Evaluation Report
proficiency test

DLA ptASW2 (2020)

Allergen Swab Test II:

Crustaceae, Egg, Milk and Fish

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

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EP-Nummer *PT-Number*	DLA ptASW2 (2020)
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (7 April 2021) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 7 April 2021
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficiency 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 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 80 – 120 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 *crustaceae* and fish and the areas C and D should be tested for egg and milk.

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-Vormischungen 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)

Zutaten *	Surface A	Surface B	Surface C	Surface D
Crustaceae: freeze-dried King Prawns (protein 87%)	positive (80 – 120)	negative	-	-
Fish: freeze-dried cod (protein 88%)	negative	positive (80 – 120)	-	-
Egg: Whole egg powder (protein 47%)	-	-	positive (80 – 120)	negative
Milk: Skimmed milk powder (protein 32%)	-	-	negativ	positive (80 – 120)

* 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*	positiv	negativ	-	-
AgraStrip® Egg	-	-	positiv	negativ
AgraStrip® Casein	-	-	negativ	positiv

* 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 44th week of 2020. The testing method was optional. The tests should be finished at December 24th 2020 the latest.

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 crustaceae, *fish, egg and milk. 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.

All 12 participants submitted their results 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 (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 fish, and the surfaces C and D should be tested for egg and milk 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		
1	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
2	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
12	positive	negative	2/2 (100%)	2/2 (100%)	BF	
4	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
3	positive	negative	2/2 (100%)	2/2 (100%)	SP	
7	positive	negative	2/2 (100%)	2/2 (100%)	SP	

	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:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

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.

4.1.2 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	
9	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
10	positive	negative	2/2 (100%)	2/2 (100%)	SFA	
11	positive	negative	2/2 (100%)	2/2 (100%)	div	

	Surface A	Surface B
Number positive	5	0
Number negative	0	5
Percent positive	100	0
Percent negative	0	100
Consensus value	positive	negative
Spiking	positive	negative

Methods:

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = no specific details / other method

Comments:
The consensus values of 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 C	Surface D	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	2/2 (100%)	2/2 (100%)	3M	Lateral Flow
1	positive	negative	2/2 (100%)	2/2 (100%)	AQ	
12	positive	negative	2/2 (100%)	2/2 (100%)	BF	
11	positive	negative	2/2 (100%)	2/2 (100%)	IL	
7	positive	negative	2/2 (100%)	2/2 (100%)	Mi	
5	positive	negative	2/2 (100%)	2/2 (100%)	RS	
4	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
8	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
9	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
10	positive	negative	2/2 (100%)	2/2 (100%)	RS-F	
3	negative	positive	1/2 (50%)	1/2 (50%)	SP	

	Surface C	Surface D
Number positive	10	1
Number negative	1	10
Percent positive	91	9
Percent negative	9	91
Consensus value	positive	negative
Spiking	positive	negative

Methods:

3M = 3M Protein ELISA Kit

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

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.

4.2.2 PCR-Results: Egg

Comments: _There are no PCR results available for the parameter Egg._

4.3 Proficiency Test Results Milk

4.3.1 ELISA- and Lateral Flow-Results: Milk

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		
1	negative	positive	2/2 (100%)	2/2 (100%)	AQ	
2	negative	positive	2/2 (100%)	2/2 (100%)	AS	Lateral Flow
4	negative	positive	2/2 (100%)	2/2 (100%)	BC	
12	negative	positive	2/2 (100%)	2/2 (100%)	BF	
8	negative	positive	2/2 (100%)	2/2 (100%)	BK	
6	negative	positive	2/2 (100%)	2/2 (100%)	IL	
11	negative	positive	2/2 (100%)	2/2 (100%)	IL	
7	negative	positive	2/2 (100%)	2/2 (100%)	Mi	
5	negative	positive	2/2 (100%)	2/2 (100%)	RS	
9	negative	positive	2/2 (100%)	2/2 (100%)	RS	
10	negative	positive	2/2 (100%)	2/2 (100%)	RS-F	
3	positive	positive	1/2 (50%)	1/2 (50%)	SP	

	Surface C	Surface D
Number positive	1	12
Number negative	11	0
Percent positive	8	100
Percent negative	92	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow), RomerLabs

