Evaluation Report

proficiency test

DLA ptASW2 (2021)

Allergen Swab Test II:

Crustaceae, Egg, Mustard and Sesame

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

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EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 23 February 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.

Contents

1. Introduction

The participation in proficiency testing (PT) 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 respective allergenic ingredients.
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 crustaceae and egg and the areas C and D should be tested for mustard and sesame.
Two sealed petri dishes were welded in into one metallized PET film bag, each.

Table 1: Composition of DLA-Samples

Ingredients	**Samples A - D**
aqueous solution containing surfactants	100 mL
allergen premixes Ingredients: - Maltodextrin (80% - 94%) - Sodium sulfate (0,0% - 7,7%) - Silicon dioxide (1,0% - 2,2%) - allergens (5,0% - 10% each)	0,16 – 0,37 g

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

Ingredients *	Surface A	Surface B	Surface C	Surface D
Crustaceae: king prawns, DLA-mixture (protein 87,0%)	positive (70-110)	negative	-	-
Egg: whole egg powder, DLA-making (protein 47,3%)	negative	positive (60-100)	-	-
Mustard: yellow mustard, DLA-premix (protein 30,6%)	-	-	negative	positive (65-110)
Sesame: commercially available white sesame (protein X%)	-	-	positive (60-95)	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 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® Crustaceae	positive	negative	-	-
AgraStrip® Egg	negative	positive	-	-
AgraStrip® Mustard	-	-	negative	positive
AgraStrip® Sesame	-	-	positive	negative

* 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 shipmend and information to the test

The portions of the test materials (sample A to D) were sent to every participating laboratory in the 42nd week of 2021. The testing method was optional. The tests should be finished at 17 December 2021 the latest.

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

There are 4 plates (each with 2 test surfaces) possibly containing the allergenic parameters Crustaceae, Egg, Mustard and Sesame. 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 remarks 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 14 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 on a test surface made of polystyrene without further processing.

3.1 Agreement with consensus values from participants

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 **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 crustaceae and egg and the surfaces C and D should be tested for mustard and sesame 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 Crustaceae

4.1.1 ELISA- and Lateral Flow Results: Crustaceae

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		
14	positive	-	1/2 (50%)	1/2 (50%)	AQ	
7	positive	negative	2/2 (100%)	2/2 (100%)	AQ-P	
10	positive	negative	2/2 (100%)	2/2 (100%)	NL-E	
5	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
1	positive	negative	2/2 (100%)	2/2 (100%)	SP	
9	positive	negative	2/2 (100%)	2/2 (100%)	SP	
3	positive	negative	2/2 (100%)	2/2 (100%)	VT	
13	positive	negative	2/2 (100%)	2/2 (100%)	VT	
6	positive	negative	2/2 (100%)	2/2 (100%)	div	

	Surface A	Surface B
Number positive	9	0
Number negative	0	8
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

AQ-P = AgraQuant Plus, RomerLabs

NL-E = nutriLinia®E Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

div = not indicated / other method

Comment:

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

4.1.2 PCR-Results: Crustaceae

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		
4	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
8	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
11	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
5	positive	negative	2/2 (100%)	2/2 (100%)	SFA-ID	
10	positive	negative	2/2 (100%)	2/2 (100%)	SFA-Q	
12	positive	negative	2/2 (100%)	2/2 (100%)	div	

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

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

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

div = not indicated / other method

Comment:

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

4.2 Proficiency Test results Egg

4.2.1 ELISA- and Lateral Flow-Results: Egg

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		
7	positive	negative	0/2 (0%)	0/2 (0%)	3M	Lateral Flow
14	-	positive	1/2 (50%)	1/2 (50%)	AQ	
13	negative	positive	2/2 (100%)	2/2 (100%)	ES	
12	negative	positive	2/2 (100%)	2/2 (100%)	IL	
9	negative	positive	2/2 (100%)	2/2 (100%)	MI	
5a	negative	positive	2/2 (100%)	2/2 (100%)	RS	
4	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
5b	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
8	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
10	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
11	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
1	negative	positive	2/2 (100%)	2/2 (100%)	SP	
3	negative	positive	2/2 (100%)	2/2 (100%)	VT	
6	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface A	Surface B
Number positive	1	13
Number negative	12	1
Percent positive	8	93
Percent negative	92	7
Consensus value	negative	positive
Spiking	negative	positive

Methods:

3M = 3M Protein Rapid Kit
AQ = AgraQuant, RomerLabs
ES = ELISA-Systems
IL = Immunolab
MI = Morinaga Institute ELISA
RS = Ridascreen®, R-Biopharm
RS-F= Ridascreen® Fast, R-Biopharm
SP = SensiSpec ELISA Kit, Eurofins
VT = Veratox, Neogen
div = not indicated / other method

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

4.2.2 PCR-Results: Egg

Qualitative valuation of results

Comment: There are no PCR results available for the parameter Egg.

