Proficiency Tests
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Evaluation Report
proficiency test

DLA 14/2018

Response PT Hazelnut:

5 Processed Samples Hazelnut (not roasted), Nut Butter (roasted), Nut Spread with Cocoa, Nut Nougat and Nut Crocant

in Potato Powder Matrix

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

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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.

Inhalt / Content

1. Introduction

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

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

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

2. Realisation

2.1 Test material

6 PT-samples for qualitative and optionally quantitative determination of hazelnut in unroasted and roasted hazelnuts, nut crocant, nut nougar and nut spread with cacao in potato powder / maltodextrin were provided.

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

Premixes with contents from approx. 1,0 - 10 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed if necessary, mixed gravimetrically, homogenized and sieved (mesh 400-600 µm). Afterwards the raw materials were mixed with further ingredients and crushed by a ball mill and homogenized.
The allergen-premixes were added to the carrier matrix of potato powder / maltodextrin (mesh < 500 µm) and homogenized. An aliquot of the carrier matrix was provided as the "blank sample".

The 6 PT-samples were portioned to approximately 20 g in metallized PET film bags.
The contents of hazelnut of the PT-samples were in the range of 50 to 54 mg/kg (see Tab. 1).

Each assigned value, here the spiked allergen-contents, is afflicted with a standard uncertainty. As uncertainties the following factors were considered: protein content of spiking materials, mixing homogeneity, homogeneity and stability of hazelnut protein.
All uncertainties were expressed in the form of their standard deviations and then added as variances. The square root from the sum of the total variances results in the combined uncertainty "Uc". Multiplied with the coverage factor k=2 the extended uncertainties of the assigned values "$U(X_{pt})$" are obtained [3, 13, 16-17].

Table 1: Composition of DLA-Samples

PT-Sample series	**Sample 1** **Hazelnut, unroasted**	**Sample 2** **Hazelnut, roasted**	**Sample 3** **Nut Crocant**	**Sample 4** **Nut Spread with Cocoa**	**Sample 5** **Nut Nougat**	**Sample 6** **„blank"**
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Potato powder Ingredients: potato, E471, E304, E223, E100 Nutrients per 100 g: Protein 8,3 g, carbohydrates 76 g, fat 0,6 g, salt 0,15 g	75	75	75	75	75	75
Maltodextrin	25	25	25	25	25	25
Allergen-Premixes Ingredients: maltodextrin (75% - 92%), sodium sulfate (< 6%), silicon dioxide (< 3%), processed allergen products (each 1,0% - 10% hazelnut)	0,051	0,054	0,11	0,50	0,16	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Hazelnut, unroasted** Protein 13,0% ** (10 products, Europe)	50,4	-	-	-	-	-
*Hazelnut, roasted** Protein 14,1% ** *(10 products, Europe)*	-	53,7	-	-	-	-
*Nut Crocant** (20% Hazelnut and sugar/glucose) Protein 3,8% ** *(5 products, Europe)*	-	-	53,6	-	-	-
*Nut Spread with Cocoa** (12% Hazelnut and other ingredients) Total protein 5,4 % *** *(7 products, Europe)*	-	-	-	51,0	-	-
*Nougat** (33% Hazelnut and other ingredients) Total protein 6,7 % *** *(6 products, Europe)*	-	-	-	-	53,2	-
- as Hazelnut	50,4	53,7	53,6	51,0	53,2	-
Extended combined uncertainty (k=2) of hazelnut-content (= ± 11 %)	± 5,54	± 5,91	± 5,90	± 5,61	± 5,85	-

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material mixtures (total nitrogen according to Kjeldahl with F=5,30 for hazelnut protein)
***Protein content calculated according to the declaration of the products (besides hazelnut, other protein sources are included)

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

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1 to 5 showed a probability of 47%, 94%, 83%, 29% and 99%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value of 1,2, 0,7, 0,9, 1,2 and 0,4 respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

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

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,31 (20,1°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 48th week of 2018. The testing method was optional. The tests should be finished at January 11th 2019 the latest.

