Proficiency Tests
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Evaluation Report
proficiency test

DLA 05/2018

Allergens V:

Peanut and Almond

in Pastry (Butter Cookies)

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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.

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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 are a common in commerce butter cookies. The basic composition of both sample A and sample B was the same (see table 1).

After crushing and sieving using an impact mill (mesh 1,5 mm) the basic mixture was homogenized. Afterwards the **spiked sample B** was produced as follows:
As an additional ingredient, cookies were baked (150°C, 30 min) with spiking material containg the allergenic ingredients peanut and almond. After crushing, sieving (mesh 1,5 mm) and homogenization the cookies were added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 2 additional steps and homogenized in each case until the total quantity had been reached.

For the **spiking level sample**, the above mentioned allergenic compounds were added during a multi-stage addition of potato powder and homogenization. Afterwards the whole sample was sieved by means of a centrifugal mill (mesh 250 µm).

The samples A and B were portioned to approximately 25 g, the spiking level sample to approximately to 10 g in metallized PET film bags.

The composition of the PT samples and the spiking level sample is given in table 1.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Butter Cookies Ingredients: Wheat flour, sugar, butter, barley malt extract, skimmed milk powder, glucose, glucose syrup, raising agent: ammonium hydrogencarbonate, salt, emulsifier lecithin Nutrients per 100 g: Fat 12 g, Carbohydrates 76 g, Protein 7,1 g, Salt 0,2 g	100 g/100 g	93,1 g/100g	-
Cookies (baked 150°C, 30 min) Ingredients: Wheat flour, sugar, butter, eggs, salt and peanuts, almonds and further ingredients (see below)	-	6,9 g/100 g	-
Potato Powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,7 g/100 g
Peanuts, roasted: milled, mixture (18 products from USA, Asia, Africa, South America) - as Peanut* - *thereof 23,2% total protein***	-	 32,0 mg/kg 7,4 mg/kg	 33,8 mg/kg 7,9 mg/kg
Almond Butter, white - as Almonds* - *thereof 16,2% total protein***	-	 68,7 mg/kg 11,1 mg/kg	 34,7 mg/kg 5,6 mg/kg
further Ingredients: Maltodextrin, sodium sulfate and silicon dioxide	-	<0,3 g/100 g	<0,3 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=5,18 for almonds)

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 sample B and the spiking level sample showed a probability of 80% and 82%. 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 HorRat values of 0,87 and 0,95, respectively. 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, AgraQuant) and almond (Immunolab, Veratox and AgraQuant) (see pages 7-8). 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].

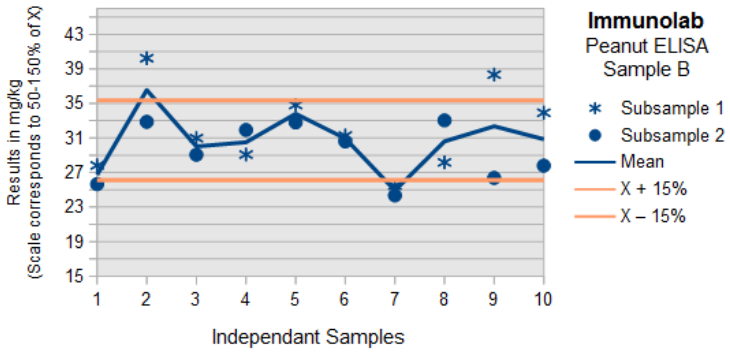
ELISA-Tests: Homogenität Erdnuss / Homogeneity Peanut

Immunolab Peanut ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 30,7 ± 2,7 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	27,8	25,7	26,7
2	40,2	32,9	36,6
3	31,0	29,1	30,0
4	29,1	31,9	30,5
5	34,8	32,8	33,8
6	31,3	30,6	30,9
7	25,5	24,3	24,9
8	28,2	33,0	30,6
9	38,3	26,4	32,4
10	33,9	27,8	30,9

General average X	30,7	
SD of sample means Sx	3,27	10,6%
SD within-samples Sw	2,65	8,6%
SD between-samples Ss	2,68	8,7%

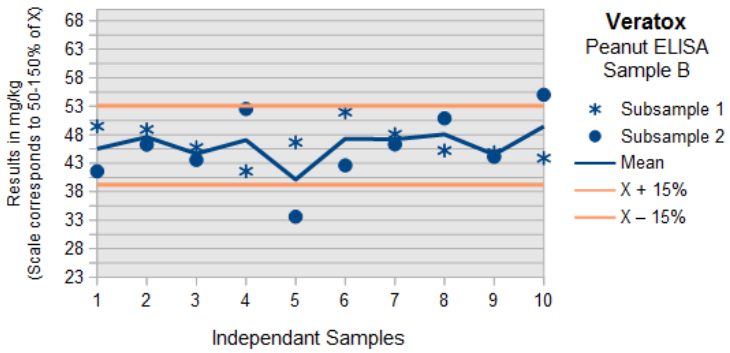


Neogen Veratox ELISA Peanut

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Peanut 46,2 ± 2,6 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	49,5	41,6	45,5
2	48,9	46,3	47,6
3	45,8	43,6	44,7
4	41,6	52,5	47,1
5	46,7	33,6	40,1
6	52,0	42,6	47,3
7	48,1	46,3	47,2
8	45,2	50,9	48,1
9	44,9	44,1	44,5
10	43,9	55,0	49,5

General average X	46,2	
SD of sample means Sx	2,61	5,7%
SD within-samples Sw	3,91	8,5%
SD between-samples Ss	< 2,61	< 5,7%

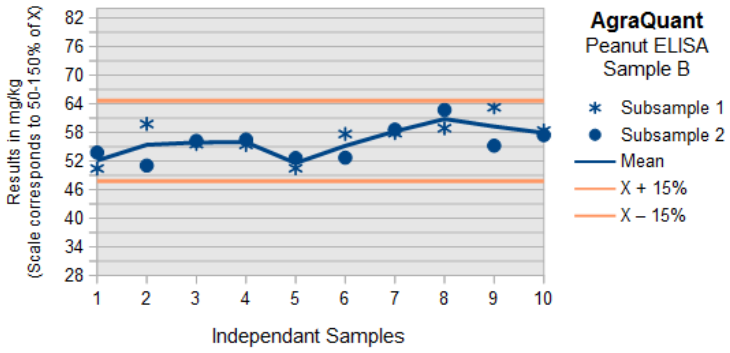


Romerlabs AgraQuant Peanut

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Peanut 56,2 ± 2,5 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	50,4	53,8	52,1
2	59,7	51,0	55,4
3	55,5	56,2	55,9
4	55,4	56,5	55,9
5	50,5	52,6	51,5
6	57,7	52,7	55,2
7	57,8	58,6	58,2
8	58,9	62,7	60,8
9	63,2	55,2	59,2
10	58,4	57,4	57,9

General average X	56,2	
SD of sample means Sx	2,94	5,2%
SD within-samples Sw	2,23	4,0%
SD between-samples Ss	2,48	4,4%



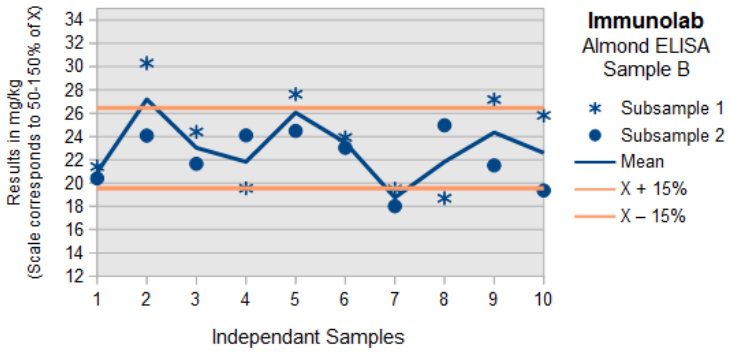
ELISA-Tests: Homogenität Mandel / Homogeneity Almond

