



Evaluation Report

proficiency test

DLA ptALR2

Response PT Sesame:

**5 processed Samples white sesame (ground),
black sesame (ground), sesame paste
(Tahini), vegetarian spread (heated) and
salt crackers (baked)**

in Potato Powder Matrix

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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].

The present proficiency test format „**Response PT Allergens**“ includes 5 differently processed samples of an allergen in a simple carrier matrix as well as a “blank sample”. Hereby it offers the possibility to prove that the analytical determination methods used by the participants are suitable to detect the respective processed allergens qualitatively and to determine its quantitative response factors.

In order to ensure comparability of the processed sample material, the allergen contents of the PT sample series were adjusted to approximately the same levels calculated as sesame contents. The evaluation of the PT-results was done qualitatively by scores from 1-5 (score 5 = all processings successfully determined). Quantitative results were given including the calculated respective recovery rate (recovery score) for information in the report.

2. Realisation

2.1 Test material

6 PT-samples for qualitative and optionally quantitative determination of almond in unroasted and roasted almonds, marzipan, almond spread and almond milk in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce partly processed almond products. For each PT-sample 5-10 products of different origin were worked up.

Premixes with contents from approx. 0,26 - 5,0 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed, mixed gravimetrically, eventually freeze-dried (sesame spread) or baked (sesame crackers), ground and homogenized. Afterwards the raw materials were mixed with further ingredients, crushed and homogenized by a ball mill.

The allergen-premixes were added to the carrier matrix of potato powder / maltodextrin (mesh < 500 µm) and homogenized. An aliquot of the carrier matrix was provided as the "blank sample".

The 6 PT-samples were portioned to approximately 20 g in metallized PET film bags.

The contents of sesame of the PT-samples were in the range of 51 to 56 mg/kg (see Tab. 1).

Each assigned value, here the spiked allergen-contents, is afflicted with a standard uncertainty. As uncertainties the following factors were considered: protein content of spiking materials, mixing homogeneity, homogeneity and stability of sesame protein.

All uncertainties were expressed in the form of their standard deviations and then added as variances. The square root from the sum of the total variances results in the combined uncertainty "Uc". Multiplied with the coverage factor k=2 the extended uncertainties of the assigned values " $U(X_{pt})$ " are obtained [3, 13, 16-17].

Table 1: Composition of DLA-Samples

PT-Sample series	Sample 1 Sesame paste	Sample 2 Sesame, white	Sample 3 Sesame, black	Sample 4 Sesame spread	Sample 5 Sesame cracker	Sample 6 „blank“
Ingredients	g/100 g	g/100g	g/100g	g/100g	g/100g	g/100g
Potato powder Ingredients: potato, E471, E304, E223, E100 Nutrients per 100 g: Protein 8,3 g, carbohydrates 76 g, fat 0,6 g, salt 0,15 g	75	75	75	75	74	75
Maltodextrin	25	25	25	25	25	25
Allergen-Premixes Ingredients (sample 1-4): malto-dextrin (88% - 98%), silicon dioxide (<3%), processed allergen products (each 0,26% - 5% sesame)	0,10	0,10	0,10	0,47	2,0	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
<i>Sesame paste (Tahini)*</i> Protein 21,0 % ** (6 products, 5 countries, Europe, Southwest Asia)	50,7	-	-	-	-	-
<i>Sesame, white* (ground)</i> Protein 20,8 % ** (10 products, Africa, Asia, South America)	-	52,3	-	-	-	-
<i>Sesame, black* (ground)</i> Protein 18,5 % ** (6 products, Asia)	-	-	51,3	-	-	-
<i>Sesame Spread*</i> Ingredients: 12% Sesame and chickpeas, water, vegetable oils and fats, conc. lemon juice, agave syrup, pineapple, salt, sugar, garlic, paprika, onions, spices and herbs and other additives Protein 2,5 % *** (5 products, Europe)	-	-	-	468	-	-
<i>Sesame Crackers*</i> (baked 200°C, 25 min) Ingredients: 0,26 % Sesame and rapeseed oil, sugar, salt, baking powder and other food additives Protein 0,054 % *** (10 products, Africa, Asia, South America)	-	-	-	-	19500	-
- as Sesame	50,7	52,3	51,3	56,2	50,6	-
Extended combined uncertainty (k=2) of sesame-content (= ± 12,5 %)	± 6,34	± 6,54	± 6,41	± 7,03	± 6,33	-

*Allergen contents as „total food“ as described in column ingredients according to gravimetric mixture

** Protein contents according to laboratory analysis of raw material mixtures (total nitrogen according to Kjeldahl with F=5,3 for sesame protein)

***Sesame protein content calculated from sesame content according to the declaration of the products or DLA manufacture

Note: The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **micro-tracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].

Before mixing dye coated iron particles of μm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of $\geq 5\%$ is equivalent to a good homogeneous mixture and of $\geq 25\%$ to an excellent mixture [14, 15].

The microtracer analysis of the present PT samples 1 to 5 showed a probability of 95%, 97%, 88%, 97% and 99%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 0,68, 0,60, 0,92, 0,67 and 0,56 respectively. The results of the microtracer analysis are given in the documentation.

2.1.2 Stability

A water activity (a_w) of $< 0,5$ is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_w value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_w value $< 0,5$).

The a_w value of the PT samples was approx. 0,28 (17 - 19°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 21st week of 2020. The testing method was optional. The tests should be finished at July 31st 2020 the latest.

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

There are 5 different samples with similar contents of the allergenic parameter Sesame, which is differently processed, contained in a simple carrier matrix as well as a "blank"-sample (carrier matrix).