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

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

- *The samples 1-5 are numbered in a random order. They contain Hazelnut (not roasted), Nut Butter (roasted), Nut Spread with Cocoa, Nut Nougat and Nut Crocant with known amounts of total hazelnut / hazelnut protein, which is the base for the response comparison of the quantitative results of the participants.*
- *Please give all your quantitative results as total hazelnut, if possible indicate the underlying total protein content in hazelnuts.*
- *Possible conversion factors for processed hazelnut products are queried separately in the result submission file.*

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website.
On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item in mg/kg were evaluated.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 11 participants submitted the results in time.

3. Evaluation

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

In the present PT five different processed products containing the allergen hazelnut, hazelnut (unroasted), nut butter (roasted), nut spread with cocoa, nut nougat and nut crocant, were provided to determine the qualitative detectability and to determine the response of the used quantitative methods.

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

3.1 Qualitative Score

The qualitative valuation of each participant's results was performed with Scores from 1-5 considering the number of "positive" or "negative" results matching the spiking of the PT-sample series (see Tab. 2).
A Score from 5 indicates, that all processed products were detected successfully.
The results of the matrix sample no. 6 ("blank"-sample) were not evaluated if the participant result is in accordance with ≥75% positive or negative results of participants (consensus value) or if the result is below the limit of quantification of the used method.

Table 2: Evaluation of results using qualitative Scores

Sample 1 Hazelnut, unroasted	Sample 2 Hazelnut, roasted	Sample 3 Nut Croc-ant	Sample 4 Nut Spread with Cocoa	Sample 5 Nut Nougat	Sample 6 „blank"	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not sucessful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

3.2 Recovery-Score (RR-Score)

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

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

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

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

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 43% - 121% for PCR methods, depending on matrix or processing and concentration (s. Table 3a and 3b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 3a: ELISA-Methods – Recovery rates and precision data from selected precision experiments[33-34].

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 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 [30].

The IRMM (Institute for Reference Materials and Measurements) proved 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 3b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations from experiments by precision and the resulting target standard deviation σ_{pt} [35-37].

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 multiplex ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR multiplex ASU 18.00-22
Brazil nut	Rice cookie	89,1 17,3 9,8	89,1 % 86,5 % 98 %	-	34,1% 36,2% 40,2%	34,4% 38,2% 41,8%	24,5% 28,4% 30,6%	rt-PCR ASU 18.00-21
Brazil nut	Wheat cookie Sauce powder	80,8 42,6	65,7 % 42,6 %	-	25,6% 27,5%	36,4% 39,7%	31,6% 34,6%	rt-PCR ASU 18.00-21
Brazil nut	Rice cookie	96,6 14,2	96,6 % 71 %	-	16,8% 54,2%	31,8% 56,5%	29,5% 41,5%	rt-PCR multiplex ASU 18.00-22
Brazil nut	Wheat cookie Sauce powder	76,5 48,4	62,2 % 48,4 %	-	15,6% 34,4%	35,8% 37,5%	34,1% 28,5%	rt-PCR multiplex ASU 18.00-22

3.2.2 Values by perception

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

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

Table 4: ELISA validation criteria

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

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

Table 5: PCR validation criteria

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

(a) = Trueness / Richtigkeit

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

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done separately for ELISA-, PCR- and LC/MS methods.

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

The **comparability of quantitative result specification** was given as all ELISA, PCR and LC/MS results were reported as hazelnut. A conversion of the results was not required.

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

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

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

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

* Recovery Rate

4.1 Proficiency Test Processed Hazelnut Products

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Sample 1 Hazelnut, unroasted	Sample 2 Hazelnut, roasted	Sample 3 Nut Cro-cant	Sample 4 Nut Spread with Cocoa	Sample 5 Nut Nougat	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Anzahl erfasster Proben 1 - 5		
10	positive	positive	positive	positive	positive	negative	5 (100%)	AQ	
1	positive	positive	positive	positive	positive	negative	5 (100%)	BF	
6a	positive	positive	positive	positive	positive	negative	5 (100%)	EF	
8	positive	positive	positive	positive	positive	negative	5 (100%)	ES	
11	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
5	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
6b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
7	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
4	positive	positive	positive	positive	positive	negative	5 (100%)	VT	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	9	9	9	9	9	0
Number negative	0	0	0	0	0	9
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

