Proficiency Tests
DLA
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cosmetics
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Evaluation Report
proficiency test

DLA 16/2017

ALM Verification:

Peanut in Cookie-Matrix

5 Samples containing roasted Peanuts (levels: 0,50 / 2,5 / 5,0 / 12,5 / 25 mg/kg)

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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* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 23. April 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 (PT) schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

The present PT-format **„Action Level Matrix - ALM Verification"** offers the possibility to prove that the analytical determination method applied by the participating laboratory is capable to reliably detect the allergen content relevant for food labelling by means of a kind of calibration row of 5 samples containing the allergen in a specific food-matrix and a blank sample.
The allergen contents of the PT-sample series vary from 1/10 to 2-fold of the action level, which is normally based on the threshold value dose (VITAL Concept 2.0) or the assessment values of the ALTS/ALS (German Food Expert Committee) (see Table 3). The evaluation of PT-results was performed qualitative in scores from 1-5 (Score 3 = Action Level successfully detected). Quantitative results were given including the recovery rate for information in the report.

2. Realisation

2.1 Test material

6 PT-samples with the food matrix cookie were provided for qualitative detection and optional quantitative detection of peanut. The peanut-levels of the PT-sample series were in the range from 0,5 mg/kg to 25 mg/kg, whereas the medial level represents the "Action Level" (see Table 1).
The food matrix of sample material was common in commerce butter cookies. The basic composition was identical for all 6 samples (see Table 1).
After crushing and sieving using an impact mill (mesh 1,5 mm) the basic mixture was homogenized and an aliquot was taken from it as blank sample.

For preparation of the peanut containing samples cookies were baked (150°C, 30 min) and dried (60°C) using roasted peanuts (further information see below). Afterwards the peanut-cookies were crushed by a knife mill and homogenized.

Afterwards the **spiked sample series** was produced as follows: After crushing and homogenization an aliquot of the peanut containing cookies was added to the basic mixture. The resulting mixture was homogenized again. Afterwards basic mixture was added stepwise (3-5 steps) including mechanical homogenization after each step until the total amount of sample material was reached.

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

For the spiking a milled mixture of roasted peanuts consisting of 18 single products out of 9 countries (USA, Asia, Africa, South America) was used. The unprocessed (not baked in the cookie) mixture of roasted peanuts gave a recovery rate for peanut of about 264 % ± 88 % (n=11) in the spiking level sample of the PT DLA 07/2017 calculated from different ELISA method results.

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „Null"	**Level 1** 0,5 mg/kg	**Level 2** 2,5 mg/kg	**Level 3** 5 mg/kg	**Level 4** 12,5mg/kg	**Level 5** 25 mg/kg
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Butter Cookies Ingredients: Wheat flour, sugar, butter, barley malt extract, skimmed milk powder, glucose, glucose syrup, raising agent ammonium carbonate, salt, emulsifier lecithin Nutrients per 100 g: Protein 7,1 g, carbo-hydrates 76 g, fat 12 g	100	100	100	99,9	99,8	99,5
Cookies (baked 150°C, 30 min) Ingredients: Wheat flour, Sugar, butter, eggs, salt and mixture of roasted peanuts and further ingredients (maltodextrin, sodium sulfate, silicon dioxide)	-	0,0100	0,0500	0,100	0,249	0,498
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
there of roasted peanuts: - as Peanuts* - *with 23,2% protein***	-	**0,51** 0,12	**2,52** 0,58	**5,05** 1,17	**12,6** 2,92	**25,3** 5,87
Extended combined uncertainty (k=2) of peanut-content (= ± 12 %)		± 0,06	± 0,30	± 0,61	± 1,5	± 3,0

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material: 23,2 ± 1,59 %, n=5 (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.*

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 material, mixing homogeneity, homogeneity and stability of peanut.
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, 16-18].

2.1.1 Characterization of the PT-Sample series

The PT-sample series was characterized by ELISA (Immunolab Peanut ELISA, n=4). All 5 spiking levels were detected with a good correlation between spiking and mean of results (see Fig. 1). The relative standard deviations (RSD) were in the range of approx. 22% to 1,4% and the recovery rates ranged from 78% to 101% (level 1 to 4) and was 141% for level 5.

Table 2: Characterization of PT-sample series peanut in cookie-matrix by ELISA determination (Immunolab Peanut, n=4).

PT-Sample	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
Spiking	0,0	0,510	2,52	5,05	12,6	25,3
Result 1	0,0	0,374	2,07	6,13	12,5	35,7
Result 2	0,0	0,413	2,84	3,92	12,8	34,3
Result 3	0,0	0,505	2,15	4,49	12,5	37,6
Result 4	0,0	0,291	1,81	5,91	13,1	35,2
Mean [mg/kg]	0,0	0,396	2,22	5,11	12,7	35,7
SD	-	0,09	0,44	1,08	0,28	1,39
RSD [%]	-	22,4	19,8	21,1	2,2	3,9
Recovery [%]	-	78	88	101	101	141

