Proficiency Tests
DLA
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Evaluation Report
proficiency test

DLA 16/2018

ALM Verification:

Hazelnut in Chocolate-Matrix

5 Samples containing roasted Hazelnuts (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* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 29 March 2019
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 5-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 rates for information in the report.
Additionally a quantitative evaluation of the results for the Action Level as well as the Level 5 using z-scores was made for information purposes.

2. Realisation

2.1 Test material

6 PT-samples with the food matrix chocolate were provided for qualitative detection and optional quantitative determination of hazelnut. The hazelnut-levels of the PT-sample series were in the range from 0,5 mg/kg to 25 mg/kg (as hazelnut), whereas the medial level represents the "Action Level" (see Table 1).

The test material is a common in commerce dark chocolate (70% cocoa). The basic composition was identical for all 6 samples (see Table 1).
After mixing and homogenizing of the basic matrix at 60°C with stirring an aliquot was taken from it as blank sample.

Afterwards the spiked sample series was produced as follows:
The spiking material containing the allergenic ingredient hazelnut was added to an aliquot of the basic mixture and then homogenized at 60°C with stirring. Subsequently, in 5 separate batches for each level basic mixture was added stepwise (2-3 steps) including homogenization after each step until the total amount of sample material was reached.

The 6 PT-samples were portioned to approximately 20 g into PE container and metallised PET film bags.

For the spiking a mixture of roasted and ground hazelnuts from a total of 10 products out of 5 countries (Europe) was used. This mixture of hazelnuts gave a mean recovery rate for hazelnut of about 73 % ± 35 % (n=9) for the food matrix sample (dark chocolate) of the PT DLA 06/2018 calculated from different ELISA method results.

Table 1: Composition of DLA-Samples

PT-Sample series	**Level 0** „blank"	**Level 1** 0,5 mg/kg	**Level 2** 2,5 mg/kg	**Level 3** 5,0 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**
Dark Chocolate (cocoa: 70% at least) Ingredients: Cocoa mass, sugar, cocoa butter, emulsifier: soy lecithin, vanilla extract Nutrients per 100 g: Fat 43 g, Carbohydrates 34 g, Protein 9,6 g	100	>99,9	>99,9	>99,9	>99,9	>99,9
further Ingredients: *Maltodextrin, Sodium Sulfate and Silicon dioxide*	-	<0,1	<0,1	<0,1	<0,1	<0,1
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
thereof roasted Hazelnuts: - as Hazelnuts* - *with 14,1% protein***	-	**0,504** 0,071	**2,53** 0,36	**5,04** 0,71	**12,5** 1,76	**25,2** 3,55
Extended combined uncertainty (k=2) of hazelnut content (= ± 11,0 %)		± 0,055	± 0,29	± 0,55	± 1,4	± 2,8

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material: 14,1 ± 0,15 %, n=5 (total nitrogen according to Kjeldahl with F=5,30 for hazelnut 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 hazelnut.
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, 18-20].

2.1.1 Characterization of the PT-Sample series

The PT-sample series was characterized by ELISA (Immunolab Hazelnut ELISA, n=5). 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. 7,3% to 16% and the recovery rates ranged from 38% to 46%.

Table 2: Characterization of PT-sample series hazelnut in chocolate by ELISA determination (Immunolab Hazelnut, n=5).
[Modifications: Skimmed milk powder was added as extraction additive. Level 1 was calculated below the LOQ.]

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	0,504	2,53	5,04	12,5	25,2
Result 1	0,0	0,20	1,24	2,27	5,36	11,8
Result 2	0,0	0,25	1,08	2,74	5,31	9,34
Result 3	0,0	0,25	1,29	2,08	4,53	7,73
Result 4	0,0	0,26	1,07	2,11	4,71	8,99
Result 5	0,0	0,18	1,19	2,16	4,92	10,3
Mean	0,0	0,23	1,17	2,27	4,97	9,63
SD	-	0,04	0,10	0,27	0,36	1,52
RSD [%]	-	15,6	8,3	11,9	7,34	15,8
Recovery [%]	-	45,2	46,4	45,1	39,7	38,2