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

4.1.2 Qualitative Scores: PCR-Methods

Evaluation number	Sample 1 Hazelnut, unroasted	Sample 2 Hazelnut, roasted	Sample 3 Nut Cro-cant	Sample 4 Nut Spread with Cocoa	Sample 5 Nut Nougat	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
7	positive	positive	negative	positive	positive	negative	4 (80%)	ASU	
8	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
3a	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
3b	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
6	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	
9	positive	positive	positive	positive	positive	negative	5 (100%)	SFA	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	6	6	5	6	6	0
Number negative	0	0	1	0	0	6
Percent positive	100	100	83	100	100	0
Percent negative	0	0	17	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method

SFA = Sure Food Allergen, R-Biopharm / Congen

Comments:

For the processed products of samples 1, 2, 4 and 5 consensus values of 100% positive results were obtained with PCR-methods. For sample 3 (nut crocant) a negative result was obtained by an ASU-method (official German method), thus the consensus value was 83% positive results.

4.1.3 Qualitative Scores: LC/MS-Methods

Evaluation number	Sample 1 Hazelnut, unroasted	Sample 2 Hazelnut, roasted	Sample 3 Nut Cro-cant	Sample 4 Nut Spread with Cocoa	Sample 5 Nut Nougat	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected samples 1 - 5		
2	positive	positive	positive	positive	positive	negative	5 (100%)	LC/MS	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Spiking	positive	positive	positive	positive	positive	negative

Methods:

LC/MS = Liquid chromatography–mass spectrometry

Comments:

For all processed products (samples 1 to 5) positive results were obtained by one participant using a LC/MS-method.

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

Evaluation number	Sample 1 Hazelnut, unroasted		Sample 2 Hazelnut, roasted		Sample 3 Nut Crocant		Sample 4 Nut Spread with Cocoa		Sample 5 Nut Nougat		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 **		
10	56,0	**111**	45,0	**84**	30,0	**56**	26,0	**51**	24,0	45	4/5 (80%)	AQ	
1	122	242	82,7	154	65,0	**121**	53,5	**105**	53,1	**100**	3/5 (60%)	BF	
6b	54,0	**107**	48,0	**89**	30,0	**56**	23,0	45	28,0	**53**	4/5 (80%)	EF	
8	51,0	**101**	35,0	**65**	41,0	**76**	29,0	**57**	23,0	43	4/5 (80%)	ES	
11	59,0	**117**	44,0	**82**	33,0	**62**	26,0	**51**	25,0	47	4/5 (80%)	IL	
5	78,0	155	64,1	**119**	51,3	**96**	36,3	**71**	34,8	**65**	4/5 (80%)	RS-F	
6a	66,0	**131**	52,0	**97**	38,0	**71**	28,0	**55**	32,0	**60**	5/5 (100%)	RS-F	
7	79,7	158	75,2	**140**	59,9	**112**	51,7	**101**	45,4	**85**	4/5 (80%)	RS-F	
4	51,0	**101**	39,0	**73**	44,0	**82**	31,0	**61**	24,0	45	4/5 (80%)	VT	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	6	Number in RA	8	Number in RA	9	Number in RA	8	Number in RA	5			
	Percent in RA	67	Percent in RA	89	Percent in RA	100	Percent in RA	89	Percent in RA	56			

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

** Acceptance range of AOAC for allergen ELISAs

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

EF = SensiSpec ELISA Kit, Eurofins

ES = ELISA-Systems

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

For sample 3 (nut crocant) 100% of the recovery rates of the ELISA methods were in the range of acceptance of 50-150%. For roasted (sample 2, exception method ES) and unroasted hazelnuts (sample 1), the response was higher, with one and three recovery rates above 150%, respectively. For sample 4 (nut spread with cocoa) and sample 5 (nougat), a lower response was observed with one and four recovery rates below 50%, respectively.
In total, 56-100% of the recovery rates of the participant results were in the range of acceptance of 50-150%.

