Proficiency Tests
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Evaluation Report
proficiency test

DLA 15/2017

Response PT Peanut:

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

in Potato Powder Matrix

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

proficiency-testing@dla-lvu.de www.dla-lvu.de

Coordinator of this PT:
Dr. Matthias Besler-Scharf

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

EP-Anbieter *PT-Provider*	**DLA - Dienstleistung Lebensmittel Analytik GbR** Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler-Scharf Waldemar-Bonsels-Weg 170, 22926 Ahrensburg, Germany Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA 15/2017
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (16 March 2018) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 16 March 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

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 in the range of 26 to 33 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** Peanuts, roasted	**Sample 2** Peanuts, unroasted	**Sample 3** Peanut Butter	**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)	0,58	0,65	0,59	1,80	2,04	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Peanuts, roasted** Protein 23,2% ** (18 products from USA, Asia, Africa and South America)	29,0	-	-	-	-	-
*Peanuts, unroasted** Protein 23,1% ** *(9 products from Africa, Asia, South America)*	-	32,2	-	-	-	-
*Peanut butter** (90% Peanut and other ingredients) Protein 21,7% ** *(6 products from USA or american style)*	-	-	29,2	-	-	-
*Peanut paste** (36% Peanut and other ingredients) Total protein 11,0% ** *(5 products seasoning sauces from Asia or asian style)*	-	-	-	90,8	-	-
*Peanut Extrudate** (32% Peanut and other ingredients) Total protein 11,2% ** *(7 products peanut puffs from Europe and USA)*	-	-	-	-	102	-
- thereof Peanut	29,0	32,2	26,3	32,7	32,5	-
Extended combined uncertainty (k=2) of peanut-content (= ± 12 %)	± 3,48	± 3,86	± 3,16	± 3,92	± 3,90	-

*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 80%, 92%, 99%, 55% and 79%. 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) [18]. This gave a HorRat value of 0,77, 0,73, 0,45, 1,1 and 0,79 respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameter peanut for comparable food matrices and water activity (a_W value <0,5). The stability of the sample material is therefore given during the investigation period under consideration of given 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 48th week of 2017. The testing method was optional. The tests should be finished at January 26th 2018 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 or protein 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.

10 participants submitted 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 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 Peanuts, roasted	Sample 2 Peanuts, unroasted	Sample 3 Peanut Butter	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[31-32].

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 [25]. 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 1 – 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 [25].

The IRMM (Institute for Reference Materials and Measurements) proved the suitability of five different ELISA-Kits for the determination of peanut [28]. 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 - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations from experiments by precision and the resulting target standard deviation σ_{pt} [34-36].

Parameter	**Matrix**	**Mean [mg/kg]**	**Reco-very**	**rob RSD_r**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
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 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 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
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9

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 [23], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [20-22], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [24] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [19].

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 [19-25]	**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 [19]	**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%.

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-, PCR- and LC/MS 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 by recalculation to total peanut content.

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.5). 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 Peanuts, roasted	Sample 2 Peanuts, unroasted	Sample 3 Peanut butter	Sample 4 Peanut paste	Sample 5 Extrudate	Sample 6 „Blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg			
1	positive	positive	positive	positive	positive	negative	5 (100%)	BF	
7b	positive	positive	positive	positive	positive	negative	5 (100%)	EF	
10	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
7a	positive	positive	positive	positive	positive	negative	5 (100%)	MI	
2	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
3	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
5	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
9	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
8	positive	positive	positive	positive	positive	negative	5 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	10	10	10	10	10	0
Number negative	0	0	0	0	0	10
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:

BF = MonoTrace ELISA, BioFront Technologies

EF = Eurofins SensiSpec

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

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 Peanuts, roasted	Sample 2 Peanuts, unroasted	Sample 3 Peanut butter	Sample 4 Peanut paste	Sample 5 Extrudate	Sample 6 „Blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg			
3	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-ID	
4	positive	positive	positive	positive	positive	positive	5/6 (83%)	SFA-ID	„Blank“ was evaluated positively
9	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-ID	
6	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-Q	
5	positive	positive	positive	negative	negative	negative	3 (60%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	5	5	5	4	4	1
Number negative	0	0	0	1	1	4
Percent positive	100	100	100	80	80	20
Percent negative	0	0	0	20	20	80
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

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

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

For the processed products of samples 1 to 3 consensus values of 100% positive results were obtained. For sample 4 (peanut paste) and sample 5 (extrudate) a negative result were obtained by an in-house-method, thus the consensus value was 80% positive results each. One participant evaluated the "Blank"-sample as positive.

