Evaluation Report
proficiency test

DLA 06/2019

Allergens VI:

Peanut and Walnut

in Spread (Cocoa Cream)

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General Information on the proficiency test (PT)

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Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

Two PT-samples with the same food matrix were provided for the detection and quantitative determination of the allergens in the range of mg/kg as well as one spiking level sample with a simple matrix. One of the samples (spiked sample) and the spiking level sample contain the respective allergenic ingredients in a similar concentration range. The results of the spiking level sample should give the possibility of a comparison with the spiked sample in respect to the detectability of the allergens with and without the influence of matrix and / or food processing.

The test material of the food matrix samples is a common in commerce spread "nut nougat cream". The basic composition of both sample A and sample B was the same (see table 1). The basic mixture was homogenized by stirring at approx. 40°C.

Afterwards the **spiked sample B** was produced as follows:
The spiking material containing the allergenic ingredients peanut and walnut was added to an aliquot of the basic mixture and the mixture was homogenized at approx. 40°C. Subsequently, the basic mixture was again added in 2 additional steps and homogenized each until the total quantity had been reached.
For the **spiking level sample**, the allergenic compounds above mentioned were added during a multi-stage addition of potato powder (mesh <500 µm) and homogenization.

After homogenization the samples A and B were portioned to approx. 25 g into PE container and metallised PET film bags. The spiking level sample was portioned to approx. 15 g in metallized PET film bags.

Table 1: Composition of DLA-Samples

Zutaten	**Probe A**	**Probe B**	**Dotierungs-niveauprobe**
Spread (Nut-Nougat Cream) Ingredients: Sugar, palm oil, hazelnuts (13%), low-fat cocoa powder, skimmed milk powder, emulsifier: lecithin, vanillin Nutrients per 100 g: Fat 31 g, Carbohydrates 58 g, Protein 6,3 g	100 g/100 g	99,9 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	–	–	99,9 g/100 g
Peanuts, roasted: ground, mixture (18 products from USA, Asia, Africa, South America) – as Peanut* – *thereof 23,2% total protein***	–	 37,5 mg/kg 8,70 mg/kg	 32,6 mg/kg 7,56 mg/kg
Walnuts, raw ground, mixture (5 countries / North- a. South America, Europe) – as Walnut* – *thereof 13,6% total protein***	–	 39,6 mg/kg 5,38 mg/kg	 38,1 mg/kg 5,18 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	–	<0,02 g/100 g	<0,02 g/100 g

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl with F=5,46 for peanuts and F=5,30 for walnuts)

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].
Because stuck solid samples can not be analysed by the microtracer method, only the spiking level sample was measured. The microtracer analysis of the present PT showed a probability of 98%. 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 0,61. The results of microtracer analysis are given in the documentation.

Homogeneity of bottled spiked sample B

Implementation of homogeneity tests
The homogeneity tests were carried out in cooperation with the laboratories of the specified test kit providers. Ten samples of the bottled spiked sample were chosen randomly by DLA, thereof 2 subsamples were weighed into previously randomly encoded sample containers, and then sent to the laboratories for analysis. The sample weights were made with a deviation of ± 10% from recommended sample weight of the test kit instructions and not communicated to the laboratories. After transmission of analysis results by the laboratories, the valid results were calculated on the basis of the exact weightings by DLA and the statistical calculation was carried out according to ISO 13528:2015 Annex B (possibly with Notes 1 and 2).

Valuation of homogeneity
The homogeneity is regarded as sufficient when the standard deviation between the samples Ss is ≤ 15% („heterogeneity standard deviation"). This criterion is fulfilled for sample B by all ELISA tests for peanut (Immunolab, Veratox and AgraQuant) and walnut (Immunolab), respectively (see page 7). Recommendations for repeatability standard deviations of ELISA and PCR methods are usually ≤ 25% [18, 19, 22, 23].

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.6 and 3.8) [3].

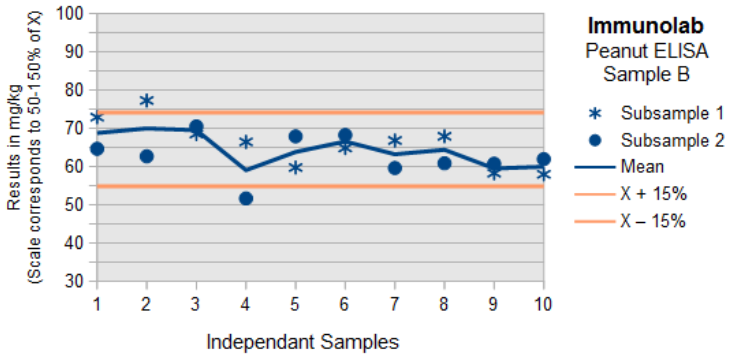
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 64,4 ± 2,9 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	72,9	64,6	68,7
2	77,2	62,6	69,9
3	68,5	70,5	69,5
4	66,4	51,6	59,0
5	59,7	67,9	63,8
6	64,8	68,2	66,5
7	66,8	59,6	63,2
8	67,9	60,8	64,3
9	58,2	60,7	59,5
10	57,9	61,9	59,9

General average X	64,4	
SD of sample means Sx	4,15	6,4%
SD within-samples Sw	4,20	6,5%
SD between-samples Ss	2,90	4,5%

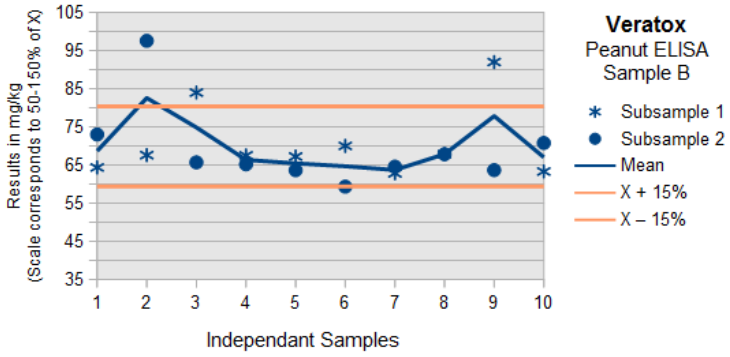


Veratox Peanut ELISA

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Peanut 69,9 ± 3,4 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	64,3	73,0	68,7
2	67,6	97,5	82,5
3	84,0	65,7	74,8
4	67,5	65,2	66,3
5	67,2	63,6	65,4
6	70,0	59,3	64,6
7	62,8	64,6	63,7
8	67,8	67,8	67,8
9	92,0	63,6	77,8
10	63,3	70,7	67,0

General average X	69,9	
SD of sample means Sx	6,33	9,1%
SD within-samples Sw	7,58	10,9%
SD between-samples Ss	3,35	4,8%

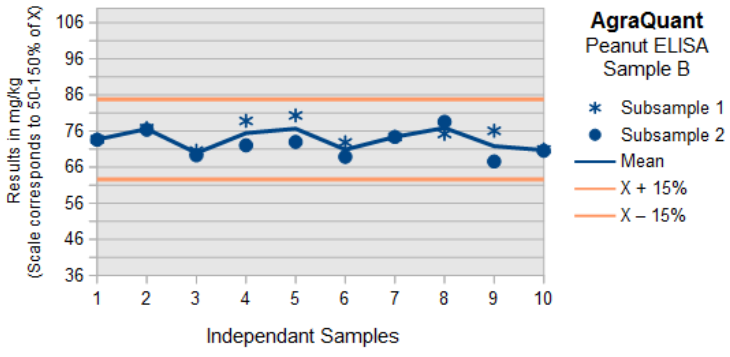


AgraQuant Peanut ELISA

Sample weights: 0,50 g (0,45 – 0,55 g)
Number of replicates: 2
Overall result: Peanut 73,7 ± 2,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	73,7	73,6	73,6
2	76,8	76,4	76,6
3	70,6	69,3	69,9
4	78,8	72,0	75,4
5	80,3	73,0	76,6
6	72,8	68,8	70,8
7	74,4	74,4	74,4
8	75,2	78,5	76,9
9	76,0	67,6	71,8
10	70,9	70,5	70,7

General average X	73,7	
SD of sample means Sx	2,70	3,7%
SD within-samples Sw	2,24	3,0%
SD between-samples Ss	2,18	3,0%



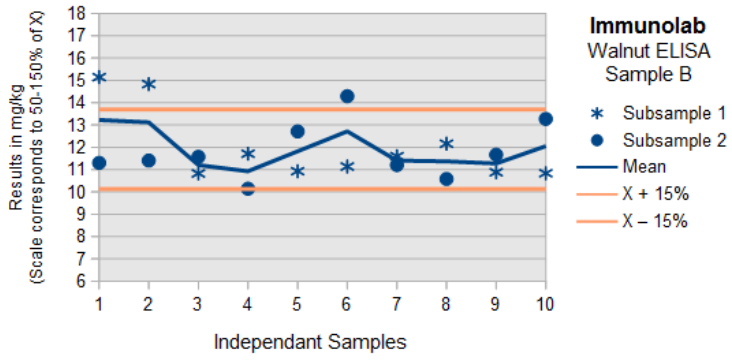
ELISA-Tests: Homogenität Walnuss / Homogeneity Walnut

Immunolab Walnut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Walnut 11,9 ± 0,2 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	15,1	11,3	13,2
2	14,8	11,4	13,1
3	10,8	11,6	11,2
4	11,7	10,1	10,9
5	10,9	12,7	11,8
6	11,1	14,3	12,7
7	11,6	11,2	11,4
8	12,2	10,6	11,4
9	10,9	11,7	11,3
10	10,8	13,3	12,1

General average X	11,9	
SD of sample means Sx	0,835	7,0%
SD within-samples Sw	1,14	9,6%
SD between-samples Ss	0,224	1,9%



2.1.2 Stability

The food matrix of the sample material is cocoa spread, 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.

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 spiking level sample was approx. 0,34 (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

The portions of test materials sample A, B and the spiking level sample were sent to every participating laboratory in the 42nd week of 2019. The testing method was optional. The tests should be finished at 29th November 2019 the latest.

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

There are two different samples A and B possibly containing the allergenic parameters ***Peanut*** *and/or* ***Walnut*** *in the range of mg/kg in the matrix of* ***Cocoa Cream****. One of these samples and the "spiking level sample" were prepared adding the allergenic ingredients. The* ***"spiking level sample"*** *contains the allergens in a simple matrix in* ***similar amounts*** *without further processing and should be analysed like a normal sample.*

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 handed out with the samples (by email).
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 quantifications, 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 24 participants submitted their results.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are eventually using different antibodies, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the content of the analyte [25, 26, 27, 28]. It is for this reason that we contrast the results of the present proficiency test with several assigned values.
Thereby it is possible to evaluate each single result in comparison to the mean of all results and/or in comparison to the mean of results obtained by a single method. For comparison the actually added amount is plotted in the figures of the results.

For quantitative results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of spiked allergens. The recovery rates were given for information only. *No* statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

ELISA- and PCR results were valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample.

3.1 Consensus value from participants (assigned value)

The **robust mean** of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the **median** may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].
In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

If possible, this is the standard procedure for the evaluation of methods for the quantitative determination of allergens:

i) ***Assigned value of all results*** - $X_{pt_{ALL}}$
ii) ***Assigned value of single methods*** - $X_{pt_{METHOD\ i}}$
with at least 5 quantitative results given.

Single results giving values outside the measuring range of the participating laboratory or given as „0" are not considered for statistical evaluation (e.g. results given as > 25 mg/kg and < 2,5 mg/kg, respectively) [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S*) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

The following robust standard deviations were considered:

i) ***Robust standard deviation of all results - S^x_{ALL}***
ii) ***Robust standard deviation of single methods - $S^x_{METHOD\ i}$***
with at least 5 quantitative results given.

3.3 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].

All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.4 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.
In the present PT the target standard deviation was determined according to 3.4.3 value by perception.

3.4.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

The target standard deviation according to Horwitz is currently not achievable by ELISA or PCR-methods for values in the mg/kg range and was therefore not considered for evaluation.

3.4.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative trial or proficiency test) the target standard deviation σ_{pt} can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2\left(m - 1/m\right)}$$

The relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) given in table 2a (ELISA) and table 2b (PCR) were obtained in precision experiments by the indicated methods. The resulting target standard deviations σ_{pt} were calculated for a number of m = 2 replicate measurements. With a number of m = 1 replicate measurements the reproducibility standard deviation σ_R is identical to the target standard deviation σ_{pt}.