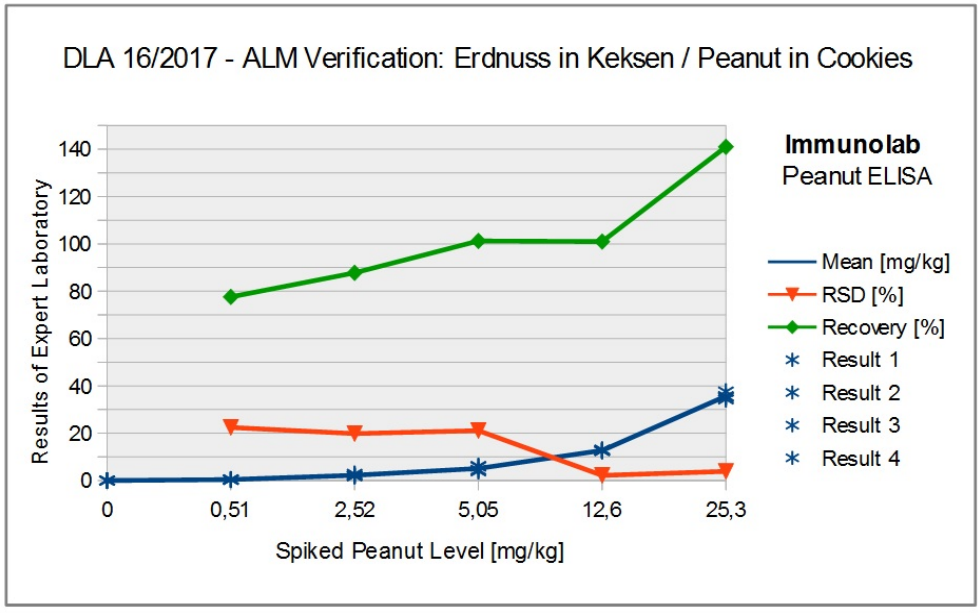


Abb./Fig. 1: ELISA results of PT-sample series peanut in cookie-matrix (Immonuloab Peanut, n=4), *Note: the x-scale is not shown linear to obtain a better recognizability of low values.*

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 level 1 to 5 showed a probability of 80%, 99%, 73%, 71% and 80%. 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 evalutation HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within a laboratory) [18]. This gave a HorRat value of 0,94, 0,58, 0,87, 0,90 and 0,96 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

The portion of test material (sample 1 to 6) were sent to every participating laboratory in the 47th week of 2017. The testing method was optional. The tests should be finished at January 19th 2018 the latest.

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

The proficiency test Action Level Matrix (ALM) – Verification consists of five different samples with specified contents of roasted peanuts as well as a „blank sample" in the matrix butter cookies.

- *The 6 samples are numbered in a random order.*
- *It is to be proven qualitatively by any suitable method that the so-called „Action Level" of 5 mg/kg peanut can be detected in the processed matrix (= Action Level 1 (VITAL concept 2.0) and judgement value of the German Commission ALTS/ALS).*
- *If possible, the indication of quantitative results is desirable in order to compare them with the levels of addition.*

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.

All 13 participants submitted results.

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 the allergenic ingredient was provided in an especially processed food matrix in a kind of a calibration line with concentrations in the range of the so called Action Level. The allergen content here referred to as the "Action Level" is highlighted by colour in Table 3.

The participant results were evaluated qualitatively with an Action Level Matrix Score (*ALM-Score*), which indicates the number of successfully detected concentration levels.
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.

Table 3: Threshold doses, judgement values and legislative maximum values.(Highlighted by colour: Action Level in the present PT)[27, 39-41]

Allergen	Threshold dose * (Vital Concept 2.0)	Judgement value ALTS/ALS	Legislative Maximum value for declaration
	mg/kg	mg/kg	mg/kg
Gluten	100	> 80	20 **
Egg (as whole egg powder)	0,66	> 1	
Peanut	***8***	***> 5***	
Soy (as Soy flour)	25	> 20	
Milk (as defatted milk powder)	2,8	> 2,5	
Hazelnut	6,4	> 5	
Cashew	106	> 50	
Almond, Walnut, Pecan, Brazil-Nut, Pistachio, Macadamia	-	> 20	
Sesame, unpeeled	11,8	> 10	
Lupine	100	> 50	
Celery seed	-	> 20	
Mustard seed	1,9	> 5	

* calculated by threshold dose considering an intake of 100 g food [40,41]
** Maximum value for declaration as „gluten free" according to EU-VO 828/2014 [39]

3.1 Action Level Matrix Score (ALM-Score)

The qualitative valuation of each participant's results was performed with the so called ALM-Scores from 1-5 considering the number of "positive" or "negative" results matching the spiking of the PT-sample series (see Tab. 4). An ALM-Score from > 3 indicates a successful detection of the Action Level. The results of the matrix sample Level 0 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 4: Evaluation of results using ALM-Scores

Level 0 „blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5 mg/kg	Level 4 12,5 mg/kg	Level 5 25 mg/kg	ALM-Score qualitative	Detection Action Level
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Levels 1 - 5	
negative	negative	negative	negative	negative	positive	1 (20%)	not successful
negative	negative	negative	negative	positive	positive	2 (40%)	not successful
negative	negative	negative	positive	positive	positive	3 (60%)	successful
negative	negative	positive	positive	positive	positive	4 (80%)	successful
negative	positive	positive	positive	positive	positive	5 (100%)	successful

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 spiked allergen (level of addition). The reference values are calculated from the values for Level 1 to 5 given in section 2.1 Sample material, 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-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 11% - 145% for PCR methods, depending on matrix or processing and concentration (s. Table 5a and 5b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 5a: ELISA-Methods – Recovery rates and precision data from chosen 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 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 [25].

