Evaluation Report
proficiency test

DLA ptALR1 (2021)

Response PT Peanut:

5 processed Samples Peanut (unroasted), Peanut (roasted), Peanut Butter, Peanut Paste and Extrudate (Peanut Puffs)

in Potato Powder Matrix

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1st Correction 10/11/2021:
On pages 17 and 18 the explanations of abbreviations for the PCR and ELISA methods were incorrectly given. This has been corrected.

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

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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: 10 November 2021
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), 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].

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 peanut 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 peanut (peanut protein) in peanut (roasted, unroasted), peanut butter, peanut paste and extrudate (peanut puffs) in potato powder / maltodextrin were provided.

The respective raw materials for the PT sample series were common in commerce processed peanut products. For each PT-sample 4-18 products of different origin were worked up. The peanut paste was dried at 60°C prior to further use.
Afterwards premixes with contents from approx. 1,6 - 5 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed if necessary, mixed gravimetrically with further ingredients, crushed by a ball mill or crushed and sieved by means of a centrifugal mill (mesh 250 µm) and homogenized.
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 peanut of the PT-samples were approx. 20 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 peanut 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** Peanut Butter	**Sample 2** Peanuts, roasted	**Sample 3** Peanuts, unroasted	**Sample 4** Peanut Paste	**Sample 5** Peanut Extrudate	**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	75	75
Maltodextrin	25	25	25	25	25	25
Allergen-Premixes Ingredients: maltodextrin (75% - 90%), sodium sulfate (< 5%), silicon dioxide (< 2,5%), processed allergen products (each 1,6% - 5% dry weight)	0,047	0,040	0,041	0,11	0,13	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Peanut butter** (90% Peanut and other ingredients) Protein 21,7% ** *(6 products from USA*	23,4	-	-	-	-	-
*Peanuts, roasted** Protein 23,2% ** (18 products from USA, Asia, Africa and South America)	-	19,9	-	-	-	-
*Peanuts, unroasted** Protein 23,1% ** *(9 products from Africa, Asia, South America)*	-	-	20,5	-	-	-
*Peanut paste** (36% Peanut and other ingredients) Total protein 11,0% ** *(5 products seasoning sauces from Asia or asian style)*	-	-	-	55,8	-	-
*Peanut Extrudate** (32% Peanut and other ingredients) Total protein 11,2% ** *(7 products peanut puffs from Europe and USA)*	-	-	-	-	65,9	-
– *thereof Peanut*	21,1	19,9	20,5	20,1	21,1	-
Extended combined uncertainty (k=2) of peanut-content (= ± 12 %)	± 2,53	± 2,34	± 2,46	± 2,41	± 2,53	-

*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=5,46 for peanut protein)

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 **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1 to 5 showed a probability of 98%, 75%, 81%, 95% and 81%. 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 0,55, 0,93, 1,1, 0,68 and 0,97 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. 0,24 - 0,25 (20 - 23°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 15th week of 2021. The testing method was optional. The tests should be finished at June 11th 2021 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 peanut, 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 peanut (unroasted), peanut (roasted), peanut butter, peanut paste ("asian" spice sauces) and extrudate (maize peanut snacks) with known amounts of total peanut / peanut protein, which is the base for the response comparison of the quantitative results of the participants.*
- *Please give all your quantitative results as total peanut, if possible indicate the underlying total protein content in peanuts.*
- *Possible conversion factors for processed peanut 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 9 participants submitted results at least for one method.

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 peanut, peanut (roasted), peanut (unroasted), peanut butter, peanut paste, and extrudate (peanut puffs), were provided to determine the qualitative detectability and to determine the response in 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 ≥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 Peanut Butter	Sample 2 Peanuts, roasted	Sample 3 Peanuts, unroasted	Sample 4 Peanut Paste	Sample 5 Extrudate	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 sucessful
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-ELISAs 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 48% - 145% 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	σpt	Method / Literature
Peanut	Milk chocolate	173,7 33,8 5,9	87 % 85 % 59 %	- - -	8,8% 5,2% 7,8%	31% 20% 31%	30,4% 19,7% 30,5%	ELISA Manuf. A ASU 00.00-69
Peanut	Milk chocolate	215,7 40,1 10,1	108 % 100 % 101 %	- - -	5,9% 7,2% 7,3%	32% 14% 16%	31,7% 13,0% 15,1%	ELISA Manuf. B ASU 00.00-69
Peanut	Dark chocolate	148,2 30,9 5,7	74 % 77 % 57 %	- - -	6,0% 13% 6,1%	22% 25% 33%	21,6% 23,2% 32,7%	ELISA Manuf. A ASU 00.00-69
Hazelnut	Dark chocolate	16,3 7,56 3,73 1,62	81 % 76 % 75 % 81 %	- - - -	4,7% 8,9% 13% 15%	12% 15% 24% 33%	11,5% 13,6% 22,2% 31,2%	ELISA Manuf. A ASU 44.00-7
Hazelnut	Dark chocolate	21,3 10,7 4,69 2,37	106 % 107 % 94 % 119 %	- - - -	7,1% 11% 11% 9,3%	14% 19% 17% 17%	13,1% 17,3% 15,1% 16,4%	ELISA Manuf. B ASU 44.00-7