Table 2a: ELISA-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [30-31]

Parameter	Matrix	Mean [mg/kg]	Recovery	rob RSD	RSD_r	RSD_R	σ_{pt}	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

From the precision data of the official German ASU §64 methods the calculated relative target standard deviations are in the range of 12 – 33% for the ELISA methods and 24 – 42% for the PCR methods depending on the matrix, processing and concentration level of allergens (s. Tab. 2a and 2b).

The Working Group on Prolamin Analysis and Toxicity (WGPAT) coordinated a collaborative study with two commercial ELISA test kits for the determination of gluten using the monoclonal R5 antibody [24]. 12 food samples with gliadin in the range of 0 - 168 mg/kg were analyzed by 20 laboratories. Recovery rates ranged between 65 and 110%, relative repeatability deviations ranged from 13 - 25% (method 1) and 11 - 22% (method 2) while the relative reproducibility standard deviations ranged from 23 - 47% (method 1) and 25 - 33% (method 2). According to the authors both ELISA test kits fulfilled therefore the current validation criteria for ELISA methods [24].

The IRMM (Institute for Reference Materials and Measurements) performed an interlaboratory comparison for five different ELISA test kits for the quantification of peanut [27]. The mean values for two matrices were in the concentration range of 0,3 - 16,1 mg/kg and 1,2 - 20,4 mg/kg, respectively. The lowest relative reproducibility standard deviations of the five test kits were for dark chocolate in the range of 20 - 42% and for cookies in the range of 23 – 61%.

Table 2b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [32-34]

Parameter	**Matrix**	**Mean [mg/kg]**	**Recov-ery**	**rob RSD**	**RSD_r**	**RSD_R**	**σ_{pt}**	**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

3.4.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].
Criteria for the level of performance of analytical methods for the quantitative determination of allergens in foods were recently elaborated e.g. by the Ministry of Health and Welfare (MHLW) in Japan [22], by the working group 12 „Food Allergens" of the technical committee CEN/TC 275 [19-21], by an international "Food Allergen Working Group" under the advice of the AOAC Presidential Task Force on Food Allergens [23] and by the Codex Alimentarius Committee (CAC/GL 74-2010) [18].

Some of the relevant ELISA and PCR validation criteria of the mentioned panels are listed in tables 3 and 4, respectively.

Table 3: ELISA-Validation

Literature [18-24]	**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 an hypothetical proficiency scheme in the range of 0,5 - 5 mg/kg

Table 4: PCR-Validation

Literature [18]	**Recovery rate**	**Repeatability standard deviation**	**Reproducibility standard deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Based on the currently achievable level of performance of ELISA and PCR methods for the quantitative determination of allergens in foods, which could be deduced from the data of precision experiments and from validation criteria, we set a relative target standard deviation σ_{pt} of 25%.
This target standard deviation was applied for the statistical evaluation of the results by z-score or if necessary by z´-Score and was used for all assigned values mentioned in 3.1.

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

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

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

For information the z-scores below are calculated with a target standard deviation of 25%:

i) ***z-Score*** - z_{ALL} *(with respect to all methods)*
ii) ***z-Score*** - $z_{METHOD\ i}$ *(with respect to single methods)*

3.5.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [3].

The calculation is performed by:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning and action signals see 3.5.1.

3.7 Quotient S*/σ_{pt}

Following the HorRat-value the results of a proficiency-test can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.8 Standard uncertainty and traceability

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(Xpt)}$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.9 Figures of assigned values

The assigned values and spiking levels are indicated as coloured lines in the figures of results. This allows the comparison of a single result with different possible target values like the spiked level, the robust mean of all results and the robust mean of a single method.

3.10 Recovery rates: Spiking

For the results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known content of added allergens. The related values of added allergens are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 50 - 150% for the recovery rates of llergen-ELISAs proposed by the AOAC was used [23]. For quantitative PCR or LC/MS determinations we use the same range of acceptance.

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done separately for ELISA and PCR-techniques. The results were grouped according to the applied methods (e.g. test kits) and sorted chronologically according to the evaluation number of the participants.
The following result sections are structured equally for the allergenic components. First all results of ELISA or PCR methods for a certain parameter are reported for samples A and B (qualitative / possibly quantitative) and afterwards for the spiking level sample (quantitative). The recovery rates of results for the spiking level sample and the spiked sample A or B are reported then.

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 **peanut protein** or **walnut protein** were converted by DLA to **total food items (peanuts, walnuts)** using the analyzed protein content of the raw materials (see page 5).

Results were valuated qualitatively with respect to the percentages of positive and negative results, respectively. If there are ≥ 75 % positive or negative results, a consensus result is determined for each sample. Each participant result is valuated qualitatively with respect to the consensus value. The valuation was given as a percentage of results in agreement with the consensus values.

When there are at least 5 quantitative results for all methods or for single methods a statistical evaluation was done.

In cases when a statistical evaluation of the quantitative values was done the result table was given as indicated below:

Evaluation number	Result	Result	z-Score Xpt_{ALL}	z-Score $Xpt_{M\,i}$	Method	Remarks
	pos/neg	[mg/kg]				

The statistical evaluation of results for each parameter was calculated in cases where at least 50% results were positive and at least 5 quantitative values were given:

Characteristics	**All Results** *[mg/kg]*	**Method i** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ i}}$
Number of results		
Number of outliers		
Mean		
Median		
Robust mean (X_{pt})		
Robust standard deviation (S*)		
Target data°:		
Target standard deviation σ_{pt} *or* σ_{pt}'		
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}'$)°		
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}'$)°		
Quotient S*/σ_{pt} *or* S*/σ_{pt}'		
Standard uncertainty $U_{(Xpt)}$		
Number of results in target range		
Percent in target range		

° Target range calculated using z-score or z'-score

After that the recovery rates of the results for the spiking level sample and the spiked sample are reported. The number of results within the range of acceptance of 50-150% is given.

4.1 Proficiency Test Peanut

4.1.1 ELISA Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
7	negative	<LOD	positive	301	2/2 (100%)	AQ	Result converted °
19	negative	<LOD	positive	64,4	2/2 (100%)	AQ	
21	negative	<LOD	positive	64,9	2/2 (100%)	AQ	
2	negative	<LOQ	positive	58,9	2/2 (100%)	BF	
18	negative	<1	positive	>40	2/2 (100%)	BF	
1	negative	<1	positive	50,0	2/2 (100%)	BK	
15a	negative	<1	positive	47,8	2/2 (100%)	BK	
16	negative	< BG	positive	41,0	2/2 (100%)	BK	
6	negative	<1	positive	52,6	2/2 (100%)	EF	
8	negative	<1,0	positive	59,1	2/2 (100%)	IL	
22	negative	0	positive	68,3	2/2 (100%)	IL	
13a	negative	< 1,34	positive	12,1	2/2 (100%)	MI-II	Result converted °
13b	negative	< 0,862	positive	51,7	2/2 (100%)	MI-III	Result converted °
3	negative	<1	positive	96,0	2/2 (100%)	RS-F	
5	negative	-	positive	90,0	2/2 (100%)	RS-F	
9	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
10	negative	<2,5	positive	81,9	2/2 (100%)	RS-F	
11	negative		positive	94,1	2/2 (100%)	RS-F	
12	negative		positive	79,0	2/2 (100%)	RS-F	
14	negative		positive	100	2/2 (100%)	RS-F	
23	negative		positive	90,0	2/2 (100%)	RS-F	
15b	negative	<2,5	positive	76,3	2/2 (100%)	VT	

° calculation see p. 19

	Sample A		Sample B	
Number positive	0		22	
Number negative	22		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
MI-III = Morinaga Institute ELISA Test Combination
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score $Xpt_{RS\text{-}F}$	Method	Remarks
	[mg/kg]				
7	301			AQ	Result converted °, Result excluded
19	64,4	-0,24		AQ	
21	64,9	-0,21		AQ	
2	58,9	-0,56		BF	
18	>40			BF	
1	50,0	-1,1		BK	
15a	47,8	-1,2		BK	
16	41,0	-1,6		BK	
6	52,6	-0,93		EF	
8	59,1	-0,55		IL	
22	68,3	-0,01		IL	
13a	12,1	-3,3		MI-II	Result converted °
13b	51,7	-0,98		MI-III	Result converted °
3	96,0	1,6	0,26	RS-F	
5	90,0	1,3	-0,01	RS-F	
9	>20			RS-F	
10	81,9	0,79	-0,37	RS-F	
11	94,1	1,5	0,18	RS-F	
12	79,0	0,62	-0,49	RS-F	
14	100	1,8	0,44	RS-F	
23	90,0	1,3	-0,01	RS-F	
15b	76,3	0,46		VT	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
MI-III = Morinaga Institute ELISA Test Combination
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

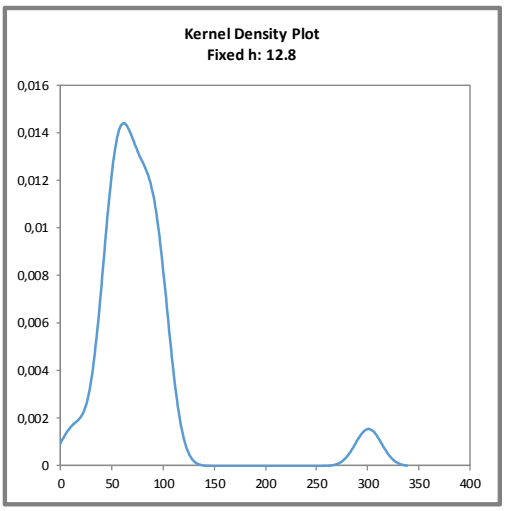


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with two shoulders below 20 mg/kg (method MI-II) and at approx. 100 mg/kg and a secondary peak at approx. 300 mg/kg (method AQ), due to an outlier above the target range.

Characteristics: Quantitative evaluation ELISA Peanut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	19°	7
Number of outliers	1	0
Mean	67,3	90,1
Median	64,9	90,0
Robust Mean (X_{pt})	**68,5**	**90,1**
Robust standard deviation (S*)	**22,5**	**8,53**
Target range:		
Target standard deviation σ_{pt}	**17,1**	**22,5**
lower limit of target range	**34,2**	**45,1**
upper limit of target range	**103**	**135**
Quotient S/σ_{pt}*	*1,3*	*0,38*
Standard uncertainty $U_{(Xpt)}$	*6,46*	*4,03*
Results in the target range	*18*	*7*
Percent in the target range	*95*	*100*

° *without result No. 7 (excluded in advance)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (one increased single value).

The evaluation of all methods and the evaluation of results from method RS-F showed a normal to low variability of results, with quotients S/σ_{pt} below 2,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 183% and 240% of the spiking level of peanut to sample B and thus above the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for Peanut").