- The samples 1-5 are numbered in a random order. They contain white sesame (ground), black sesame (ground), sesame paste (Tahini, roasted), vegetarian spread (heated) and salt crackers (baked).*
- Please give all your quantitative results as total Sesame, if possible indicate the underlying total protein content in Sesame.*
- Possible conversion factors for processed Sesame products are queried separately in the result submission file.*

Please note the attached information on the proficiency test.

(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website.

On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item in mg/kg were evaluated.

Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 13 participants submitted the results in time.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are using different antibodies, which are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the analyte content [26-29, 40]. Furthermore matrix- and/or processing of samples can have a strong impact on the detectability of allergens by ELISA and/or PCR methods.

In the present PT five different processed products containing the allergen sesame, *white sesame (ground)*, *black sesame (ground)*, *sesame paste (Tahini)*, *vegetarian spread (heated)* and *sesame crackers (baked)*, were provided to determine the qualitative detectability and to determine the response of the used quantitative methods.

The participant results were evaluated *qualitatively* with a score from 1-5 indicating the number of successfully detected processed products. The quantitative results were evaluated with a Recovery-Score (*RR-Score*), which indicates the number of results with a recovery rate in the range of 50 - 150% of the spiking level.

3.1 Qualitative Score

The qualitative valuation of each participant's results was performed with Scores from 1-5 considering the number of "positive" or "negative" results matching the **spiking of the PT-sample series** (see Tab. 2).

A Score from 5 indicates, that all processed products were detected successfully.

The results of the matrix sample no. 6 ("blank"-sample) were not evaluated if the participant result is in accordance with $\geq 75\%$ positive or negative results of participants (consensus value) or if the result is below the limit of quantification of the used method.

Table 2: Evaluation of results using qualitative Scores

Sample 1 Sesame paste	Sample 2 Sesame, white	Sample 3 Sesame, black	Sample 4 Sesame spread	Sample 5 Sesame cracker	Sample 6 „blank“	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not successful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

3.2 Recovery-Score (RR-Score)

The evaluation of the quantitative participant results for the spiked **PT-samples** was done by recovery scores (RR-Scores) which are related to the number of recovery rates in the range of acceptance. The RR-Scores are calculated by counting the number of results in the range of acceptance (s. below) per number of quantitatively determined samples. Further the percentage is given in the brackets behind.

The recovery rates were calculated considering the content of the spiked allergen (level of addition). The reference values are calculated from the values for samples 1 to 5 given in section 2.1 Sample material in Table 1. As range of acceptance RA for the evaluation of the participant results the range of the AOAC-recommendation of 50-150% for allergen-EL-ISAs was used [21]. This range was also used in the present PT for quantitative PCR- and LC/MS-results.

Only exact quantitative results were considered. Single results outside the given measuring range (e.g. indicated with > 25 mg/kg or $< 2,5$ mg/kg) or indicated with "0" were not considered.

The given recovery rates enable inter alia an assessment of matrix and/or processing influences.

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 12% - 176% for PCR methods, depending on matrix or processing and concentration (s. Table 3a and 3b). The given target standard deviation σ_{pt} was calculated for a number of $m = 2$ repeated measurements.

Table 3a: ELISA-Methods – Recovery rates and precision data from selected precision experiments [30-31].

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD _r	RSD _r	RSD _R	opt	Method / Literature
Peanut	Milk chocolate	173,7	87 %	-	8,8%	31%	30,4%	ELISA Manuf. A ASU 00.00-69
		33,8	85 %	-	5,2%	20%	19,7%	
		5,9	59 %	-	7,8%	31%	30,5%	
Peanut	Milk chocolate	215,7	108 %	-	5,9%	32%	31,7%	ELISA Manuf. B ASU 00.00-69
		40,1	100 %	-	7,2%	14%	13,0%	
		10,1	101 %	-	7,3%	16%	15,1%	
Peanut	Dark chocolate	148,2	74 %	-	6,0%	22%	21,6%	ELISA Manuf. A ASU 00.00-69
		30,9	77 %	-	13%	25%	23,2%	
		5,7	57 %	-	6,1%	33%	32,7%	
Hazelnut	Dark chocolate	16,3	81 %	-	4,7%	12%	11,5%	ELISA Manuf. A ASU 44.00-7
		7,56	76 %	-	8,9%	15%	13,6%	
		3,73	75 %	-	13%	24%	22,2%	
		1,62	81 %	-	15%	33%	31,2%	
Hazelnut	Dark chocolate	21,3	106 %	-	7,1%	14%	13,1%	ELISA Manuf. B ASU 44.00-7
		10,7	107 %	-	11%	19%	17,3%	
		4,69	94 %	-	11%	17%	15,1%	
		2,37	119 %	-	9,3%	17%	16,4%	

The Working Group on Prolamin Analysis and Toxicity (WGPAT) performed ring trials for validation of two commercial ELISA-Kits for determination of gluten using monoclonal R5 antibodies [24]. 12 food samples with gliadin contents in the range if 0 - 168 mg/kg were analysed by 20 laboratories. The obtained recovery rates were in the range between 65 and 110%, the relative repeatability standard deviation was between 13 - 25% (1. method) and 11 - 22% (2. method) and the relative reproducibility standard deviation between 23 - 47 % (1. method) and 25 - 33% (2. method). The authors concludes that both ELISA-Kits fulfil the validation criteria for ELISA methods [24].

The IRMM (Institute for Reference Materials and Measurements) proved the suitability of five different ELISA-Kits for the determination of peanut [27]. The mean values were in the concentration range of 0,3 - 16,1 mg/kg and/or 1,2 - 20,4 mg/kg. The smallest relative reproducibility standard deviation for each Kit was obtained for dark chocolate at 20 - 42% and cookies at 23 - 61%.

Table 3b: PCR-Methods - Recovery rates and precision data from selected precision experiments [32-36].