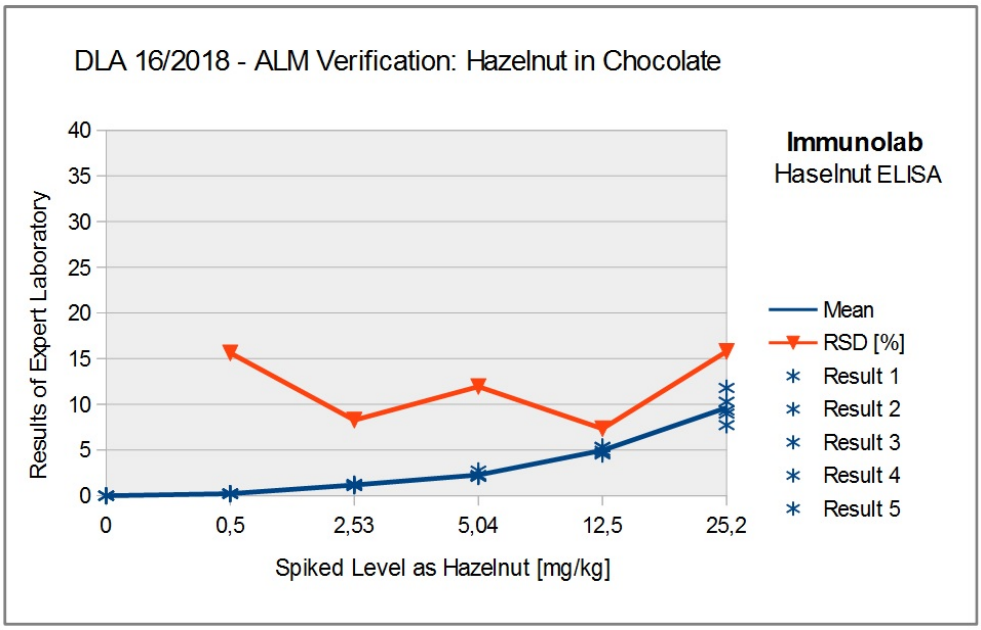


Abb./Fig. 1: ELISA results of PT-sample series hazelnut in chocolate (Immunolab Hazelnut, n=5), *Note: the x-scale is not shown linear to obtain a better recognizability of low values.*

2.1.2 Homogeneity

For testing of homogeneity 5 independent samples per level were analysed by ELISA (results see section 2.1.1).

2.1.3 Stability

The food matrix of the sample material is chocolate, which is known to be stable for years because of its low water content. The storage stability and durability of the samples (microbial spoilage) was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test material (sample 1 to 6) were sent to every participating laboratory in the 46th week of 2018. The testing method was optional. The tests should be finished at December 28th 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 Hazelnut as well as a „blank sample" in the matrix Chocolate.

- *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 hazelnut 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 5.2 Information on the PT)

2.3 Submission of results

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

9 of 10 participants submitted results in time. One participant submitted results late in consultation with DLA.

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 [31-34]. 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)[21-23, 32]

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 [22,23]
** Maximum value for declaration as „gluten free" according to EU-VO 828/2014 [21]

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) 5,0 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 [29]. 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 experiments

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 48% - 105% 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[36-37].

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 [30]. 12 food samples with gliadin contents in the range of 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 [30].

THE IRMM (Institute for Reference Materials and Measurements) proofed the suitability of five different ELISA-Kits for the determination of peanut [33]. 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} [39-40].

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**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 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 [28], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [25-27], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [29] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [24].

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 [24-29]	**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 [24]	**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.
The **qualitative and quantitative evaluations** were done **separately** for ELISA and PCR methods. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.

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

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

ELISA results given as hazelnut protein were converted to hazelnut using the experimentally determined protein content of 14,1% in the hazelnut mixture (see p.6).