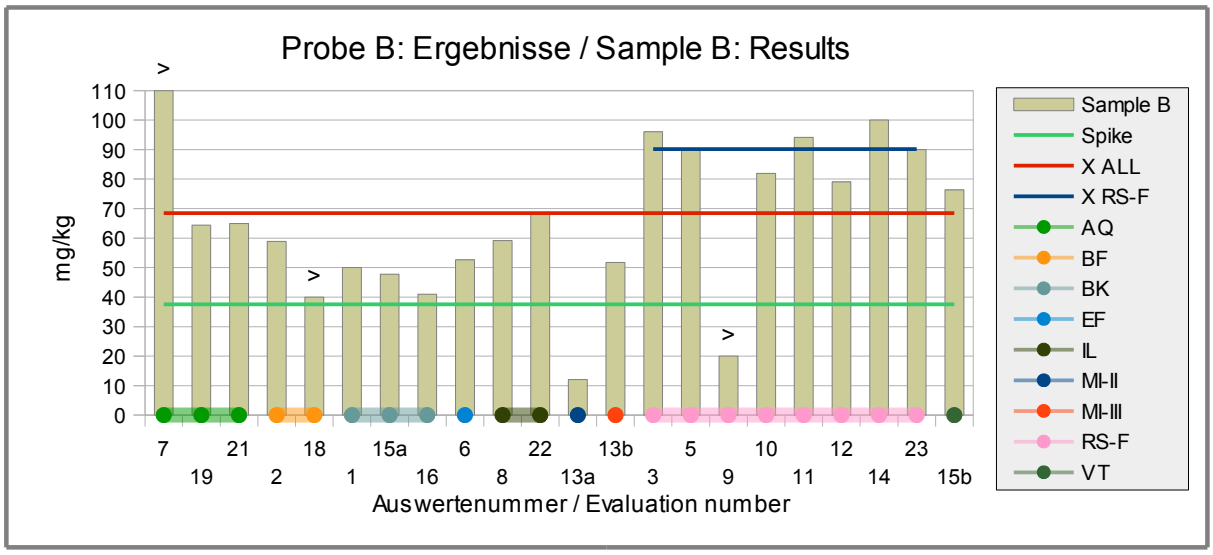


Abb./Fig. 2: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

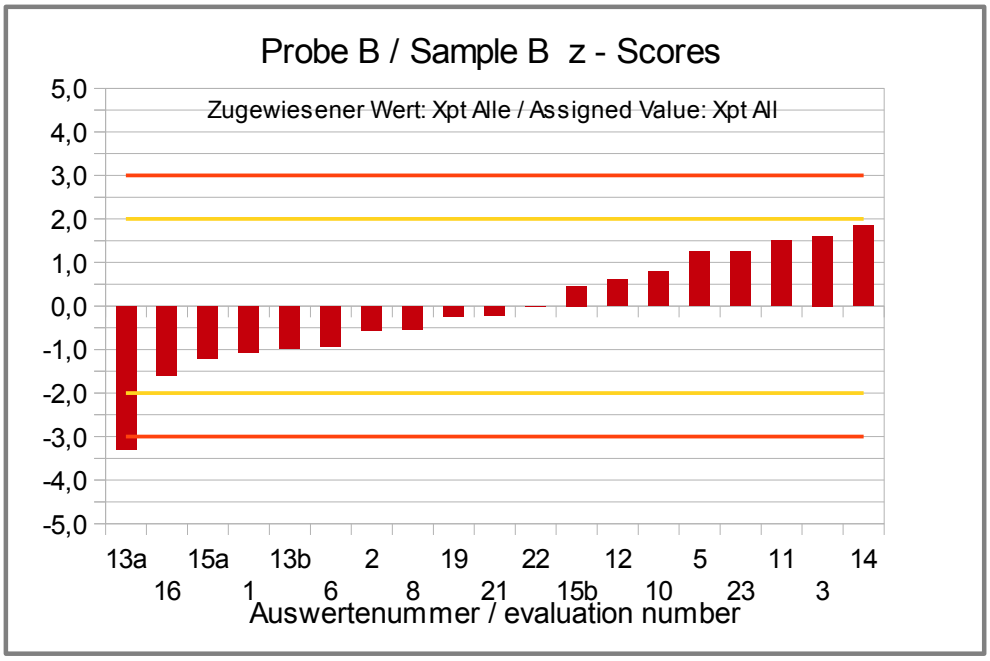


Abb./Fig. 3:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

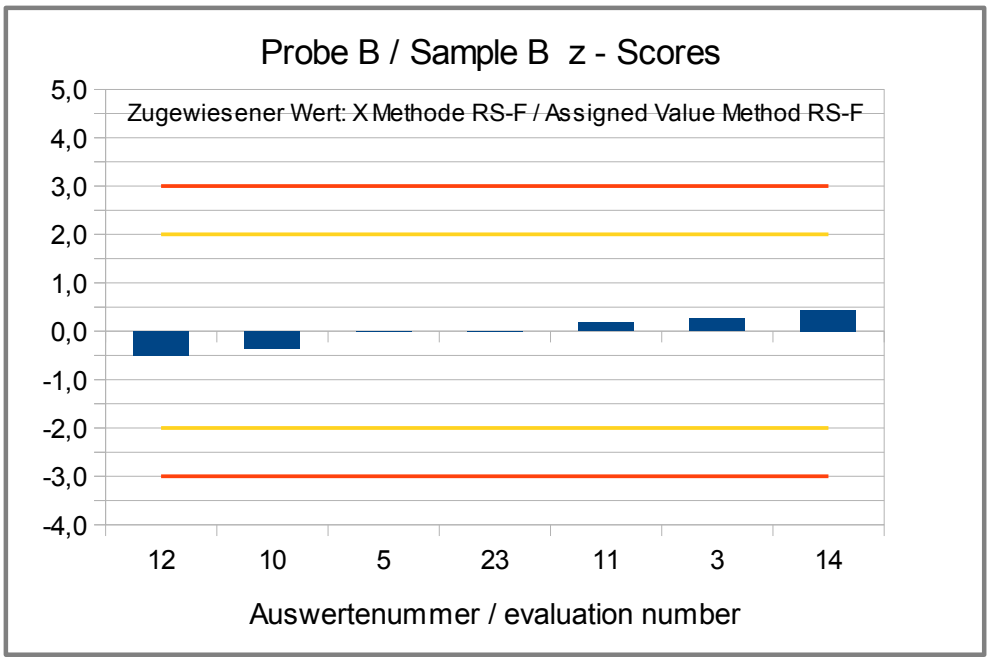


Abb./Fig. 4:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of ELISA-results: Spiking Level Sample

Evaluation number	Peanut	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
7	239			AQ	Result converted °, Result excluded
19	93,7	0,31		AQ	
21	70,0	-0,78		AQ	
2	94,3	0,33		BF	
18	>40			BF	
1	100	0,59		BK	
15a	64,0	-1,1		BK	
16	75,0	-0,55		BK	
6	97,6	0,48		EF	
8	74,8	-0,56		IL	
22	85,8	-0,06		IL	
13a	13,4	-3,4		MI-II	Result converted °
13b	47,4	-1,8		MI-III	Result converted °
3	110	1,1	0,36	RS-F	
5	>90			RS-F	
9	>20			RS-F	
10	90,8	0,17	-0,41	RS-F	
11	94,0	0,32	-0,28	RS-F	
12	97,0	0,46	-0,17	RS-F	
14	120	1,5	0,74	RS-F	
23	95,0	0,37	-0,25	RS-F	
15b	91,4	0,20		VT	

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
MI-III = Morinaga Institute ELISA Test Combination
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

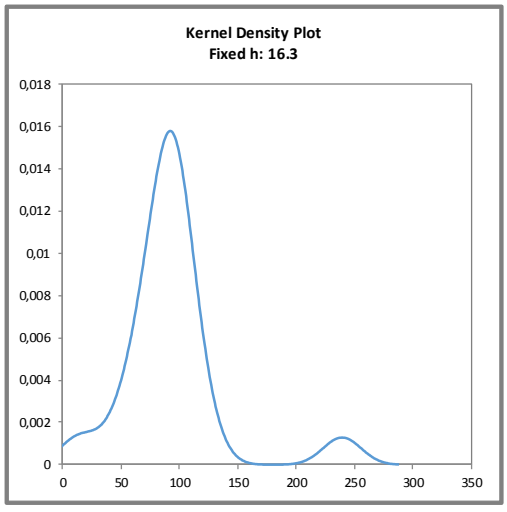


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with a shoulder below 20 mg/kg (method MI-II) and a secondary peak at about 240 mg/kg (method AQ), due to an outlier above the target range.

Characteristics: Quantitative evaluation ELISA Peanut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method RS-F** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ RS\text{-}F}}$
Number of results	18°	6
Number of outliers	1	0
Mean	84,1	101
Median	92,6	96,0
Robust Mean (X_{pt})	**87,1**	**101**
Robust standard deviation (S*)	**18,7**	**13,0**
Target range:		
Target standard deviation σ_{pt}	**21,8**	**25,3**
lower limit of target range	**43,5**	**50,6**
upper limit of target range	**131**	**152**
Quotient S/σ_{pt}*	*0,86*	*0,51*
Standard uncertainty U(X_{pt})	*5,52*	*6,62*
Results in the target range	*17*	*6*
Percent in the target range	*94*	*100*

° *without result No. 7 (excluded in advance)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (one increased single value).

The evaluation of all methods and the evaluation of results from method RS-F showed a low variability of results, with quotients S/σpt below 1,0. The robust standard deviations are in the range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were 267% and 310% of the spiking level of peanut to the spiking level sample and were above the range of the recommendations for the applied methods (s. 3.4.3 and p.30 "Recovery rates ELISA for Peanut").

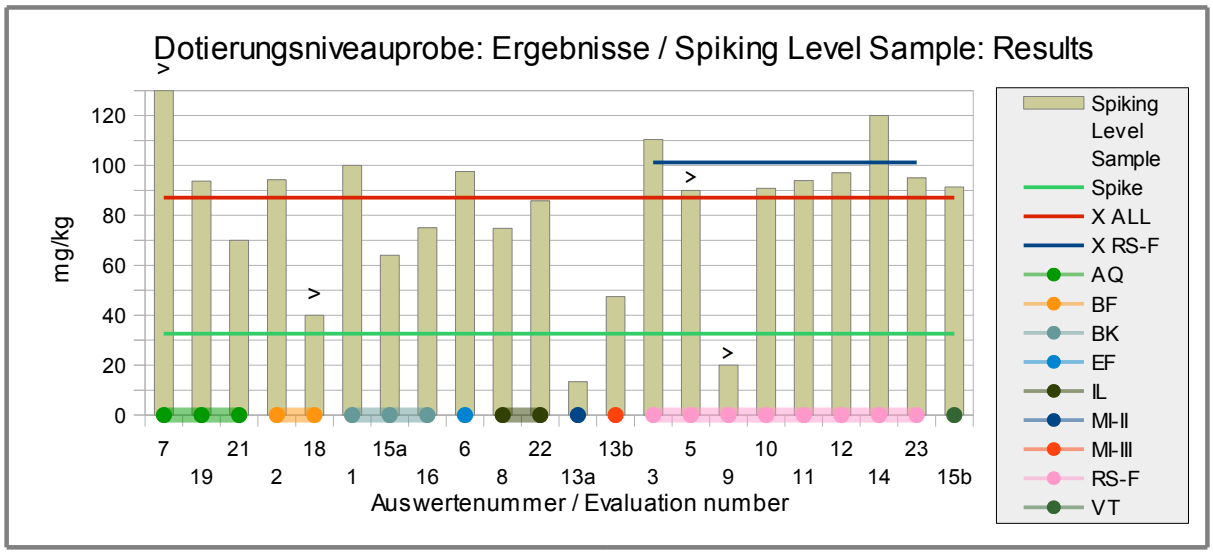


Abb./Fig. 6: ELISA Results Peanut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