THE IRMM (Institute for Reference Materials and Measurements) proofed 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 5b: PCR-Methods - Relative repeated standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) according to chosen evaluation from experiments by precision and the resulting target standard deviation σ_{pt} [33-38].

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 nuts	Rice cakes	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 nuts	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 nuts	Rice cakes	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 nuts	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

3.2.1 Recovery rates by precision experiment

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 6 and 7.

Table 6: 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 7: 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.
There was a separate evaluation of ELISA 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 was converted to the total food peanut using the experimentally determined protein content of the raw material for roasted peanuts of 23% (see Ta. 1, p. 6). All other ELISA and PCR results were given as peanut, therefore no recalculation was necessary.

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

Participant	Level 0 „blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5 mg/kg	Level 4 12,5 mg/kg	Level 5 25 mg/kg	ALM-Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Levels 1 - 5		

In cases when quantitative values were submitted the result table are given as indicated below:

Participant	Level 1 - 0,5 mg/kg		Level 2 - 2,5 mg/kg		Level 3 - 5,0 mg/kg (Action Level)		Level 4 - 12,5mg/kg		Level 5 - 25 mg/kg		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**		

* RR = Recovery Rate (RR)

4.1 Proficiency Test Peanut

4.1.1 Qualitative: Action Level Matrix-Scores (ELISA-methods)

Evaluation number	Level 0 „blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 mg/kg	ALM-Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of recorded Level 1 - 6		
1	negative	positive	positive	positive	positive	positive	5 (100%)	BF	
13a	negative	positive	positive	positive	positive	positive	5 (100%)	BK	
5b	negative	negative	positive	positive	positive	positive	4 (80%)	EF	
12	negative	positive	positive	positive	positive	positive	5 (100%)	IL	
5a	negative	negative	negative	positive	positive	positive	3 (60%)	MI	
2	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
3	positive	negative	positive	negative	positive	positive	3 (60%)	RS-F	Level 0 and Level 3 mixed up ?
6	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
7	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
9	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	
11	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	
13b	negative	negative	positive	positive	positive	positive	4 (80%)	RS-F	
8	negative	negative	positive	positive	positive	positive	4 (80%)	VT	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	1	5	12	12	13	13
Number negative	12	8	1	1	0	0
Percent positive	8	38	92	92	100	100
Percent negative	92	62	8	8	0	0
Consensus value	negative	none	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

BF = MonoTrace ELISA, BioFront Technologies

BK = BioKits, Neogen

EF = Eurofins Sensispec

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

With one exception, all participants detected the Action Level of 5 mg/kg successfully. Also for level 2 (1/2 of the action level) 92% positive results were obtained. The lowest content of 0,5 mg/kg (1/10 of the action level) was still detected by 38% of participants. This value is below the limits of quantification given by the participants for the applied methods.

4.1.2 Qualitative: Action Level Matrix-Scores (PCR-methods)

Evaluation number	Level 0 „blank“	Level 1 0,5 mg/kg	Level 2 2,5 mg/kg	Level 3 (Action Level) 5,0 mg/kg	Level 4 12,5 mg/kg	Level 5 25 mg/kg	ALM-Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of recorded Level 1 – 6		
13	negative	negative	negative	negative	positive	positive	2 (40%)	ASU	LOD < 10 mg/kg
4a	negative	positive	positive	positive	positive	positive	5 (100%)	SFA-4p	
4b	negative	positive	positive	positive	positive	positive	5 (100%)	SFA-ID	
2	negative	negative	positive	positive	positive	positive	4 (80%)	SFA-ID	
6	negative	negative	positive	positive	positive	positive	4 (80%)	SFA-ID	
11	negative	negative	positive	positive	positive	positive	4 (80%)	SFA-ID	
7	negative	negative	positive	positive	positive	positive	4 (80%)	SFA-Q	
10	negative	negative	positive	positive	positive	positive	4 (80%)	div	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	2	7	7	8	8
Number negative	8	6	1	1	0	0
Percent positive	0	25	88	88	100	100
Percent negative	100	75	13	13	0	0
Consensus value	negative	negative	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method

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

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

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

div = not indicated / other method

Comments:

With one exception, all participants detected the Action Level of 5 mg/kg successfully. Also for level 2 (1/2 of the action level) 88% positive results were obtained. The lowest content of 0,5 mg/kg (1/10 of the Action Level) was still detected by 25% of participants. This value is below the limits of detection given by the participants for the applied methods.