Immunolab Almond ELISA

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 23,0 ± 1,9 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	21,4	20,4	20,9
2	30,3	24,1	27,2
3	24,4	21,7	23,0
4	19,5	24,1	21,8
5	27,6	24,5	26,1
6	23,9	23,0	23,5
7	19,5	18,0	18,8
8	18,7	25,0	21,8
9	27,2	21,5	24,3
10	25,8	19,4	22,6

General average X	23,0	
SD of sample means Sx	2,46	10,7%
SD within-samples Sw	2,20	9,6%
SD between-samples Ss	1,90	8,3%

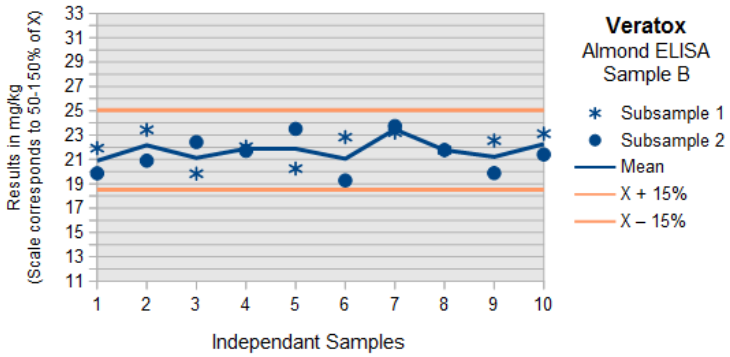


Neogen Veratox ELISA Almond

Sample weights: 5,0 g (4,5 – 5,5 g)
Number of replicates: 2
Overall result: Almond 21,8 ± 0,8 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	21,9	19,9	20,9
2	23,4	20,9	22,2
3	19,8	22,4	21,1
4	22,1	21,7	21,9
5	20,2	23,5	21,9
6	22,8	19,3	21,1
7	23,2	23,7	23,5
8	21,8	21,8	21,8
9	22,5	19,9	21,2
10	23,1	21,4	22,3

General average X	21,8	
SD of sample means Sx	0,77	3,5%
SD within-samples Sw	1,13	5,2%
SD between-samples Ss	< 0,77	< 3,5%

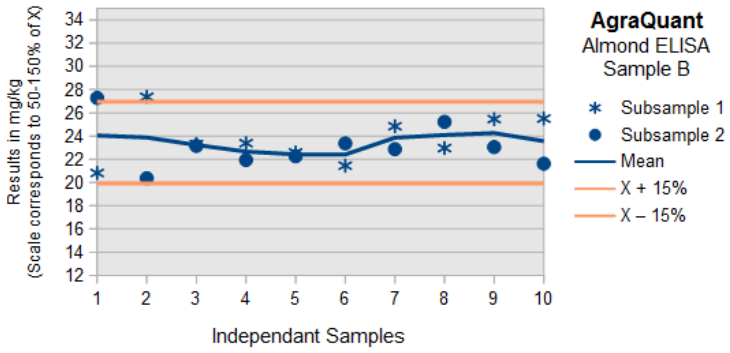


Romerlabs AgraQuant Almond

Sample weights: 1,0 g (0,9 – 1,1 g)
Number of replicates: 2
Overall result: Almond 23,4 ± 0,71 mg/kg

Sample B	Subsample 1 mg/kg	Subsample 2 mg/kg	Mean mg/kg
1	20,8	27,3	24,0
2	27,4	20,4	23,9
3	23,3	23,1	23,2
4	23,4	21,9	22,7
5	22,6	22,3	22,4
6	21,5	23,4	22,4
7	24,8	22,9	23,9
8	23,0	25,2	24,1
9	25,5	23,1	24,3
10	25,5	21,6	23,6

General average X	23,4	
SD of sample means Sx	0,71	3,0%
SD within-samples Sw	1,78	7,6%
SD between-samples Ss	< 0,71	< 3,0%



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. 0,19 (21,2°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 38th week of 2018. The testing method was optional. The tests should be finished at November 2nd 2018.

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 Almond in the range of mg/kg in the matrix of butter cookies. 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.*

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

All 19 participants submitted their results in time.

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 ELISA methods for the 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^x) 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, and results for a 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 for the allergens peanut, hazelnut, almond and brazil nut are in the range of 11 – 32% 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 process, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision, and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [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 (xi) 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 (Ux_{pt}) [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 (PT) 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

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 allergen-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** were converted by DLA to the **total food item (peanut)** using the analyzed protein content of the raw material (see page 5).

ELISA results given as **almond protein** were converted by DLA to the **total food item (almond)** using the analyzed protein content of the raw material (see page 5).

In the present PT, the quantitative PCR results e.g. for celery were sometimes given unclear or implausible as DNA, seed, tuber and/or only as celery, mustard and sesame. It was therefore not intended to standardize the PCR results.

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})	$\boldsymbol{X_{pt_{ALL}}}$	$\boldsymbol{X_{pt_{METHOD\ i}}}$
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 is calculated with 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		
3a	negative	<1	positive	50,8	2/2 (100%)	BF	
16	negative	0	positive	35,2	2/2 (100%)	BF	
1	negative	<1	positive	52,1	2/2 (100%)	BK	
6	negative	<LOQ	positive	31,0	2/2 (100%)	BK	
5	negative	< 0,1	positive	35,0	2/2 (100%)	IL	
9	negative	<1,0	positive	37,5	2/2 (100%)	IL	
15	negative	<LOD	positive	37,6	2/2 (100%)	IL	
18	negative	<1,3	positive	5,60	2/2 (100%)	MI-II	result converted °
3b	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
7	negative		positive	50,9	2/2 (100%)	RS-F	
10	negative	<11	positive	224	2/2 (100%)	RS-F	result converted °
11	negative		positive	48,8	2/2 (100%)	RS-F	
12	negative	< 0,13	positive	42,7	2/2 (100%)	RS-F	
14	negative	<2.5	positive	48,5	2/2 (100%)	RS-F	
17	negative	<2,50	positive	48,5	2/2 (100%)	RS-F	
1	negative	<2,5	positive	41,2	2/2 (100%)	VT	
8	negative		positive	154	2/2 (100%)	VT	result converted °

° calculation p. 19

	Sample A		Sample B	
Number positive	0		17	
Number negative	17		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

BF = MonoTrace ELISA, BioFront Technologies
BK = BioKits, Neogen
IL = Immunolab
MI-II = Morinaga Institute ELISA Kit II
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-F}	Method	Remarks
	pos/neg				
3a	50,8	0,72		BF	
16	35,2	-0,73		BF	
1	52,1	0,84		BK	
6	31,0	-1,1		BK	
5	35,0	-0,75		IL	
9	37,5	-0,52		IL	
15	37,6	-0,51		IL	
18	5,60	-3,5		MI-II	result converted ° / outlier excluded
3b	>20			RS-F	
7	50,9	0,73	0,20	RS-F	
10	224	17	14	RS-F	result converted ° / outlier excluded
11	48,8	0,53	0,03	RS-F	
12	42,7	-0,03	-0,48	RS-F	
14	48,5	0,51	0,00	RS-F	
17	48,5	0,51	0,00	RS-F	
1	41,2	-0,17		VT	
8	154	10		VT	result converted ° / outlier excluded

° calculation S. 19

Methods:

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

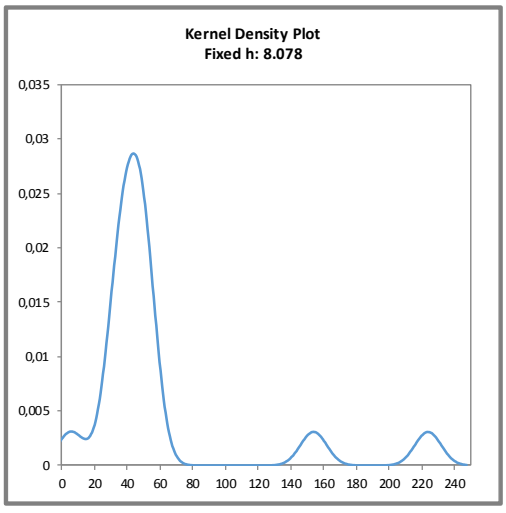


Abb. / Fig. 1:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a side-peak caused by one result at 5-6 mg/kg (method MI-II) and two peaks > 100 mg/kg due to outliers (possibly the higher results were submitted as peanut protein by mistake).