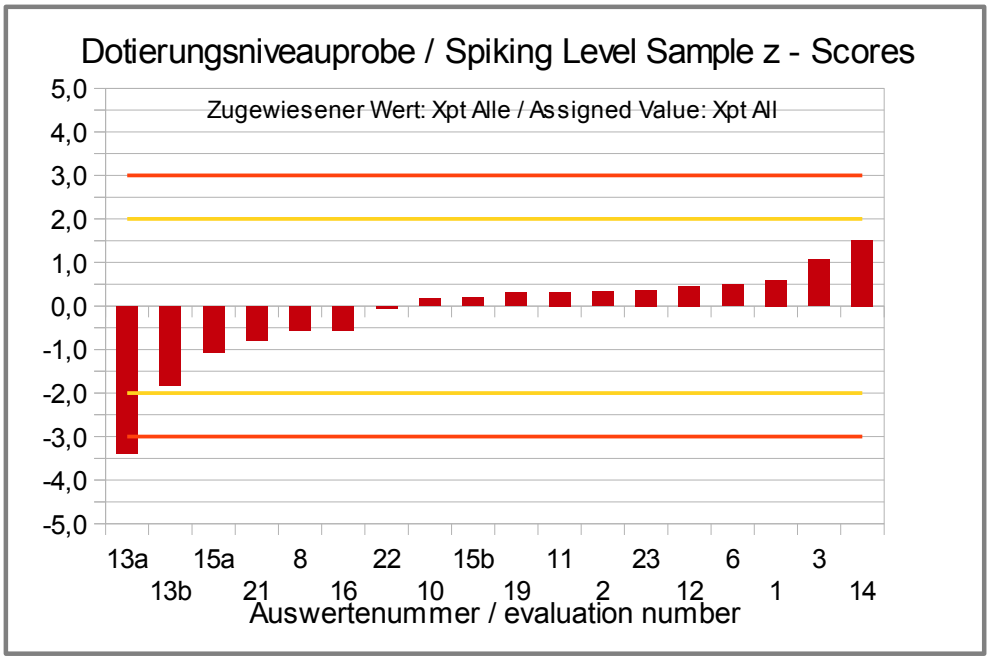


Abb./Fig. 7:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of all results

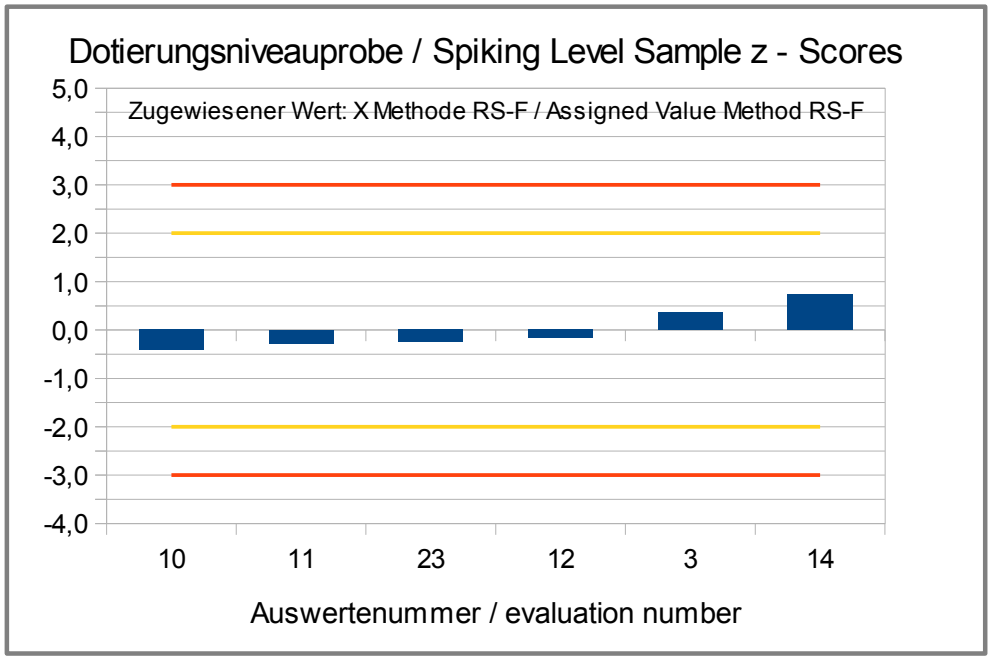


Abb./Fig. 8:
z-Scores (ELISA Results Peanut)
Assigned value robust mean of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates ELISA for Peanut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
7	239	734	301	802	AQ	Result converted °
19	93,7	287	64,4	172	AQ	
21	70,0	215	64,9	173	AQ	
2	94,3	289	58,9	157	BF	
18	>40		>40		BF	
1	100	307	50,0	**133**	BK	
15a	64,0	196	47,8	**127**	BK	
16	75,0	230	41,0	**109**	BK	
6	97,6	299	52,6	**140**	EF	
8	74,8	229	59,1	158	IL	
22	85,8	263	68,3	182	IL	
13a	13,4	41	12,1	32	MI-II	Result converted °
13b	47,4	**145**	51,7	**138**	MI-III	Result converted °
3	110	339	96,0	256	RS-F	
5	>90		90,0	240	RS-F	
9	>20		>20		RS-F	
10	90,8	279	81,9	218	RS-F	
11	94,0	288	94,1	251	RS-F	
12	97,0	298	79,0	211	RS-F	
14	120	368	100	267	RS-F	
23	95,0	291	90,0	240	RS-F	
15b	91,4	280	76,3	203	VT	

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**1**	Number in RA	**5**
Percent in RA	**5**	Percent in RA	**25**

* Recovery rate 100% relative size: peanut, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
MI-III = Morinaga Institute ELISA Test Combination
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

Comments:

One participant obtained for the spiking level sample a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B 25% (5) of the recovery rates were within the range of acceptance. With one exception, all other results were well above this range for both samples.

4.1.2 PCR Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
16	negative		positive		2/2 (100%)	ASU	
24	negative		positive		2/2 (100%)	ASU	
14	negative		positive	50,0	2/2 (100%)	MS	
3	negative	<1	positive	52,3	2/2 (100%)	SFA	
18	negative	<0,4	positive		2/2 (100%)	SFA	
20	negative		positive		2/2 (100%)	SFA-4p	
1	negative		positive		2/2 (100%)	div	
13	negative		positive		2/2 (100%)	div	
23	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		9	
Number negative	9		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.

Quantitative Valuation PCR: Sample B

No quantitative valuation was done, because there were too few results available.

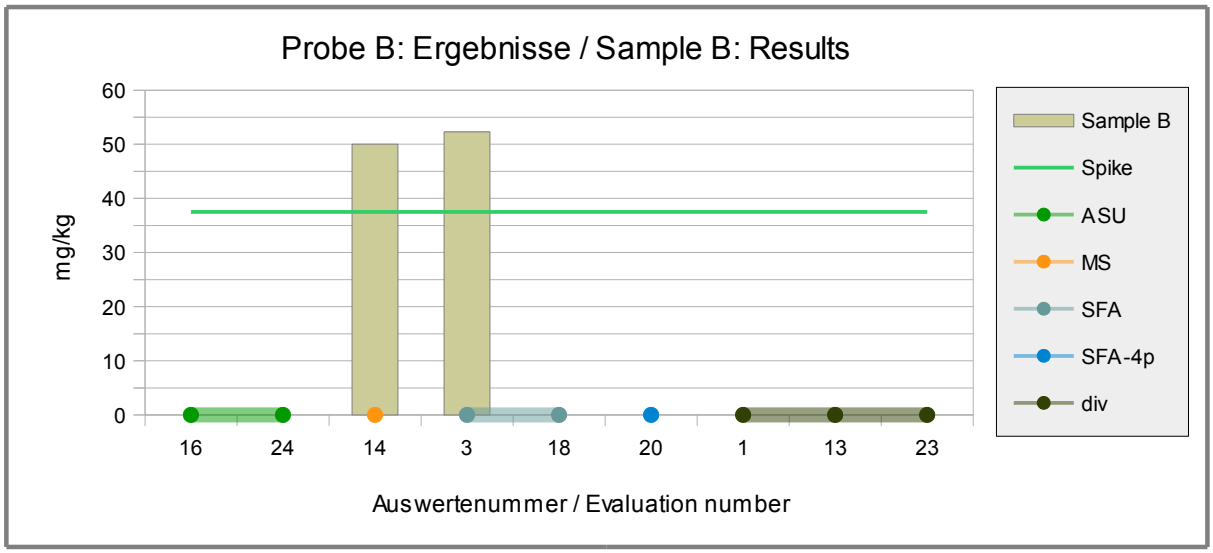


Abb./Fig. 9: PCR Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation PCR: Spiking Level Sample

No quantitative valuation was done, because there were too few results available.

Evaluation number	Peanut	Spiking Level Sample	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
16	positive			ASU	
24	positive			ASU	
14	positive	40,0		MS	
3	positive	47,4		SFA	
18	positive			SFA	
20	positive			SFA-4p	
1	positive			div	
13	positive			div	
23	positive			div	

	Sample B
Number positive	9
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:
ASU = ASU §64 Methode/method
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comment:
100% positive results were obtained for the spiking level sample.

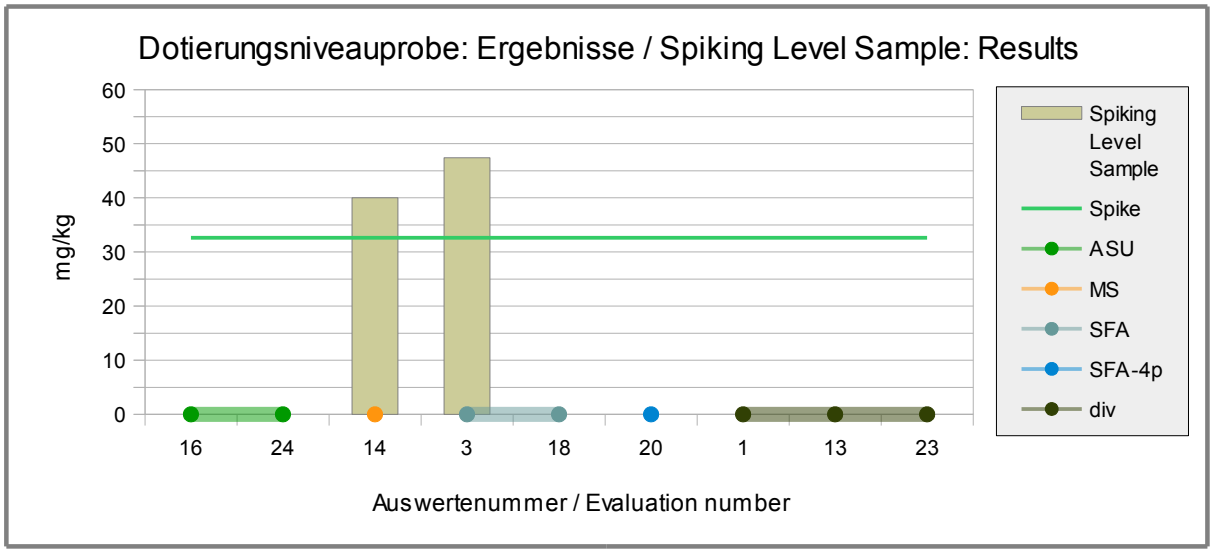


Abb./Fig. 10: PCR Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Peanut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
16					ASU	
24					ASU	
14	40,0	**123**	50,0	**133**	MS	
3	47,4	**145**	52,3	**139**	SFA	
18					SFA	
20					SFA-4p	
1					div	
13					div	
23					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	2	Number in RA	2
Percent in RA	100	Percent in RA	100

* Recovery rate 100% relative size: peanut, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

Both participants obtained with both the spiking level sample and the spiked food matrix sample B recovery rates by PCR methods within the range of the AOAC-recommendation of 50-150% by PCR-methods.

4.1.3 LC-MS/MS Results: Peanut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
4	negative	< 10	positive	36,5	2/2 (100%)	LC-MS/MS	

Methods:
LC-MS/MS = Liquid Chromatography Mass Spectrometry

Comments:
Only one set of results was submitted using a LC-MS/MS method. The results are in qualitative agreement with the spiking of sample B.

Quantitative Valuation LC-MS/MS: Sample B

No quantitative valuation was done, because there were too few results available.

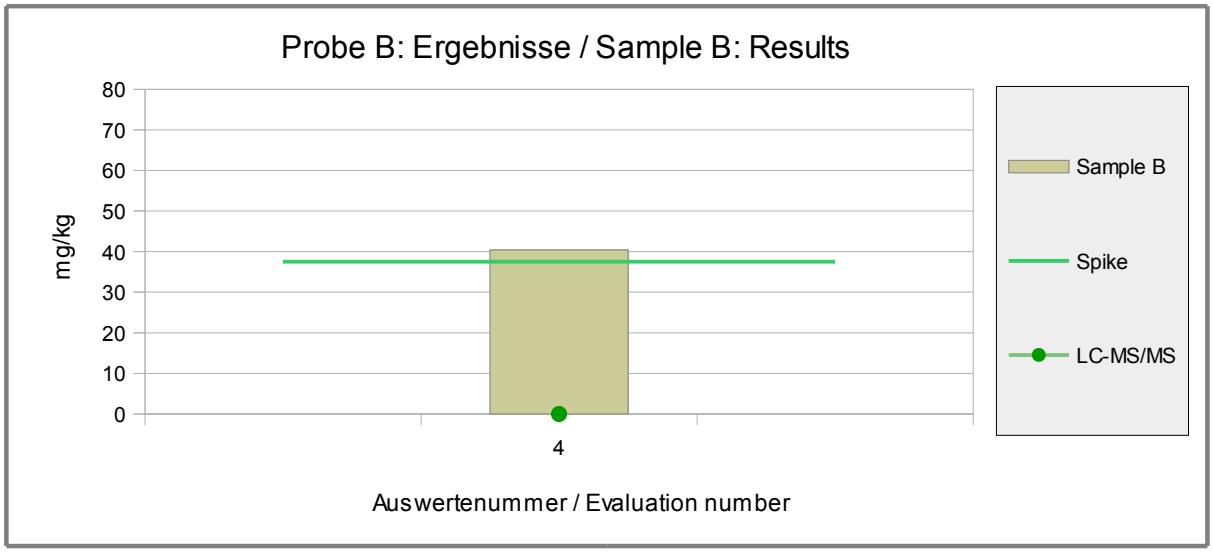


Abb./Fig. 11: LC-MS/MS Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation LC-MS/MS: Spiking Level Sample

No quantitative valuation was done, because there were too few results available.

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
4	positive	30,6		LC-MS/MS	

Methods:
LC-MS/MS = Liquid Chromatography Mass Spectrometry

Comment:
A positive result was obtained for the spiking level sample.