4.1.3 Quantitative: Recovery-Scores (ELISA-methods)

Evaluation number	Level 1 - 0,5 mg/kg		Level 2 - 2,5 mg/kg		Level 3 - 5,0 mg/kg (Action Level)		Level 4 - 12,5 mg/kg		Level 5 - 25 mg/kg		WFR-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	1,10	216	2,02	**80**	6,00	**119**	12,5	**99**	27,5	**109**	4/5 (80%)	BF	
13a	0,7	**137**	2,90	**115**	6,00	**119**	17,0	**135**	30,0	**119**	5/5 (100%)	BK	result Level 1 < LOQ
5b	<1		3,70	**147**	8,00	158	20,0	158	34,0	**135**	2/4 (50%)	EF	
12	0,395	**77**	2,22	**88**	5,11	**101**	12,7	**101**	35,7	**141**	5/5 (100%)	IL	result Level 1 < LOQ
5a	<4,2		<4,2		4,20	**83**	6,80	**54**	15,0	**59**	3/3 (100%)	MI	
2	<1.5		4,80	190	8,10	160	21,0	166	38,0	**150**	0/4 (0%)	RS-F	
3	0,399	**78**	3,03	**120**	< 2,5		16,7	**132**	43,4	172	3/4 (75%)	RS-F	result Level 1 < LOQ
6	< 2,5		3,40	**135**	7,60	**150**	19,4	154	29,8	**118**	2/4 (50%)	RS-F	
7			14,7 (3,38)	(134)	30,4 (7,00)	(139)	97,8 (22,5)	(178)	141 (32,5)	(129)	0/4 (0%)	RS-F	Result converted ° (in parentheses given as peanut protein)
9	<2,5		2,70	**107**	6,30	**125**	18,0	**143**	30,0	**119**	4/4 (100%)	RS-F	
11	0,650	**127**	2,50	**99**	6,70	**133**	19,8	157	37,2	**147**	4/5 (80%)	RS-F	result Level 1 < LOQ
13b	0,44	**86**	3,60	**143**	9,00	178	26,0	206	50,0	198	2/5 (40%)	RS-F	result Level 1 < LOD
8	<2.5		3,00	**119**	6,70	**133**	15,0	**119**	53,0	210	3/4 (75%)	VT	
	RA**	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**	**RA****	**50-150 %**			
	Number in RA	**5**	**Number in RA**	**10**	**Number in RA**	**8**	**Number in RA**	**7**	**Number in RA**	**9**			
	Prozent in RA	**83**	**Prozent in RA**	**91**	**Prozent in RA**	**73**	**Prozent in RA**	**58**	**Prozent in RA**	**75**			

° calculation see p. 15

Methods:

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

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

** Acceptance range of AOAC for allergen ELISAs

Comments:

For levels 2 to 5, 58% to 91% of the recovery rates of the participant results were within the range of acceptance of 50-150%. The quantitative results for Level 1 were all below the limits of quantification of the methods as given by the participants (exception: Method BF limit of quantification 1 mg / kg) (Representation Level 2-4 in Fig. 2).

4.1.4 Quantitative: Recovery-Scores (PCR-methods)

Evaluation number	Level 1 - 0,5 mg/kg		Level 2 - 2,5 mg/kg		Level 3 - 5,0 mg/kg (Action Level)		Level 4 - 12,5 mg/kg		Level 5 - 25 mg/kg		WFR-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**		
13	<10		<10		<10		<100		<100			ASU	
4a	0,200	39	4,00	158	12,0	238	51,0	404	78,0	309	0/5 (0%)	SFA-4p	
4b	0,100	20	1,20	48	2,00	40	6,80	**54**	10,4	41	1/5 (20%)	SFA-ID	
2	<1		>1		>1		>1		>1			SFA-ID	
6	< 1,0											SFA-ID	
11												SFA-ID	
7			< 4		< 4		< 4		5,40	21	0/1 (0%)	SFA-Q	
10	< 1		< 2		2,20	44	5,50	44	7,40	29	0/3 (0%)	div	
	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	1	Number in RA	0			
	Prozent in RA	0	Prozent in RA	0	Prozent in RA	0	Prozent in RA	33	Prozent in RA	0			

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

** Acceptance range of AOAC for allergen ELISAs

Methods:

ASU = ASU §64 Methode/method

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

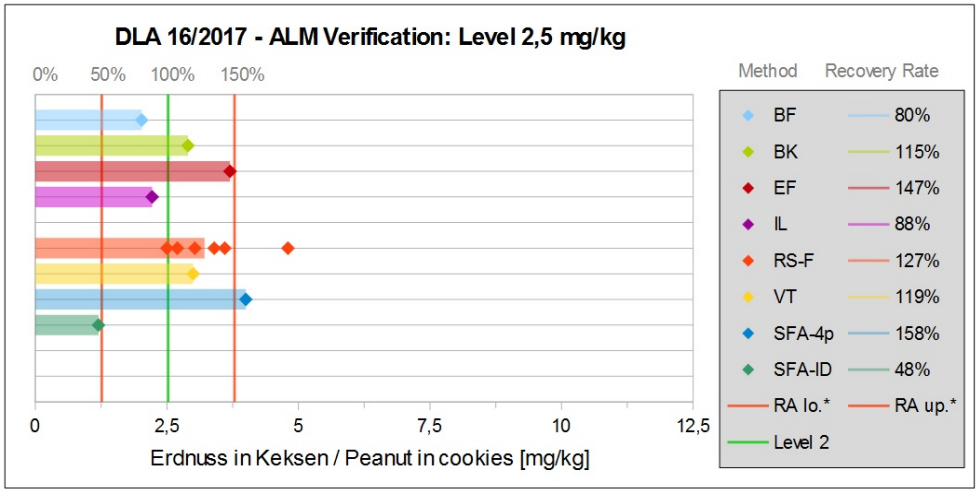
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