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 [32-35].

Parameter	Matrix	Mean [mg/kg]	Recov-ery	rob RSD	RSD_r	RSD_R	σpt	Method / Literature
Peanut	Rice cookie	23,4 5,19	113 % 99,7 %	15,6% 15,0%	11,6% 14,7%	14,4% 18,1%	11,8% 14,8%	rt-PCR ASU 00.00-169
Peanut	Wheat cookie (DLA)	1,97	39,3 %	16,2%	16,0%	19,5%	15,8%	rt-PCR ASU 00.00-169
Peanut	Milk powder Boiled sausa-ge	3,66 2,44	73,2 % 49,4 %	15,8% 15,6%	12,8% 11,9%	14,8% 15,9%	11,7% 13,5%	rt-PCR ASU 00.00-169
Almond	Rice cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR multiplex ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR multiplex ASU 18.00-22
Brazil nut	Rice cookie	89,1 17,3 9,8	89,1 % 86,5 % 98 %	-	34,1% 36,2% 40,2%	34,4% 38,2% 41,8%	24,5% 28,4% 30,6%	rt-PCR ASU 18.00-21
Brazil nut	Wheat cookie Sauce powder	80,8 42,6	65,7 % 42,6 %	-	25,6% 27,5%	36,4% 39,7%	31,6% 34,6%	rt-PCR ASU 18.00-21
Brazil nut	Rice cookie	96,6 14,2	96,6 % 71 %	-	16,8% 54,2%	31,8% 56,5%	29,5% 41,5%	rt-PCR multiplex ASU 18.00-22
Brazil nut	Wheat cookie Sauce powder	76,5 48,4	62,2 % 48,4 %	-	15,6% 34,4%	35,8% 37,5%	34,1% 28,5%	rt-PCR multiplex ASU 18.00-22

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 Commitee (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:

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

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 ($U_{(Xpt)}$) [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.

In the present PT one result was given as peanut protein and converted into the total peanut-content with the experimentally determined protein content of raw materials for roasted and unroasted peanuts of 23% (see Table 1, p.6). All other ELISA and PCR results were submitted as peanut, therefore no recalculation was necessary.

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 *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		

* Recovery Rate

4.1 Proficiency Test Processed Peanut Products

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Sample 1 Peanut Butter	Sample 2 Peanuts, roasted	Sample 3 Peanuts, unroasted	Sample 4 Peanut Paste	Sample 5 Peanut Extrudate	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%)	BK	
4	positive	positive	positive	positive	positive	negative	5 (100%)	MI-II	
2a	positive	positive	positive	positive	positive	negative	5 (100%)	RS	
2b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
8	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
7	positive	positive	positive	positive	positive	negative	5 (100%)	SP	
1	positive	positive	positive	positive	positive	negative	5 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	8	8	8	8	8	0
Number negative	0	0	0	0	0	8
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:

BK = BioKits, Neogen

MI-II = Morinaga Institute ELISA Kit II

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

VT = Veratox, Neogen

Comments:

For all processed products (samples 1 to 5) consensus values of 100% positive results were obtained by the ELISA-methods.

4.1.2 Qualitative Scores: PCR-Methods

Evaluation number	Sample 1 Peanut Butter	Sample 2 Peanuts, roasted	Sample 3 Peanuts, unroasted	Sample 4 Peanut Paste	Sample 5 Peanut Extrudate	Sample 6 „blank“	Score qualitativee	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
2	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
4	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	Sample 4: traces at LOD
9	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
3	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
5	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
8	positive	positive	positive	positive	positive	negative	5 (100%)	div	Samples 4+5 w eakly positivee

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positivee	6	6	6	6	6	0
Number negativee	0	0	0	0	0	6
Percent positivee	100	100	100	100	100	0
Percent negativee	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 / Cong

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. Two participants reported a qualitatively lower response for samples 4 or 4 and 5 (peanut paste and peanut extrudate) ("at LOD" or "weakly positive").