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 Hazelnut

4.1.1 Qualitativ: Action Level Matrix-Scores

4.1.1.1 ELISA-Methods

Evaluation number	Level 0 „blank“	Level 1 0,50 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	Anzahl erfasster Level 1 - 5		
6	negative	negative	positive	positive	positive	positive	4 (80%)	AQ	
5	negative	positive	positive	positive	positive	positive	5 (100%)	BF	
2	negative	negative	negative	positive	positive	positive	3 (60%)	ES	
9	negative	positive	positive	positive	positive	positive	5 (100%)	IL	
4	negative	negative	positive	positive	positive	positive	4 (80%)	NL	Level 1 < LOD w eak colouring
7	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	
8	negative	positive	positive	positive	positive	positive	5 (100%)	RS-F	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	4	6	7	7	7
Number negative	7	3	1	0	0	0
Percent positive	0	57	86	100	100	100
Percent negative	100	43	14	0	0	0
Consensus value	negative	none	positive	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methodes:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

ES = ELISA-Systems

IL = Immunolab

NL = nutriLinia® Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

The Action Level (5 mg/kg) as well as the levels 4 and 5 were successfully detected by all participants. Level 2 was detected by 86% (6) and level 1 by 57% (4) of the participants. The negative results are in agreement with the limits of quantification according to the test kit instructions (AQ and NL with 1 mg/kg and ES with approx. 3-5 mg/kg as hazelnut).

4.1.1.2 PCR-Methoden

Evaluation number	Level 0 „blank“	Level 1 0,50 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	Anzahl erfasster Level 1 - 5		
1	negative	negative	negative	positive	positive	positive	3 (60%)	GI	
3	negative	positive	positive	positive	positive	positive	5 (100%)	SFA-ID	
10	negative	positive	positive	positive	positive	positive	5 (100%)	SFA	

	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
Number positive	0	2	2	3	3	3
Number negative	3	1	1	0	0	0
Percent positive	0	67	67	100	100	100
Percent negative	100	33	33	0	0	0
Consensus value	negative	none	none	positive	positive	positive
Spiking	negative	positive	positive	positive	positive	positive

Methods:

GI = GEN-IAL First Allergen

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

SFA = Sure Food Allergen, R-Biopharm / Congen

Comments:

The Action Level (5 mg/kg) as well as the levels 4 and 5 were successfully detected by all participants. Level 2 and level 1 were detected by 67% (2) of the participants. The negative results for levels 1 and 2 are in agreement with the limit of detection according to the test kit information (GI with 10 mg/kg).

4.1.2 Quantitative: Recovery-Scores

4.1.2.1 ELISA-Results

Evaluation number	Level 1 - 0,50 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		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *			
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA **		
6	<LOD		<LOQ		1,34	27	2,92	23	6,25	25	0/3 (0%)	AQ	
5	1,50	298	2,60	**103**	5,24	**104**	10,1	**81**	22,5	**89**	4/5 (80%)	BF	
2	< 3,55		< 3,55		< BG		7,10	**57**	13,48	**54**	2/2 (100%)	ES	result converted °
9	0,165	33	0,940	37	2,22	44	5,52	44	11,9	47	0/5 (0%)	IL	
4												NL	
7	0,421	**84**	1,91	**75**	4,21	**84**	12,3	**98**	24,0	**95**	5/5 (100%)	RS-F	
8	< 2.5		2,70	**107**	4,00	**79**	9,20	**74**	17,5	**69**	4/5 (80%)	RS-F	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	**1**	Number in RA	**3**	Number in RA	**3**	Number in RA	**4**	Number in RA	**4**			
	Percent in RA	**33**	Percent in RA	**75**	Percent in RA	**60**	Percent in RA	**67**	Percent in RA	**67**			

° calculation p. 15

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

** Acceptance range of AOAC for allergen ELISAs

Methods:
AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
ES = ELISA-Systems
IL = Immunolab
NL = nutriLinia® Allergen-ELISA
RS-F= Ridascreen® Fast, R-Biopharm

Comments:
For the levels 2 to 5 60% to 75% of the recovery rates of the participants' results were within the AOAC recommendations of 50-150%. The results for level 1 were with one exception outside this range of acceptance.