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

Evaluation number	Sample 1 Hazelnut, unroasted		Sample 2 Hazelnut, roasted		Sample 3 Nut Crocant		Sample 4 Nut Spread with Cocoa		Sample 5 Nut Nougat		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 **		
7												ASU	
8												ASU	
3a	114	226	74,0	**138**	71,5	**133**	60,7	**119**	45,4	**85**	4/5 (80%)	SFA	calibration see documentation
3b	68,5	**136**	47,5	**88**	37,0	**69**	40,0	**78**	34,0	**64**	5/5 (100%)	SFA	calibration see documentation
6												SFA	
9												SFA	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	1	Number in RA	2	Number in RA	2	Number in RA	2	Number in RA	2			
	Percent in RA	50	Percent in RA	100	Percent in RA	100	Percent in RA	100	Percent in RA	100			

Methods:
ASU = ASU §64 Methode/method
SFA = Sure Food Allergen, R-Biopharm / Congen

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

** Acceptance range of AOAC for allergen ELISAs

Comments:

With one exception, the recovery rates of the PCR methods were in the range of acceptance of 50-150%. Only for the unroasted hazelnuts (sample 1) was a recovery rate well above 150% obtained.

4.1.6 Quantitative: LC/MS-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Hazelnut, unroasted		Sample 2 Hazelnut, roasted		Sample 3 Nut Crocant		Sample 4 Nut Spread with Cocoa		Sample 5 Nut Nougat		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 **		
2	72	143	92,9	173	104,7	195	77	151	82,4	155	1/5 (20%)	LC/MS	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	1	Number in RA	0	Number in RA	0	Number in RA	0	Number in RA	0			
	Percent in RA	100	Percent in RA	0	Percent in RA	0	Percent in RA	0	Percent in RA	0			

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

** Acceptance range of AOAC for allergen ELISAs

Methods:
LC/MS = Liquid chromatography– mass spectrometry

Comments:

The recovery rates for unroasted hazelnuts (sample 1), nut spread with cocoa (sample 4) and nougat (sample 5) were each close to 150%, the upper limit of the range of acceptance. For roasted hazelnuts (sample 2) and nut crocant (sample 3), higher recovery rates were obtained.