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD	RSD _r	RSD _R	σ_{pt}	Method / Literature
Sesame	Rice cookie	94,6 15,7 9,8	95 % 79 % 98 %	-	22,5% 26,0% 20,9%	27,5% 39,5% 33,5%	22,4% 35,0% 30,0%	rt-PCR ASU 18.00-19
Sesame	Wheat cookie Sauce powder	96,9 59,8	79 % 60 %	-	21,8% 22,2%	33,0% 43,2%	29,2% 40,2%	rt-PCR ASU 18.00-19
Sesame	Rice cookie	88,9 17,8 9,8	89 % 89 % 98 %	-	18,2% 34,2% 26,2%	30,5% 37,8% 37,0%	27,7% 29,1% 32,0%	rt-PCR ^{multiplex} ASU 18.00-22
Sesame	Wheat cookie Sauce powder	115 58,5	93 % 59 %	-	16,7% 30,8%	41,1% 44,4%	39,4% 38,7%	rt-PCR ^{multiplex} ASU 18.00-22
Mustard, brown / black	Sausage, autoclaved	146,7 50,0 15,8	147 % 125 % 158 %	-	12,3% 17,2% 15,4%	22,0% 31,6% 27,1%	20,2% 29,2% 24,8%	rt-PCR ASU 08.00-64
Mustard, brown / black	Sausage, autoclaved	168,3 52,9 17,6	168 % 132 % 176 %	-	11,4% 10,0% 23,1%	31,6% 23,1% 46,3%	29,5% 21,9% 43,3%	rt-PCR ASU 08.00-65
Mustard, white	Boiled Sausage (100°C, 60min)	79,9 37,0 18,0 8,0	80 % 93 % 90 % 80 %	-	13,6% 15,7% 14,4% 15,4%	23,6% 29,2% 30,6% 26,1%	21,6% 27,0% 28,9% 23,7%	rt-PCR ASU 08.00-59
Mustard, weiß	Boiled Sausage (100°C, 60 min)	103,3 45,9	103 % 115 %	- -	11,8% 14,7%	17,1% 21,8%	14,9% 19,2%	rt-PCR ASU 08.00-65
Mustard, weiß	Sausage, autoclaved	11,7	11,7 %	-	24,1%	34,3%	29,8%	rt-PCR ASU 08.00-65

3.2.2 Values by perception

Requirements to the performance of analysis methods for quantitative determination of allergens in food were compiled for example from the Ministry of Health and Welfare (MHLW) in Japan [25], by the Working Group 12 „Food allergens“ of the Technician Committee CEN/TC 275 [22-24], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [26] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [21].

The following relevant ELISA and/or PCR validation criteria of the committees are given in Table 4 and 5.

Table 4: ELISA validation criteria

Literature [21-26]	Recovery Rate	Repeatability Standard Deviation	Reproducibility Standard Deviation
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% ^(a)	19,5 - 57,2% ^(a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

(a) = Example from hypothetical ring trail in the concentration range of 0,5 - 5 mg/kg

Table 5: PCR validation criteria

Literature [20]	Recovery Rate	Repeatability Standard Deviation	Reproducibility Standard Deviation
CAC 2010	± 25% ^(a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Due to the current performance of ELISA and PCR methods for quantitative determination of allergens in food, which can be derived from precision data by experiments and from validation criteria mentioned above, a common relative target standard deviation (σ_{pt} value) from 25% was defined. The recovery rate was set to 50-150%.

3.3 z-Score (Spiking Levels)

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}), here the spiking levels [3].

Participants' z-scores are derived from:

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 .$$

The z-scores corresponding to the recovery rates were calculated with the target standard deviation of 25% (see 3.2.2).

3.4 z'-Score (Spiking Levels)

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered. The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(x_{pt})}$) [3].

The calculation is performed by:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}' .

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 .$$

4. Results

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

Evaluation was done separately for ELISA- (and Lateral Flow) and PCR-methods.

In the result chapter all quantitative results of the participants are displayed formatted to 3 decimal places. In the documentation, all results are given as they were transmitted by the participants.

To ensure the **comparability of quantitative results** DLA harmonized participants' results giving different specifications (e.g. as protein or as allergenic food) as far as possible.

ELISA results given as **sesame protein** were converted by DLA to **total food items (sesame seed)** using the analyzed protein content of the raw materials (see page 6).

The qualitative results are presented in the corresponding evaluation table as indicated below:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5		

The quantitative results are presented in the corresponding evaluation table as indicated below:

Evaluation number	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *			
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		

* Recovery Rate

4.1 Proficiency Test Processed Sesame Products

4.1.1 Qualitative Scores: ELISA-Methods

Evaluation number	Sample 1 Sesame paste	Sample 2 Sesame, white	Sample 3 Sesame, black	Sample 4 Sesame spread	Sample 5 Sesame crackers	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
6	positive	positive	positive	positive	positive	negative	5 (100%)	AQ-P	
7	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
13a	positive	positive	positive	negative	positive	negative	4 (80%)	BF	
13b	positive	positive	positive	negative	positive	negative	4 (80%)	BF-LF	Lateral Flow
12	positive	positive	positive	positive	positive	negative	5 (100%)	MI-II	
2	positive	positive	positive	positive	positive	negative	5 (100%)	NL-E	
3	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
4	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
5	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
10	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
11	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
1	positive	positive	positive	positive	positive	negative	5 (100%)	SP	
8	positive	positive	positive	positive	positive	negative	5 (100%)	SP	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	13	13	13	11	13	0
Number negative	0	0	0	2	0	13
Percent positive	100	100	100	85	100	0
Percent negative	0	0	0	15	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

- AQ-P = AgraQuant Plus, RomerLabs
- AQ = AgraQuant, RomerLabs
- BF = MonoTrace ELISA, BioFront Technologies
- BF = MonoTrace ELISA, BioFront Technologies
- MI-II = Morinaga Institute ELISA Kit II
- NL-E = nutriLinia®E Allergen-ELISA
- RS-F= Ridascreeen® Fast, R-Biopharm
- SP = SensiSpec ELISA Kit, Eurofins

Comments:

For the samples 1 to 3 and 5 consensus values of 100% positive results were obtained by the ELISA-methods. For the processed sample 4 (sesame spread) two negative results were obtained.