4.1.2.2 PCR-Results

Evaluation number	Level 1 - 0,50 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		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *			
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Anzahl im AB**		
1												GI	
10	< 1		< 1		3,10	**62**	4,70	38	7,90	31	1/3 (33%)	SFA	
3	1,00	198	1,00	40	4,00	**79**	9,00	**72**	15,0	**60**	3/5 (60%)	SFA-ID	

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	**2**	Number in RA	**1**	Number in RA	**1**
Percent in RA	**0**	Percent in RA	**0**	Percent in RA	**100**	Percent in RA	**50**	Percent in RA	**50**

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

** Acceptance range of AOAC for allergen ELISAs

Methods:

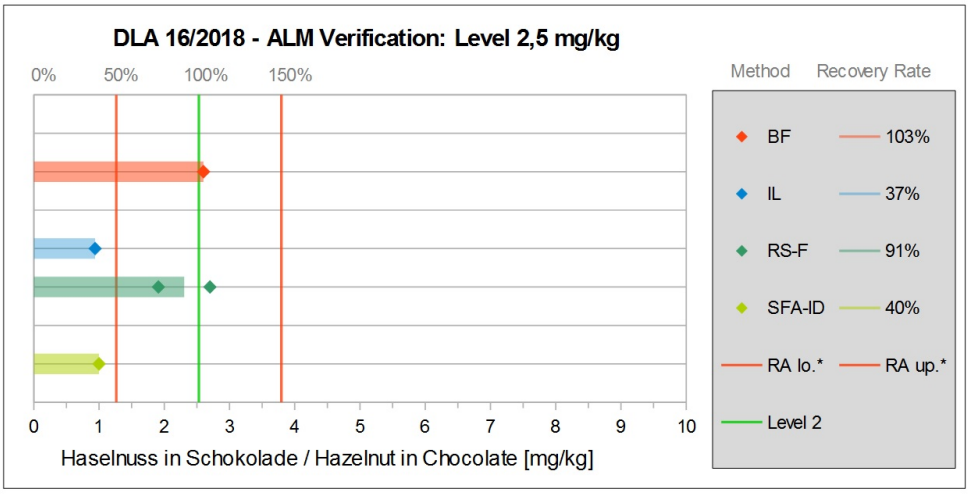
GI = GEN-IAL First Allergen

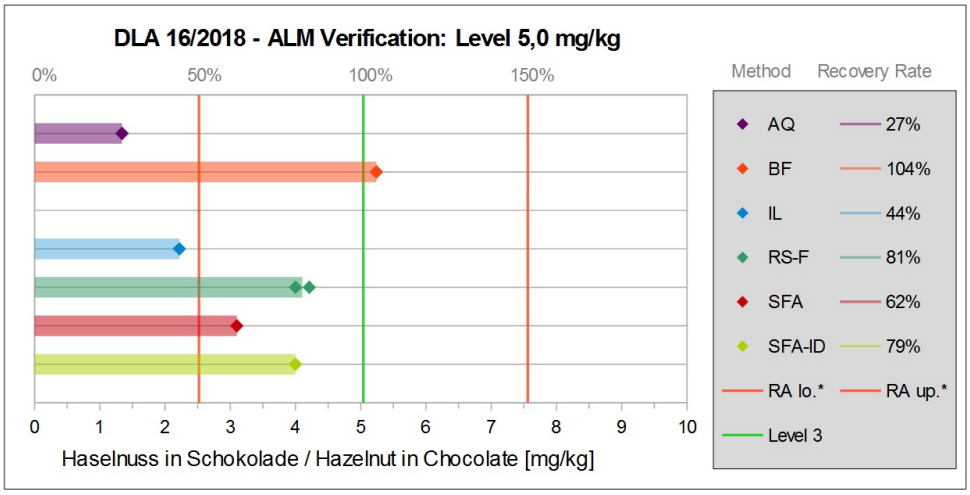
SFA = Sure Food Allergen, R-Biopharm / Congen

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

Comments:

For the action level (level3) the recovery rates of the two participants' results were within the AOAC recommendations of 50-150%. One participant obtained recovery rates within this range of acceptance for levels 4 and 5 too.