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

Evaluation number	Sample 1 Peanut Butter			Sample 2 Peanuts roasted			Sample 3 Peanuts unroasted			Sample 4 Peanut Paste			Sample 5 Peanut Extrudate			RR- score	Method	Remarks
	Result	RR *		Result	RR *		Result	RR *		Result	RR *		Result	RR *		RR *		
	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	[mg/kg]	[%]	[Z_{WFR}]	Number in RA**		
3	37,4	177	3,1	42,1	212	4,5	82,5	402	12	9,43	47	-2,1	9,21	44	-2,3	0/5 (0%)	BK	
4	29,0	**137**	**1,5**	37,4	188	3,5	33,9	165	2,6	13,9	**69**	**-1,2**	20,9	**99**	**-0,04**	3/5 (60%)	MI-II	result converted °
2a	34,7	164	2,6	38,4	193	3,7	84,9	414	13	11,5	**57**	**-1,7**	9,50	45	-2,2	1/5 (20%)	RS	
2b	46,2	219	4,8	52,3	263	6,5	98,5	480	15	16,7	**83**	**-0,68**	13,1	**62**	**-1,5**	2/5 (40%)	RS-F	
6	67,4	319	8,8	65,7	330	9,2	151	737	25	19,8	**99**	**-0,06**	12,1	**57**	**-1,7**	2/5 (40%)	RS-F	
8	47,0	223	4,9	52,3	263	6,5	59,3	289	7,6	11,6	**58**	**-1,7**	12,2	**58**	**-1,7**	2/5 (40%)	RS-F	
7	40,8	193	3,7	42,3	213	4,5	74,4	363	11	11,6	**58**	**-1,7**	12,7	**60**	**-1,6**	2/5 (40%)	SP	
1	34,5	164	2,5	40,0	201	4,0	69,6	339	10	7,36	37	-2,5	8,99	43	-2,3	0/5 (0%)	VT	

° calculation p. 15

RA**	50-150 %		RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %
Number in RA	1	0	Number in RA	0	Number in RA	0	Number in RA	6	Number in RA	5
Percent in RA	13	0	Percent in RA	0	Percent in RA	0	Percent in RA	75	Percent in RA	63

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

** Acceptance range of AOAC for allergen ELISAs

Methods:

BK = BioKits, Neogen

MI-II = Morinaga Institute ELISA Kit II

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

SP = SensiSpec ELISA Kit, Eurofins

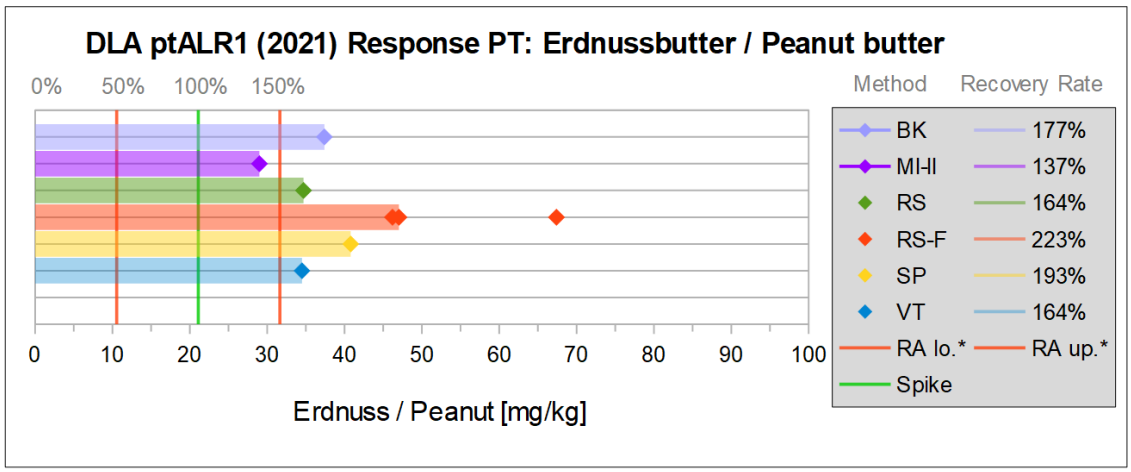
VT = Veratox, Neogen

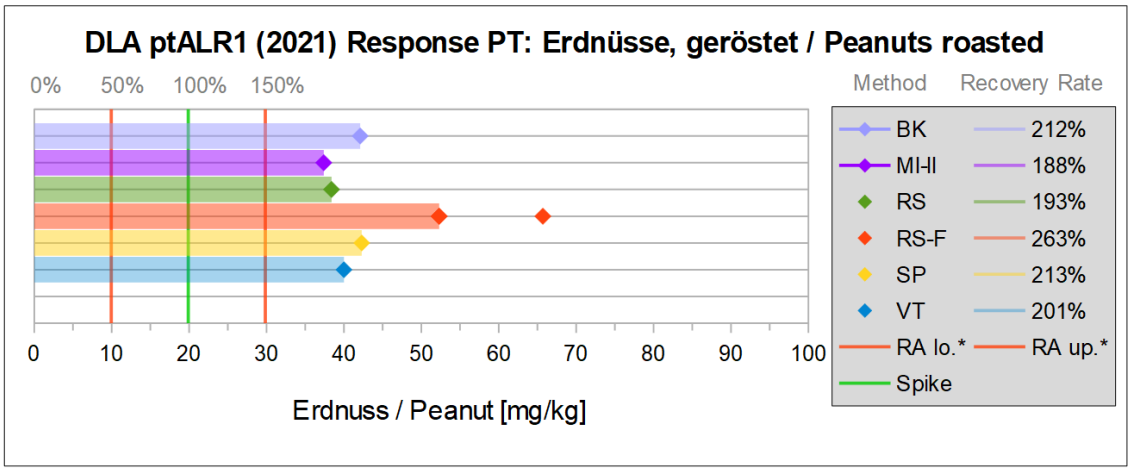
Comments:

With one exception, for the samples 1 (peanut butter), 2 (peanuts, roasted) and 3 (peanuts, unroasted) none of the recovery rates of the participant results were in the range of acceptance of 50-150%.
Clearly higher values were predominatly obtained. The recovery rate of method MI for sample 1 was within the range of acceptance. For samples 4 (peanut paste) and 5 (peanut extrudate), 75% and 63% of the recovery rates of the participant results were in the range of acceptance of 50-150%. The other recoveries were below 50%.

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

No quantitative results were submitted for the PCR methods.





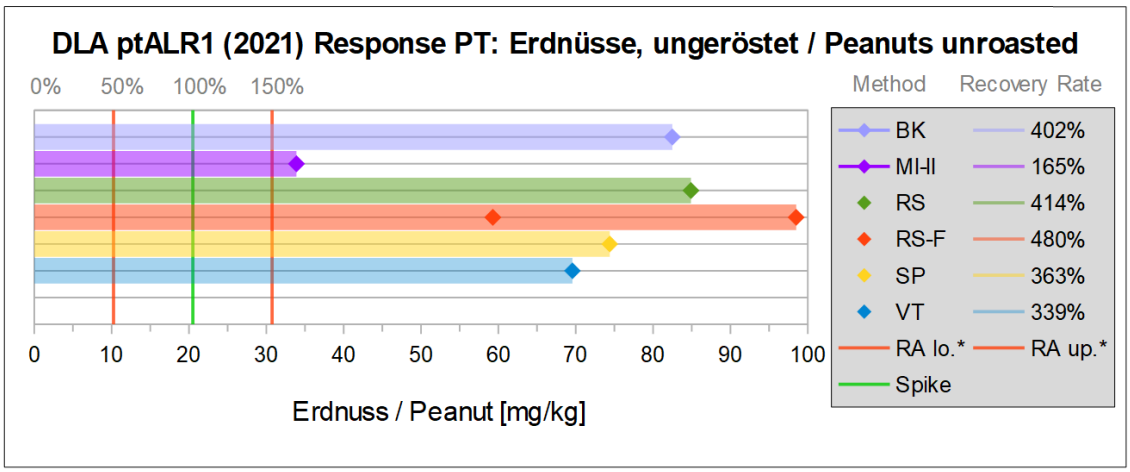
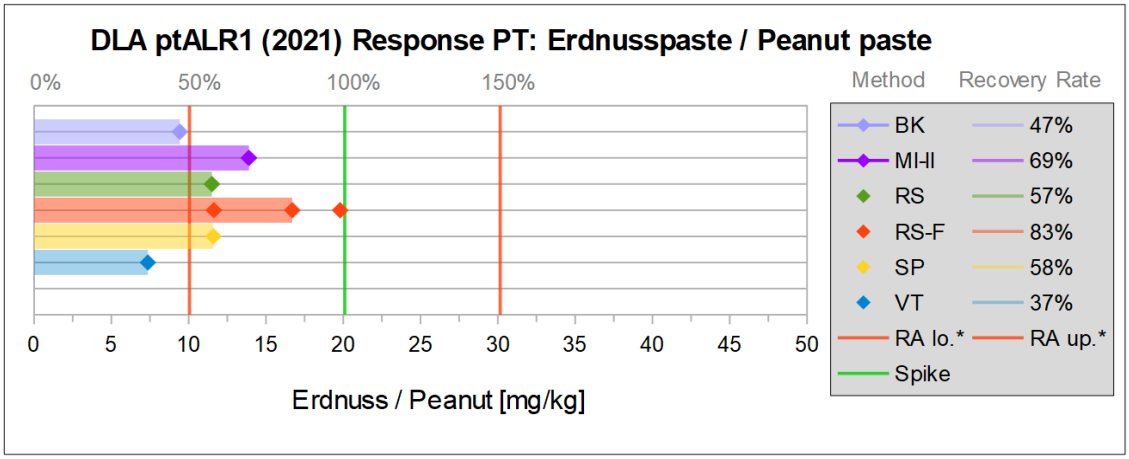