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

Evaluation number	Sample 1 Peanuts roasted		Sample 2 peanuts unroasted		Sample 3 Peanut Butter		Sample 4 Peanut Paste		Sample 5 Extrudate (Peanut-flips)		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**		
1	88,9	307	183	568	56,0	213	7,50	23	23,6	**73**	1/5 (20%)	BF	
7b	74,0	255	150	466	57,0	217	19,0	**58**	21,0	**65**	2/5 (40%)	EF	
10	59,2	204	98,0	304	44,5	169	14,4	44	14,6	45	0/5 (0%)	IL	
7a	10,4	36	13,2	41,0	7,60	29	3,20	10	6,40	20	0/5 (0%)	MI	
2	69,9	241	173	537,3	50,3	191	17,7	**54**	13,6	42	1/5 (20%)	RS-F	
3	79,0	272	160	496,9	48,0	183	19,0	**58**	18,0	**55**	2/5 (40%)	RS-F	
5	60,0	207	147	456,5	49,0	186	27,0	**83**	19,0	**58**	2/5 (40%)	RS-F	
6	465 (107)	(369)	782 (180)	(559)	217 (50)	(190)	117 (27)	(83)	65,2 (15)	(46)	0/5 (0%)	RS-F	result converted ° (in parentheses given as peanut protein)
9	81,6	281	190	589,8	51,0	194	20,6	**63**	17,2	**53**	2/5 (40%)	RS-F	
8	78,0	269	158	490,7	63,0	240	20,0	**61**	22,0	**68**	2/5 (40%)	VT	

° Calculation p. 14

RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %
number in RA	**0**	number in RA	**0**	number in RA	**0**	number in RA	**6**	number in RA	**6**
percent in RA	**0**	percent in RA	**0**	percent in RA	**0**	percent in RA	**67**	percent in RA	**67**

*Recovery rate 100% Reference value: Peanut, see page 6

** Acceptance range of AOAC for allergen ELISAs

Methods:

BF = MonoTrace ELISA, BioFront Technologies
EF = Eurofins Sensispec
IL = Immunolab
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

For the samples 1 (peanuts, roasted), 2 (peanuts, unroasted) and 3 (peanut butter) none of the recovery rates of the participants' results were in the range of acceptance of 50-150%. Clearly higher values were obtained, with the exception of the results of method MI, where the recovery rates were below the range of acceptance. For samples 4 (peanut paste) and 5 (peanut extrudate), 67% of the recovery rates of the participants' results were in the range of acceptance of 50-150%. The remaining recoveries were below 50%.

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

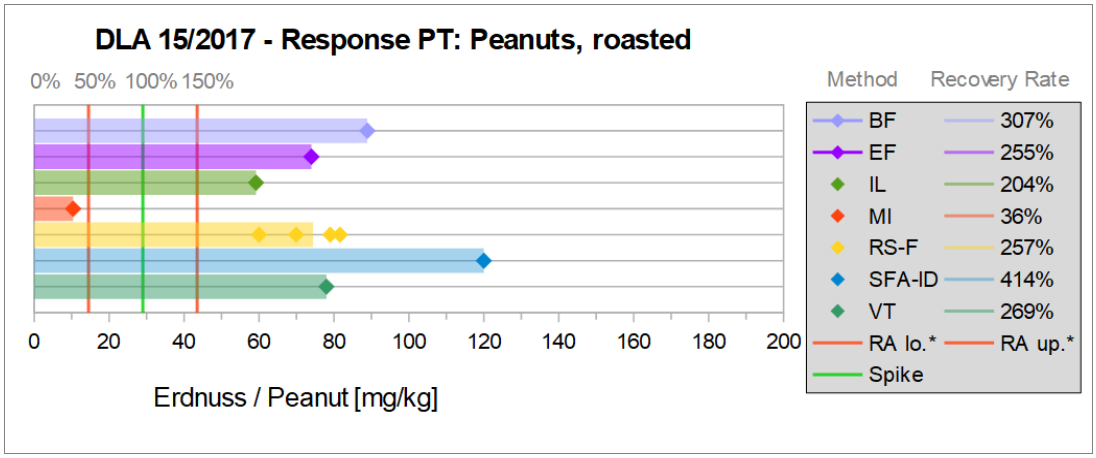
Evaluation number	Sample 1 Peanuts, roasted		Sample 2 Peanuts, unroasted		Sample 3 Peanut Butter		Sample 4 Peanut Paste		Sample 5 Extrudate (Peanut-flips)		RR- score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA **		
3	>1		>1		>1		>1		>1			SFA-ID	
4	120	414	40,0	**124**	12,0	46	1,00	3,1	5,80	18	1/5 (20%)	SFA-ID	Blank-sample positive: 2,9 mg/kg
9												SFA-ID	
6	<4		<4		<4		<4		<4			SFA-Q	
5												div	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	number in RA	0	number in RA	1	number in RA	0	number in RA	0	number in RA	0			
	percent in RA	0	percent in RA	100	percent in RA	0	percent in RA	0	percent in RA	0			