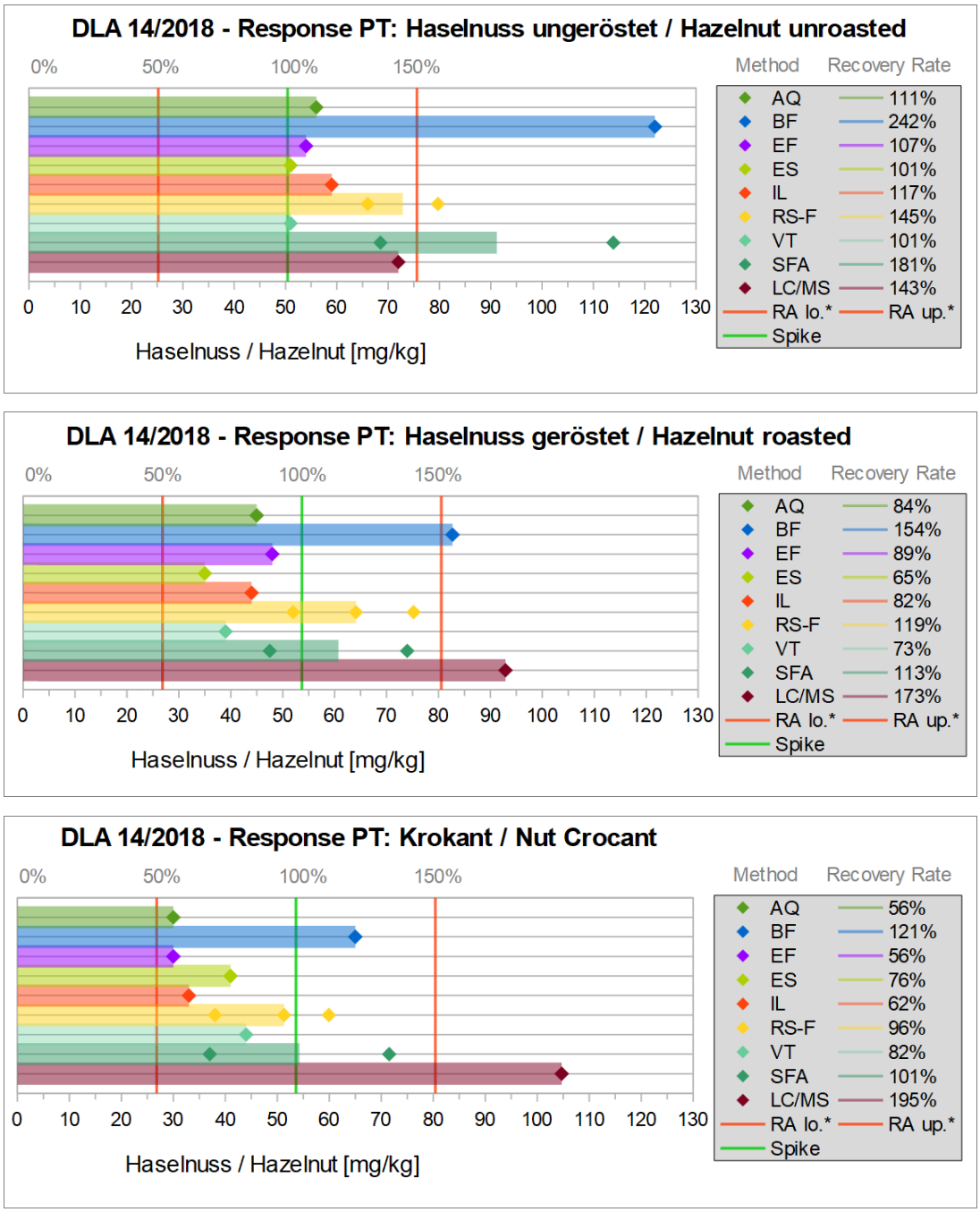
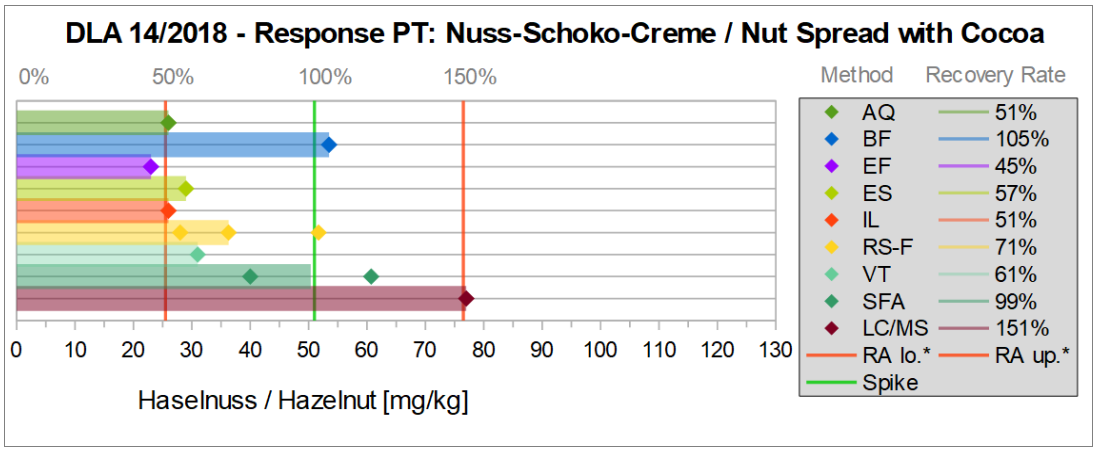


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale hazelnut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