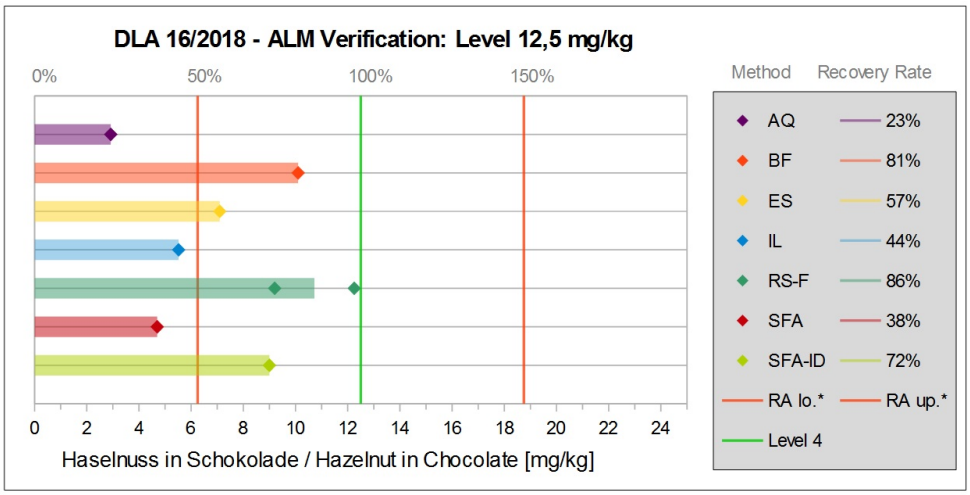


Abb./Fig. 2: Graphs of single results (Level 2-4) separated by methods with corresponding mean recovery rates, lower scale hazelnut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

4.1.3 Informative Data: Statistical characteristics hazelnut

4.1.3.1 ELISA-Methods

Sample: Action Level 5,0 mg/kg

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	5
Number of outliers	0
Mean	3,40
Robust Mean	3,40
Median (X_{pt})	**4,00**
Robust standard deviation (S*)	**1,80**
Target range:	
Target standard deviation σ_{pt}	**1,00**
lower limit of target range	**2,00**
upper limit of target range	**6,00**
Quotient S/σ_{pt}*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*1,00*
Results in the target range	*4*
Percent in the target range	*80*

Comments on the statistic data:

Assigned value was the median of all results. The calculation of the z-scores was based on a target standard deviation of 25% (see Fig. 3, p. 23).

All data are for information only.

Sample: Level 25 mg/kg

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results	6
Number of outliers	0
Mean	15,9
Median	15,5
Robust Mean (X_{pt})	**15,9**
Robust standard deviation (S*)	**7,62**
Target range:	
Target standard deviation σ_{pt}	**3,98**
lower limit of target range	**7,97**
upper limit of target range	**23,9**
Quotient S/σ_{pt}*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*3,89*
Results in the target range	*4*
Percent in the target range	*67*

Comments on the statistic data and comparison of the reference values:

Assigned value was the robust mean of all results (algorithm A). The calculation of the z-scores was based on a target standard deviation of 25% (see Fig. 4, p. 23).

All data are for information only.