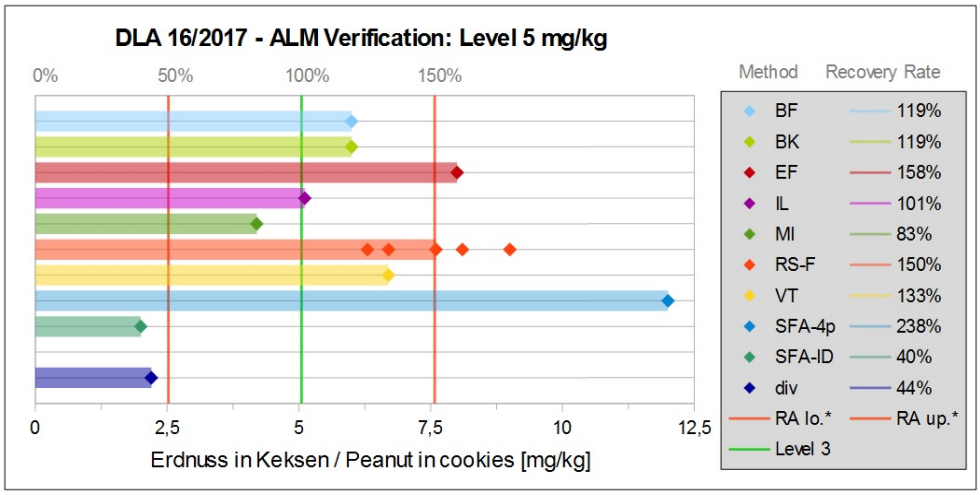
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

div = not indicated / other method

Comments:

Using PCR, four participants reported quantitative results for at least one level. A recovery rate for level 4 was in the acceptance range of 50-150% (presentation level 2-4 in Fig. 2).





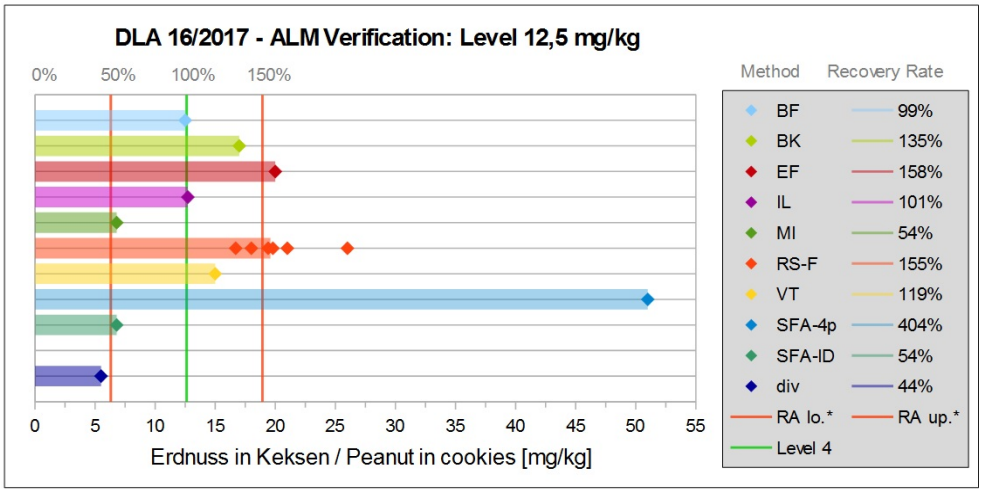


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale peanut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.1.5 Informative Data: Statistical characteristics (ELISA-Methods)

Sample: Action Level 5 mg/kg

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS-F}}$
Number of results	11	5
Number of outliers	0	0
Mean	6,70	7,54
Median	6,70	7,60
Robust Mean (X)	**6,72**	**7,54**
Robust standard deviation (S*)	**1,55**	**1,23**
Target range:		
Target standard deviation σ_{pt}	**1,68**	**1,89**
lower limit of target range	**3,36**	**3,77**
upper limit of target range	**10,1**	**11,3**
Quotient S/σ_{pt}*	*0,92*	*0,65*
Standard uncertainty $U_{(Xpt)}$	*0,585*	*0,687*
Results in the target range	*11*	*5*
Percent in the target range	*100*	*100*

Methods:
RS-F = R-Biopharm, Ridascreen® Fast

Comments:

Assigned values were the robust means (Algorithm A) of all results and of the indicated single methods. The determination of the z-scores was based on a target standard deviation of 25% (see Fig. 3 and 4).

All data are for information only.

