instructions of SENSISwab Swab Test Kit, Swab extracted and solution used for testing	
VT-R5	5	285264		as per test-kit instuction	
VT-R5	8		R5		(**) detection limit value is corresponding to food samples

5.1.2 ELISA: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
BF	13a		negative	positive	X	X	0,24	Peanut	BF = MonoTrace ELISA, BioFront Technologies
BF-LF	13b		negative	positive	X	X	2	Peanut	BF = AllerTrace LFD, BioFront Technologies
IL	5	21.05.20	negative	positive	X	X	0,1	Peanut	IL = Immunolab
MI	9	29.05.20	negative	positive	X	X	0,01	Peanut protein	MI = Morinaga Institute ELISA
RS-F	2	15.04.	negative	positive	X	X	0,13	Peanut	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11	08.05.20	negative	positive	X	X	0,6	Peanut protein	AOAC RI PTM 030404
RS-F	12	28.05.20	negative	positive	X	X	<0,125µg/ Fläche	Peanut	RS-F= Ridascreen® Fast, R-Biopharm
SP	14	11.04.20	negative	positive	X	X	0.1	Peanut	SP = SensiSpec, Eurofins Technologies
VT	8	29.05.20	negative	positive	X	X	2.5 (**)	Peanut	VT = Veratox, Neogen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	
BF	13a		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 1 ml swab solution. 4 ml of extraction buffer added and incubated for 10 minutes at 60C.	
BF-LF	13b		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 2 ml of extraction buffer. Shake for 20 seconds, incubate for 1 minute at room temp.	
IL	5	ERN-159		as per test-kit instuction	
MI	9	MloBS Test-Combination M2120	detects peanut proteins	Area swabbed according to instructions of SENSISwab Swab Test Kit, Swab extracted and solution used for testing	
RS-F	2	R6202	Ara-h (i.a.)	Allergen extraction buffer (Kit)	
RS-F	11	AOAC RI PTM 030404 / RIDASCREEN - FAST Peanut Art. No. R6202			
RS-F	12	R6202		Petri dish sampled with swab soaked in extraction buffer, then rinsed Petri dish with 2 ml diluted extraction buffer. Continue according to the manufacturer's instructions!	Area B = 19,2µg/area
SP	14			Application of Eurofins Technologies SENSISwab kit	
VT	8				(**) detection limit value is corresponding to food samples

5.1.3 ELISA: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	5	26.06.20	X	X	positive	negative	0,2	Sesame	AQ = AgraQuant, RomerLabs
BF	13a		X	X	positive	negative	0,16	Sesame	Selection ELISA-Kits:
BF-LF	13b		X	X	positive	negative	2	Sesame	BF = AllerTrace LFD, BioFront Technologies
IL	7	13.05.20	X	X	positive	negative	2	Sesame protein	IL = Immunolab
RS-F	2	15.04.	X	X	positive	negative	0,14	Sesame	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11	11.05.20	X	X	positive	negative	1,2	Sesame protein	LFOD-TST-SOP-8867
SP	9	28.05.20	X	X	positive	negative	0,1	Sesame	SP = SensiSpec, Eurofins Technologies
SP	14	11.04.20	X	X	positive	negative	0.2	Sesame	SP = SensiSpec, Eurofins Technologies
VT	8	29.05.20	X	X	positive	negative	2.5 (**)	Sesame	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 / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	
AQ	5	SE1018-1907		as per test-kit instuction	
BF	13a		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 1 ml swab solution. 4 ml of extraction buffer added and incubated for 10 minutes at 60C.	
BF-LF	13b		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 2 ml of extraction buffer. Shake for 20 seconds, incubate for 1 minute at room temp.	
IL	7	MEI10,01 / SES-E01	ND	Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24	
RS-F	2	R7202	Sesame proteins (o.A.)	Allergen extraction buffer (Kit) plus skimmed milk powder	
RS-F	11	LFOD-TST-SOP-8867 / RIDASCREEN - FAST Sesame Art.No. R7202			
SP	9	HU0030022:2	detects sesame proteins	Area swabbed according to instructions of SENSISwab Swab Test Kit, Swab extracted and solution used for testing	
SP	14			Application of Eurofins Technologies SENSISwab kit	
VT	8				(**) detection limit value is corresponding to food samples