4.1.2 Qualitative Scores: PCR-Methods

Evaluation number	Sample 1 Sesame paste	Sample 2 Sesame, white	Sample 3 Sesame, black	Sample 4 Sesame spread	Sample 5 Sesame crackers	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
3	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
8	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
12	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
11	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
2	positive	positive	positive	positive	positive	negative	5 (100%)	div	
4	positive	positive	positive	positive	positive	negative	5 (100%)	div	
9	positive	positive	positive	positive	positive	negative	5 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	7	7	7	7	7	0
Number negative	0	0	0	0	0	7
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method
 SFA = Sure Food Allergen, R-Biopharm / Congen
 div = keine genaue Angabe / andere Methode
 div = not indicated / other method

Comments:

For all samples 1-5 consensus values of 100% positive results were obtained by PCR-methods.

4.1.4 Quantitative: ELISA-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Sesame paste			Sample 2 Sesame, white			Sample 3 Sesame, black			Sample 4 Sesame spread			Sample 5 Sesame crackers			RR- score	Method	Remarks
	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]			
	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	Number in RA**		
6	123	243	5,7	156	298	7,9	230	448	14	13,4	24	-3,0	55,0	109	0,35	1/5 (20%)	AQ-P	
7	31,0	61	-1,6	52,0	99	-0,02	53,0	103	0,13	24,0	43	-2,3	19,0	38	-2,5	2/5 (40%)	AQ	
13a	57,4	113	0,53	92,8	177	3,1	70,9	138	1,5				28,4	56	-1,8	2/4 (50%)	BF	
13b																	BF-LF	Lateral Flow
12	57,6	114	0,55	58,2	111	0,45	59,5	116	0,64	59,6	106	0,24	49,5	98	-0,09		MI-II	result converted °
2	38,0	75	-1,0	57,0	109	0,36	74,0	144	1,8	29,0	52	-1,9	16,4	32	-2,7	4/5 (80%)	NL-E	
3	115	227	5,1	153	293	7,7	152	296	7,9	72,0	128	1,1	74,0	146	1,8	2/5 (40%)	RS-F	
4	137	269	6,8	195	372	11	186	362	10	87,5	156	2,2	88,4	175	3,0	0/5 (0%)	RS-F	
5	112	220	4,8	128	245	5,8	122	237	5,5	47,8	85	-0,60	52,3	103	0,13	2/5 (40%)	RS-F	
10	85,0	168	2,7				145	283	7,3	68,0	121	0,84	67,0	132	1,3	2/4 (50%)	RS-F	
11	127	250	6,0	178	340	9,6	165	322	8,9	77,3	138	1,5	72,8	144	1,8	2/5 (40%)	RS-F	
1	38,0	75	-1,0	51,0	98	-0,10	50,0	97	-0,10	31,0	55	-1,8	27,0	53	-1,9	5/5 (100%)	SP	
8	26,0	51	-1,9	40,0	76	-0,94	46,0	90	-0,41	22,0	39	-2,4	12,0	24	-3,1	3/5 (60%)	SP	

° calculation p. 14

RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %
Number in RA	6	Number in RA	5	Number in RA	6	Number in RA	7	Number in RA	8
Percent in RA	50	Percent in RA	45	Percent in RA	50	Percent in RA	64	Percent in RA	67

* Recovery rate 100% Reference value: Sesame, s. Page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:

AQ-P = AgraQuant Plus, RomerLabs

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

BF = MonoTrace ELISA, BioFront Technologies

MI-II = Morinaga Institute ELISA Kit II

NL-E = nutriLinia®E Allergen-ELISA

RS-F = Ridascreeen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

Comments:

For samples 1 - 5 45% to 67% of the recovery rates of the participants results were in the range of acceptance of 50-150%.

4.1.5 Quantitative: PCR-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Sesame paste			Sample 2 Sesame, white			Sample 3 Sesame, black			Sample 4 Sesame spread			Sample 5 Sesame crackers			RR- score	Method	Remarks	
	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]	Result	RR *	[Z _{RR}]				RR *
	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	[mg/kg]	[%]	[Z _{RR}]	Number in RA **			
3																	ASU		
8																	ASU		
12																	ASU		
11	1,57	3,1	-3,9	5,16	9,9	-3,6	5,35	10,4	-3,6	0,875	1,6	-3,9	0,865	1,7	-3,9	0/5 (0%)	SFA	Samples 4 and 5 < LOQ	
2																	div		
4																	div		
9																	div		
	RA**			50-150 %			RA**			50-150 %			RA**			50-150 %			Methods: ASU = ASU §64 Methode/method SFA = Sure Food Allergen, R-Biopharm / Congen div = keine genaue Angabe / andere Methode div = not indicated / other method
	Number in RA			0			Number in RA			0			Number in RA			0			
	Percent in RA			0			Percent in RA			0			Percent in RA			0			

* Recovery rate 100% Reference value: Sesame, s. Page 6

** Acceptance range of AOAC for allergen ELISAs

Comments:

One participant has determined quantitative results using PCR methods. All recovery rates obtained were below the range of acceptance of 50-150%.