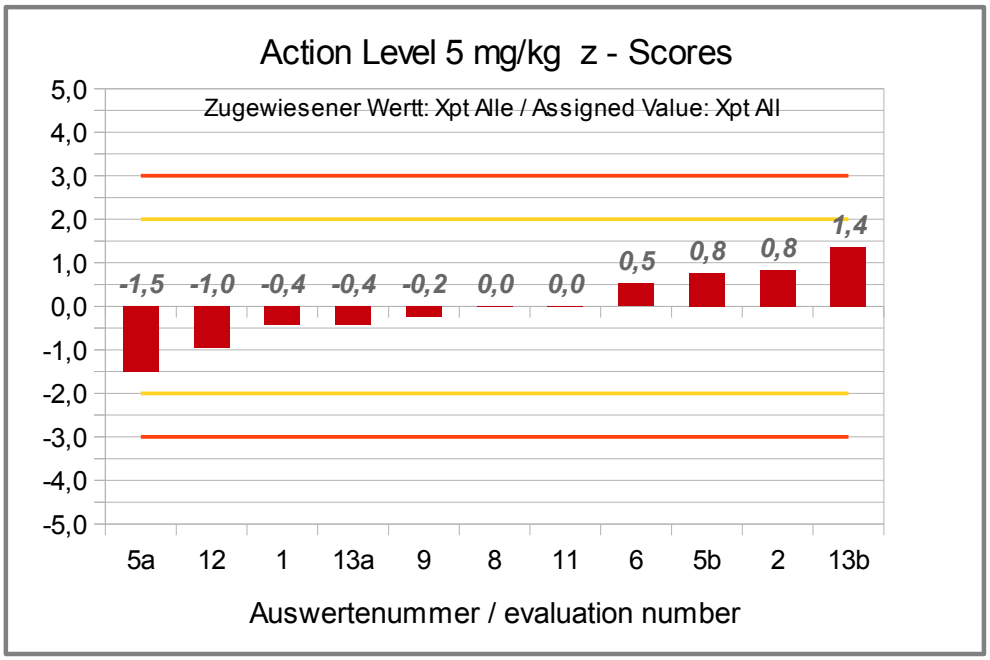


Abb./Fig. 3:
z-Scores (ELISA-results as peanut)
Assigned value: robust mean of all results

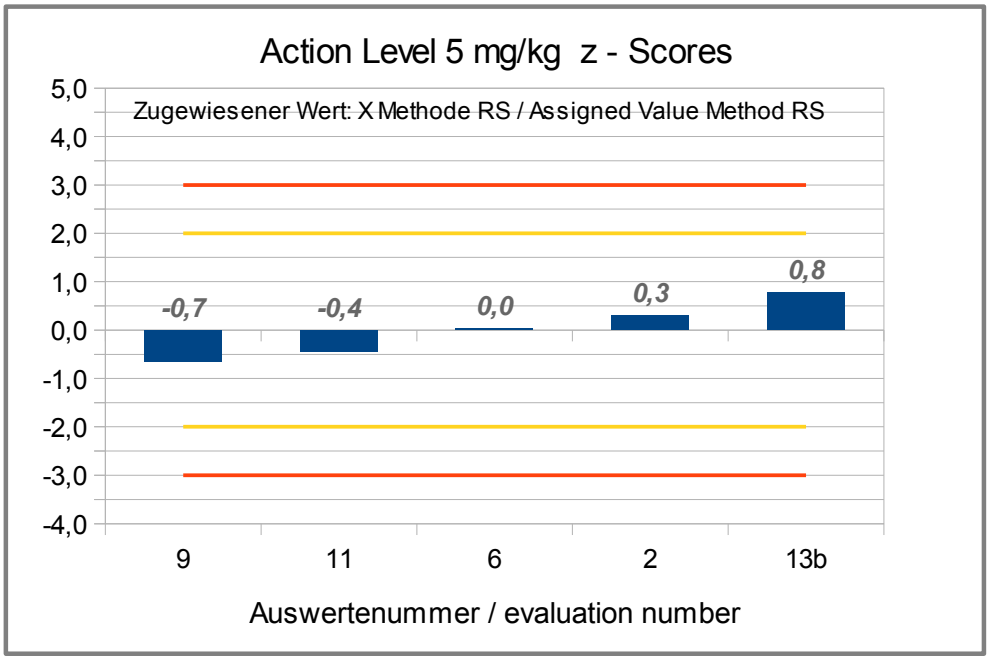


Abb./Fig. 4:
z-Scores (ELISA-results as peanut) Reference value robust average results Method RS-F(R-Biopharm, Ridascreen Fast)

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 Abk.	Evaluation number	Date of Analysis	Result Sample 2 blank		Result Sample 5 0,5 mg/kg Level		Result Sample 4 2,5 mg/kg Level		Result Sample 3 5,0 mg/kg Level		Result Sample 1 12,5 mg/kg Level		Result Sample 6 25 mg/kg Level		NWG / LOD *	BG / LOQ *	Result given 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	e.g. Food / Protein
BF	1	07.12.18	negative	0	positive	1,1	positive	2,02	positive	6	positive	12,5	positive	27,5	0,24	1	Peanut
BK	13a	28/29.12/19.1	negative	< 0.5	positive	<1 (0.7)	positive	2,9	positive	6	positive	17	positive	30	0,5	1	Peanut
EF	5b	10.01.18	negative	<1	negative	<1	positive	3,7	positive	8	positive	20	positive	34	0,1	1	Peanut
IL	12	28.11.17	negative	0	positive	0,395	positive	2,22	positive	5,11	positive	12,72	positive	35,7	0,1	1	Peanut
MI	5a	08.12.17	negative	<4,2	negative	<4,2	negative	<4,2	positive	4,2	positive	6,8	positive	15	1,6	4,2	Peanut
RS-F	2		negative	<1.5	negative	<1.5	positive	4,8	positive	8,1	positive	21	positive	38	1,5	2,5	Peanut
RS-F	3	09.01.	positive	4,85	negative	< 2,5	positive	3,03	negative	< 2,5	positive	16,7	positive	43,4	0,13	2,5	Peanut
RS-F	6	15.01.18	negative	< 2,5	negative	< 2,5	positive	3,4	positive	7,6	positive	19,4	positive	29,8	2,5	2,5	Peanut
RS-F	7	03.01.18	negative		negative		positive	3,375	positive	7	positive	22,5	positive	32,5	0,8	2,4	Peanutprotein
RS-F	9	02.01.18	negative		positive	<2,5	positive	2,7	positive	6,3	positive	18	positive	30	1,5	2,5	Peanut
RS-F	11		negative		positive	0,65	positive	2,5	positive	6,7	positive	19,8	positive	37,2	0,13	2,5	Peanut
RS-F	13b	29.12./19.1.	negative	<1.5	negative	<1.5 (0.44)	positive	3,6	positive	9	positive	26	positive	50	1,5	2,5	Peanut
VT	8	08.12.	-	<2.5	-	<2.5	-	3	-	6,7	-	15	-	53	2,5	2,5	Peanut