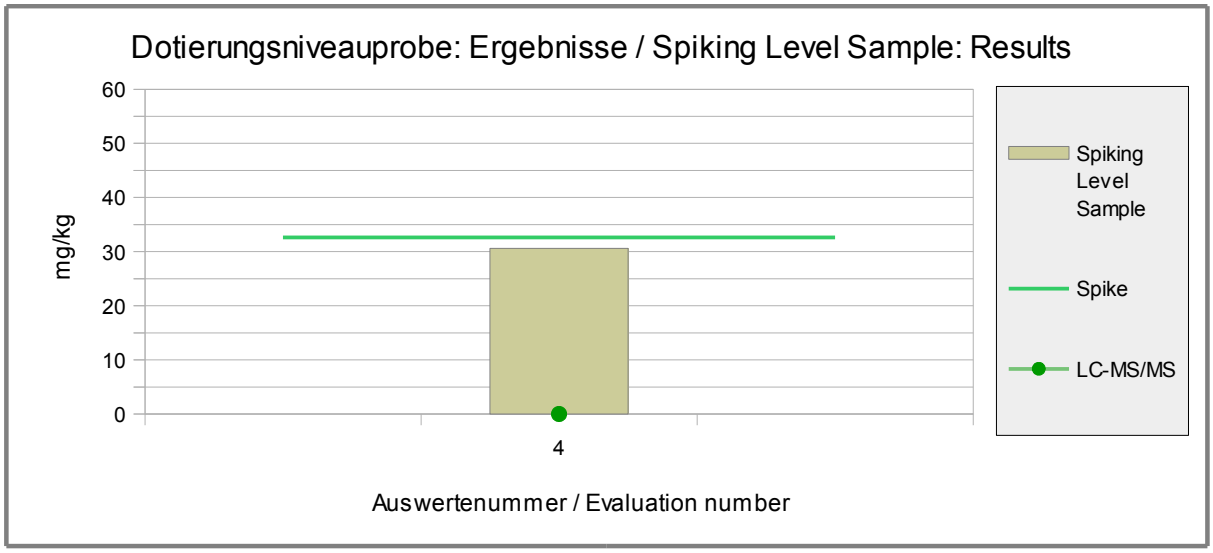


Abb./Fig. 12: LC-MS/MS Results Peanut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates LC-MS/MS for Peanut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4	30,6	**94**	40,4	**108**	LC-MS/MS	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	100	Percent in RA	100

Methods:
LC-MS/MS = Liquid Chromatography Mass Spectrometry

* Recovery rate 100% relative size: peanut, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Comments:
The participant obtained with both the spiking level sample and the spiked food matrix sample B recovery rates by LC-MS/MS within the range of the AOAC-recommendation of 50-150% by PCR-methods.

4.2 Proficiency Test Walut

4.2.1 ELISA Results: Walnut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
5	negative	-	positive	17,0	2/2 (100%)	AQ	
7	negative	<LOD	positive	80,5	2/2 (100%)	AQ	Result converted °
10	negative	<1.9	positive	7,20	2/2 (100%)	AQ	
15a	negative	<2	positive	2,80	2/2 (100%)	AQ	
16	negative	< BG	positive	10,6	2/2 (100%)	AQ	
19	negative	<LOD	positive	10,9	2/2 (100%)	AQ	
21	negative	<LOD	positive	10,2	2/2 (100%)	AQ	
3	negative	<2	positive	6,19	2/2 (100%)	BC	
2	negative	<LOQ	positive	65,8	2/2 (100%)	BF	
9	negative	<2,0	positive	71,0	2/2 (100%)	BF	
18	negative	<1	positive	>40	2/2 (100%)	BF	
1	negative	<2.4	positive	40,0	2/2 (100%)	BK	
15b	negative	<2,4	positive	13,5	2/2 (100%)	BK	
6	negative	<2	positive	9,90	2/2 (100%)	EF	
13	negative	<2	positive	15,0	2/2 (100%)	EF	
8	negative	<2,0	positive	9,10	2/2 (100%)	IL	
11	negative		positive	9,32	2/2 (100%)	IL	
22	negative	0	positive	13,3	2/2 (100%)	IL	
23	negative		positive	6,00	2/2 (100%)	IL	
17	negative	< 14,7	positive	307	2/2 (100%)	OS	Result converted °

° calculation see p. 19

	Sample A		Sample B	
Number positive	0		20	
Number negative	20		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
OS = Orsell

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.

Quantitative valuation of ELISA-results: Sample B

Evaluation number	Walnut	z-Score Xpt_{ALL}	z'-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
5	17,0	2,4	2,0	AQ	
7	80,5			AQ	Result converted °, Result excluded
10	7,20	-1,3	-0,71	AQ	
15a	2,80	-2,9	-1,9	AQ	
16	10,6	-0,01	0,22	AQ	
19	10,9	0,10	0,31	AQ	
21	10,2	-0,16	0,11	AQ	
3	6,19	-1,7		BC	
2	65,8			BF	Result excluded
9	71,0			BF	Result excluded
18	>40			BF	
1	40,0	11		BK	
15b	13,5	1,1		BK	
6	9,90	-0,28		EF	
13	15,0	1,6		EF	
8	9,10	-0,58		IL	
11	9,32	-0,49		IL	
22	13,3	1,0		IL	
23	6,00	-1,7		IL	
17	307			OS	Result converted °, Result excluded

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
OS = Orsell

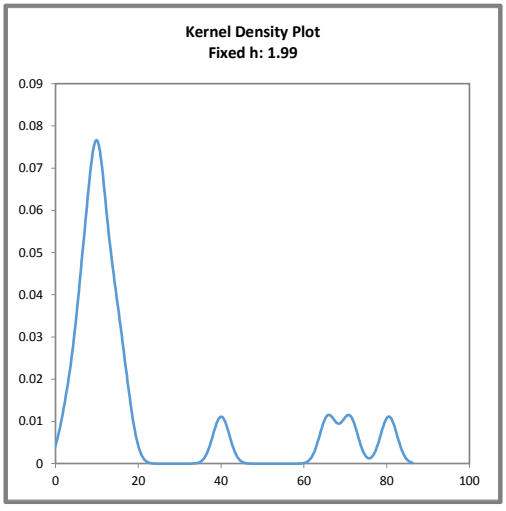


Abb. / Fig. 13:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σpt of Xpt_{ALL})

Comments:
The kernel density estimation shows nearly a symmetric distribution of results with 5 secondary peaks above 30 mg/kg, due to single results of the methods AQ, BK, and OS and two results of method BF. The results of the method BF were excluded, because they can not be evaluated with the rob. mean of all methods. The outlier at 307 mg/kg is not shown in Fig. 13.

Characteristics: Quantitative evaluation ELISA Walnut

Sample B

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	15°	6°°
Number of outliers	4	1
Mean	12,1	9,78
Median	10,2	10,4
Robust Mean (X_{pt})	**10,6**	**9,78**
Robust standard deviation (S*)	**4,56**	**5,31**
Target range:		
Target standard deviation σ_{pt} or $\sigma_{pt'}$	**2,66**	**3,65**
lower limit of target range	**5,32**	**2,48**
upper limit of target range	**15,9**	**17,1**
Quotient S/σ_{pt} or S*/$\sigma_{pt'}$*	*1,7*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*1,47*	*2,71*
Results in the target range	*12*	*6*
Percent in the target range	*80*	*100*

° *without results No. 2, 7, 9 and 17 (excluded in advance)*
°° *without result No. 7 (excluded in advance)*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed method-dependent differences regarding method BF, which was therefore excluded from the evaluation.

The evaluation of all methods showed a normal variability of results, with a quotient S/σ_{pt} below 2,0. The distribution of the results of method AQ showed a slightly increased variability with a quotient S*/σ_{pt} of 2,2. Thus evaluation was done by z'-score considering the standard uncertainty. The quotient S*/$\sigma_{pt'}$ was below 2,0.*
The robust standard deviation is in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.

The robust means of the evaluations were 27% and 25% of the spiking level of walnut to sample B below the range of the recommendations for the applied methods (s. 3.4.3 and p.46 "Recovery rates ELISA for walnut")

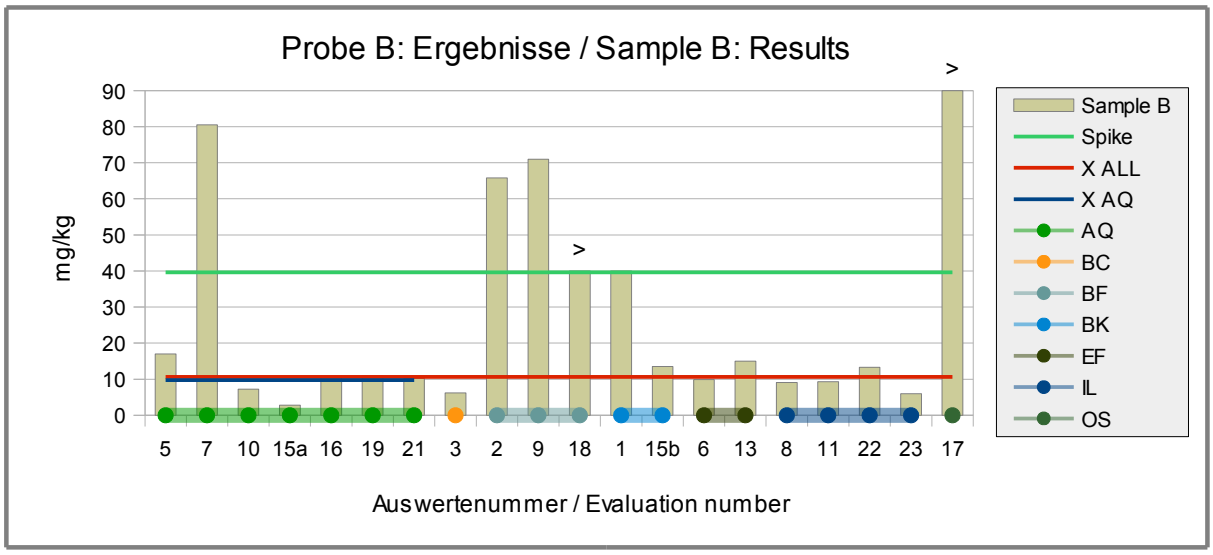


Abb./Fig. 14: ELISA Results Walnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

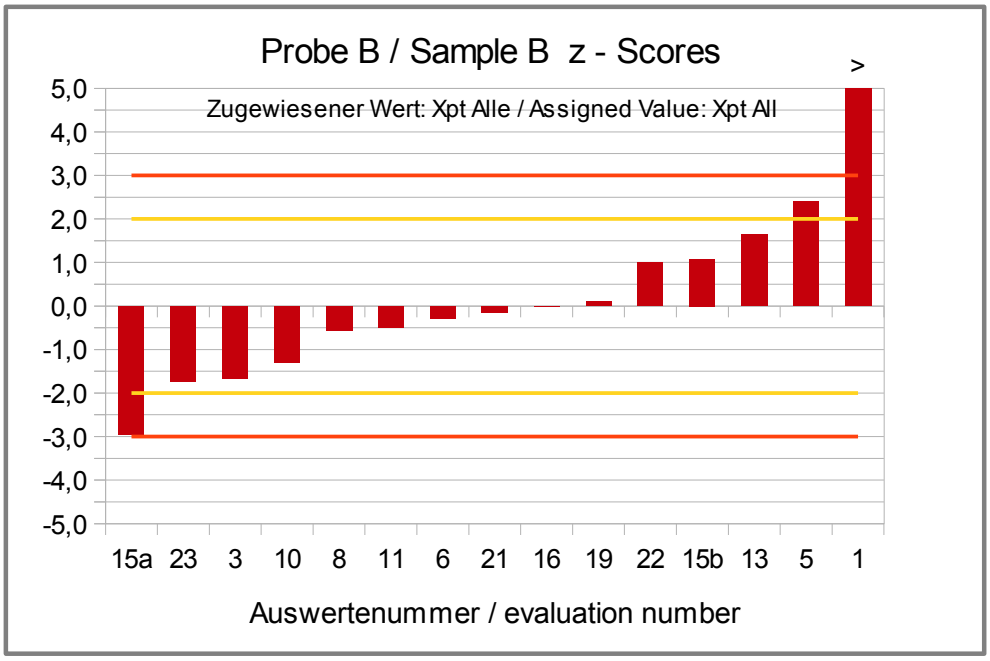


Abb./Fig. 15:
z-Scores (ELISA Results Walnut)
Assigned value robust mean of all results