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°	13	5
Number of outliers	3	1
Mean	43,1	47,9
Median	42,7	48,5
Robust Mean (X_{pt})	**43,1**	**48,5**
Robust standard deviation (S*)	**8,23**	**2,08**
Target range:		
Target standard deviation σ_{pt}	**10,8**	**12,1**
lower limit of target range	**21,5**	**24,2**
upper limit of target range	**64,6**	**73**
Quotient S/σ_{pt}*	*0,76*	*0,17*
Standard uncertainty $U_{(Xpt)}$	*2,9*	*1,20*
Results in the target range	*13*	*5*
Percent in the target range	*100*	*100*

° *results without outliers (evaluation no. 8, 10 and 18)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results. The outliers were excluded from statistical calculations.

The evaluation of all methods and the evaluation of results from method RS-F showed a low variability of results. The quotients S/σ_{pt} were 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 assigned values of the evaluation of all results and method RS-F were 134% and 152% of the spiking level of peanut to sample B and thus in the range and close to the upper limit of the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Peanut" p.29).

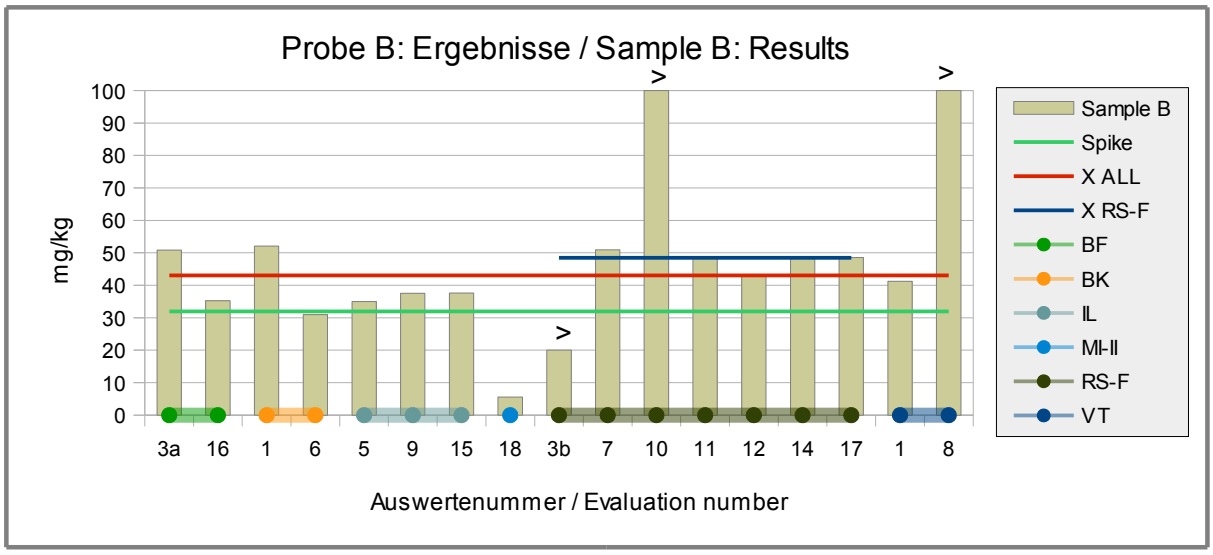


Abb./Fig. 2: ELISA Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

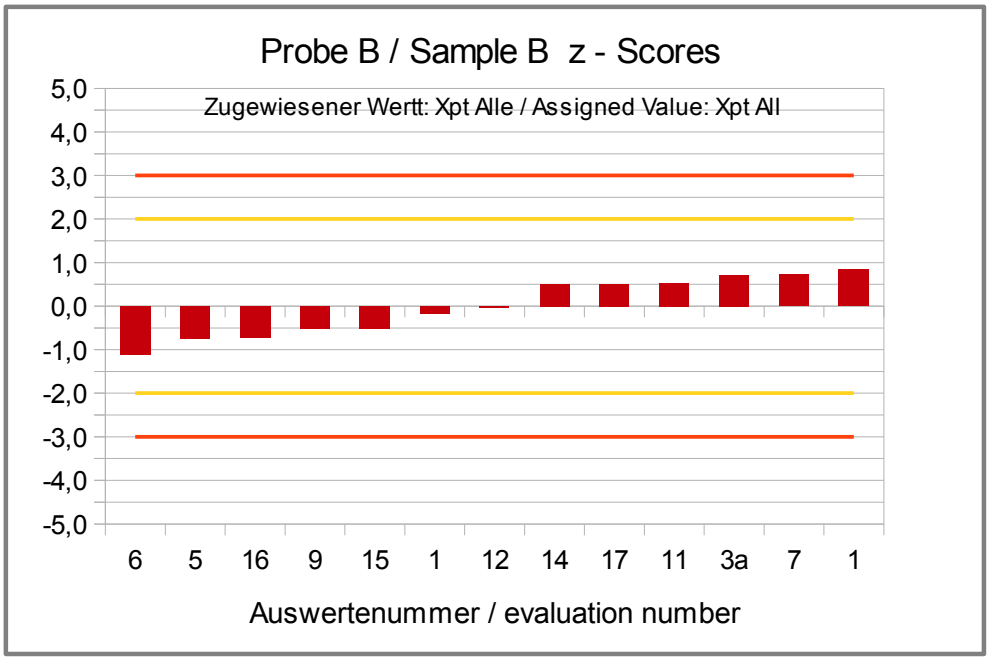


Abb./Fig. 3:
z-Scores (ELISA Results Peanut)
Assigned value robust mean (algorithm A) of all results

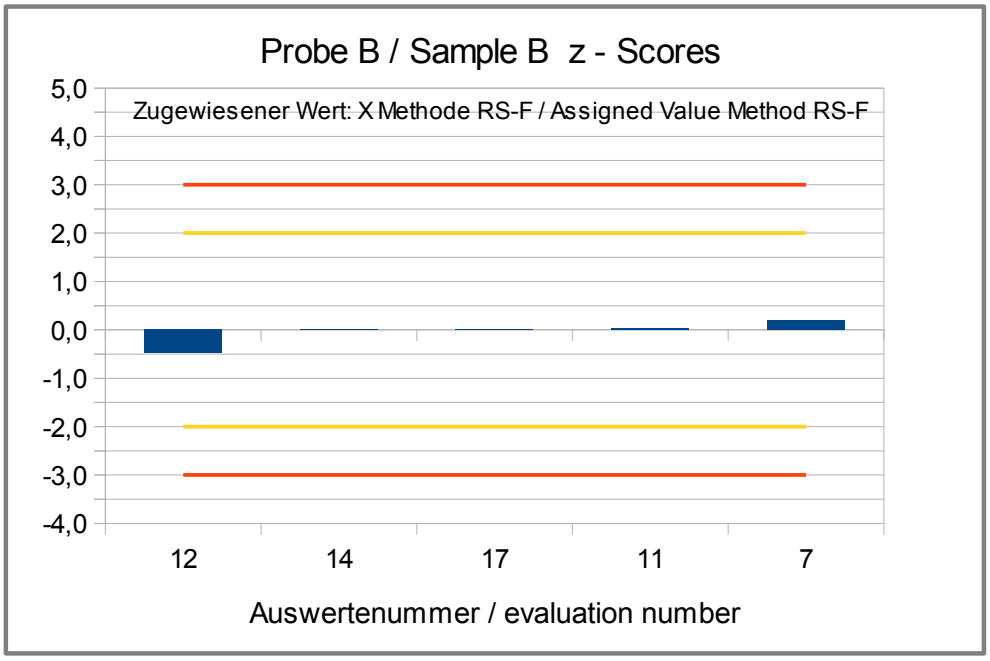


Abb./Fig. 4:
z-Scores (ELISA Results Peanut)
Assigned value: robust mean (algorithm A) of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Spiking Level Sample	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
3a	216	3,9		BF	
16	118	0,32		BF	
1	175	2,4		BK	
6	85,0	-0,89		BK	
5	71,0	-1,4		IL	
9	100	-0,34		IL	
15	109	-0,01		IL	
18	12,1	-3,6		MI-II	result converted °
3b	>20			RS-F	
7	104	-0,20		RS-F	
10	362	9,3		RS-F	result converted ° / outlier excluded
11				RS-F	
12	125	0,59		RS-F	
14	83,8	-0,93		RS-F	
17	79,5	-1,1		RS-F	
1	73,7	-1,3		VT	
8	206	3,5		VT	result converted °