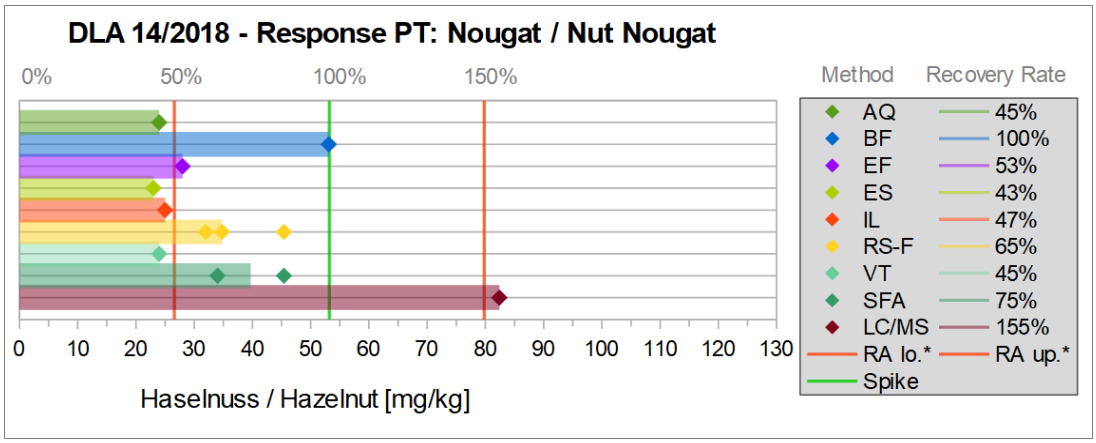


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale hazelnut content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

5. Documentation

5.1 Details by the participants

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

5.1.1 ELISA-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as hazelnut
AQ	10	03.12.18	-	56	-	45	-	30	-	26	-	24	negative		0,3	1		Hazelnut
BF	1	11.01.19	-	122	positive	82,7	positive	65	positive	53,5	positive	53,1	negative	0	1	0,04		Hazelnut
EF	6b	12.12.18	-	54	-	48	-	30	-	23	-	28	-	<0.3	0,3			Hazelnut
ES	8	12.12.18	-	51	positive	35	positive	41	positive	29	positive	23	negative	<3,7	1,85	3,7		Hazelnut
IL	11	10.12.18	-	59	positive	44	positive	33	positive	26	positive	25	negative	<0.3	0.3	1		Hazelnut
RS-F	5	17.12.	positive	78,0	positive	64,1	positive	51,3	positive	36,3	positive	34,8	negative	< 2,5	0,19	2,5		Hazelnut
RS-F	6a	12.12.18	-	66		52		38	-	28	-	32	-	<1.5	1,5			Hazelnut
RS-F	7	14.12.18	-	79,7	positive	75,2	positive	59,9	positive	51,7	positive	45,4	negative		2,5	2,5	0,38	Hazelnut
VT	4	20.12.18	-	51	positive	39	positive	44	positive	31	positive	24	negative	0		2,5		Hazelnut

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

Continuation details by participants: ELISA-Methods

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in hazelnut (According to method prescription)	Conversion for processed hazelnut	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	%	Recalculation from X to Y (factor or %)	e.g. Extraction solution / time / temperature	yes/no	
AQ	10	AgraQuant ELISA Hazelnut COKAL0348, RomerLabs					yes	
BF	1	MonoTrace Hazelnut ELISA kit, BioFront Technologies	monoclonal antibody-based kit	1:20 extraction ratio, 10 minutes at 60C	not provided	1:20 extraction ratio, 10 minutes at 60C	no	
EF	6b	Eurofins SensiSpec Hazelnut ELISA Kit					no	
ES	8	ELISA Systems Hazelnut ESHRD-48	detects hazelnut proteins	12-15		according to manufacturer's instructions	yes	Sample 1 qualitative: positive (cannot be entered), 1mg/kg hazelnut protein equivalent to 7,4mg/kg hazelnut according to provider
IL	11	Immunolab Hazelnut ELISA	polyclonal					
RS-F	5	Ridascreen® FAST Hazelnut R6802, R-Biopharm				according to manufacturer's instructions, with skimmed milk powder	yes	
RS-F	6a	Ridascreen® FAST Hazelnut R6802, R-Biopharm					yes	
RS-F	7	Ridascreen® FAST Hazelnut R6802, R-Biopharm		unknown	no conversion	according to manual with addition of milk powder	yes	For sample 1 the input box of the qualitative result is locked. MU of the method indicated.
VT	4	Veratox Hazelnut, Neogen				Phosphate Buffered Salt Solution / 15 mins / 60oC	yes	Single Result