BC = BioCheck ELISA

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

IL = Immunolab

MI = Morinaga Institute ELISA

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

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

4.3.2 PCR-Results: Milk

Qualitative valuation of results

<u>*Comments:*</u> *There are no PCR results available for the parameter Milk.*

4.4 Proficiency Test Results Fish

4.4.1 ELISA- and Lateral Flow-Results: Fish

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		
2	negative	positive	2/2 (100%)	2/2 (100%)	3M	Lateral Flow
1	negative	positive	2/2 (100%)	2/2 (100%)	AQ	
4	negative	positive	2/2 (100%)	2/2 (100%)	BC	
3	negative	positive	2/2 (100%)	2/2 (100%)	SP	

	Surface A	Surface B
Number positive	0	4
Number negative	4	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

3M = 3M Protein ELISA Kit

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

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: Fish

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%)	IG	
4	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
8	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
9	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
10	negative	positive	2/2 (100%)	2/2 (100%)	SFA	
7	negative	positive	2/2 (100%)	2/2 (100%)	div	
11	negative	positive	2/2 (100%)	2/2 (100%)	div	

	Surface A	Surface B
Number positive	0	7
Number negative	7	0
Percent positive	0	100
Percent negative	100	0
Consensus value	negative	positive
Spiking	negative	positive

Methods:

IG = Imegen

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

div = no specific details / other methods

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: 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	mg/kg	e.g. food / food protein	Test-Kit + Provider
AQ	1		positive	negative	X	X	0,000225	Crustaceae protein	AQ = AgraQuant, RomerLabs
AQ	2		positive	negative	X	X		Crustaceae protein	AQ = AgraQuant, RomerLabs
BF	12	24/12	positive	negative	X	X	0,07	Crustaceae, fresh	BF = MonoTrace ELISA, BioFront Technologies
RS-F	4	11.04.20	positive	negative	X	X	20ug/sw ab	Crustaceae, fresh	RS-F= Ridascreen® Fast, R-Biopharm
SP	3	13.05.20	positive	negative	X	X	0.020 (LOQ)	Tropomyosin	SP = SensiSpec, Eurofins Technologies
SP	7	19.11.20	positive	negative	X	X	0,001	Tropomyosin Crustaceae	Eurofins Technologies Sensispec

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	1	10002076			
AQ	2	10002076			
BF	12	CR1-EK	Monoclonal; anti-tropomyosin	1:10 extraction ration	
RS-F	4	R7312	As per kit instructions	As per kit instructions	
SP	3	R6202	Tropomyosin		
SP	7	HU 0030006/HU 003	detects crustaceae-Tropomyosin	As per kit instructions	

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 detec-tion given as	Method
		Day/ Month	qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
div	11	22.12.20	positive	negative	X	X		LD PCR=15 pg DNA (<10mg / kg for reference material) LD PCR=15 pg DNA (<10mg / kg for reference material)	Real Time PCR Internal Method: MEB241Real Time PCR Internal Method: MEB241
SFA	4		positive	negative	X	X	1ug/sw ab	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	04.11.20	positive	negative	X	X	0,4	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9		positive	negative	X	X	0,4	Crustacean, DNA	LFOD-TST-SOP-8852
SFA	10	17.11.	positive	negative	X	X	0,4	Food-DNA	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	
div	11	Internal Method: MEB241	16S RNA	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (45 cycles of amplification)	Internal Method: MEB241
SFA	4	R3612	As per kit instructions	As per kit instructions	
SFA	8	S3612	Crustacea	Extraction by SureFood® Prep Advanced Protokoll 1 (S1053), using the w hole sw ab	QE for Abalone (Haliotis) 100 %, K01
SFA	9	LFOD-TST-SOP-8852 Surefood Allergen Crustacean S3612LFOD-TST-SOP-8852 Surefood Allergen Crustacean S3612			
SFA	10	S3612		Preparation via SureFood PREP Advanced, as per kit instructions	