° calculation S. 19

Methods:

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

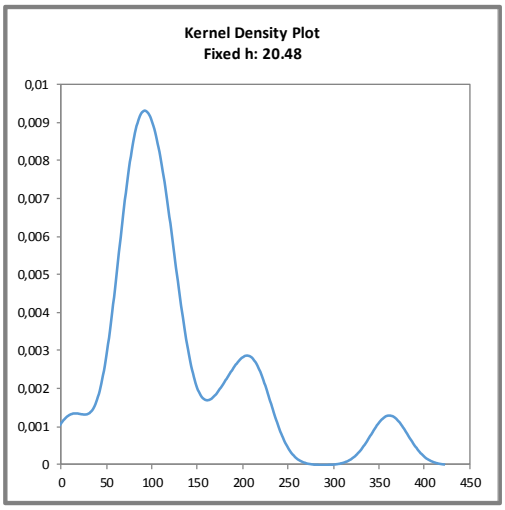


Abb. / Fig. 5:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a side-peak caused by one result at approx. 12 mg/kg (method MI-II) and two peaks > 150 mg/kg due to one outlier and three results outside the target range (possibly the higher results were submitted as peanut protein by mistake).

Characteristics: Quantitative evaluation Peanut

Spiking level sample

Statistic Data	**All Results** *[mg/kg]*
Assigned value (X_{pt})	$X_{pt_{ALL}}$
Number of results°	14
Number of outliers	1
Mean	111
Median	102
Robust Mean (X_{pt})	**109**
Robust standard deviation (S*)	**52,5**
Target range:	
Target standard deviation σ_{pt}	**27,3**
lower limit of target range	**54,6**
upper limit of target range	**164**
Quotient S/σ_{pt}*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*17,5*
Results in the target range	*10*
Percent in the target range	*71*

° *results without outliers (evaluation no. 10)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results. The outlier was excluded from statistical calculations.

The evaluation of all methods showed a normal variability of results. The quotient S/σ_{pt} was 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 assigned value of the evaluation of all results was 322% of the spiking level of peanut to the spiking level sample and thus clearly above the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Peanut" p.29).

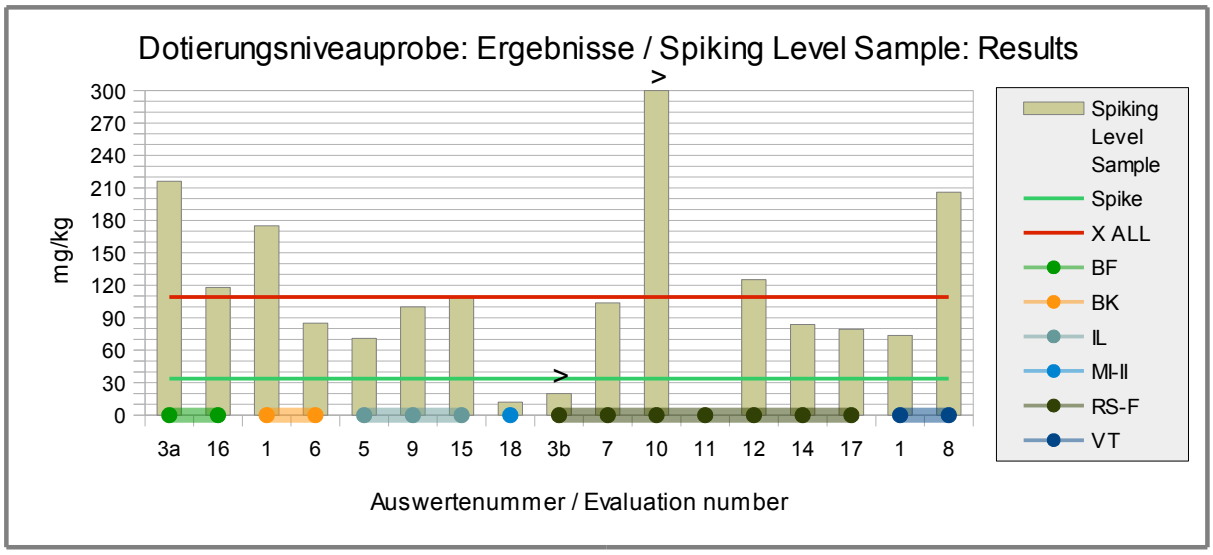


Abb./Fig. 6: ELISA Results Peanut
green line = Spiking level
red line = Assigned value robust mean all results
round symbols = Applied methods (see legend)

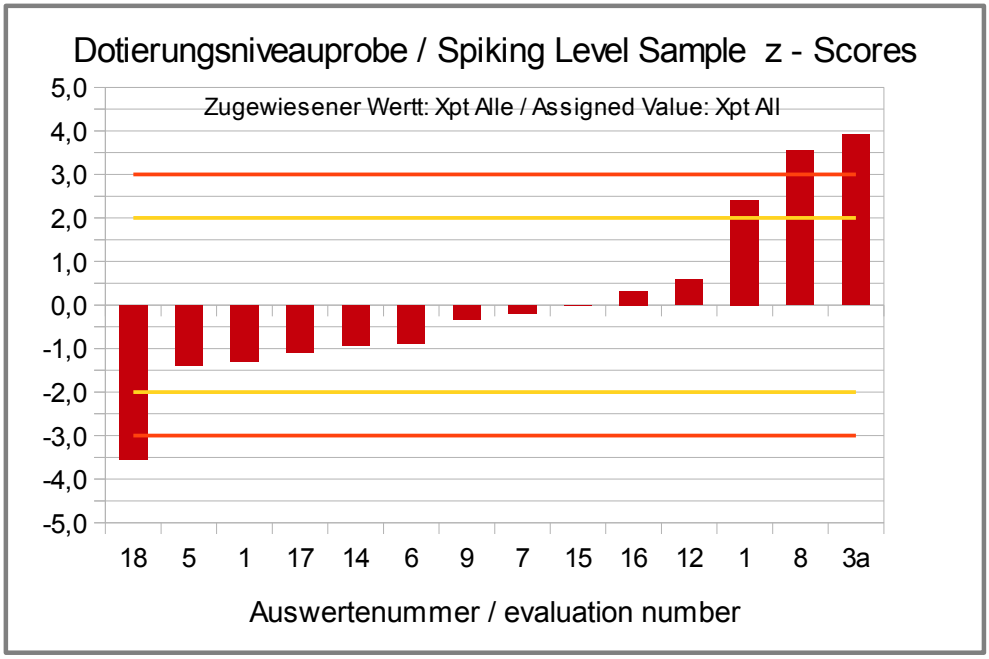


Abb./Fig. 7:
z-Scores (ELISA Results Peanut)
Assigned value: robust mean (algorithm A) of all results

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]	[%]	pos/neg	[%]		
3a	216	638	50,8	159	BF	
16	118	349	35,2	**110**	BF	
1	175	518	52,1	163	BK	
6	85,0	251	31,0	**97**	BK	
5	71,0	210	35,0	**110**	IL	
9	100	296	37,5	**117**	IL	
15	109	322	37,6	**118**	IL	
18	12,1	36	5,60	18	MI-II	result converted °
3b	>20		>20		RS-F	
7	104	307	50,9	159	RS-F	
10	362	1070	224	701	RS-F	result converted °
11			48,8	153	RS-F	
12	125	370	42,7	**134**	RS-F	
14	83,8	248	48,5	152	RS-F	
17	79,5	235	48,5	152	RS-F	
1	73,7	218	41,2	**129**	VT	
8	206	609	154	482	VT	result converted °

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**0**	Number in RA	**7**
Percent in RA	**0**	Percent in RA	**44**

* Recovery rate 100% relative size: Peanut, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

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

Comments:

For the spiking level sample all the recovery rates of the ELISA methods, with one exception, were above the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B 44% (7) of the recovery rates were within the range of acceptance.