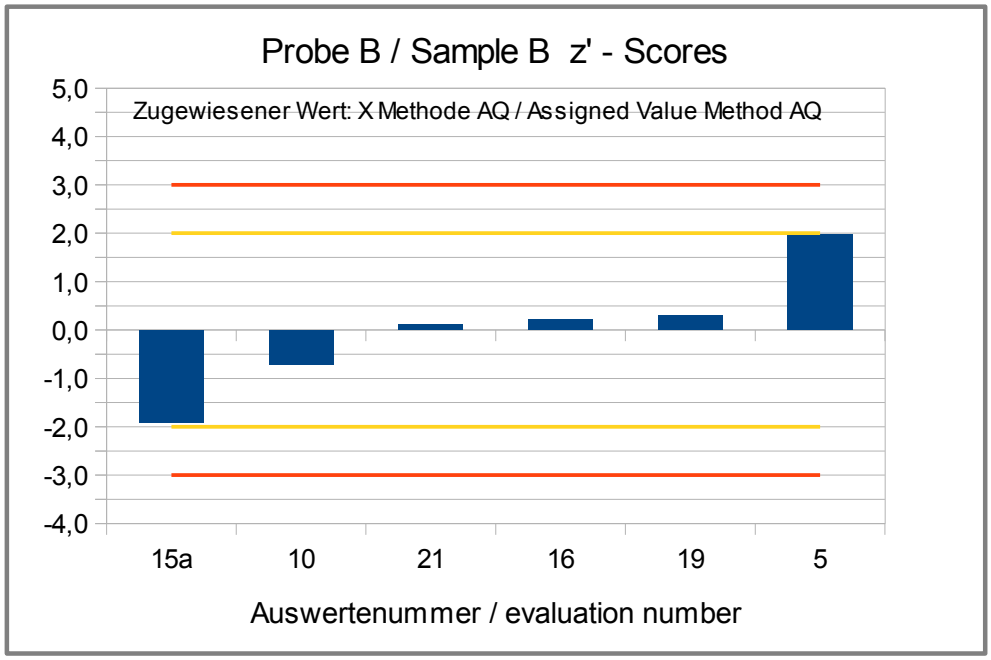


Abb./Fig. 16:
z'-Scores (ELISA Results Walnut)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Quantitative valuation of ELISA: Spiking Level Sample

Evaluation number	Walnut	z-Score Xpt_{ALL}	z-Score Xpt_{AQ}	Method	Remarks
	[mg/kg]				
5	122	-0,37	-0,35	AQ	
7	684			AQ	Result converted °, Result excluded
10	155	0,60	0,62	AQ	
15a	94,2	-1,2	-1,2	AQ	
16	132	-0,07	-0,06	AQ	
19	174	1,2	1,2	AQ	
21	126	-0,24	-0,23	AQ	
3	191	1,7		BC	
2	73,5	-1,8		BF	
9	74,0	-1,8		BF	
18	>40			BF	
1	260	3,7		BK	
15b	108	-0,80		BK	
6	114	-0,61		EF	
13	120	-0,43		EF	
8	150	0,46		IL	
11	167	0,98		IL	
22	140	0,15		IL	
23	149	0,43		IL	
17	1108			OS	Result converted °, Result excluded

° calculation see p. 19

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
OS = Orsell

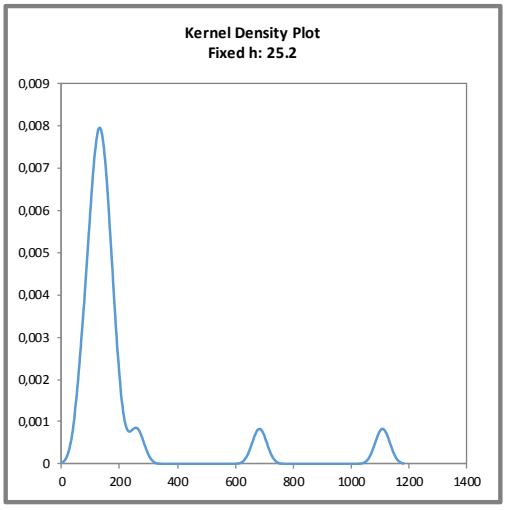


Abb. / Fig. 17:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

Kernel density plot of all ELISA results (with h = 0,75 x σpt of Xpt_{ALL})

Comment:
The kernel density estimation shows nearly a symmetric distribution of results with three secondary peaks at > 200 mg/kg, due to single values of the methods AQ, BK and OS. They are above the target range.

Characteristics: Quantitative evaluation ELISA Walnut

Spiking Level Sample

Statistic Data	**All Results** *[mg/kg]*	**Method AQ** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$	$X_{pt_{METHOD\ AQ}}$
Number of results	17°	6°°
Number of outliers	2	1
Mean	138	134
Median	132	129
Robust Mean (X_{pt})	**134**	**134**
Robust standard deviation (S*)	**41,2**	**31,3**
Target range:		
Target standard deviation σ_{pt}	**33,6**	**33,5**
lower limit of target range	**67,2**	**66,9**
upper limit of target range	**202**	**201**
Quotient S/σ_{pt}*	*1,2*	*0,94*
Standard uncertainty $U_{(Xpt)}$	*12,5*	*16,0*
Results in the target range	*16*	*6*
Percent in the target range	*94*	*100*

° *without results No. 7 and 17 (excluded in advance)*
°° *without result No. 7 (excluded in advance)*

Method:
AQ = AgraQuant, RomerLabs

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed no clear method-dependent differences (three increased single values).

The evaluation of all methods and for the method AQ showed a normal variability of results each, with quotients S/σ_{pt} below 2,0. The robust standard deviations are in the upper range of established values for the reproducibility standard deviation of the applied methods (see 3.4.2 value by precision experiments and 3.4.3 value by perception). The comparability of results is given. This conclusion is limited for the evaluation across the methods, because there were only a few results for some methods.*

The robust means of the evaluations were each 352% of the spiking level of walnut to the spiking level sample and were well above the range of the recommendations for the applied methods (s. 3.4.3 and p.46 "Recovery rates ELISA for walnut").

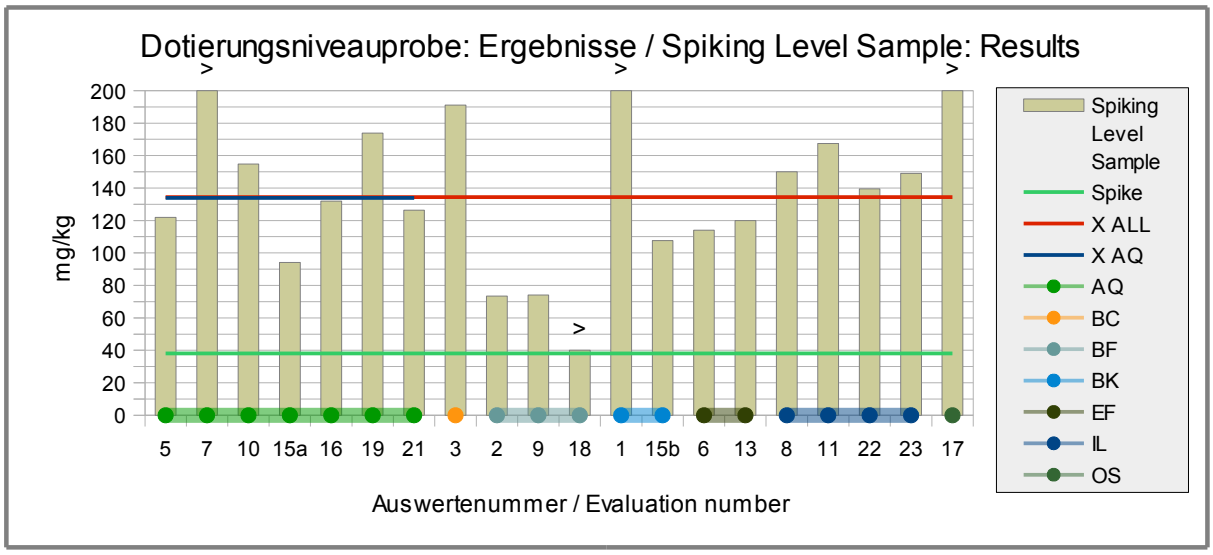


Abb./Fig. 18: ELISA Results Walnut
green line = Spiking level (Spike)
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method AQ
round symbols = Applied methods (see legend)

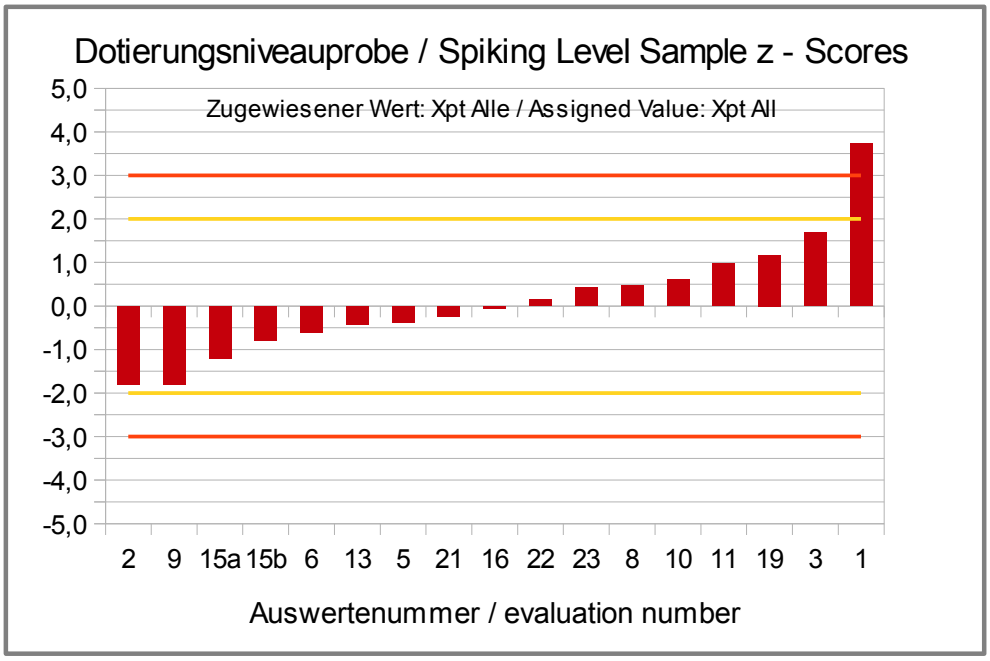


Abb./Fig. 19:
z-Scores (ELISA Results Walnut)
Assigned value robust mean of all results

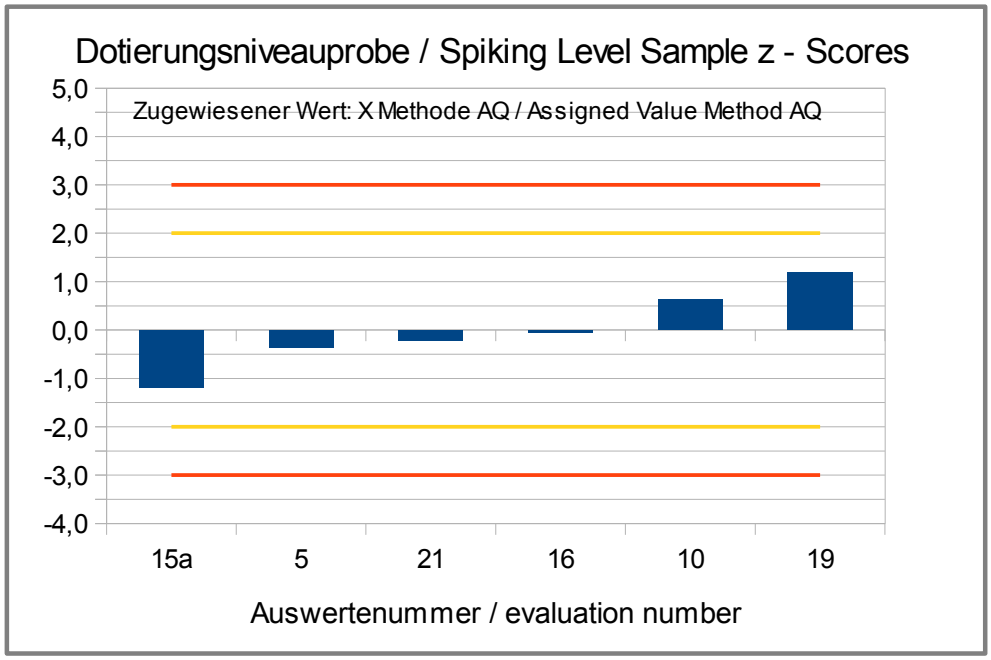


Abb./Fig. 20:
z-Scores (ELISA Results Walnut)
Assigned value robust mean of method AQ (AgraQuant, RomerLabs)

Recovery Rates ELISA for Walnut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
5	122	320	17,0	43	AQ	
7	684	1795	80,5	203	AQ	Result converted °
10	155	406	7,20	18	AQ	
15a	94,2	247	2,80	7	AQ	
16	132	346	10,6	27	AQ	
19	174	456	10,9	28	AQ	
21	126	331	10,2	26	AQ	
3	191	501	6,19	16	BC	
2	73,5	193	65,8	166	BF	
9	74,0	194	71,0	179	BF	
18	>40		>40		BF	
1	260	682	40,0	**101**	BK	
15b	108	282	13,5	34	BK	
6	114	299	9,90	25	EF	
13	120	315	15,0	38	EF	
8	150	394	9,10	23	IL	
11	167	439	9,32	24	IL	
22	140	366	13,3	34	IL	
23	149	391	6,00	15	IL	
17	1108	2908	307	776	OS	Result converted °

° calculation see p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**1**
Percent in RA	**0**	Percent in RA	**5**

* Recovery rate 100% relative size: walnut, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs
BC = BioCheck ELISA
BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
EF = SensiSpec ELISA Kit, Eurofins
IL = Immunolab
OS = Orsell

Comments:

For the spiking level sample none of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the spiked food matrix sample B one of the recovery rates was within the range of acceptance.