5.1.3 ELISA: 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	mg/kg	e.g. food / food protein	Test-Kit + Provider
3M	2		-	-	positive	negative		Protein	3M Allergen Protein Rapid Kit-Egg
AQ	1		X	X	positive	negative	0,00005	Whole Egg Protein	AQ = AgraQuant, RomerLabs
BF	12	24/12	X	X	positive	negative	0,3	Whole Egg Powder	BF = MonoTrace ELISA, BioFront Technologies
IL	11		X	X	positive	negative	0.4	Egg White Proteins	IL = Immunolab
Mi	7	10.11.20	X	X	positive	negative	0,0155	Whole Egg Powder	MI = Morinaga Institute ELISA
RS	5		X	X	positive	negative	0,1	Whole Egg Powder	RS = Ridascreen®, R-Biopharm
RS-F	4		X	X	positive	negative	0.13ug/swab	Egg White Proteins	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	8	04.11.20	X	X	positive	negative	0,27	Whole Egg Powder	RS-F= Ridascreen® Fast, R-Biopharm
RS-F	9		X	X	positive	negative	0,9	Whole Egg Powder	LFOD-TST-SOP-8966
RS-F	10	16.11.	X	X	positive	negative	0,5	Whole Egg Powder	RS-F= Ridascreen® Fast, R-Biopharm
SP	3		X	X	negative	positive	0.4 (LOQ)	Egg White Proteins	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 solution / time / temperature	
3M	2	3M Allergen Protein Rapid Kit-Egg	L25EGG		
AQ	1	10002060			
BF	12	EOM-EK	Monoclonal; anti-ovomucoid	1:20 extraction ratio	
IL	11	MEI10.01/EGG-E01	ND	Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24 Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24	
Mi	7	M2111	detects egg white protein Ovalbumin	As per kit instructions	
RS	5				
RS-F	4	R6402	As per kit instructions	As per kit instructions	
RS-F	8	R6402		as per manual instructions, result only qualitative	QE for quail egg, duck egg, turkey egg
RS-F	9	LFOD-TST-SOP-8966 RIDASCREEN FAST Egg protein R6402LFOD-TST-SOP-8966 RIDASCREEN FAST Egg protein R6402			
RS-F	10	R6402		As per kit instructions	
SP	3		Ovomucoid		

5.1.4 ELISA: Milk

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	1		X	X	negative	positive	0,0025	Milkprotein	AQ = AgraQuant, RomerLabs
AS	2		-	-	negative	postive		Protein	Romer Labs AgraStrip Milk Test Kit
BC	4		X	X	negative	positive	0.2ug/sw ab	other: please fill in!	BC = BioCheck ELISA
BF	12	24/12	X	X	negative	positive	0,48	Skimmed Milk Powder	BF = MonoTrace ELISA, BioFront Technologies
BK	8	05.11.20	X	X	negative	positive	< 1	Skimmed Milk Powder	BK = BioKits, Neogen
IL	6	26.11.2020r.	X	X	negative	positive	0,05	Milkprotein	IL = Immunolab
IL	11		X	X	negative	positive	0.4	Milkprotein	IL = Immunolab
Mi	7	11.11.20	X	X	negative	positive	0,0125	Casein	MI = Morinaga Institute ELISA
RS	5		X	X	negative	positive	0,7	Milkprotein	RS = Ridascreen®, R-Biopharm
RS	9		X	X	negative	positive	0,7	Milkprotein	J.AOAC Int.99 (2016) 495-502
RS-F	10	07.12.	X	X	negative	positive	2,5	Milkprotein	RS-F= Ridascreen® Fast, R-Biopharm
SP	3		X	X	positive	positive	0.4 (LOQ)	Casein + BLG	SP = SensiSpec, Eurofins Technologies