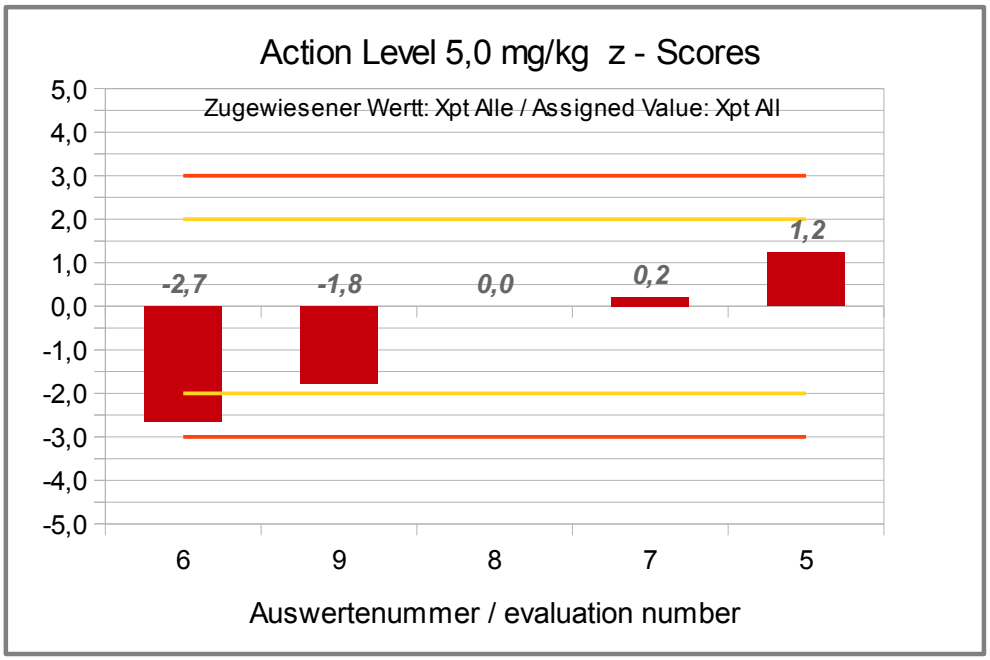


Abb./Fig. 3:
z-Scores action level 5,0 mg/kg (ELISA-results as hazelnut)
Assigned value: median of all results

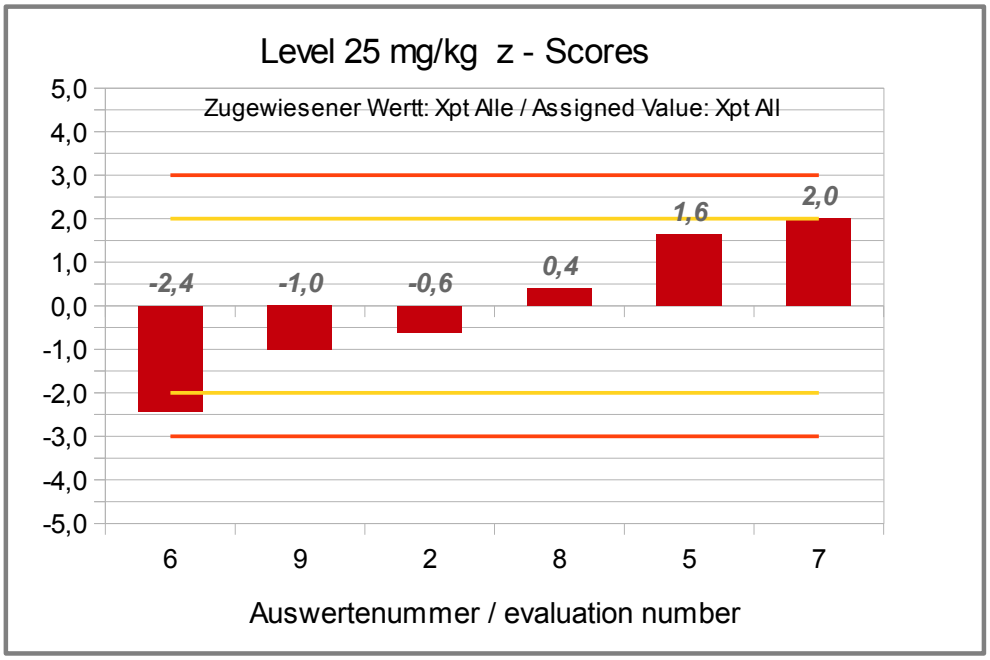


Abb./Fig. 4:
z-Scores level 25 mg/kg (ELISA-results as hazelnut)
Assigned value: robust mean (alg. A) of all results

4.1.3.2 PCR-Methods

There were only two results obtained by PCR methods, thus no statistical valuation was done.