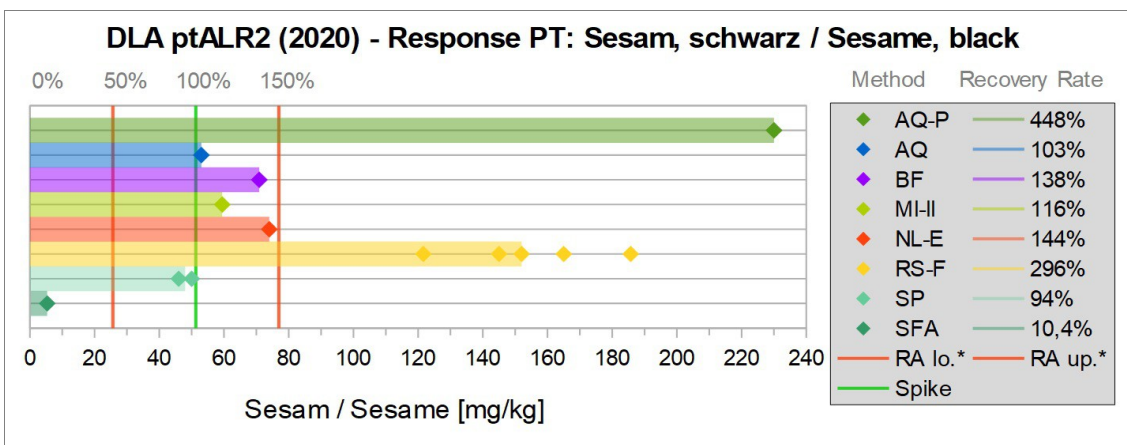
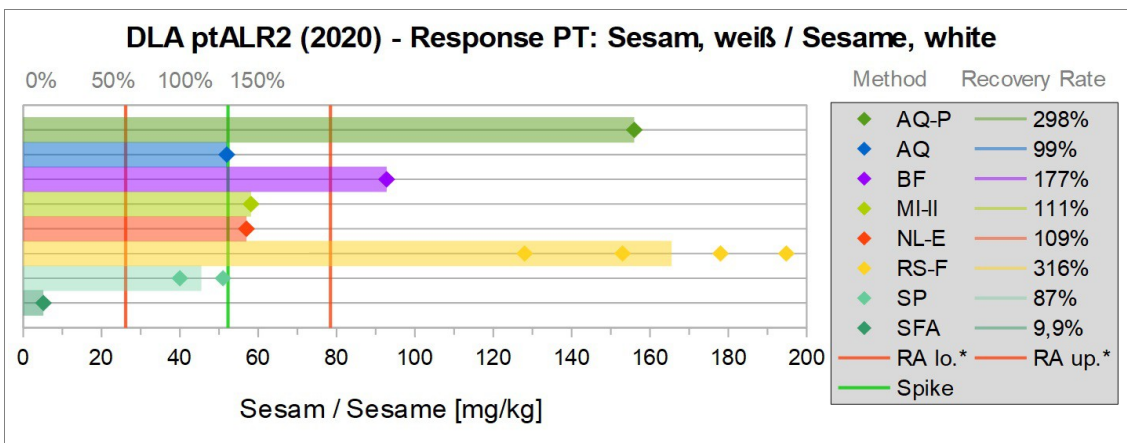
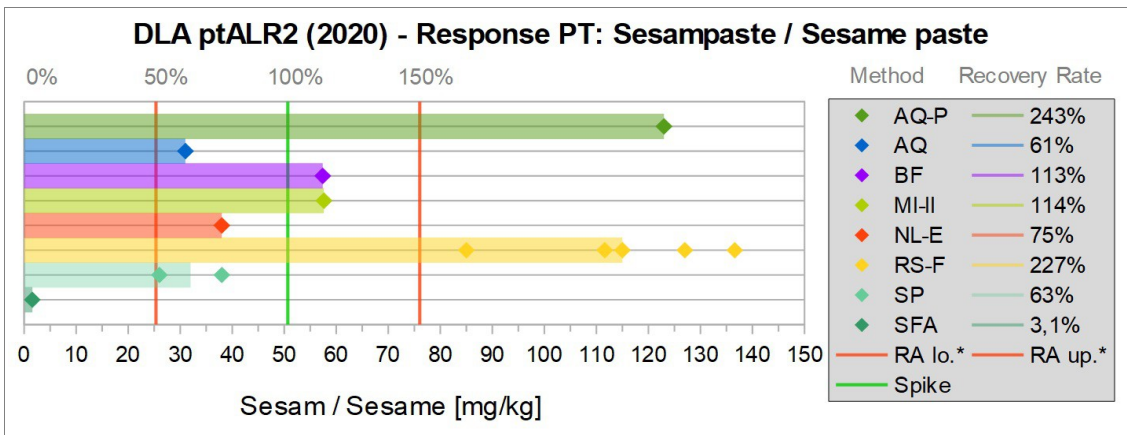


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale sesame content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