5.1.2 PCR-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as hazelnut
ASU	7	14.12.18	positive		positive		negative		positive		positive		negative					Hazelnut-DNA
ASU	8	06.12.18	positive		positive		positive		positive		positive		negative		10			Hazelnut-DNA
SFA	3a	17.12.18	+	113,88	+	73,97	+	71,51	+	60,72	+	45,41	-	0	< 0,4	1		
SFA	3b	17.12.18	+	68,5	+	47,5	+	37	+	40	+	34	-	0	< 0,4	1		
SFA	6	12.12.18	positive		positive		positive		positive		positive		negative		0,4			Hazelnut DNA
SFA	9	28.12.18	positive		positive		positive		positive		positive		negative		0,4	1	0,3	Hazelnut

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in hazelnut (According to method prescription)	Conversion for processed hazelnut	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider	Target sequence / DNA	%	Recalculation from X to Y (factor or %)	e.g. Extraction / Enzyme / Clean-Up / Real Time PCR / Gel Electrophoresis / Cycles	yes/no	
ASU	7	ASU §64 Methode/method	Cor a 1			Extraction with 2 g lysis with the Maxwell FFS Kit.	yes	ASU method based on PCR multiplex with other nuts. Sample 3 not reproducible positive.
ASU	8	ASU §64 Methode/method				CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	§ 64 LFGB L 44.00-08:2010-01
SFA	3a	Sure Food Allergen, R-Biopharm / Congen				CTAB / Quiaquick	yes	Quantitative evaluation by copy standard
SFA	3b	Sure Food Allergen, R-Biopharm / Congen				CTAB / Quiaquick	yes	Quantitative evaluation by Quantard Allergen 40 dilutions
SFA	6	Sure Food Allergen, R-Biopharm / Congen					yes	
SFA	9	Sure Food Allergen, R-Biopharm / Congen	Corylus			Sure Food Prep Advanced Protokoll 1	yes	Article no. S3602

5.1.3 LC/MS-Methods

Method Abr.	Evaluation Number	Date of Analysis	Result Sample 1		Result Sample 2		Result Sample 3		Result Sample 4		Result Sample 5		Result Sample 6		NWG / LOD *	BG / LOQ *	MU*	Specification of quantitative result as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	mg/kg	prefered as hazelnut
LC/MS	2		positive	72	positive	92,9	positive	104,7	positive	77	positive	82,4	negative	< 10	3	10	0,2	Hazelnut

Method Abr.	Evaluation Number	Method	Specificity	Total protein content in hazelnut (According to method prescription)	Conversion for processed hazelnut	Remarks to the Method (Extraction and Determination)	Method accredited to ISO / IEC 17025	Further remarks
		Test-Kit + Provider + Literature			Recalculation from X to Y (factor or %)		yes/no	
LC/MS	2	UHPLC-MS/MS	specific peptide sequences				yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 14-2018 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	21,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	77	30,6
2	4,97	71	28,6
3	5,54	72	26,0
4	4,99	79	31,7
5	5,06	58	22,9
6	5,05	69	27,3
7	5,02	61	24,3
8	4,99	77	30,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	70,6	Particles
Standard deviation	8,15	Particles
χ^2 (CHI-Quadrat)	6,58	
Probability	**47**	%
Recovery rate	132	%

Normal distribution		
Number of samples	8	
Mean	27,8	mg/kg
Standard deviation	3,21	mg/kg
rel. Standard deviaton	11,5	%
Horwitz standard deviation	9,70	%
HorRat-value	**1,2**	
Recovery rate	132	%