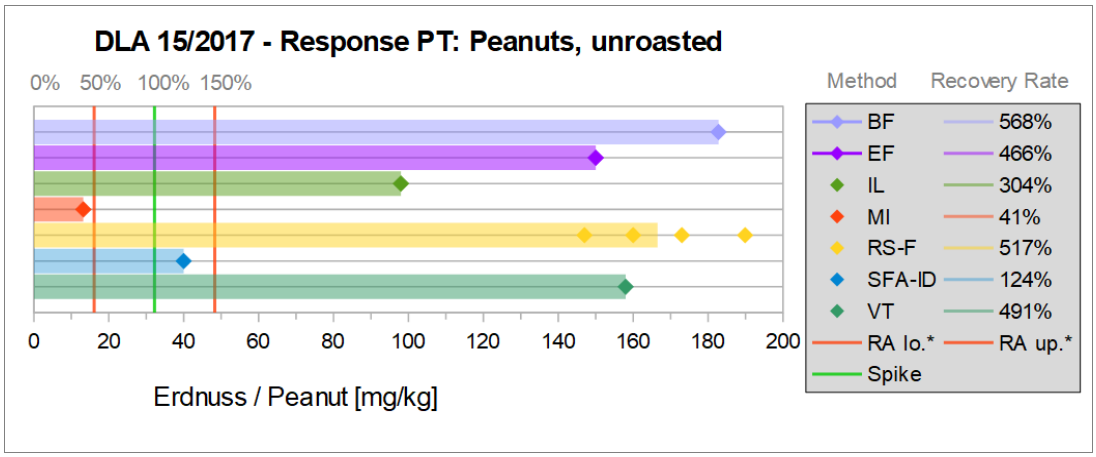
Methods:
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

* Recovery rate 100% Reference value: Peanut, see page 6

** Acceptance range of AOAC for allergen ELISAs

Comments:
Only one participant has indicated quantitative results by PCR-method. The recovery rate for sample 2 (peanuts, unroasted) was in the range of acceptance of 50 - 150%.