4.3 Proficiency Test Results Mustard

4.3.1 ELISA- and Lateral Flow-Results: Mustard

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		
14	negative	positive	2/2 (100%)	2/2 (100%)	AQ	
5	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
1	negative	positive	2/2 (100%)	2/2 (100%)	SP	
9	negative	positive	2/2 (100%)	2/2 (100%)	SP	
3	negative	positive	2/2 (100%)	2/2 (100%)	VT	
13	negative	positive	2/2 (100%)	2/2 (100%)	VT	
6	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface C	Surface D
Number positive	0	7
Number negative	7	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

AQ = AgraQuant, RomerLabs

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

div = not indicated / other method

Comment:

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

4.3.2 PCR-Results: Mustard

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	negative	positive	2/2 (100%)	2/2 (100%)	ASU	
4	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
5	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
11	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
2	negative	positive	2/2 (100%)	2/2 (100%)	SFA-4p	
8	negative	positive	2/2 (100%)	2/2 (100%)	SFA-4p	
10	negative	positive	2/2 (100%)	2/2 (100%)	div	
12	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface C	Surface D
Number positive	0	8
Number negative	8	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = not indicated / other method

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

4.4 Proficiency Test Results Sesame

4.4.1 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		
7	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
14	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
5	positive	negative	2/2 (100%)	2/2 (100%)	BC	
13	positive	negative	2/2 (100%)	2/2 (100%)	ES	
12	positive	negative	2/2 (100%)	2/2 (100%)	IL	
10	positive	negative	2/2 (100%)	2/2 (100%)	NL-E	
3	positive	negative	2/2 (100%)	2/2 (100%)	RS	
4	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
1	positive	negative	2/2 (100%)	2/2 (100%)	SP	
9	positive	negative	2/2 (100%)	2/2 (100%)	SP	
6	positive	negative	2/2 (100%)	2/2 (100%)	div	

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

Methods:

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

ES = ELISA-Systems

IL = Immunolab

NL-E = nutriLinia®E Allergen-ELISA

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

div = not indicated / other method

Comment:

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

4.4.2 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	
8	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
11	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
5	positive	negative	2/2 (100%)	2/2 (100%)	SFA-ID	
10	positive	negative	2/2 (100%)	2/2 (100%)	div	

	Surface 3	Surface 4
Number positive	5	0
Number negative	0	5
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food ALLERGEN, 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 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 and Lateral Flow: Crustaceae

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	µg/cm2		Test-Kit + Provider
AQ	14	13.12.21	pos	-	X	X	0,0002	Tropomyosin	AQ = AgraQuant, RomerLabs
AQ-P	7		positive	negative	X	X		Tropomyosin	AQ-P = AgraQuant Plus, RomerLabs
NL-E	10	08.12.21	positive	negative	X	X		Tropomyosin	NL-E = nutriLinia®E Allergen-ELISA
RS-F	5	17.11.21	positive	negative	X	X	20	Crustaceae	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		positive	negative	X	X		Tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	9	10.12.	positive	negative	X	X	0,001	Tropomyosin	SP = SensiSpec, Eurofins Technologies
VT	3	29.10.21	positive	negative	X	X	2,5	Crustaceae	VT = Veratox, Neogen
VT	13	11.02.21	positive	negative	X	X	0,023	Crustaceae protein	VT = Veratox, Neogen
div	6		positive	negative	X	X		Please select!	Selection ELISA-Kits:

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	
AQ	14				
AQ-P	7	10002076/1000003572			
NL-E	10	NC-6051		lt. Manual	
RS-F	5	R7312	As Per Kit Instructions	As Per Kit Instructions	
SP	1		Tropomyosin		
SP	9	HU0030006/HU0030030	detects crustacean tropomyosin	Swab the surface, extract the swab, carry out the test according to the manufacturer's instructions	>0,50mg/l
VT	3				
VT	13	CHEM-035 / 8520		Extraction: 30C pre-heated PBS extraction buffer & additive / vortex for 30 seconds. Centrifugation. Determination: 4 parameter curve	
div	6				

5.1.2 PCR: Crustaceae

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	µg/cm2		Test-Kit + Provider
SFA	4	8.11.	positive	negative	X	X		Food item, DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	29.11.21	positive	negative	X	X	0.4 mg/kg	Food item, DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		positive	negative	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	5	12.11.21	positive	negative	X	X	1	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	10	08.12.21	positive	negative	X	X		Food item, total	SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div	12		positive	negative	X	X	LD PCR=15 pg DNA (<10mg / kg for reference material)LD PCR=15 pg DNA (<10mg / kg for reference material)	Food item, DNA	Real Time PCR Internal Method: MEB241Real Time PCR Internal Method: MEB241