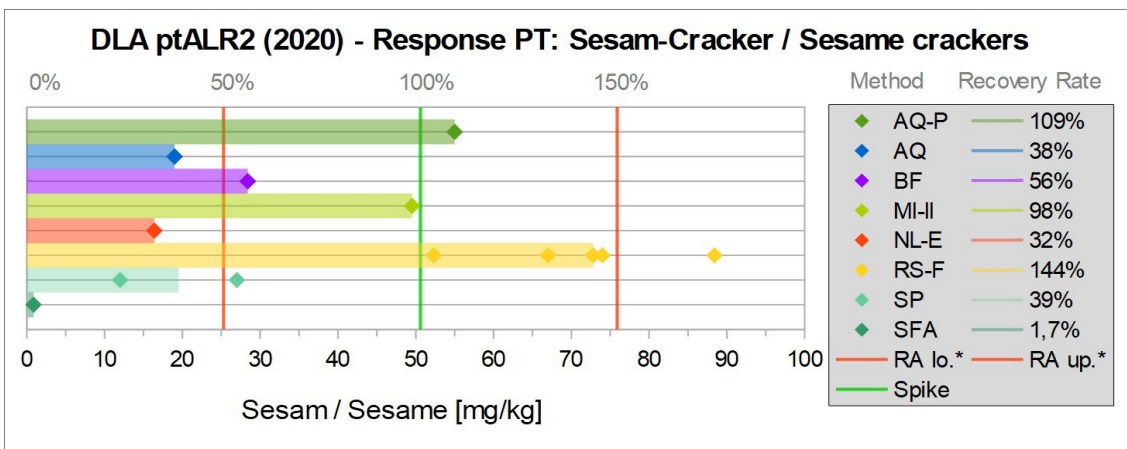
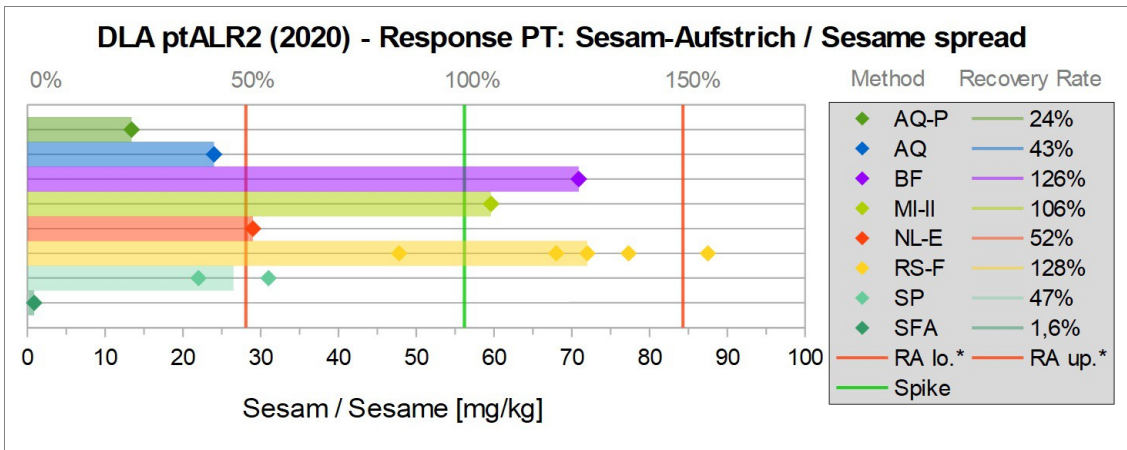


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale sesame content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.2 Participant z-Scores: overview table

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	ELISA Sesame					PCR Sesame				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	-1,0	-0,10	-0,10	-1,8	-1,9					
2	-1,0	0,36	1,8	-1,9	-2,7					
3	5,1	7,7	7,9	1,1	1,8					
4	6,8	11	10	2,2	3,0					
5	4,8	5,8	5,5	-0,60	0,13					
6	5,7	7,9	13,9	-3,0	0,35					
7	-1,6	-0,02	0,13	-2,3	-2,5					
8	-1,9	-0,94	-0,41	-2,4	-3,1					
9										
10	2,7		7,3	0,84	1,3					
11	6,0	9,6	8,9	1,5	1,8	-3,9	-3,6	-3,6	-3,9	-3,9
12	0,55	0,45	0,64	0,24	-0,09					
13a	0,53	3,1	1,5		-1,8					
13b										

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):

- 2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
- 2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
- 3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 ELISA-Methods

Method Abr.	Evalu- ation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantita- tive result as
			qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg				
AQ-P	6	28.05.20	positive	123	positive	156	positive	230	positive	13,4	positive	55	negative	< LOQ	1	1		Sesame
AQ	7	08.06.20	-	31	-	52	-	53	-	24	-	19	-	<LOD	0,2	2	0,5	Sesame
BF	13a	31.07.20	positive	57,4	positive	92,8	positive	70,9	negative		positive	28,4	negative		0,16	1		Sesame
BF-LF	13b	31.07.20	positive		positive		positive		negative		positive		negative		2	-		Sesame
MI-II	12	29.07.20	positive	12,1	positive	12,1	positive	11	positive	12,4	positive	10,3	negative	<0,16	0,16	0,16		Sesame protein
NL-E	2	25/05; 26/05	-	38	-	57	-	74	-	29	-	16,4	-	< LOQ		2		Sesame
RS-F	3		positive	115	positive	153	positive	152	positive	72	positive	74	negative		2,5	2,5		Sesame
RS-F	4	05.06.20	positive	136,6	positive	194,8	positive	185,7	positive	87,5	positive	88,4	negative	<2,5	0,2	2,5		Sesame
RS-F	5	24.06.20	-	111,6	-	128	-	121,6	-	47,8	-	52,3	-	<2,5	<0,14	<2,5		Sesame
RS-F	10	17.07.20	positive	85	positive	N/A	positive	145	positive	68	positive	67	negative	0	N/A	2,5	N/A	Sesame
RS-F	11		positive	127	positive	178	positive	165	positive	77,3	positive	72,8	negative	< 2,5	0,14	2,5		Sesame
SP	1	22.05.20	positive	38	positive	51	positive	50	positive	31	positive	27	negative	0	0,2	2		Sesame
SP	8	28.05.20	positive	26	positive	40	positive	46	positive	22	positive	12	negative	<2	1,5	2		Sesame

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation details by participants: ELISA-Methods