5.1.4 ELISA: Soya

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	5	13.05.20	X	X	negative	positive	0,016	STI – Soy Trypsin Inhibitor	AQ = AgraQuant, RomerLabs
BF	13a		X	X	negative	positive	0,16	Soya	Selection ELISA-Kits:
BF-LF	13b		X	X	negative	positive	5	Soya	BF = AllerTrace LFD, BioFront Technologies
Mi	9	29.05.20	X	X	negative	positive	0,0155		MI = Morinaga Institute ELISA
RS-F	1		X	X	negative	positive	4	Soya	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	2	15.04.	X	X	negative	positive	0,24	Soya	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	3		X	X	negative	positive	0,24ppm	Soyprotein	Auswahl ELISA-Kits:
RS-F	8	29.05.20	X	X	negative	positive	2.5 (**)	Soyprotein	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11	11.05.20	X	X	negative	positive	0,6	Soyprotein	LFOD-TST-SOP-8989
RS-F	12	28.05.20	X	X	negative	positive	<0,125µg/ Fläche	Soyprotein	RS-F= Ridascreen® Fast, R-Biopharm
SP	14	11.04.20	X	X	negative	positive	0.2	Soyprotein	SP = SensiSpec, Eurofins Technologies

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 / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	
AQ	5	1000000822		as per test-kit instuction	
BF	13a		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 1 ml swab solution. 4 ml of extraction buffer added and incubated for 10 minutes while boiling	
BF-LF	13b		monoclonal antibody-based assay	Swab collection performed and swab tip broken off into 2 ml of extraction buffer. Shake for 20 seconds, incubate for 1 minute while heating.	
Mi	9	MioBS Test-Combination M2117	detects the soyprotein beta-conglycinin	Area swabbed according to instructions of SENSISwab Swab Test Kit, Swab extracted and solution used for testing	
RS-F	1				
RS-F	2	R7102	heated soyproteins	Allergen extraction buffer (Kit)	
RS-F	3	R7102	specific heated soyproteins	Extractor3 +diluted AEP, 100°C,10min	
RS-F	8				(**) detection limit value is corresponding to food samples
RS-F	11	LFOD-TST-SOP-8989 / RIDASCREEN - FAST Soya Art. No. R7102			
RS-F	12	R7102		Petri dish sampled with swab soaked in extraction buffer, then rinsed Petri dish with 2 ml diluted extraction buffer. Continue according to the manufacturer's instructions!	Area D = 4,76µg/area
SP	14			Application of Eurofins Technologies SENSISwab kit	

5.1.5 PCR: Cereals containing gluten (wheat)

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
4L	5	13.05.20	positive	negative	X	X	< 5 DNA copies	Food item, DNA	4L = 4LAB Diagnostics
SFA	4		positive	negative	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	19.05.20	positive	negative	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
4L	5	GR 19294		real-time PCR method	
SFA	4	S3606			
SFA	6	S3606	gluten-containing cereals (wheat including spelt and khorasan-wheat, rye, barley and oat)	Sure Food Prep Advanced Protokoll 1	K01

5.1.6 PCR: Peanut

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
GI	5	12.05.20	negative	positive	X	X	< 5 DNA copies	Food item, DNA	GI = GEN-IAL First Allergen
SFA	3		negative	positive	X	X	0,4		SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	4		negative	positive	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	19.05.20	negative	positive	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7	14.05.20	negative	positive	X	X		LD PCR=15 pg DNA (<10mg / kg for reference material)	Real Time PCR Internal Method: MEB66
div	9	27.05.20	negative	positive	X	X		Food item, DNA	internal method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
GI	5	00.18.215		real-time PCR method	
SFA	3	S3603	Peanut	Extraction by SureFood PREP Advanced Kit	
SFA	4	S3603			
SFA	6	S3603	Arachis hypogae	Sure Food Prep Advanced Protocol 1	K01
div	7	Internal Method: MEB66	Ara h 2 gene	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (50 cycles of amplification)	
div	9			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime PCR / 45 Cycles	