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No./ Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-Sequence/ DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	
SFA	4	S3612		processing w ith SureFood PREP Advanced, according to the manufacturer's instructions	
SFA	8	Art No.S3612		Realtime PCR	
SFA	11	S3612			
SFA-ID	5	S3612	As Per Kit Instructions	As Per Kit Instructions	
SFA-Q	10			Real Time PCR 35 Cyclen	
div	12	Internal Method: MEB241 Internal Method: MEB241	16S RNA	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (45 cycles of amplification)	

5.1.3 ELISA and Lateral Flow: Egg

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	µg/cm2		Test-Kit + Provider
3M°	7		positive	negative	X	X			3M Egg White Protein Rapid Kit
AQ	14	13.12.21	-	pos	X	X	0,00005	Egg proteins, total	AQ = AgraQuant, RomerLabs
ES	13	05.11.21	negative	positive	X	X	0,1	Whole egg powder	ES = ELISA-Systems
IL	12		negative	positive	X	X	0,4	Egg white proteins	IL = Immunolab
MI	9	15.11.	negative	positive	X	X	0,0155	whole egg protein	MI = Morinaga Institute ELISA
RS	5a	19.11.21	negative	positive	X	X	0,25	Whole egg powder	RS = Ridascreen®, R-Biopharm
RS-F	4	17.11.	negative	positive	X	X		Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	5b	11.11.21	negative	positive	X	X	0,5	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	8	29.11.21	negative	positive	X	X	0.1 ppm	Egg proteins, total	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	10	08.12.21	negative	positive	X	X		Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	11		negative	positive	X	X	0,025	Whole egg powder	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		negative	positive	X	X		Egg white proteins	SP = SensiSpec, Eurofins Technologies
VT	3	11.01.21	negative	positive	X	X	2,5	Whole egg powder	VT = Veratox, Neogen
div	6		negative	positive	X	X		Please select!	Selection ELISA-Kits:

** Lateral Flow*

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	
3M*	7	L25EGG/EA0002TA			
AQ	14				
ES	13	IMC-412 / ESEGG-48	Anti-ovomucoid	Extraction: Room temperature PBS extraction buffer, vortex for 30 seconds, extract in shaking w aterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	
IL	12	MEI10.01/EGG-E01	ND	Short Application Protocol for Sw ab Test in Combination w ith Immunolab Food Allergen ELISAs Version: 2013-04-24Short Application Protocol for Sw ab Test in Combination w ith Immunolab Food Allergen ELISAs Version: 2013-04-24	
MI	9	M2111	detects egg w hite protein ovalbumin	Sw ab the surface, extract the sw ab, carry out the test according to the manufacturer's instructions	2,5mg/l
RS	5a	R6411	As Per Kit Instructions	As Per Kit Instructions	
RS-F	4	R6402		according to manufacturer instructions	
RS-F	5b	R4602	As Per Kit Instructions	As Per Kit Instructions	
RS-F	8	Art.No.R6402			
RS-F	10	R6402		lt. Manual	
RS-F	11	R6402	The specific antibodies detected the egg w hite proteins ovalbumin and ovomucoid		
SP	1		Ovomucoid		
VT	3				
div	6				

** Lateral Flow*

5.1.4 ELISA and Lateral Flow: Mustard

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	µg/cm2		Test-Kit + Provider
AQ	14	13.12.21	X	X	neg	pos	0,05	Mustard	AQ = AgraQuant, RomerLabs
RS-F	5	17.11.21	X	X	negative	positive	0,5	Mustard	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		X	X	negative	positive		Mustard	SP = SensiSpec, Eurofins Technologies
SP	9	12.11.	X	X	negative	positive	0,1	Mustard	SP = SensiSpec, Eurofins Technologies
VT	3	11.02.21	X	X	negative	positive	2,5	Mustard	VT = Veratox, Neogen
VT	13	11.11.21	X	X	negative	positive	0,5	Mustard	VT = Veratox, Neogen
div	6		X	X	negative	positive		Please select!	Selection ELISA-Kits:

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	
AQ	14				
RS-F	5	R6152	As Per Kit Instructions	As Per Kit Instructions	
SP	1		Total Protein		
SP	9	HU0030016/HU0030040	detects mustard proteins	Swab the surface, extract the swab, carry out the test according to the manufacturer's instructions	0,90mg/l
VT	3				
VT	13	CHEM-255 / 8400		Extraction: 60C pre-heated TRIS extraction buffer & additive / vortex for 30 seconds. Centrifugation. Determination: 4 parameter curve	
div	6				