Method Abr.	Evaluation number	Method	Specificity	Total protein content in sesame (According to method prescription)	Conversion for processed sesame	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	%	Recalculation from X to Y (factor or %)	e.g. Extraction solution / time / temperature	yes/no	
AQ-P	6	AgraQuant Plus Sesame						
AQ	7	AgraQuant ELISA Sesame COKAL1948, RomerLabs					yes	
BF	13a	MonoTrace Sesame ELISA kit, BioFront Technologies	Monoclonal antibody-based assay			1:20 extraction ratio/10 minutes/60C		
BF-LF	13b	AllerTrace Sesame - BioFront Technologies	Monoclonal antibody-based assay			1:10 extraction ratio/1 minute at room temperature		
MI-II	12	Morinaga Sesam Elisa Test Kit II (M2121)	11S globulin			Short Time Extraction Method	no	
NL-E	2	Sesam-E nutriLinia über Romer Labs	Ab against sesame proteins			as per kit instructions	yes	
RS-F	3	Ridascreen® FAST Sesame R7202, R-Biopharm					yes	
RS-F	4	Ridascreen® FAST Sesame R7202, R-Biopharm	Sesame protein			as per kit instructions	yes	
RS-F	5	Ridascreen® FAST Sesame R7202, R-Biopharm		69,7/80,0/76,0/29,9/32,7/<0,6 mg/kg sesameprotein	25%protein (factor 0,625)		NO	
RS-F	10	Ridascreen® FAST Sesame R7202, R-Biopharm	NA	21	response according to method instructions	as per kit insert, extraction with 5% milk powder	yes	
RS-F	11	Ridascreen® FAST Sesame R7202, R-Biopharm				as per kit instructions	no	
SP	1	SensiSpec ELISA Sesame, Eurofins						
SP	8	SensiSpec ELISA Sesame, Eurofins	detects sesame proteins	16-32%		as per kit instructions	yes	

5.1.2 PCR-Methods

Method Abr.	Evalu- ation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantita- tive result as
			qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg				
ASU	3		positive		positive		positive		positive		positive		negative		10			prefered as almond
ASU	8	28.05.20	positive		positive		positive		positive		positive		negative		10			Sesame-DNA
ASU	12	06.08.20	positive		positive		positive		positive		positive		negative					Sesame-DNA
SFA	11		positive	1,57	positive	5,16	positive	5,35	positive	0,875	positive	0,865	negative	< 1	0,4	1		Sesame
div	2	06.03.20	positive		positive		positive		positive		positive		negative					
div	4	02.06.20	positive		positive		positive		positive		positive		negative		10 haploide genomic copies			Sesame-DNA
div	9	03.07.20	positive		positive		positive		positive		positive		negative		25			Please select!

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation details by participants: PCR-Methods

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in sesame (According to method prescription)	Conversion for processed sesame	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	%	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
ASU	3	ASU §64 Methode/method	66 bp from 2S Albumin				yes	
ASU	8	ASU §64 Methode/method				CTAB, Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	§64 LFGB L 18.00-19:2014-08
ASU	12	ASU §64 Methode/method	2S Albumin			MericonFood Kit (Qiagen)	yes	
SFA	11	Sure Food ALLERGEN, R-Biopharm / Congen				DNA-Isolation by SureFood PREP Advanced, Protocol 1, RealTime-PCR as per kit instructions	yes	
div	2	Anlehnung an Methode Mustorb et al 2007	64 bp long sequence fragment of genes from 2S Albumin of Sesame			Extraction: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
div	4	Hausmethode (Mustorp et al., 2008; Eur Food Res Technol 226:771-778)	2S-Albumin Gen			DNA-Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/ Guanidinium chloride buffer w ith Proteinase K, clean-up by Wizard-Kit from Promega); qualitative Real-time PCR w ith 45 Cycles	yes	
div	9		Sesamum indicum oleosin mRNA			DNA extraction w ith Dnaesy mericon Food Kit	yes	in house real time PCR method based on article "Two tetraplex real-time PCR for the detection and quantification of DNA from eight allergens in food, Eur Food Res Technol (2010) 230:367-374"

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test

DLA ptALR2 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	71	28,6
2	5,03	81	32,2
3	4,99	73	29,3
4	5,01	79	31,5
5	5,00	77	30,8
6	5,05	72	28,5
7	5,02	76	30,3
8	5,03	66	26,2

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	74,4	Particles
Standard deviation	4,83	Particles
χ^2 (CHI-Quadrat)	2,20	
Probability	95	%
Recovery rate	114	%

Normal distribution

Number of samples	8	
Mean	29,7	mg/kg
Standard deviation	1,93	mg/kg
rel. Standard deviation	6,5	%
Horwitz standard deviation	9,6	%
HorRat-value	0,68	
Recovery rate	114	%

Microtracer Homogeneity Test

DLA ptALR2 Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	83	32,9
2	5,00	74	29,6
3	5,02	76	30,3
4	5,04	77	30,6
5	4,97	68	27,4
6	5,01	77	30,7
7	5,03	75	29,8
8	5,02	70	27,9

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	75,0	Particles
Standard deviation	4,35	Particles
χ^2 (CHI-Quadrat)	1,77	
Probability	97	%
Recovery rate	144	%

Normal distribution

Number of samples	8	
Mean	29,9	mg/kg
Standard deviation	1,73	mg/kg
rel. Standard deviation	5,8	%
Horwitz standard deviation	9,6	%
HorRat-value	0,60	
Recovery rate	144	%

Microtracer Homogeneity Test**DLA ptALR2 Sample 3**

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	49	19,6
2	5,01	47	18,8
3	5,03	43	17,1
4	5,04	59	23,4
5	5,04	51	20,2
6	5,00	50	20,0
7	4,97	46	18,5
8	5,05	52	20,6

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	49,6	Particles
Standard deviation	4,65	Particles
χ^2 (CHI-Quadrat)	3,05	
Probability	88	%
Recovery rate	75	%