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		
6	negative		positive		2/2 (100%)	ASU	
13	negative		positive	14	2/2 (100%)	ASU	
15	negative		positive		2/2 (100%)	GI	
3	negative	<0,4	positive	>0,4	2/2 (100%)	SFA	
12	negative	<0,4	positive	22,7	2/2 (100%)	SFA	
14a	negative	<1	positive	41,0	2/2 (100%)	SFA	
14b	negative	<1	positive	35,1	2/2 (100%)	SFA-ID	
11	negative		positive		2/2 (100%)	SFA-Q	
4	negative		positive	6,50	2/2 (100%)	div	
7	negative		negative		1/2 (50%)	div	no positive sample detected
18	negative		positive		2/2 (100%)	div	

	Sample A		Sample B	
Number positive	0		10	
Number negative	11		1	
Percent positive	0		91	
Percent negative	100		9	
Consensus value	negative		positive	

Methods:
ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

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

Quantitative Valuation PCR: Sample B

No quantitative evaluation was done, because there were not enough results per single methods. A joint evaluation of all methods was not done because the two individual results of the ASU and another method (div) were well below the three results of the SFA methods.

(Quantitative) Valuation PCR: Spiking Level Sample

No quantitative evaluation was done, because there were not enough results per single PCR methods. A joint evaluation of all methods was not done because the two individual results of the ASU and another method (div) were well below the three results of the SFA methods.

Evaluation number	Peanut	Peanut	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]	[mg/kg]			
6	positive			ASU	
13	positive	27,7		ASU	
15	positive			GI	
3	positive	>0,4		SFA	
12	positive	78,5		SFA	
14a	positive	74,1		SFA	
14b	positive	42,4		SFA-ID	
11	positive			SFA-Q	
4	positive	28,0		div	
7	negative			div	
18	positive			div	

Number positive	10
Number negative	1
Percent positive	91
Percent negative	9
Consensus value	positive

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Comments:

For the spiking level sample there were 91% positive results.

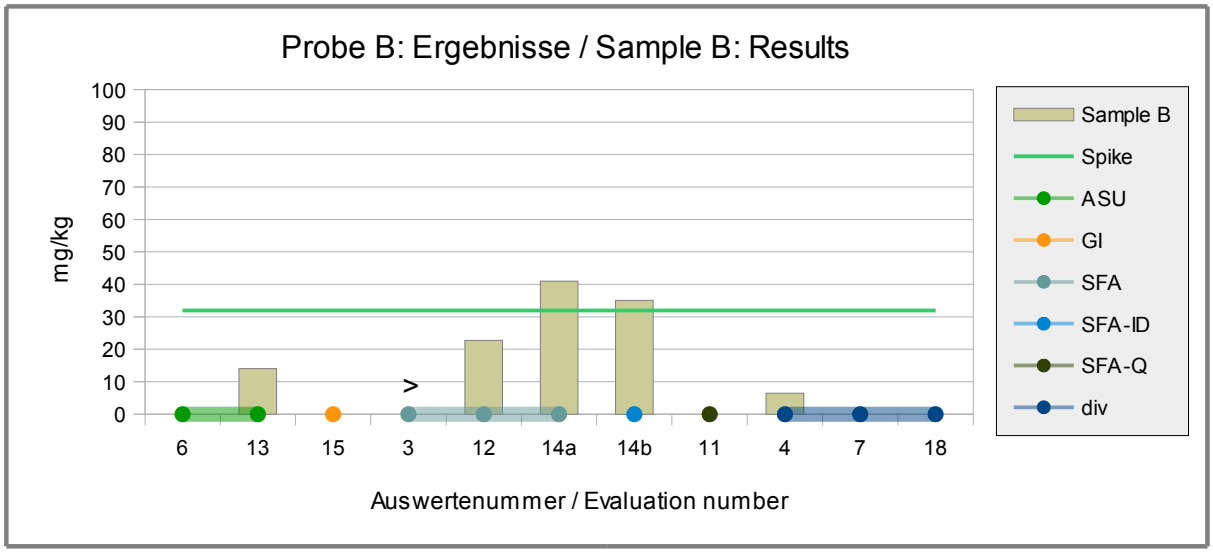


Abb./Fig. 8: PCR Results Peanut Sample B
green line = Spiking level
round symbols = Applied methods (see legend)

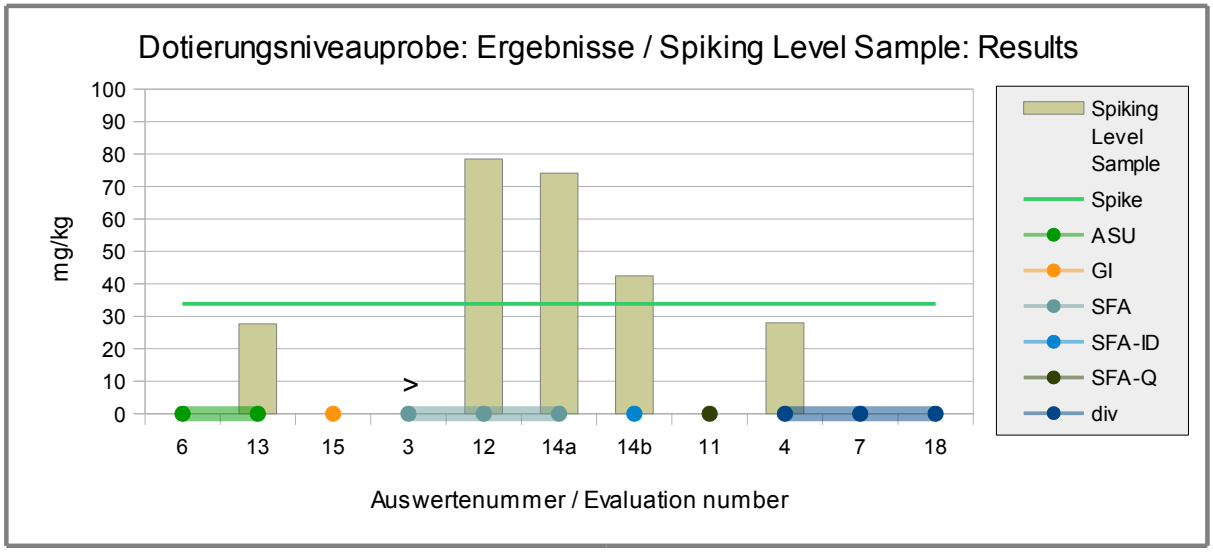


Abb./Fig. 9: PCR Results Peanut Spiking Level Sample
green line = Spiking level
round symbols = Applied methods (see legend)

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]	[%]	pos/neg	[%]		
6					ASU	
13	27,7	**82**	14	44	ASU	
15					GI	
3	>0,4		>0,4		SFA	
12	78,5	232	22,7	**71**	SFA	
14a	74,1	219	41,0	**128**	SFA	
14b	42,4	**125**	35,1	**110**	SFA-ID	
11					SFA-Q	
4	28,0	**83**	6,50	20	div	
7					div	
18					div	

RA**	50-150 %	RA**	50-150 %
Number in RA	**3**	Number in RA	**3**
Percent in RA	**60**	Percent in RA	**60**

* Recovery rate 100% relative size: Peanut, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen
div = not indicated / other method

Comments:

One participant (evaluation no. 14b) obtained recovery rates by PCR for the spiking level sample and for the processed spiked food matrix sample B both within the range of the AOAC-recommendation of 50-150%. Overall 60% (3) of the recovery rates were within the range of acceptance for the spiking level sample and sample B each.