4.2.2 PCR Results: Walnut

Qualitative valuation of results: Samples A and B

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Method	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value		
14	negative		positive	50,0	2/2 (100%)	MS	
3	negative	<1	positive	82,8	2/2 (100%)	SFA	
16	negative		positive		2/2 (100%)	SFA	
18	negative	<0,4	positive		2/2 (100%)	SFA	
20	negative		positive		2/2 (100%)	SFA-4p	
1	negative		positive		2/2 (100%)	div	
13	negative		positive		2/2 (100%)	div	
23	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		8	
Number negative	8		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comments:
The consensus values are in qualitative agreement with the spiking of sample B.

Quantitative valuation of PCR-results: Sample B

No quantitative valuation was done, because there were too few results available.

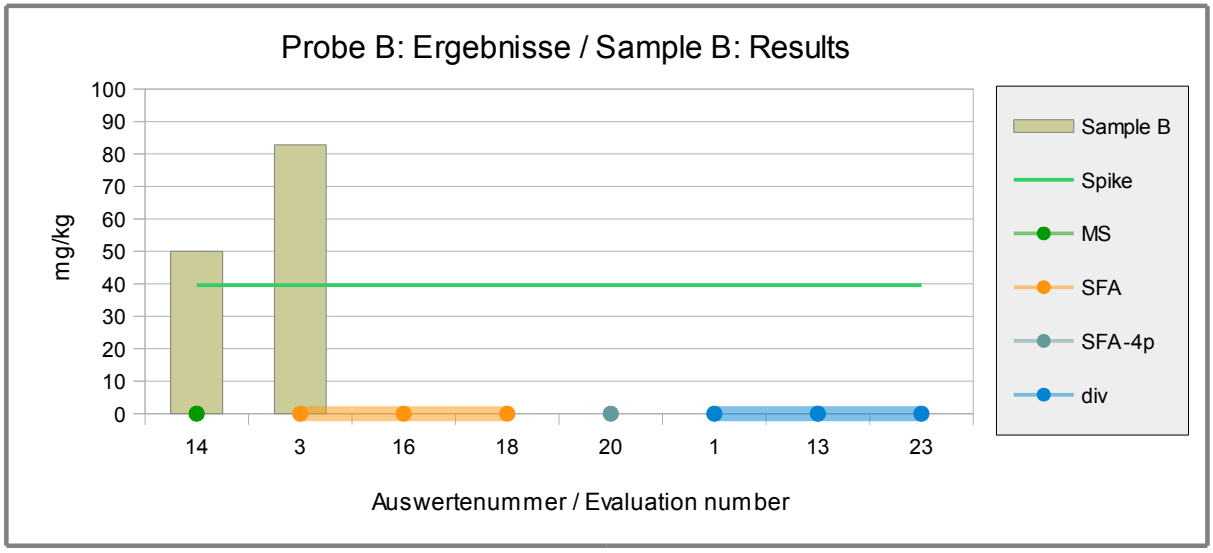


Abb./Fig. 21: PCR Results Walnut
green line = Spiking level
round symbols = Applied methods (see legend)

Quantitative Valuation of PCR: Spiking level sample

No quantitative valuation was done, because there were too few results available.

Evaluation number	Walnut	Walnut	z-Score Xpt_{ALL}	Method	Remarks
	pos/neg	[mg/kg]			
14	positive	50,0		MS	
3	positive	82,9		SFA	
16	positive			SFA	
18	positive			SFA	
20	positive			SFA-4p	
1	positive			div	
13	positive			div	
23	positive			div	

	Sample B
Number positive	8
Number negative	0
Percent positive	100
Percent negative	0
Consensus value	positive

Methods:
MS = Microsynth
SFA = Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen
div = keine genaue Angabe / andere Methode
div = not indicated / other method

Comment:
For the spiking level sample 100% positive results were obtained.

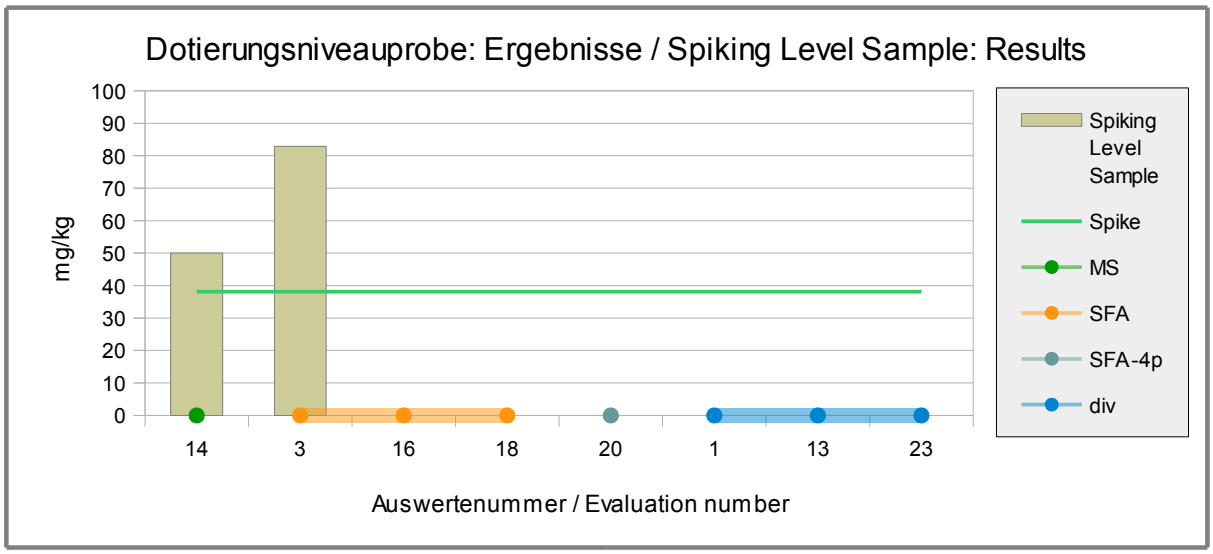


Abb./Fig. 22: PCR-Results Walnut
green line = Spiking level
round symbols = Applied methods (see legend)

Recovery Rates PCR for Walnut: Spiking Level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
14	50,0	**131**	50,0	**126**	MS	
3	82,9	218	82,8	209	SFA	
16					SFA	
18					SFA	
20					SFA-4p	
1					div	
13					div	
23					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	1	Number in RA	1
Percent in RA	50	Percent in RA	50

* Recovery rate 100% relative size: walnut, s. Page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

MS = Microsynth

SFA = Sure Food ALLERGEN, R-Biopharm / Congen

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

One participant obtained for the spiking level sample and the spiked food matrix sample B recovery rates by PCR methods within the range of the AOAC-recommendation of 50-150%.

4.3 Participant z-Scores: overview table

Evalutaion number	ELISA Peanut : Xpt_{ALL}		ELISA Peanut : Xpt_{RS-F}		ELISA Walnut : Xpt_{ALL}		ELISA Walnut : Xpt_{AQ}	
Method	Sample B	Spiking L. Sample	Sample B	Spiking L. Sample	Sample B	Spiking L. Sample	Sample B*	Spiking L. Sample
1	-1,1	0,59	-	-	11	3,7	-	-
2	-0,56	0,33	-	-	-	-1,8	-	-
3	1,6	1,1	0,26	0,36	-1,7	1,7	-	-
4	-	-	-	-	-	-	-	-
5	1,3	-	-0,01	-	2,4	-0,37	2,0	-0,35
6	-0,93	0,48	-	-	-0,28	-0,61	-	-
7	-	-	-	-	-	-	-	-
8	-0,55	-0,56	-	-	-0,58	0,46	-	-
9	-	-	-	-	-	-1,8	-	-
10	0,79	0,17	-0,37	-0,41	-1,3	0,60	-0,71	0,62
11	1,5	0,32	0,18	-0,28	-0,49	0,98	-	-
12	0,62	0,46	-0,49	-0,17	-	-	-	-
13a/ 13	-3,3	-3,4	-	-	1,6	-0,43	-	-
13b	-0,98	-1,8	-	-	-	-	-	-
14	1,8	1,5	0,44	0,74	-	-	-	-
15a	-1,2	-1,1	-	-	-2,9	-1,2	-1,9	-1,2
15b	0,46	0,20	-	-	1,1	-0,80	-	-
16	-1,6	-0,55	-	-	-0,01	-0,07	0,22	-0,06
17	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-
19	-0,24	0,31	-	-	0,10	1,2	0,31	1,2
20	-	-	-	-	-	-	-	-
21	-0,21	-0,78	-	-	-0,16	-0,24	0,11	-0,23
22	-0,01	-0,06	-	-	1,0	0,15	-	-
23	1,3	0,37	-0,01	-0,25	-1,7	0,43	-	-
24	-	-	-	-	-	-	-	-

* z'-Score

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: Peanut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	7	06.11.2019	-	<LOD	-	69,75	-	55,5	0,1	1		Peanut protein	AgraQuant ELISA Peanut COKAL0148, RomerLabs
AQ	19	23.10.19	negative	<LOD	positive	64,4	positive	93,7	0,1	1	50	Peanut	AgraQuant ELISA Peanut COKAL0148, RomerLabs
AQ	21	31.10.19	negative	<LOD	positive	64,9	positive	70	0,5	1	50	Peanut	AgraQuant Plus ELISA Peanut COKAL0148F, RomerLabs
BF	2	27/11	negative	bLOQ	positive	58,9	positive	94,3	0,24	1		Peanut	MonoTrace Peanut ELISA kit, BioFront Technologies
BF	18		negative	<1	positive	>40	positive	>40		1		Peanut	MonoTrace Peanut ELISA kit, BioFront Technologies
BK	1	23./31.10., 29.11.19	negative	<1	positive	50	positive	100	1	1		Peanut	BioKits Peanut Assay Kit, Neogen
BK	15a	15.11.2019	negative	<1	positive	47,8	positive	64		1		peanut	BioKits Peanut Assay Kit, Neogen
BK	16	04.11.19	-	< BG	-	41	-	75		1		Food	BioKits Peanut Assay Kit, Neogen
EF	6		-	<1	-	52,6	-	97,6		<1		Peanut	Eurofins SensiSpec Peanut ELISA Kit
IL	8	22. Nov	-	<1,0	-	59,1	-	74,8		1		Peanut	Immunolab Peanut ELISA
IL	22	29.10.19	negative	0	positive	68,3	positive	85,8				Peanut	Immunolab Peanut ELISA
MI-II	13a	23.10.19	negative	<0,31	positive	2,8	positive	3,1	0,12	0,31		Peanutprotein	Peanut ELISA Kit-II, Morinaga
MI-III	13b	30.10.19	negative	<0,2	positive	12	positive	11	0,2	0,2		Peanutprotein	MIoBS Test Combination M2120:2019-02
RS-F	3	30.10.2019	negative	<1	positive	96,01	positive	110,44	1	1	31,4	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	5	26.11.19	NN	-	-	90	-	>90	0,3	1		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	9	13. Nov	-	<2,5	-	>20	-	>20		2,5		Please select!	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	10	25.10.19	-	<2.5	-	81,9	-	90,8	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	11	26.11.2019	negative		positive	94,1	positive	94	0,8	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	12	20. Nov	negative		positive	79	positive	97	2,5	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	18.11.19	negative		positive	100	positive	120	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	23		neg		pos	90	pos	95	2,5	2,5		Please select!	r-biopharm R6202
VT	15b	13.11.2019	negative	<2,5	positive	76,3	positive	91,4		2,5		peanut	Veratox Peanut, Neogen