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

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 12,5 mg/kg Level		Result Sample 2 „Nullprobe“		Result Sample 3 25 mg/kg Level		Result Sample 4 5,0 mg/kg Level		Result Sample 5 2,5 mg/kg Level		Result Sample 6 0,5 mg/kg Level		NWG / LOD *	BG / LOQ *	MU*	Quantitative Result given as	Method
		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	Test-Kit + Provider
AQ	6	03.12.18	-	2,92	negative		-	6,25	-	1,34	positive	<LOQ	negative		0,3	1		Hazelnut	AgraQuant ELISA Hazelnut COKAL0348, RomerLabs
BF	5	27/12	positive	10,1	negative	0	positive	22,5	positive	5,24	positive	2,6	positive	1,5	0,04	1		Hazelnut	MonoTrace Hazelnut ELISA kit, BioFront Technologies
ES	2	26.11.18	positive	1	negative	<0,5	positive	1,9	positive	positive <LOQ	negative	<0,5	negative	<0,5	0,25	0,5		Hazelnut protein	ELISA Systems Hazelnut ESHRD-48
IL	9	26.11.18	positive	5,52	negative	0.0	positive	11,9	positive	2,22	positive	0,94	positive	0,165	0,3	1		Hazelnut	Immunolab Hazelnut ELISA
NL	4	13.12.18	positive		negative		positive		positive		positive		negative		0,3	1			nutriLinia® Hazelnut-ELISA
RS-F	7	21.11.	positive	12,25	negative	< 0,19	positive	23,96	positive	4,21	positive	1,91	positive	0,421	0,19	2,5		Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm
RS-F	8	27.11.18	-	9,2	-	< 1.5	-	17,5	-	4	-	2,7	-	Pos. < 2.5	1,5	2,5	30% at measured level (higher than LOQ)	Hazelnut	Ridascreen® FAST Hazelnut R6802, R-Biopharm

Continuation details by participants:

Method Abbr.	Evaluation number	Specificity	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Antibody	e.g. Extraction solution / Time / Temperature	yes/no	
AQ	6			yes	<LOQ; measured value = 0,66ppm
BF	5	Monoclonal antibody	1:20 extraction ratio/10 min/60°C	no	
ES	2	detects hazelnut proteins	according to test kit manufacturer	yes	Sample 4: non-quantifiable traces betw een LOD and LOQ
IL	9		use of extraction additive + skimmed milk pow der		LOD refers to pure buffer, because blank matrix w as identified as sample 2, sample 6 w as judged as positive
NL	4	Cor a9 / a11	according to test kit manual	yes	Probe 6 < NWG leichte Färbung
RS-F	7		according to manual, w ith skimmed milk pow der	yes	
RS-F	8			yes	

5.1.2 PCR Methods

Meth. Abbr.	Evaluation number	Date of Analysis	Result Sample 1 12,5 mg/kg Level		Result Sample 2 „Nullprobe“		Result Sample 3 25 mg/kg Level		Result Sample 4 5,0 mg/kg Level		Result Sample 5 2,5 mg/kg Level		Result Sample 6 0,5 mg/kg Level		NWG / LOD *	BG / LOQ *	MU*	Quantitativee Result given as	Method
		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	Test-Kit + Provider
GI	1		positive		negative		positive		positive		negative		negative						GEN-IAL First-Hazelnut /RomerLabs
SFA	10	15.01.19	positive	4,7	negative		positive	7,9	positive	3,1	positive	< 1	positive	< 1	0,4	1		Hazelnut	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	3	04.12.18	positive	9	negative	0	positive	15	positive	4	positive	1	positive	1	< 0,4	1		Hazelnut	Sure Food Allergen ID, R-Biopharm / Congen

Continuation details by participants:

Method Abk.	Evaluation number	Specificity	Remarks to the Method (Extraction and Determination)	**Method** accred. accord. ISO/IEC 17025	Further remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
GI	1	CorA1-Gen	§64 LFGB L44.00-8	yes	Sample 4 w eakly positive
SFA	10	Corylus	Sure Food Prep Advanced Protocol 1	yes (qualitative)	Art. no. S3602
SFA-ID	3		CTAB / Quiaquick	yes	Semiquantitative valuation by SureFood Quantard Allergen 40

5.2 Information on the Proficiency Test (PT)

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

PT number	***DLA 16-2018***
PT name	***ALM-Verification Hazelnut: 5 calibration samples containing roasted Hazelnut in Chocolate-Matrix*** *(and a "blank sample")*
Sample matrix (processing)	***Samples 1-6:*** *Chocolate 70%/ ingredients: Cocoa mass, sugar, cocoa butter, emulsifier: lecithins, vanilla extract other food additives and the allergenic food hazelnut*
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):* *Hazelnut (Hazelnut protein / DNA)* *Levels: 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 December 28th 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-Scharf, 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
		Germany
		USA
		Germany
		Germany
		Germany
		ITALY
		Germany
		Germany
		Germany
		AUSTRIA