4.2 Proficiency Test Almond

4.2.1 ELISA Results: Almond

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		
2	negative	<0,4	positive	22,3	2/2 (100%)	AQ	
7	negative		positive	148	2/2 (100%)	AQ	result converted °
15	negative	<LOD	positive	22,0	2/2 (100%)	AQ	
3a	negative	<1	positive	110	2/2 (100%)	BF	
16	negative	0	positive	26,8	2/2 (100%)	BF	
5	negative	< 0,2	positive	24,0	2/2 (100%)	IL	
9	negative	<0,4	positive	24,4	2/2 (100%)	IL	
3b	negative	<2,5	positive	>20	2/2 (100%)	RS-F	
6	negative	<LOQ	positive	30,0	2/2 (100%)	RS-F	
10	negative	<15	positive	179	2/2 (100%)	RS-F	result converted °
11	negative		positive	32,1	2/2 (100%)	RS-F	
14	negative	<2.5	positive	36,5	2/2 (100%)	RS-F	
17	negative	<2,50	positive	36,6	2/2 (100%)	RS-F	
18	negative	<2,5	positive	37,0	2/2 (100%)	RS-F	
19	negative	<2,5	positive	42,0	2/2 (100%)	RS-F	
1	negative	<2,5	positive	27,2	2/2 (100%)	VT	
8	negative		positive	94,4	2/2 (100%)	VT	result converted °

° calculation p. 19

	Sample A		Sample B	
Number positive	0		17	
Number negative	17		0	
Percent positive	0		100	
Percent negative	100		0	
Consensus value	negative		positive	

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

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	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	22,3	-1,0		AQ	
7	148	16		AQ	result converted ° / outlier excluded
15	22,0	-1,1		AQ	
3a	110	11		BF	outlier excluded
16	26,8	-0,43		BF	
5	24,0	-0,80		IL	
9	24,4	-0,75		IL	
3b	>20			RS-F	
6	30,0	0,00	-0,64	RS-F	
10	179	20	16	RS-F	result converted ° / outlier excluded
11	32,1	0,28	-0,40	RS-F	
14	36,5	0,87	0,09	RS-F	
17	36,6	0,88	0,10	RS-F	
18	37,0	0,93	0,15	RS-F	
19	42,0	1,6	0,71	RS-F	
1	27,2	-0,37		VT	
8	94,4	8,6		VT	result converted ° / outlier excluded

° calculation S. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

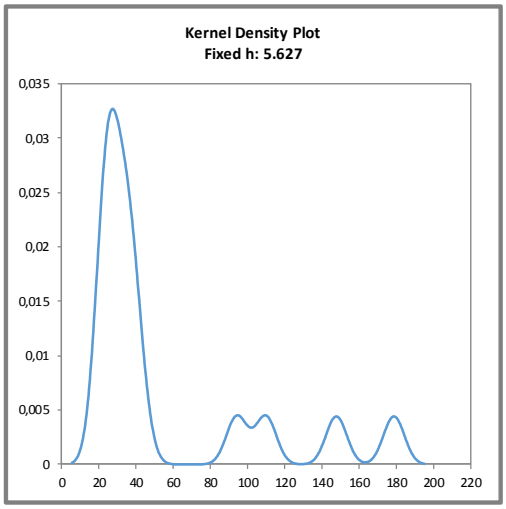


Abb. / Fig. 10:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with several smaller side-peaks > 90 mg/kg due to outliers (possibly the higher results were submitted as almond protein by mistake).

Characteristics: Quantitative evaluation ELISA: Almond

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°	12	6
Number of outliers	4	1
Mean	30,1	35,7
Median	28,6	36,6
Robust Mean (X_{pt})	**30,0**	**35,7**
Robust standard deviation (S*)	**7,43**	**4,77**
Target range:		
Target standard deviation σ_{pt}	**7,50**	**8,93**
lower limit of target range	**15,0**	**17,9**
upper limit of target range	**45,0**	**53,6**
Quotient S/σ_{pt}*	*0,99*	*0,53*
Standard uncertainty $U_{(Xpt)}$	*2,68*	*2,43*
Results in the target range	*12*	*6*
Percent in the target range	*100*	*100*

° *results without outliers (evaluation no.3a, 7, 8 and 10)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results. The outliers were excluded from statistical calculations.

The evaluation of all methods and the evaluation of results from method RS-F showed a low variability of results. The quotients S/σ_{pt} were approx. 1,0 and 0,5, respectively. 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 assigned values of the evaluation of all results and method RS-F were 44% and 52% of the spiking level of almond to sample B and thus slightly below and in the lower range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Almond" p.43).

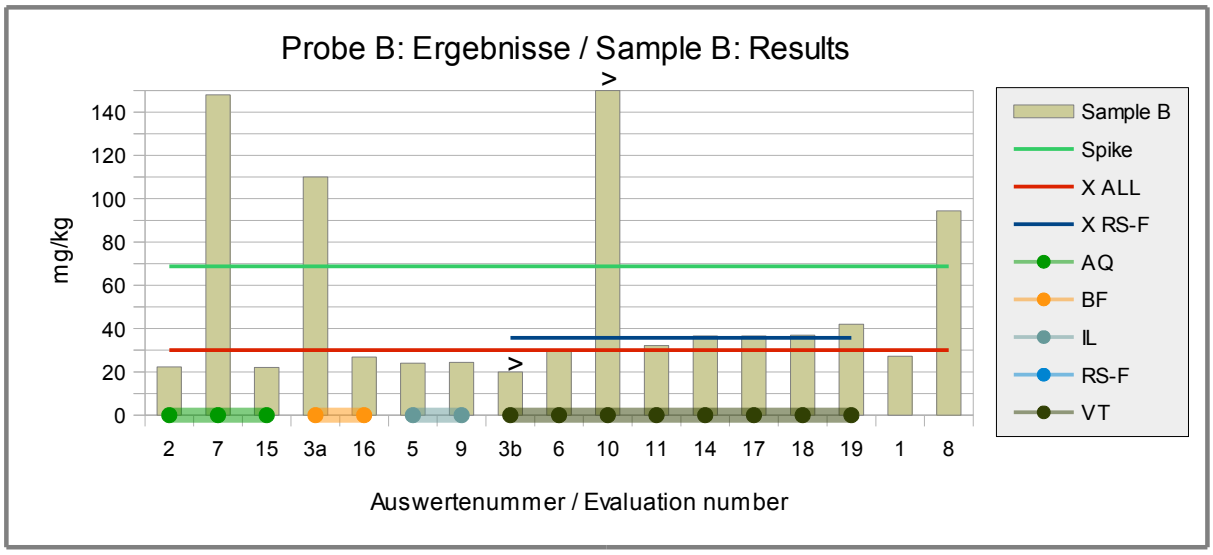


Abb./Fig. 11: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

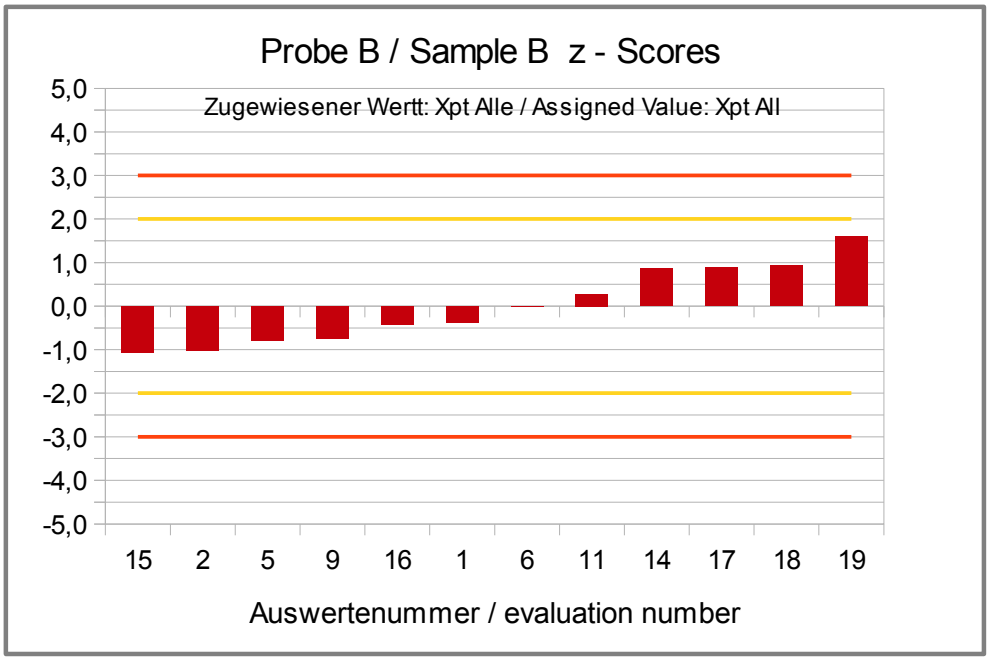


Abb./Fig. 12:
z-Scores (ELISA Results Almond)
Assigned value: robust mean (algorithm A) of all results