5.1.4 ELISA: Milk

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	1	10002080			
AS	2	10002078			
BC	4	R6022	As per kit instructions	As per kit instructions	Casein
BF	12	CAS-EK	Monoclonal; anti-casein	1:10 extraction ratio	
BK	8	8470		Extraction downscaled according to manual, indication only as qualitative results	
IL	6	Cat.-No.: MIL-E01/ Lot: MIL-160	Milk protein	Extraction: Pipet 1 mL of prediluted extraction buffer into the reaction tube/ Dip the swab into the extraction buffer in the reaction tube/ Swab the area first in horizontal then in vertical lines, rotate the stick while swabbing the area/ Dip the stick back into the tube with the extraction buffer and shake thoroughly/ Directly apply the solution as a sample in the corresponding assay Determination: 100 µL of particle-free solution, ready-to-use standards applied per well/ 20 minutes incubation at room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL conjugate into each well/ 20 minutes incubation in room temperature/ x3 Plate wash with 300 µL pre-diluted wash solution/ add 100 µL substrate solution into each well/ 20 minutes incubation in the dark, at room temperature/ add 100 µL Stop enzyme solution into each well/ Measure absorbance at 450 nm (reference 620 nm)	
IL	11	MEI10.01/MILK-E01	ND	Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24 Short Application Protocol for Swab Test in Combination with Immunolab Food Allergen ELISAs Version: 2013-04-24	
Mi	7	M2113	detects cow milk-Casein	As per kit instructions	
RS	5				
RS	9	J.AOAC Int.99 (2016) 495-502 RIDASCREEN FAST Milk protein R4652J.AOAC Int.99 (2016) 495-502 RIDASCREEN FAST Milk protein R4652			
RS-F	10	R4652		As per kit instructions	
SP	3		Casein, BLG		

5.1.5 ELISA: Fish

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
3M	2		negative	postive	-	-		Protein	3M Allergen Protein Rapid Kit-Fish
AQ	1		negative	positive	X	X	0,07	Fish protein	AQ = AgraQuant, RomerLabs
BC	4		negative	positive	X	X	5ug/sw ab	other: please fill in!	BC = BioCheck ELISA
SP	3		negative	positive	X	X	4 (LOQ)	cod	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 solution / time / temperature	
3M	2	L25Fsh			
AQ	1	10002083			
BC	4	R6010	As per kit instructions	As per kit instructions	LOD as Cod, Fresh
SP	3		Parvalbumin		

5.1.6 PCR: Fish

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
div	7	19.11.20	negative	positive	X	X	20	Food item, total	internal Method
div	11	22.12.20	negative	positive	X	X	LD PCR=15 pg DNA (<10mg / kg for reference material) LD PCR=15 pg DNA (<10mg / kg for reference material)		Real Time PCR Internal Method: MEB73Real Time PCR Internal Method: MEB73
I	5		negative	positive	X	X	4	Please select!	other: Imegen
SFA	4		negative	positive	X	X	5ug/swab	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	8	04.11.20	negative	positive	X	X	1	Food item, total	SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA	9		negative	positive	X	X	1	Fish, DNA	LFOD-TST-SOP-8852
SFA	10	17.11.	negative	positive	X	X	0,4	Food-DNA	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	
div	7			CTAB / Proteinase K / Rnase A / Maxwell / Real-time PCR 45 cycles	
div	11	Internal Method: MEB73	18S RNA	Extraction performed using the DNeasy Mericon Qiacube HT kit. Detection performed by Real-Time PCR (45 cycles of amplification)	Internal Method: MEB73
I	5			CTAB/ kit /PCR real time	
SFA	4	R3610	As per kit instructions	As per kit instructions	
SFA	8	S3610	Osteichthyes	Extraction with SureFood® Prep Advanced Protokoll 1 (S1053), usage of the whole swab	QE zu flying duck (Cairina moschata) 100 %, K01
SFA	9	LFOD-TST-SOP-8852 Surefood Allergen Fish S3610LFOD-TST-SOP-8852 Surefood Allergen Fish S3610			
SFA	10	S3610		preparation with SureFood PREP Advanced, as per kit instructions	

5.2 Information on the Proficiency Test (PT)

Vor der LVU wurden den Teilnehmern im Proben-Anschreiben folgende Informationen mitgeteilt:

PT number	*DLA ptASW2 (2020)*
PT name	*Allergen Swab Test II: Crustaceae, Egg, Milk and Fish*
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 Fish** *(Plates A and B)* *qualitative:* **Egg and Milk** *(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 24th 2020*
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		SPANIEN/SPAIN
		Deutschland/Germany
		USA
		Deutschland/Germany
		PORTUGAL
		Deutschland/Germany
		POLEN/POLAND
		Deutschland/Germany
		GROSSBRITANNIEN/ GREAT BRITAN
		USA
		USA
		VIETNAM

[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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