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale peanut content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



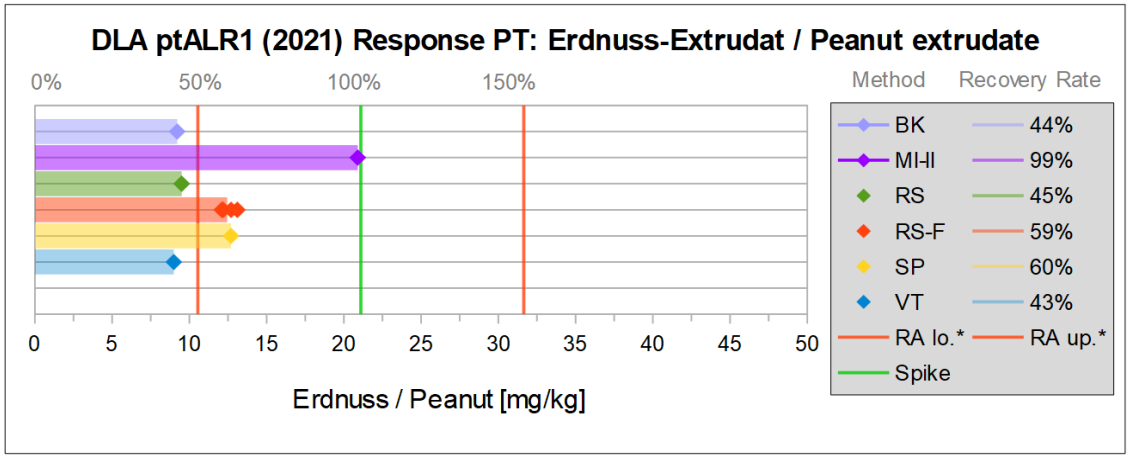


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale peanut content in mg/kg, upper scale recovery rate in %, with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.2 Participant z-Scores: overview table

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

Evaluation number	ELISA Peanut					PCR Peanut				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	2,5	4,0	10	-2,5	-2,3					
2a	2,6	3,7	13	-1,7	-2,2					
2b	4,8	6,5	15	-0,68	-1,5					
3	3,1	4,5	12	-2,1	-2,3					
4	1,5	3,5	2,6	-1,2	-0,04					
5										
6	8,8	9,2	25	-0,06	-1,7					
7	3,7	4,5	11	-1,7	-1,6					
8	4,9	6,5	7,6	-1,7	-1,7					
9										