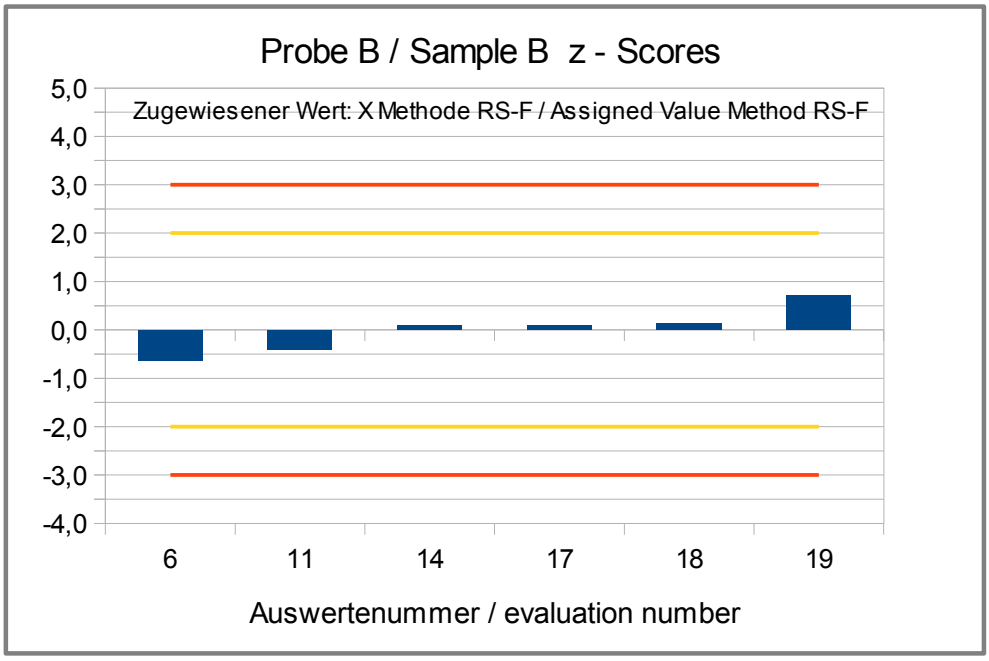


Abb./Fig. 13:
z-Scores (ELISA Results Almond)
Assigned value: robust mean (algorithm A) of method RS-F (R-Biopharm, Ridascreen® Fast)

Quantitative valuation of results: Spiking level sample

Evaluation number	Almond	z-Score Xpt_{ALL}	z-Score Xpt_{RS-F}	Method	Remarks
	[mg/kg]				
2	31,6	-0,73		AQ	
7	215	18		AQ	result converted ° / outlier excluded
15	31,0	-0,79		AQ	
3a	141	11		BF	outlier excluded
16	66,3	2,9		BF	
5	27,0	-1,2		IL	
9	56,3	1,8		IL	
3b	>20			RS-F	
6	30,0	-0,89	-0,80	RS-F	
10	198	17	17	RS-F	result converted ° / outlier excluded
11				RS-F	
14	46,5	0,81	0,95	RS-F	
17	37,6	-0,10	0,01	RS-F	
18	38,0	-0,06	0,05	RS-F	
19	37,0	-0,17	-0,06	RS-F	
1	36,9	-0,18		VT	
8	188	15		VT	result converted ° / outlier excluded

° calculation S. 19

Methods:

AQ = AgraQuant, RomerLabs
BF = MonoTrace ELISA, BioFront Technologies
IL = Immunolab
RS-F= Ridascreen® Fast, R-Biopharm
VT = Veratox, Neogen

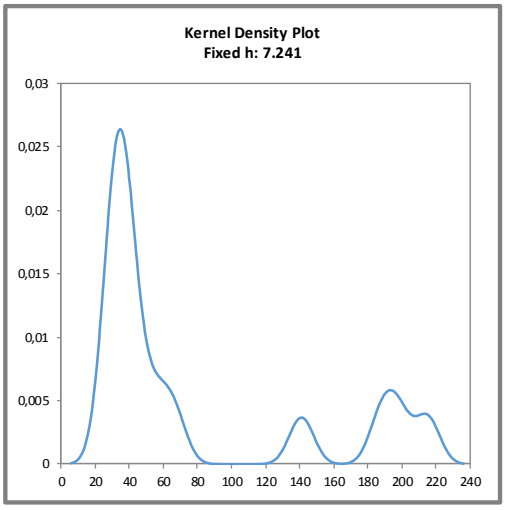


Abb. / Fig. 14:
Kerndichte-Schätzung aller ELISA-Ergebnisse (mit h = 0,75 x σpt von Xpt_{ALL})

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

Comments:
The kernel density estimation shows nearly a symmetrical distribution of results with a shoulder at approx. 60 mg/kg. Several smaller side-peaks > 120 mg/kg are due to outliers (possibly the higher results were submitted as almond protein by mistake).

Characteristics: Quantitative evaluation Almond

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°	11	5
Number of outliers	4	1
Mean	39,8	37,8
Median	37,0	37,6
Robust Mean (X_{pt})	**38,6**	**37,5**
Robust standard deviation (S*)	**10,7**	**5,84**
Target range:		
Target standard deviation σ_{pt}	**9,66**	**9,39**
lower limit of target range	**19,3**	**18,8**
upper limit of target range	**57,9**	**56,3**
Quotient S/σ_{pt}*	*1,1*	*0,62*
Standard uncertainty $U_{(Xpt)}$	*4,05*	*3,27*
Results in the target range	*10*	*5*
Percent in the target range	*91*	*100*

° *results without outliers (evaluation no.3a, 7, 8 and 10)*

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

Comments to the statistical characteristics and assigned values:

The kernel density estimation showed almost a symmetrical distribution of results. The outliers were excluded from statistical calculations.

The evaluation of all methods and the evaluation of results from method RS-F showed a low variability of results. The quotients S/σ_{pt} were approx. 1,1 and 0,6, respectively. 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 assigned values of the evaluation of all results and method RS-F were 111% and 108% of the spiking level of almond to the spiking level sample and thus within the range of the recommendations for the applied methods (s. 3.4.3 and "Recovery rates of Almond" p.43).