5.1.5 PCR: Mustard

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	µg/cm2		Test-Kit + Provider
ASU	9	12.11.	X	X	negative	positive	5	Please select!	Selection PCR-Methods
SFA	4	8.11.	X	X	negative	positive		Food item, DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	5	14.11.21	X	X	negative	positive	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		X	X	negative	positive	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	2	03.11.21	X	X	negative	positive		Please select!	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
SFA-4p	8	30.11.21	X	X	negative	positive	0.4 mg/kg	Food item, DNA	SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div	10	08.12.21	X	X	negative	positive		Food item, total	In-house method, multicopy
div	12		X	X	negative	positive	LD PCR=15 pg DNA (<10mg / kg for reference material)LD PCR=15 pg DNA (<10mg / kg for reference material)	Food item, DNA	Real Time PCR Internal Method: MEB67Real Time PCR Internal Method: MEB67

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 08.00-65		Swab surface, extract swab, CTAB / Proteinase K / Rnase A / Promega Maxwell / real-time PCR	in post material
SFA	4	S3609		processing with SureFood PREP Advanced, according to the manufacturer's instructions	
SFA	5	S3609	As Per Kit Instructions	As Per Kit Instructions	
SFA	11	S3609			
SFA-4p	2	S3401		CTAB Isolation/Real Time PCR	Surface swab with liquid swab
SFA-4p	8	Art.No.S3401		Realtime PCR (4 plex)	
div	10			Real Time PCR 45 Cyclen	
div	12	Internal Method: MEB67 Internal Method: MEB67	16S RNA	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (45 cycles of amplification)	

5.1.6 ELISA and Lateral Flow: 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	µg/cm2		Test-Kit + Provider
AQ	7		X	X	positive	negative		Sesame	AQ = AgraQuant, RomerLabs
AQ	14	13.12.21	X	X	pos	neg	0,01	Sesame	AQ = AgraQuant, RomerLabs
BC	5	14.11.21	X	X	positive	negative	2	Sesame	BC = BioCheck ELISA
ES	13	05.11.21	X	X	positive	negative	0,025	Sesame protein	ES = ELISA-Systems
IL	12		X	X	positive	negative	2	Sesame protein	IL = Immunolab
NL-E	10	08.12.21	X	X	positive	negative		Sesame	NL-E = nutriLinia®E Allergen-ELISA
RS	3	29.10.21	X	X	positive	negative	2,5	Crustaceae	RS = Ridascreen®, R-Biopharm
RS-F	4	17.11.	X	X	positive	negative		Sesame	RS-F= Ridascreen® Fast, R-Biopharm
SP	1		X	X	positive	negative		Sesame	SP = SensiSpec, Eurofins Technologies
SP	9	17.11.	X	X	positive	negative	0,1	Sesame	SP = SensiSpec, Eurofins Technologies
div	6		X	X	positive	negative		Please select!	Selection ELISA-Kits:

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	
AQ	7	10002064/1000007125			
AQ	14				
BC	5	R6028	As Per Kit Instructions	As Per Kit Instructions	
ES	13	CHEM-241 / ESSESE-48	Anti-sesame seed 2S-albumin	Extraction: Room temperature PBS extraction buffer, vortex for 30 seconds, extract in shaking waterbath @ 60C for 15 min. Centrifugation. Determination: 4 parameter curve	
IL	12	MEI08.01/SES-E01	ND	Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24	
NL-E	10	NC-6005		lt. Manual	
RS	3				
RS-F	4	R7202		according to manufacturer instructions	
SP	1		Total protein		
SP	9	HU0030022/HU0030046	detects sesame proteins	Swab the surface, extract the swab, carry out the test according to the manufacturer's instructions	14mg/l
div	6				

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	µg/cm2		Test-Kit + Provider
ASU	9	12.11.	X	X	positive	negative	10	Please select!	Selection PCR-Methods
SFA	8	30.11.21	X	X	positive	negative	0.4 mg/kg	Food item, DNA	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	11		X	X	positive	negative	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-ID	5	16.11.21	X	X	positive	negative	1	Food item, total	SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
div	10	10.12.21	X	X	positive	negative		Food item, total	In-house method, multicopy

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		Swab surface, extract swab, CTAB / Proteinase K / Rnase A / Promega Maxwell / real-time PCR	in post material
SFA	8	Art. No.S3608		Realtime PCR	
SFA	11	S3608			
SFA-ID	5	S3608	As Per Kit Instructions	As Per Kit Instructions	
div	10			Real Time PCR 45 Cyclen	

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 ptASW2 (2021)***
PT name	***Allergen Swab Test II: Crustaceae, Egg, Mustard and Sesame***
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: Crustaceae and Egg (Plates A and B)* *qualitative: Mustard and Sesame (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 December 17th 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
		Germany
		PORTUGAL
		Germany
		CANADA
		Germany
		Germany
		Germany
		GREAT BRITAIN
		VIETNAM
		Germany
		USA
		USA
		CANADA
		ITALY

[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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