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 quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	preferred as Peanut
BK	3	04.05.21	-	37,41	-	42,14	-	82,49	-	9,43	-	9,21	-	< 1		1		Peanut
MI-II	4	07.05.21	positive	6,7	positive	8,6	positive	7,8	positive	3,2	positive	4,8	negative	<0,2	0,2	0,2		Peanutprotein
RS	2a	02.06.21	positive	34,7	positive	38,4	positive	84,9	positive	11,5	positive	9,5	negative		-	-		Peanut
RS-F	2b	22.04.21	positive	46,2	positive	52,3	positive	98,5	positive	16,7	positive	13,1	negative		2,5	2,5		Peanut
RS-F	6	01.06.	positive	67,4	positive	65,7	positive	151	positive	19,8	positive	12,1	negative	< 2,5	0,13	2,5		Peanut
RS-F	8	07.05.21	positive	47,03	positive	52,31	positive	59,28	positive	11,63	positive	12,19	negative	<2,5	0,13	2,5		Peanut
SP	7	20.04.21	positive	40,8	positive	42,3	positive	74,4	positive	11,6	positive	12,7	negative	0	0,1	1		Peanut
VT	1	03.05.2021	positive	34,5	positive	40	positive	69,58	positive	7,36	positive	8,99	negative	0	N/A	2,5	N/A	Peanut

* NWG Nachw eisgrenze / BG Bestimmungsgrenze
* LOD limit of detection / LOQ limit of quantitation
* MU Messunsicherheit / MU measurement uncertainty

Continuation details by participants: ELISA-Methods

Method Abr.	Evalu-ation number	Method	Specificity	Total protein content in peanut (According to method instructions)	Conversion for processed peanut	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	
BK	3	BioKits Peanut Assay Kit, Neogen	Conarachin (Ara h1)			as per kit instructions	yes	
MI-II	4	Peanut ELISA Kit-II, Morinaga	detects peanut proteins	approx. 25		as per kit instructions	yes	M2120
RS	2a	Ridascreen Peanut (R6811), r-biopharm					yes	New RB Kit ref No. 6811. not validated
RS-F	2b	Ridascreen Fast Peanut (R6202), r-Biopharm					yes	
RS-F	6	Ridascreen Fast Peanut (R6202), r-Biopharm				as per kit instructions w ith skimmed milk pow der	yes	
RS-F	8	Ridascreen Fast Peanut (R6202), r-Biopharm	Ara h1 Ara h2	25		as per kit instructions	yes	
SP	7	Eurofins SensiSpec Peanut ELISA Kit						
VT	1	Veratox Peanut, Neogen	N/A	25,8	N/A	as stipulated in kit insert	yes	recovery in sample 6 (111%)

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 quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as peanut
ASU	2		positive		positive		positive		positive		positive		negative		2			Peanut-DNA
ASU	4	28.05.21	positive		positive		positive		positive*		positive		negative		5			Peanut-DNA
ASU	9	02.06.21	positive		positive		positive		positive		positive		negative		0,1 pg			Peanut-DNA
SFA	3	10./12./18. 05.21	positive		positive		positive		positive		positive		negative					Peanut-DNA
SFA	5	07.05.21	positive		positive		positive		positive		positive		negative		0,4	1	30%	Peanut
div	8	04.05.21	positive		positive		positive		positive		positive		negative					Peanut-DNA

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation details by participants: PCR-Methods

Method Abr.	Evalu-ation Number	Method	Specificity	Total protein content in peanut (According to method instructions)	Conversion for processed peanut	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	2	ASU §64 Methode/method				Macherey & Nagel NucleoSpin Food Kit	yes	ASU L 00.00-169 (2019-07)
ASU	4	ASU §64 Methode/method				CTAB / Proteinase K / Rnase A / Promega Maxw ell / Real Time PCR / 45 Cycles	yes	§ 64 LFGB L 00.00-169:2019-07, Sample 4: traces at LOD
ASU	9	ASU §64 Methode/method	apt6			DNeasy mericon Food Kit (Qiagen)		
SFA	3	Sure Food ALLERGEN, R-Biopharm / Congen	characteristic sequence part of peanut DNA			SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
SFA	5	Sure Food ALLERGEN, R-Biopharm / Congen	Arachis hypogae			Sure Food Prep Advanced Protokoll 2 + 200 µl LB	no	Article no. S3603, K01
div	8	Literature method according to Hird et al. (2003) Eur. Food Res technol 217:265-268, modifiziert	Arah2 Gene			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); Real-time PCR w ith 50 Cycles	yes	Samples 4 and 5 w eakly positive