Normal distribution

Number of samples	8	
Mean	19,8	mg/kg
Standard deviation	1,85	mg/kg
rel. Standard deviaton	9,4	%
Horwitz standard deviation	10,2	%
HorRat-value	0,92	
Recovery rate	75	%

Microtracer Homogeneity Test**DLA ptALR2 Sample 4**

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,05	56	22,2
2	5,05	59	23,4
3	5,03	60	23,9
4	5,00	59	23,6
5	5,00	53	21,2
6	5,01	58	23,2
7	5,02	49	19,5
8	4,95	53	21,4

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	55,9	Particles
Standard deviation	3,76	Particles
χ^2 (CHI-Quadrat)	1,77	
Probability	97	%
Recovery rate	103	%

Normal distribution

Number of samples	8	
Mean	22,3	mg/kg
Standard deviation	1,50	mg/kg
rel. Standard deviaton	6,7	%
Horwitz standard deviation	10,0	%
HorRat-value	0,67	
Recovery rate	103	%

Microtracer Homogeneity Test

DLA ptALR2 Sample 5

Weight whole sample	1,02	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	54	21,6
2	4,98	55	22,1
3	4,98	61	24,5
4	5,01	62	24,8
5	5,05	56	22,2
6	5,01	55	22,0
7	4,98	60	24,1
8	4,98	59	23,7

Poisson distribution

Number of samples	8	
Degree of freedom	7	
Mean	57,8	Particles
Standard deviation	3,20	Particles
χ^2 (CHI-Quadrat)	1,24	
Probability	99	%
Recovery rate	83	%

Normal distribution

Number of samples	8	
Mean	23,1	mg/kg
Standard deviation	1,28	mg/kg
rel. Standard deviaton	5,5	%
Horwitz standard deviation	10,0	%
HorRat-value	0,56	
Recovery rate	83	%

5.3 Information on the Proficiency Test (PT)

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

<i>PT number</i>	DLA ptALR2 (2020)
<i>PT name</i>	Response PT Sesame: Processed samples white sesame (ground), black sesame (ground), sesame paste (Tahini, roasted), vegetarian spread (heated) and salt crackers (baked) in potato powder matrix (levels: 25 - 150 mg/kg)
<i>Sample matrix (processing)</i>	Samples 1-6: Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)
<i>Number of samples and sample amount</i>	5 different Samples: 20 g each + 1 "Blank" Sample: 20 g
<i>Storage</i>	Samples 1-6: room temperature (PT period), cooled 2 - 10°C (long term)
<i>Intentional use</i>	Laboratory use only (quality control samples)
<i>Parameter</i>	qualitative + quantitative: Sesame / Sesame Protein / DNA from Sesame Samples 1-5: approx. 25 - 150 mg/kg (as total sesame)
<i>Methods of analysis</i>	Analytical methods are optional
<i>Notes to analysis</i>	The analysis of PT samples should be performed like a routine laboratory analysis. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.
<i>Result sheet</i>	One result each should be determined for Samples 1 - 6 and the results should be filled in the result submission file. In case of several determinations the mean.
<i>Units</i>	mg/kg
<i>Number of digits</i>	at least 2
<i>Result submission</i>	The result submission file should be sent by e-mail to: pt@dla-lvu.de
<i>Deadline</i>	the latest <u>July 31st 2020</u>
<i>Evaluation report</i>	The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.
<i>Coordinator and contact person of PT</i>	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 in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		Germany
		Germany
		USA
		CANADA
		Germany
		Germany
		Germany
		Germany
		FINLAND
		Germany
		AUSTRIA
		AUSTRIA

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswertebereichs nicht angegeben.]

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung - Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment - General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Analytical Laboratories ; J.AOAC Int., 76(4), 926 - 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 - 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. DIN EN ISO 15633-1:2009; Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
20. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
21. DIN EN ISO 15842:2010 Lebensmittel - Nachweis von Lebensmittelallergenen - Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
22. Ministry of Health and Welfare, JSM, Japan 2006
23. Working Group Food Allergens, Abbott et al., Validation Procedures for

- Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices
JAOAC Int. 93:442-50 (2010)
24. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
 25. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
 26. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes¹, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
 27. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
 28. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
 29. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
 30. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contaminations in foodstuffs by ELISA in microtiterplates]
 31. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contaminations in chocolate and chocolate products by ELISA in microtiterplates]
 32. ASU §64 LFGB L 18.00-19 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Sesam (Sesamum indicum) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of sesame (Sesamum indicum) in rice and wheat cookies and sauce powders by PCR]
 33. ASU §64 LFGB L 18.00-22 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]
 34. ASU §64 LFGB L 08.00-59 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Senf (Sinapis alba) sowie Soja (Glycine max) in Brühwürsten mittels real-time PCR (2013) [Foodstuffs, detection and determination of mustard (Sinapis alba) and soya (Glycine max) in boiled sausages by real-time PCR]
 35. ASU §64 LFGB L 08.00-64 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von von schwarzem Senf (Brassica nigra L.) und braunem Senf (Brassica juncea L.) in Brühwurst mittels real-time PCR (2016) [Foodstuffs, detection and determination of black mustard (Brassica nigra L.) and brown mustard (Brassica juncea L.) in boiled sausages by real-time PCR]
 36. ASU §64 LFGB L 08.00-65 Untersuchung von Lebensmitteln - Simultaner Nachweis und Bestimmung von schwarzem Senf (Brassica nigra L.), braunem Senf (Brassica juncea L.), weißem Senf (Sinapis alba), Sellerie (Apium graveolens) und Soja (Glycine max) in Brühwurst mittels real-time PCR (2017) [Foodstuffs, simultaneous detection and determination of black mustard (Brassica nigra L.), brown mustard (Brassica juncea L.), white mustard (Sinapis alba), celery (Apium graveolens) and soya (Glycine max) in boiled sausages by real-time PCR]