[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
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. EN ISO/IEC 17034:2016; Konformitätsbewertung - Allgemeine Anforderungen an die Kompetenz von Referenzmaterialherstellern / General requirements for the competence of reference material producers
19. ISO Guide 34:2000; General requirements for the competence of reference material producers
20. DAkkS 71 SD 1/4 016; Ermittlung und Angabe der Messunsicherheit nach Forderungen der DIN EN ISO/IEC 17025 (2011) [Estimation and indication of the measurement uncertainty]
21. Durchführungsverordnung der Kommission/ Commission Implementing Regulation EU 828/2014; über die Anforderungen an die Bereitstellung von Informationen für Verbraucher über das Nichtvorhandensein oder das reduzierte Vorhandensein von Gluten in Lebensmitteln / on the requirements for the provision of information

to consumers on the absence or reduced presence of gluten in food
22. Taylor et al. (2014) Establishment of reference doses for residues of allergenic foods: report of the VITAL Expert Panel, Food Chem Toxicol 63: 9-17
23. Demmel et al. (2015) Kap. 4.1 Existierende Aktionswerte, in: Allergene in Lebensmitteln, Behr's Verlag, Hamburg [Chapter 4.1 Existing Action Levels, in Allergens in Foods]
24. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, CAC/GL 74-2010
25. 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
26. 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
27. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
28. Ministry of Health and Welfare, JSM, Japan 2006
29. Working Group Food Allergens, Abbott et al., Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices JAOAC Int. 93:442-50 (2010)
30. Working Group on Prolamin Analysis and Toxicity (WGPAT): Méndez et al. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol. 17:1053-63 (2005)
31. DLA Publikation: Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.); Scharf et al.; J Agric Food Chem. 61(43):10261-72 (2013)
32. EFSA (2014) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes1, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(11):3894
33. IRMM, Poms et al.; Inter-laboratory validation study of five different commercial ELISA test kits for determination of peanut residues in cookie and dark chocolate; European Commission, Joint Research Centre, Belgium; GE/R/FSQ/D08/05/2004
34. Jayasena et al. (2015) Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. J Agric Food Chem. 2015 Feb 18;63(6):1849-55
35. ASU §64 LFGB L 06.00-56 Bestimmung von Sojaprotein in Fleisch und Fleischerzeugnissen Enzymimmunologisches Verfahren (2007) [Determination of soyprotein in meat and meat products by enzyme immunoassay]
36. ASU §64 LFGB L 00.00-69 Bestimmung von Erdnuss-Kontaminationen in Lebensmitteln mittels ELISA im Mikrotiterplattensystem (2003) [Foodstuffs, determination of peanut contamintions in foodstuffs by ELISA in microtiterplates]
37. ASU §64 LFGB L 44.00-7 Bestimmung von Haselnuss-Kontaminationen in Schokolade und Schokoladenwaren mittels ELISA im Mikrotiterplattensystem (2006) [Foodstuffs, determination of hazelnut contamintions in chocolate and chocolate products by ELISA in microtiterplates]
38. ASU §64 LFGB L 18.00-20 Untersuchung von Lebenmitteln - 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]
39. ASU §64 LFGB L 18.00-21 Untersuchung von Lebenmitteln - Nachweis und Bestimmung von Paranuss (Bertholletia exceisa) in Reis- und Weizenkeksen sowe

in Soßenpulver mittels real-time PCR (2014) [Foodstuffs, detection and determination of brazil nut (Bertholletia exceisa) in rice and wheat cookies and sauce powders by PCR]

40. ASU §64 LFGB L 18.00-22 Untersuchung von Lebenmitteln - Simultaner Nachweis und Bestimmung von Lupine, Mandel, Paranuss und Sesam in Reis- und Weizenkeksen sowie Soßenpulver mittels real-time PCR (2014) [Foodstuffs, simultaneous detection and determination of lupin, almond, brazil nut and sesame in rice and wheat cookies and sauce powders by PCR]