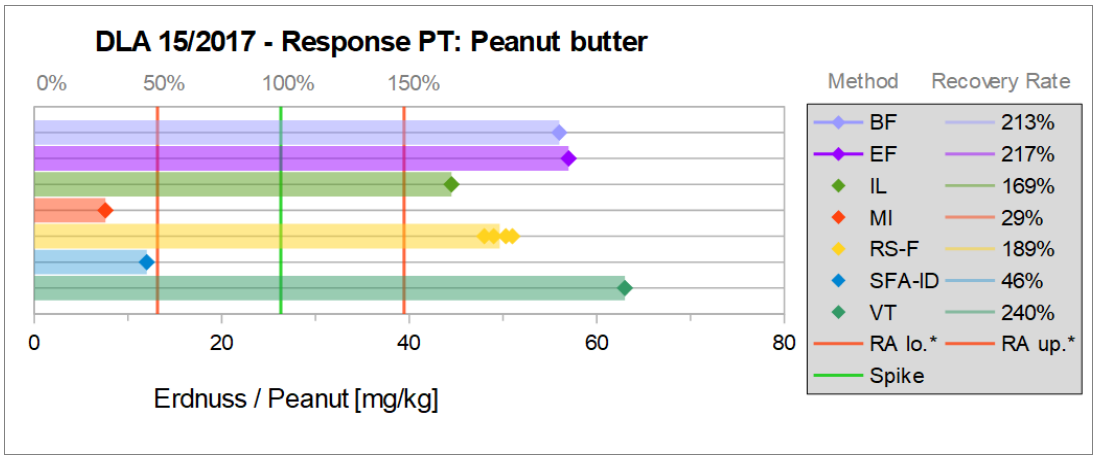
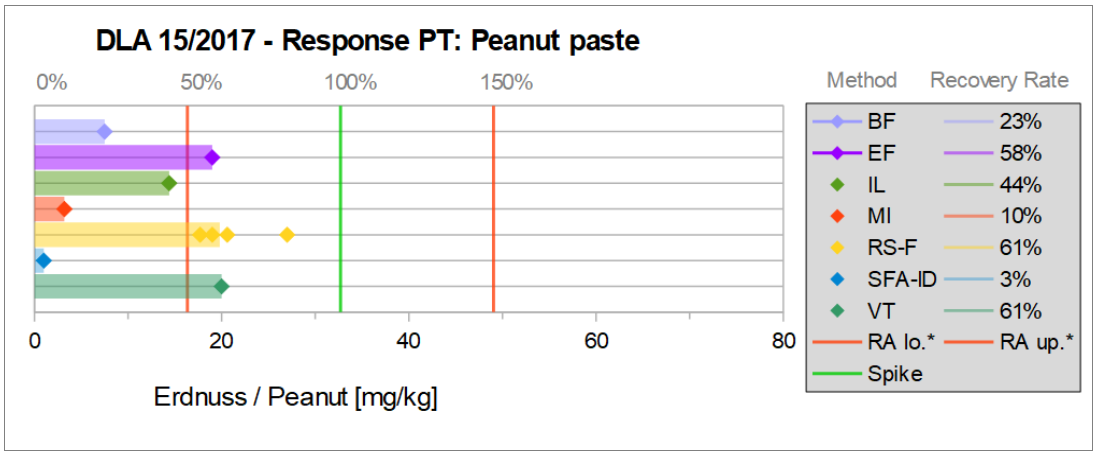


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale peanut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



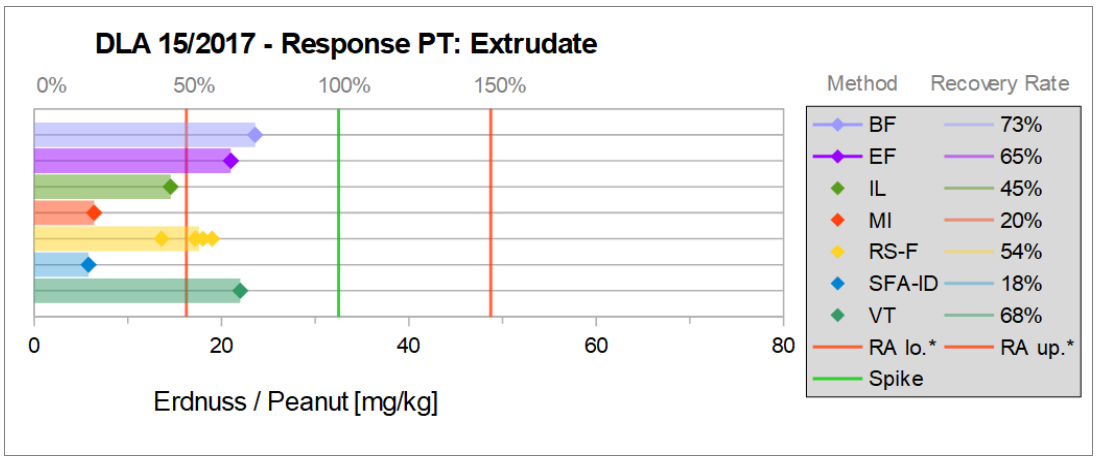


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale peanut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