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Peanut:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	7		Extracted with the kit's supply of extraction buffer. Extracted for 15 minutes at 60 degrees Celsius.	Yes	
AQ	19	whole peanut		yes	AgraQuant Kits have new article numbers. Peanut: 10001990
AQ	21	Peanut	Water, extraction additives, 15 seconds shaking	no	
BF	2	Monoclonal antibody-based assay	1:10 extraction ratio @ 62C for 10 minutes	N/A	Product # PA3-EK
BF	18			yes	
BK	1	Conarachin (Ara h1)	as per kit instructions	yes	
BK	15a		as stipulated in kit insert	yes	low recovery in sample A (32%)
BK	16	Polyclonal AB against Conarachin (Ara h1)	according to instructions	yes	
EF	6			yes	
IL	8			yes	
IL	22				
MI-II	13a	recognizes peanut proteins	according to manufacturer's instructions	yes	M2116
MI-III	13b	recognizes peanut proteins	according to manufacturer's instructions	yes	
RS-F	3	As Per Kit Instructions	As Per Kit Instructions	Yes	With 1ppm LOD adaptation
RS-F	5	see instruction	see instruction	yes	
RS-F	9			yes	
RS-F	10	not known	according to kit instruction	yes	Cross reactivity to green peas, lentils, semolina and fenugreek
RS-F	11			yes	dillution 1:10 for samples B,Sp
RS-F	12			no	
RS-F	14			yes	
RS-F	23		1 g sample weight, according to kit instruction	yes	
VT	15b		as stipulated in kit insert	yes	good recovery in sample A (105%)

5.1.2 ELISA: Walnut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	ELISA Test-Kit+Manufacturer
AQ	5	20.11.19	NN	-	-	17	-	122	0,35	2		Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	7	06.11.2019	-	<LOD	-	10,95	-	93	0,35	2		Walnut protein	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	10	25.11.19	-	<1.9	-	7,2	-	154,7	0,35	2		Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	15	24.10.2019	negative	<2	positive	2,8	positive	94,2		2		walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	16	07.11.19	-	< BG	-	10,6	-	132		2		Food	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	19	23.10.19	negative	<LOD	positive	10,9	positive	173,9	0,35	2	40	Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
AQ	21	31.10.19	negative	<LOD	positive	10,2	negative	126,3	0,35	2	50	Walnut	AgraQuant ELISA Walnut COKAL0948, RomerLabs
BC	3	25.11.2019	negative	<2	positive	6,19	positive	191,01	2	2	30,15	Walnut	BioCheck ELISA Walnut-Check
BF	2	27/11	negative	bLOQ	positive	65,8	positive	73,5	0,22	1		Walnut	MonoTrace Walnut ELISA kit, BioFront Technologies
BF	9	07. Nov	-	<2,0	-	71	-	74		2		Please select!	MonoTrace Peanut ELISA kit, BioFront Technologies
BF	18		negative	<1	positive	>40	positive	>40		1		Walnut	MonoTrace Walnut ELISA kit, BioFront Technologies
BK	1	23./31.10., 29.11.19	negative	<2.4	positive	40	positive	260	2,4	2,4		Walnut	BioKits Walnut Assay Kit, Neogen
BK	15	15.11.2019	negative	<2,4	positive	13,5	positive	107,6		2,4		walnut	BioKits Walnut Assay Kit, Neogen
EF	6		-	<2	-	9,9	-	114,1		<2		Walnut	Eurofins SensiSpec Walnut ELISA Kit
EF	13	28.10.19	negative	<2	positive	15	positive	120	2	2		Walnut	Eurofins SensiSpec Walnut ELISA Kit
IL	8	22. Nov	-	<2,0	-	9,1	-	150		2		Walnut	Immunolab Walnut ELISA
IL	11	04.11.2019	negative		positive	9,32	-	167,4	0,35	2		Walnut	Immunolab Walnut ELISA
IL	22	29.10.19	negative	0	positive	13,3	positive	139,5				Walnut	Immunolab Walnut ELISA
IL	23		neg		pos	6	pos	149	n.a.			Please select!	Immunolab Wal-137
OS	17	28.10.2019	negative	< 2	positive	41,8	positive	150,7		2		Walnut protein	other: EZ-PLATE WALNUT 2-60 ppm - ORSELL

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Continuation ELISA Walnut:

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
AQ	5	s. instruction	s. instruction	yes	
AQ	7		Extracted w ith the kit's supply of extraction buffer. Extracted for 15 minutes at 60 degrees Celsius.	Yes	
AQ	10	not know n	as per kit instuctions	yes	Cross reactivity to cashew nuts, chicken, pecan nuts
AQ	15		as stipulated in kit insert	yes	Spiking of the chocolat cream matrix give signal under LOQ for the non-detected sample
AQ	16	Anti-w alnutprotein	according to instruction	yes	
AQ	19	w hole w alnut		yes	AgraQuant Kits have new article numbers. Walnut: 10002030
AQ	21	w alnut	aqueous buffer, shaker for 15 minutes	no	
BC	3	As Per Kit Instructions	As Per Kit Instructions	Yes	
BF	2	Monoclonal antibody-based assay	1:10 extraction ratio @ 62C for 10 minutes	N/A	Product # WJ4-EK
BF	9			yes	
BF	18			yes	
BK	1		as per kit instruction	yes	
BK	15		as stipulated in kit insert	yes	low recovery in sample A (6%)
EF	6			yes	
EF	13	recognizes w alnutprotein	according to manufacturer's instructions	yes	HU0030024:2
IL	8			yes	
IL	11				dillution 1:10 for sample spiking level
IL	22				
IL	23		1 g sample w eight, according to kit instructions		Method not established
OS	17		1g in 20 mL of Buffer solution; incubation time 15 minute at 60°C	Yes	

5.1.3 PCR: Peanut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
ASU	16	29.10.19	negative		positive		positive					Peanut-DNA	ASU §64 Methode/method
ASU	24	18. Dez	negative		positive		positive		10			Please select!	ASU §64 Methode/method
MS	14	7.11.19	negative		positive	50	positive	40	10	50	50	Peanut	Microsynth
SFA	3	31.10.2019	negative	<1	positive	52,29	positive	47,42	1	1	73,57	Peanut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18		negative	<0,4	positive		positive		0,4			Peanut DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	20	30.10.19	negative		positive		positive		1		30	Peanut	Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	07.11.19	negative		positive		positive					Peanut-DNA	Selection PCR methods
div	13	23.10.19	negative		positive		positive		5			Peanut-DNA	internal method
div	23		neg		pos		pos					Please select!	Köppel et al., Eur Food Res Technol, 2012

* NWG Nachw eisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	16	86bp long sequence section of the gene for Ara h2	spiking level sample: SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles Sample A+B: Dneasy Mericon Food-Kit,QIAquick PCR Purification-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
ASU	24	Ara H2	acc. to ISO 15634-4:2016	No	
MS	14	Ara h2	CTAB-Extraction / ProtK / Promega Wizard DNA CleanUp / Real-time PCR: 45 cycles	yes	
SFA	3	As Per Kit Instructions	As Per Kit Instructions	Yes	
SFA	18			yes	
SFA-4p	20	Arachis hypogae	SureFood Prep Advanced Protokoll 1	no	Article No. S3402 (K01)
div	1	MT-ATP6	Wizard Genomic DNA isolation	no	
div	13		CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Realtime PCR / 45 cycles	yes	
div	23		Qiagen Mericon extraction Kit, 45 cycles, Real-Time P CR	yes	

5.1.4 PCR: Walnut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	PCR Test-Kit+Manufacturer
MS	14	7.11.19	negative		positive	50	positive	50	5	25	50	Walnut	Microsynth
SFA	3	31.10.2019	negative	<1	positive	82,79	positive	82,9	1	1	N/A	Walnut	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	16	28.10.19	negative		positive		positive					Walnut-DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA	18		negative	<0,4	positive		positive		0,4			Walnut DNA	Sure Food ALLERGEN, R-Biopharm / Congen
SFA-4p	20	30.10.19	negative		positive		positive		0,4		30	Walnut	Sure Food Allergen 4plex, R-Biopharm / Congen
div	1	07.11.19	negative		positive		positive					Walnut-DNA	Selection PCR methods
div	13	23.10.19	negative		positive		positive		5			Walnut-DNA	internal method
div	23		neg		pos		pos					Please select!	Köppel et al., Eur Food Res Technol, 2012

* NWG Nachweisgrenze / BG Bestimmungsgrenze

* LOD limit of detection / LOQ limit of quantitation

* MU Messunsicherheit / MU measurement uncertainty

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
MS	14	jug r2	CTAB-Extraction / ProtK / Promega Wizard DNA CleanUp / Real-time PCR: 45 cycles	yes	
SFA	3	As Per Kit Instructions	As Per Kit Instructions	No	
SFA	16	characteristic sequence section of the walnut DNA	Spiking level sample: SureFood Prep Advanced, r-biopharm/ Proteinase K/ Real Time PCR/ 45 cycles; Sample A+B: Dneasy Mericon Food-Kit,QIAquick PCR Purification-Kit Qiagen/ Proteinase K/ Real Time PCR/ 45 cycles	yes	
SFA	18			yes	
SFA-4p	20	Juglans	SureFood Prep Advanced Protokoll 1	yes	Article No. S3402 (K01)
div	1	Juglans regia sucrose transporter	Wizard Genomic DNA isolation	no	
div	13		CTAB / Proteinase K / Promega Wizard DNA-CleanUp / Realtime PCR / 45 cycles	yes	
div	23		Qiagen Mericon extraction Kit, 45 cycles, Real-Time PCR	yes	

5.1.5 LC-MS/MS: Peanut

Meth. Abr.	Evaluation no.	Date of Analysis	Result Sample A		Result Sample B		Result Spiking Sample		NWG / LOD *	BG / LOQ *	MU*	quantitative Result given as	Method
		day/month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	%	e.g. food /protein	Test-Kit+Manufacturer
LC-MS/MS	4	18./20.11. 2019	negative	< 10	positive	36,5	positive	30,6	S/N > 3	10	40	Food	LC-MS/MS

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

Meth. Abr.	Evaluation no.	Specifity	Remarks to the Method (Extraction and Determination)	Method Accredited ISO/IEC 17025	Further Remarks
LC-MS/MS	4	specific peptides	Protein extraction follow ed by enzymatic digestion	no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 06-2019 Spiking Level Sample

Weight whole sample	1,51	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	24,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,01	65	25,9
2	5,12	64	25,0
3	5,14	67	26,1
4	5,13	60	23,4
5	5,06	61	24,1
6	5,06	70	27,7
7	5,11	71	27,8
8	5,05	65	25,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	65,4	Particles
Standard deviation	3,94	Particles
χ^2 (CHI-Quadrat)	1,66	
Probability	**98**	%
Recovery rate	105	%

Normal distribution		
Number of samples	8	
Mean	25,7	mg/kg
Standard deviation	1,55	mg/kg
rel. Standard deviaton	6,02	%
Horwitz standard deviation	9,81	%
HorRat-value	**0,61**	
Recovery rate	105	%

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 06-2019***
PT name	***Allergens VI: Peanut and Walnut in Spread (Cocoa Cream)***
Sample matrix (processing)	***Samples A + B:*** *Nut nougat cream (spread)/ ingredients: Sugar, palm oil, hazelnuts (13%), skimmed milk powder, low-fat cocoa, emulsifier lecithin (soy), vanillin other food additives and allergenic foods (one of both samples)* ***Spiking Level Sample:*** *potato powder, other food additives and allergenic foods*
Number of samples and sample amount	*2 different Samples A + B: 25 g each + 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term cooled 2 - 10°C) Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Peanut (Peanut protein, DNA), Walnut (Walnut protein, DNA) Samples A + B: < 500 mg/kg Spiking Level Sample: < 500 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 is homogenized.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample. The results should be filled in the result submission file.*
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 November 29th 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
		SPAIN
		SWITZERLAND
		Germany
		USA
		SWITZERLAND
		CANADA
		ITALY
		Germany
		Germany
		Germany
		GREAT BRITAIN
		Germany
		Germany
		SERBIA
		SWITZERLAND
		SWITZERLAND
		SPAIN
		ITALY
		GREAT BRITAIN
		AUSTRIA
		AUSTRIA
		USA
		SPAIN
		GREECE

[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)
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