Continuation details by participants

Method Abk.	Evaluation number	Method	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	e.g. Extraction solution / Time / Temperature	yes/no	
BF	1	MonoTrace Peanut ELISA kit, BioFront Technologies	Ara h 3	Extraction for 10 minutes at 60°C	no	
BK	13a	BioKits Peanut Assay Kit, Neogen				
EF	5b	Eurofins SensiSpec Peanut ELISA Kit	peanutprotein	As per kit instructions	no	
IL	12	Immunolab Peanut ELISA				
MI	5a	Morinaga Peanut ELISA Kit II, M2116	peanutprotein	As per kit instructions	yes	converted to peanut calibrated against NIST material (protein content about 25%, conversion factor for processed peanut 3,4)
RS-F	2	R6202 Ridascreen Fast Peanut			yes	
RS-F	3	Ridascreen Fast Peanut (R6202), r-Biopharm		As per kit instructions, w ith skimmed milk pow der	yes	Sample 5: value below determination limit (0.399 mg / kg, determined w ith further dilutions of the standards)
RS-F	6	Ridascreen Fast Peanut (R6202), r-Biopharm			yes	
RS-F	7	Ridascreen Fast Peanut (R6202), r-Biopharm		Cross sensitivity to lentils, green peas, fenugreek	yes	
RS-F	9	Ridascreen Fast Peanut (R6202), r-Biopharm	Ara h 1 and Ara h 2	As per kit instructions	yes	Sample 5: w eak positive, < BG 2,5 mg/kg
RS-F	11	Ridascreen Fast Peanut (R6202), r-Biopharm		allergen extraction buffer/ 10 min / 60°C	no	
RS-F	13b	Ridascreen Fast Peanut (R6202), r-Biopharm				
VT	8	Veratox Peanut, Neogen				

5.1.2 PCR-Methoden

Method Abk.	Evaluation number	Date of Analysis	Result Sample 2 blank		Result Sample 5 0,5 mg/kg Level		Result Sample 4 2,5 mg/kg Level		Result Sample 3 5,0 mg/kg Level		Result Sample 1 12,5 mg/kg Level		Result Sample 6 25 mg/kg Level		NWG / LOD *	BG / LOQ *	Result given as
		Day/Month	qualitativee	mg/kg	qualitativee	mg/kg	qualitativee	mg/kg	qualitativee	mg/kg	qualitativee	mg/kg	qualitativee	mg/kg	mg/kg	mg/kg	e.g. Food / Protein
ASU	13	18./19.1.18	negative	<10	negative	<10	negative	<10	negative	<10	positive	<100	positive	<100	10		
SFA-4p	4a		negative	0	positive	0,2	positive	4	positive	12	positive	51	positive	78	< 1mg/kg		Peanut-DNA
SFA-ID	4b		negative	0	positive	ca. 0,1	positive	1,2	positive	2	positive	6,8	positive	10,4	< 1 mg/kg	< 5ppm	Peanut-DNA
SFA-ID	2		negative	<1	negative	<1	positive	>1	positive	>1	positive	>1	positive	>1	1		DNA Peanut
SFA-ID	6	30.11.18	negative	< 1,0	negative	< 1,0	positive		positive		positive		positive		1		Peanut
SFA-ID	11		negative		negative		positive		positive		positive		positive		1		Peanut-DNA
SFA-Q	7	04.01.18	negative		negative		positive	< 4	positive	< 4	positive	< 4	positive	5,4	1	4	Peanut-DNA
div	4c		negative	0	positive	0,25	positive	2,5	positive	5	positive	12,5	positive	25			Peanut-DNA
div	10	27.12.17	negative	< 1	negative	< 1	positive	< 2	positive	2,2	positive	5,5	positive	7,4	1	2	Peanut

Continuation details by participants

Method Abk.	Evaluation number	Method	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	e.g. Extraction solution / Time / Temperature	yes/no	
ASU	13	ASU §64 Methode/method	Ara H 2 Trypsin-Inhibitor			
SFA-4p	4a	Sure Food Allergen 4plex, R-Biopharm / Congen		CTAB / Qiaquick	yes	Semiquantitative evaluation on unprocessed standard material (quantitative data correspond to the average of 5 measurements)
SFA-ID	4b	Sure Food Allergen ID, R-Biopharm / Congen		CTAB / Qiaquick	yes	Semiquantitative evaluation on unprocessed standard material (Quantard / Congen) undervaluation (quantitative data correspond to the mean of 5 measurements)
SFA-ID	2	SureFood® ALLERGEN Peanut Art.-No. S3103 Congen			yes	
SFA-ID	6	Sure Food Allergen ID, R-Biopharm / Congen			yes	
SFA-ID	11	Sure Food Allergen ID, R-Biopharm / Congen			no	
SFA-Q	7	Sure Food Allergen Quant, R-Biopharm / Congen			yes	Articlenr. S3203
div	4c			CTAB / Qiaquick		Assignment to the spiked contents
div	10	other: Ladenburger et al (2017) J AOAC Intern 101 (1), modifiziert	atp6 (104 bp)	CTAB-precipitation method, s. e.g. ASU L 18.00-22	yes	Calibration / quantification using matrix standards, doped material: peanut, roasted, degreased. Sample 5 gave amplifications with high Ct values, which were rated as "negative" (<1 mg / kg)