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.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		LOD	LOQ	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	preferred as Peanut
BF	1	26.01.18	positive	88,9	positive	182,8	positive	56	positive	7,5	positive	23,6	negative	0	0,24	1	Peanut
EF	7b	10.01.18	positive	74	positive	150	positive	57	positive	19	positive	21	negative	<1	0,1	1	Peanut
IL	10	05.12.17	positive	59,2175	positive	97,98	positive	44,5075	positive	14,397	positive	14,57075	negative	0	0,1	1	Peanut
MI	7a	13.12.17	positive	10,4	positive	13,2	positive	7,6	positive	3,2	positive	6,4	negative	<1,2	0,48	1,2	Peanut
RS-F	2	10.01.18	positive	69,9	positive	173	positive	50,3	positive	17,7	positive	13,6	negative	< 2,5	0,13	< 2,5	Peanut
RS-F	3	19.01.18	positive	79	positive	160	positive	48	positive	19	positive	18	negative	<1.5	1,5	2,5	Peanut
RS-F	5	12.12.17	positive	60	positive	147	positive	49	positive	27	positive	19	negative	<2,5	2,5		Peanut
RS-F	6	09.01.18	positive	107	positive	180	positive	50	positive	27	positive	15	negative	-	1,5	2,5	Peanutprotein
RS-F	9		positive	81,6	positive	189,9	positive	51	positive	20,6	positive	17,2	negative		0,13	2,5	Peanut
VT	8	09.01.18	-	78	-	158	-	63	-	20	-	22	-	<2.5	2,5	2,5	Peanut

Continuation details by participants: ELISA-Methods

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in peanut (According to method prescription)	Conversion for processed peanuts (Example: Response for raw or roasted peanuts according to method prescription)	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	
BF	1	MonoTrace Peanut ELISA kit, BioFront Technologies	Ara h 3	N/A	N/A	Extracted for 10 minutes at 60C	no	
EF	7b	Eurofins SensiSpec Peanut ELISA Kit	Peanutprotein	25		according to manufacturer's instructions	no	
IL	10	Immunolab Peanut ELISA	polyclonal	25% (USDA Data)	-			
MI	7a	Peanut ELISA Kit-II, Morinaga	Peanutprotein	25	3,4	according to manufacturer's instructions	yes	
RS-F	2	RIDASCREEN Fast Peanut (R6201), R-Biopharm				according to manufacturer's instructions, with skimmed milk powder	yes	
RS-F	3	R6202 Ridascreen Fast Peanut						
RS-F	5	Ridascreen Fast Peanut (R6202), r-Biopharm				according to manufacturer's instructions, without skimmed milk powder	yes	Reference material NIST SRM 2387 Peanutbutter
RS-F	6	Ridascreen Fast Peanut (R6202), r-Biopharm					yes	
RS-F	9	Ridascreen Fast Peanut (R6202), r-Biopharm				Allergenextraction-buffer / 10 min / 60°C	no	
VT	8	Veratox Peanut, Neogen					no	

5.1.2 PCR-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		LOD	LOQ	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	preferred as Peanut
SFA-ID	3	19.01.18	positive	>1	positive	>1	positive	>1	positive	>1	positive	>1	negative	<1	1		Peanut - DNA
SFA-ID	4	16.01.18	positive	120	positive	40	positive	12	positive	1	positive	5,8	positive	2,9	< 1mg/kg		Peanut
SFA-ID	9		positive		positive		positive		positive		positive		negative		1		Peanut -DNA
SFA-Q	6	05.12.17	positive	<4	positive	<4	positive	<4	positive	<4	positive	<4	negative	-	1	4	Peanut
div	5	10.01.18	positive		positive		positive		negative		negative		negative				Peanut DNA

Continuation details by participants: PCR-Methods

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in peanut (According to method prescription)	Conversion for processed peanuts (Example: Response for raw or roasted peanuts according to method prescription)	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	
SFA-ID	3	SureFood® ALLERGEN Peanut Art.-No. S3103 Congen						
SFA-ID	4	Sure Food Allergen ID, R-Biopharm / Congen				CTAB / Qiaquick	yes	Quantification by standard addition to Eugster
SFA-ID	9	Sure Food Allergen ID, R-Biopharm / Congen					no	
SFA-Q	6	Sure Food Allergen Quant, R-Biopharm / Congen				Sure Food ® Allergen Quant Peanut (S3202)	no	
div	5	Selection PCR methods	Ara h 2 L77197			ASU 44.00-11 (01/2013) as a multiplex / extraction of 2 g of material with Maxwell	yes	LOD: 0,01 ng/µl DNA in the PCR approach