5.1.7 PCR: Sesame

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	9	27.05.20	X	X	positive	negative		Food item, DNA	ASU = ASU §64 Methode/method
GI	5	12.05.20	X	X	positive	negative	< 5 DNA copies	Food item, DNA	GI = GEN-IAL First Allergen
SFA	3		X	X	positive	negative	0,4 ppm		SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	4		X	X	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	19.05.20	X	X	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	12	08.06.20	X	X	positive	negative		Sesame	SFA = Sure Food ALLERGEN, R-Biopharm / Congen

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	9	§64 LFGB L 18.00-19:2014-08		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime PCR / 45 Cycles	
GI	5	0028.65		real-time PCR method	
SFA	3	S3208	Sesame	Extraction by SureFood PREP Advanced Kit	
SFA	4	S3608			
SFA	6	S3608	Sesamum indicum	Sure Food Prep Advanced Protokoll 1	K01
SFA	12	S3608		Petri dish sampled with water-soaked swab, then removed "remnants" with second swab. Continue according to the manufacturer's instructions!	Area C = 3,36µg/area

5.1.8 PCR: Soya

Primary data

Meth. Abr.	Evaluation number	Date of analysis	Result Surface A	Result Surface B	Result Surface C	Result Surface D	Limit of detection	Limit of detection given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
GI	5	12.05.20	X	X	negative	positive	< 5 DNA copies	Food item, DNA	GI = GEN-IAL First Allergen
SFA	3		X	X	negative	positive	0,4		SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	4		X	X	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	6	19.05.20	X	X	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
div	7	14.05.20	X	X	negative	positive		LD PCR=150 pg DNA (0.1% relative to reference material)	Real Time PCR Internal Method: MEB61
div	9	27.05.20	X	X	negative	positive		Food item, DNA	internal method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
GI	5	0035.95		real-time PCR method	
SFA	3	3601	Soya	Extraction by SureFood PREP Advanced Kit	
SFA	4	S3601			
SFA	6	S3601	Glycine max	Sure Food Prep Advanced Protocol 1	K02
div	7	Internal Method: MEB61	Le1 Gen	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (45 cycles of amplification)	
div	9			CTAB / Proteinase K / Promega Wizard DNA CleanUp / Realtime PCR / 45 Cycles	

5.2 Information on the Proficiency Test (PT)

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

PT number	***DLA ptASW1 (2020)***
PT name	***Allergen Swab Test I: Gluten, Peanut, Sesame and Soya***
Sample matrix	*Plates A, B, C and D: 2 x 4 Test areas* *Plastic trays / ingredients: additives and allergenic foods*
Number of samples and sample amount	*4 Plates with 8 different test areas of approx. 30 cm².*
Storage	*Samples A + B:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative: **Gluten and Peanut** (Plates A and B)* *qualitative: **Sesame and Soya** (Plates C and D)* *Levels: approx. 10 - 100 µg / test area*
Methods of analysis	*Swab test with optional analytical method.*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis. The test areas are labeled with the allergen to be tested. It is recommended to sample the entire test area (half the area of a plate) according to the instructions of the swab test method applied.*
Result sheet	*For each parameter two different test areas should be examined and one result each should be determined per test area.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection in µg/cm²)*
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 June 12th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD / Alexandra Scharf M.Sc.*

** 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
		Germany
		USA
		SWITZERLAND
		SWITZERLAND
		Germany
		PORTUGAL
		Germany
		FRANCE
		Germany
		POLAND
		BELGIUM
		GREAT BRITAIN
		Germany
		VIETNAM
		Germany

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

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

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