Microtracer Homogeneity Test
DLA 14-2018 Sample 2

Weight whole sample	1,010	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	82	33,0
2	5,06	83	32,8
3	5,05	78	30,9
4	5,00	80	32,0
5	4,96	70	28,2
6	5,02	84	33,5
7	5,03	72	28,6
8	5,02	83	33,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	79,0	Particles
Standard deviation	5,18	Particles
χ^2 (CHI-Quadrat)	2,38	
Probability	**94**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	31,5	mg/kg
Standard deviation	2,07	mg/kg
rel. Standard deviaton	6,55	%
Horwitz standard deviation	9,52	%
HorRat-value	**0,69**	
Recovery rate	107	%

Microtracer Homogeneity Test
DLA 14-2018 Sample 3

Weight whole sample	1,000	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	35,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,07	63	24,9
2	5,02	66	26,3
3	5,05	71	28,1
4	5,00	73	29,2
5	5,03	55	21,9
6	5,03	63	25,0
7	4,96	66	26,6
8	4,98	69	27,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,8	Particles
Standard deviation	5,77	Particles
χ^2 (CHI-Quadrat)	3,55	
Probability	**83**	%
Recovery rate	75	%

Normal distribution		
Number of samples	8	
Mean	26,2	mg/kg
Standard deviation	2,30	mg/kg
rel. Standard deviaton	8,78	%
Horwitz standard deviation	9,79	%
HorRat-value	**0,90**	
Recovery rate	75	%

Microtracer Homogeneity Test
DLA 14-2018 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	29,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,97	76	30,6
2	4,99	86	34,5
4	5,01	101	40,3
5	4,96	83	33,5
6	4,98	90	36,1
8	5,01	100	39,9
9	4,99	102	40,9
10	5,04	78	31,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	89,5	Particles
Standard deviation	10,39	Particles
χ^2 (CHI-Quadrat)	8,45	
Probability	**29**	%
Recovery rate	122	%

Normal distribution		
Number of samples	8	
Mean	35,8	mg/kg
Standard deviation	4,16	mg/kg
rel. Standard deviaton	11,6	%
Horwitz standard deviation	9,3	%
HorRat-value	**1,2**	
Recovery rate	122	%

Microtracer Homogeneity Test
DLA 14-2018 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	24,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,04	98	38,9
2	4,99	99	39,7
3	5,01	92	36,7
4	4,99	96	38,5
5	5,06	101	39,9
6	5,05	99	39,2
7	4,99	105	42,1
8	5,00	94	37,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	98,0	Particles
Standard deviation	4,05	Particles
χ^2 (CHI-Quadrat)	1,17	
Probability	**99**	%
Recovery rate	158	%

Normal distribution		
Number of samples	8	
Mean	39,1	mg/kg
Standard deviation	1,61	mg/kg
rel. Standard deviaton	4,1	%
Horwitz standard deviation	9,2	%
HorRat-value	**0,4**	
Recovery rate	158	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA 14-2018***
PT name	***Response PT Hazelnut: Processed samples Hazelnut (not roasted), Nut Butter (roasted), Nut Spread with Cocoa, Nut Nougat and Nut Crocant in potato powder matrix***
Sample matrix (processing)	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 "Blank" Sample: 20 g*
Storage	*Samples 1-6: room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Hazelnut / Hazelnutprotein / DNA from Hazelnut (not roasted), Nut Butter (roasted), Nut Spread with Cocoa, Nut Nougat and Nut Crocant* *Samples 1-5: approx. 25 - 150 mg/kg (as total hazelnut)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. It is the best to homogenize the whole sample.*
Result sheet	*One result each should be determined for Samples 1 - 6 and the* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of 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 11th 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Deutschland
		USA
		Deutschland
		Deutschland
		Deutschland
		Deutschland
		ITALIEN
		Deutschland
		Deutschland
		CANADA
		ÖSTERREICH

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

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
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19. ISO Guide 34:2000; General requirements for the competence of reference material producers
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23. DIN EN ISO 15634-1:2009; Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen / Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
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