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA ptALR1 (2021) 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	33,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	75	30,0
2	5,00	75	30,0
3	4,97	74	29,8
4	5,01	70	27,9
5	5,03	75	29,8
6	5,02	77	30,7
7	4,98	66	26,5
8	5,03	79	31,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	73,9	Particles
Standard deviation	3,91	Particles
χ^2 (CHI-Quadrat)	1,45	
Probability	**98**	%
Recovery rate	88	%

Normal distribution		
Number of samples	8	
Mean	29,5	mg/kg
Standard deviation	1,56	mg/kg
rel. Standard deviaton	5,30	%
Horwitz standard deviation	9,61	%
HorRat-value	**0,55**	
Recovery rate	88	%

Microtracer Homogeneity Test
DLA ptALR1 (2021) 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	33,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	77	30,9
2	5,01	80	31,9
3	5,00	68	27,2
4	5,01	76	30,3
5	5,02	84	33,5
6	5,03	69	27,4
7	4,99	72	28,9
8	5,01	87	34,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	76,6	Particles
Standard deviation	6,81	Particles
χ^2 (CHI-Quadrat)	4,24	
Probability	**75**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	30,6	mg/kg
Standard deviation	2,72	mg/kg
rel. Standard deviaton	8,89	%
Horwitz standard deviation	9,56	%
HorRat-value	**0,93**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA ptALR1 (2021) 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	22,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	39	15,6
2	5,02	46	18,3
3	4,98	39	15,7
4	5,02	52	20,7
5	5,01	42	16,8
6	4,98	50	20,1
7	5,01	49	19,6
8	5,01	44	17,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,1	Particles
Standard deviation	4,91	Particles
χ^2 (CHI-Quadrat)	3,75	
Probability	**81**	%
Recovery rate	81	%

Normal distribution		
Number of samples	8	
Mean	18,0	mg/kg
Standard deviation	1,96	mg/kg
rel. Standard deviaton	10,9	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,1**	
Recovery rate	81	%

Microtracer Homogeneity Test
DLA ptALR1 (2021) Sample 4

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	30,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	73	29,2
2	4,99	62	24,8
3	4,98	71	28,5
4	5,02	76	30,3
5	5,00	72	28,8
6	5,00	64	25,6
7	4,98	68	27,3
8	4,98	68	27,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	69,2	Particles
Standard deviation	4,59	Particles
χ^2 (CHI-Quadrat)	2,13	
Probability	**95**	%
Recovery rate	91	%

Normal distribution		
Number of samples	8	
Mean	27,7	mg/kg
Standard deviation	1,84	mg/kg
rel. Standard deviaton	6,62	%
Horwitz standard deviation	9,70	%
HorRat-value	**0,68**	
Recovery rate	91	%

Microtracer Homogeneity Test
DLA ptALR1 (2021) Sample 5

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	54	21,6
2	5,03	52	20,7
3	5,02	55	21,9
4	5,01	70	27,9
5	5,00	55	22,0
6	5,02	58	23,1
7	4,99	57	22,8
8	5,00	56	22,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	57,1	Particles
Standard deviation	5,52	Particles
χ^2 (CHI-Quadrat)	3,73	
Probability	**81**	%
Recovery rate	103	%

Normal distribution		
Number of samples	8	
Mean	22,8	mg/kg
Standard deviation	2,20	mg/kg
rel. Standard deviaton	9,66	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,97**	
Recovery rate	103	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptALR1-2021***
PT name	***Response PT Peanut: Processed samples Peanut (unroasted), Peanut (roasted), Peanut Butter, Peanut Paste and Extrudate (Peanut-Flips) in potato powder matrix***
*Sample matrix**	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 "Blank" Sample: 20 g*
Storage	*Samples 1 - 6:* *room temperature (PT period), cooled 2 - 10°C (long term)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Peanut/ Peanut protein* *from Peanut (unroasted), Peanut (roasted), Peanut Butter, Peanut Paste and Extrudate (Peanut-Flips)* *Samples 1-5: approx. 15 - 150 mg/kg (as total peanut)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples 1 - 6 and the* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of significant digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest June 11th 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 in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		Germany
		CANADA
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany

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

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

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
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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
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Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)

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33. ASU §64 LFGB L 18.00-20 Untersuchung von Lebensmitteln - Nachweis und Bestimmung von Mandel (Prunus dulcis) in Reis- und Weizenkeksen sowie in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of almond (Prunus dulcis) in rice and wheat cookies and sauce powders by PCR]
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