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 15-2017 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	40,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,09	102	40,1
2	5,07	103	40,6
3	5,10	110	43,1
4	5,05	119	47,1
5	5,02	116	46,2
6	5,03	123	48,9
7	4,99	114	45,7
8	5,03	113	44,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	113,0	Particles
Standard deviation	7,82	Particles
χ^2 (CHI-Quadrat)	3,80	
Probability	**80**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	44,6	mg/kg
Standard deviation	3,10	mg/kg
rel. Standard deviaton	6,95	%
Horwitz standard deviation	9,03	%
HorRat-value	**0,77**	
Recovery rate	110	%

Microtracer Homogeneity Test
DLA 15-2017 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	31,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	72	28,8
2	4,97	73	29,4
3	4,98	81	32,5
4	5,06	77	30,4
5	4,96	76	30,6
6	4,94	85	34,4
7	5,03	74	29,4
8	5,03	70	27,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	76,0	Particles
Standard deviation	5,33	Particles
χ^2 (CHI-Quadrat)	2,61	
Probability	**92**	%
Recovery rate	97	%

Normal distribution		
Number of samples	8	
Mean	30,4	mg/kg
Standard deviation	2,13	mg/kg
rel. Standard deviaton	7,01	%
Horwitz standard deviation	9,57	%
HorRat-value	**0,73**	
Recovery rate	97	%

Microtracer Homogeneity Test
DLA 15-2017 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	32,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	92	36,7
2	5,07	83	32,7
3	5,00	84	33,6
4	5,05	81	32,1
5	5,06	85	33,6
6	5,00	82	32,8
7	5,02	83	33,1
8	4,99	86	34,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	84,5	Particles
Standard deviation	3,62	Particles
χ^2 (CHI-Quadrat)	1,08	
Probability	**99**	%
Recovery rate	104	%

Normal distribution		
Number of samples	8	
Mean	33,6	mg/kg
Standard deviation	1,44	mg/kg
rel. Standard deviaton	4,28	%
Horwitz standard deviation	9,43	%
HorRat-value	**0,45**	
Recovery rate	104	%

Microtracer Homogeneity Test
DLA 15-2017 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	25,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	81	32,3
2	4,99	90	36,1
3	5,00	77	30,8
4	4,97	80	32,2
5	4,96	73	29,4
6	4,99	67	26,9
7	5,03	73	29,1
8	4,99	90	36,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	78,9	Particles
Standard deviation	8,18	Particles
χ^2 (CHI-Quadrat)	5,94	
Probability	**55**	%
Recovery rate	123	%

Normal distribution		
Number of samples	8	
Mean	31,6	mg/kg
Standard deviation	3,28	mg/kg
rel. Standard deviaton	10,4	%
Horwitz standard deviation	9,51	%
HorRat-value	**1,1**	
Recovery rate	123	%

Microtracer Homogeneity Test
DLA 15-2017 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	37,2	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	113	45,5
2	4,99	101	40,5
3	4,99	111	44,5
4	5,05	123	48,7
5	5,06	108	42,7
6	5,07	118	46,5
7	5,06	101	39,9
8	5,06	119	47,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	111,7	Particles
Standard deviation	7,94	Particles
χ^2 (CHI-Quadrat)	3,95	
Probability	**79**	%
Recovery rate	119	%

Normal distribution		
Number of samples	8	
Mean	44,4	mg/kg
Standard deviation	3,15	mg/kg
rel. Standard deviaton	7,10	%
Horwitz standard deviation	9,04	%
HorRat-value	**0,79**	
Recovery rate	119	%

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 15-2017***
PT name	***Response PT Peanut: Processed samples Peanut (unroasted), Peanut (roasted), Peanut Butter, Peanut Paste and Extrudate (Peanut-Flips)*** *in potato powder matrix (levels: 25 - 150 mg/kg)*
Sample matrix (processing)	***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 (long term: cooled 2 - 10°C)*
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. 25 - 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. Preferably the total sample amount should be homogenized.*
Result sheet	*One result each should be determined for Samples 1-6.* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of digits	*at least 2 significant digits*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest January 26th 2018***
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, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. 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

[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
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21. 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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