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 16-2017 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	22,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	52	20,8
2	4,95	66	26,7
3	5,05	60	23,8
4	5,05	69	27,3
5	5,06	64	25,3
6	4,99	60	24,0
7	4,97	70	28,2
8	5,00	65	26,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	63,3	Particles
Standard deviation	5,87	Particles
χ^2 (CHI-Quadrat)	3,81	
Probability	**80**	%
Recovery rate	115	%

Normal distribution		
Number of samples	8	
Mean	25,3	mg/kg
Standard deviation	2,35	mg/kg
rel. Standard deviaton	9,28	%
Horwitz standard deviation	9,84	%
HorRat-value	**0,94**	
Recovery rate	115	%

Microtracer Homogeneity Test
DLA 16-2017 Sample 3

Weight whole sample	1,10	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	18,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	58	23,2
2	5,09	58	22,8
3	4,98	53	21,3
4	5,14	62	24,1
5	5,01	57	22,8
6	5,04	64	25,4
7	5,10	64	25,1
8	4,96	60	24,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,5	Particles
Standard deviation	3,43	Particles
χ^2 (CHI-Quadrat)	1,38	
Probability	**99**	%
Recovery rate	127	%

Normal distribution		
Number of samples	8	
Mean	23,6	mg/kg
Standard deviation	1,36	mg/kg
rel. Standard deviaton	5,76	%
Horwitz standard deviation	9,94	%
HorRat-value	**0,58**	
Recovery rate	127	%

Microtracer Homogeneity Test
DLA 16-2017 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	38,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,10	107	42,0
2	4,99	89	35,7
3	5,09	92	36,1
4	5,08	109	42,9
5	5,02	94	37,5
6	4,97	97	39,0
7	5,01	108	43,1
8	4,99	91	36,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	98,4	Particles
Standard deviation	7,89	Particles
χ^2 (CHI-Quadrat)	4,44	
Probability	**73**	%
Recovery rate	101	%

Normal distribution		
Number of samples	8	
Mean	39,1	mg/kg
Standard deviation	3,14	mg/kg
rel. Standard deviaton	8,03	%
Horwitz standard deviation	9,21	%
HorRat-value	**0,87**	
Recovery rate	101	%

Microtracer Homogeneity Test
DLA 16-2017 Sample 5

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	39,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,96	101	40,7
2	5,02	105	41,8
3	5,10	91	35,7
4	5,05	85	33,7
5	5,03	87	34,6
6	5,07	102	40,2
7	5,09	93	36,5
8	5,00	89	35,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	94,1	Particles
Standard deviation	7,83	Particles
χ^2 (CHI-Quadrat)	4,55	
Probability	**71**	%
Recovery rate	95	%

Normal distribution		
Number of samples	8	
Mean	37,4	mg/kg
Standard deviation	3,11	mg/kg
rel. Standard deviaton	8,31	%
Horwitz standard deviation	9,28	%
HorRat-value	**0,90**	
Recovery rate	95	%

Microtracer Homogeneity Test
DLA 16-2017 Sample6

Weight whole sample	1,20	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	25,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	62	24,6
2	4,95	54	21,8
3	5,03	73	29,0
4	5,03	55	21,9
5	4,97	57	22,9
6	5,00	62	24,8
7	5,03	63	25,0
8	5,06	61	24,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	60,9	Particles
Standard deviation	5,78	Particles
χ^2 (CHI-Quadrat)	3,84	
Probability	**80**	%
Recovery rate	96	%

Normal distribution		
Number of samples	8	
Mean	24,3	mg/kg
Standard deviation	2,31	mg/kg
rel. Standard deviaton	9,50	%
Horwitz standard deviation	9,90	%
HorRat-value	**0,96**	
Recovery rate	96	%

5.3 Information on the Proficiency Test (PT)

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

Information on the Proficiency Test (PT)

PT number	***DLA 16-2017***
PT name	***ALM-Verification Peanut: 5 calibration samples containing roasted Peanuts in Cookie-Matrix*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Butter Cookies (baked at appr. 160°C)/ ingredients: Wheat flour, sugar, butter, barley malt extract, glucose syrup, baking agent ammonium carbonate, salt, emulsifier lecithins, other food additives, egg and roasted peanuts (except "blank sample")*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 „blank sample" : 20 g*
Storage	*Samples : room temperature (long term 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative (optional: quantitative):* *Peanut / Peanut protein* *Levels (Peanut): 0,50 / 2,5 / 5,0 / 12,5 / 25 mg/kg*
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 qualitative (and optional quantitative) result each should be determined for Samples 1-6.* *The results should be filled in the result submission file.*
Units	*positive / negative (optional: mg/kg)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest January 19th 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.]

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