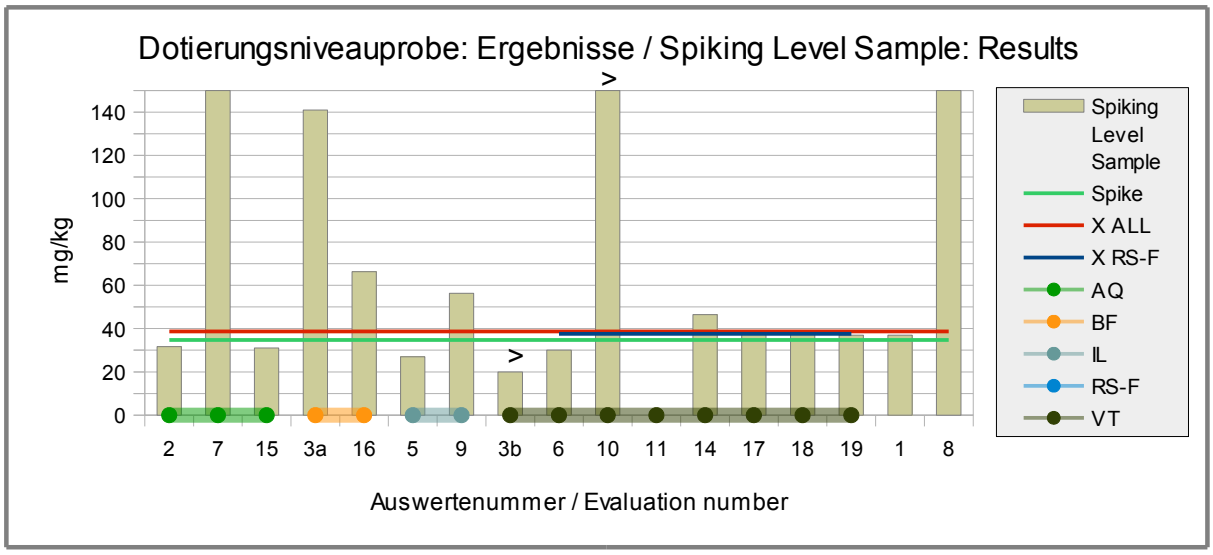


Abb./Fig. 15: ELISA Results Almond
green line = Spiking level
red line = Assigned value robust mean all results
blue line = Assigned value robust mean results method RS-F
round symbols = Applied methods (see legend)

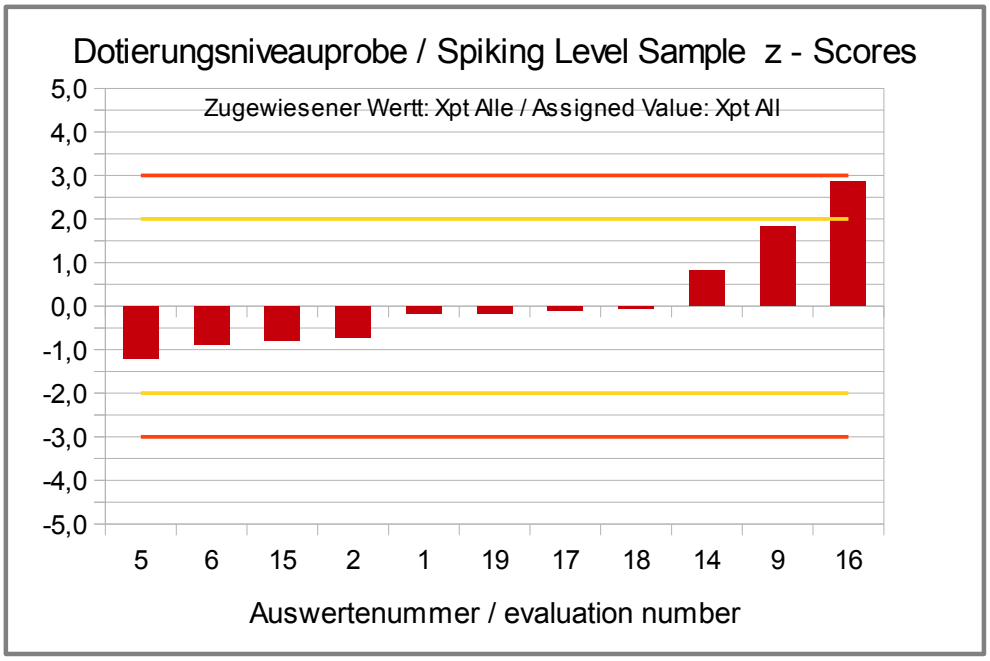


Abb./Fig. 16:
z-Scores (ELISA Results Almond)
Assigned value: robust mean (algorithm A) of all results

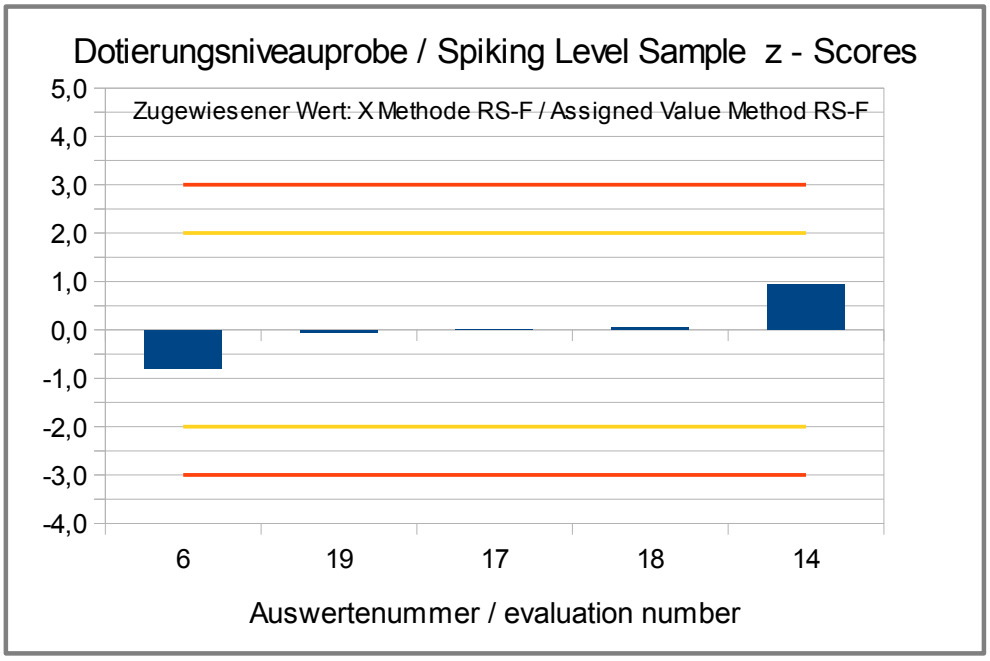


Abb./Fig. 17:
z-Scores (ELISA Results Almond)
Assigned value: robust mean (algorithm A) of method RS-F (R-Biopharm, Ridascreen® Fast)

Recovery Rates ELISA for Almond: Spiking level Sample and Sample B

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
2	31,6	**91**	22,3	32	AQ	
7	215	620	148	215	AQ	result converted °
15	31,0	**89**	22,0	32	AQ	
3a	141	406	110	160	BF	
16	66,3	191	26,8	39	BF	
5	27,0	**78**	24,0	35	IL	
9	56,3	162	24,4	36	IL	
3b	>20		> 20		RS-F	
6	30,0	**86**	30,0	44	RS-F	
10	198	571	179	261	RS-F	result converted °
11			32,1	47	RS-F	
14	46,5	**134**	36,5	**53**	RS-F	
17	37,6	**108**	36,6	**53**	RS-F	
18	38,0	**110**	37,0	**54**	RS-F	
19	37,0	**107**	42,0	**61**	RS-F	
1	36,9	**106**	27,2	40	VT	
8	188	542	94,4	**137**	VT	result converted °

° calculation p. 19

RA**	50-150 %	RA**	50-150 %
Number in RA	**9**	Number in RA	**5**
Percent in RA	**60**	Percent in RA	**31**

* Recovery rate 100% relative size: Almond, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:

AQ = AgraQuant, RomerLabs

BF = MonoTrace ELISA, BioFront Technologies

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

Comments:

For the spiking level sample 60% (9) of the participants obtained a recovery rate by ELISA methods within the range of the AOAC-recommendation of 50-150%. For the processed spiked food matrix sample B 31% (5) of the recovery rates were within the range of acceptance.

4.2.2 PCR Results: Almond

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		positive	< 20	2/2 (100%)	ASU	
7	negative		negative		1/2 (50%)	ASU	no positive sample detected
13	negative		positive	19,6	2/2 (100%)	ASU	
15	negative		positive		2/2 (100%)	GI	
6	negative		positive		2/2 (100%)	SFA	
14	negative	<1	positive	15,1	2/2 (100%)	SFA-ID	
11	negative		positive		2/2 (100%)	SFA-Q	

	Sample A		Sample B	
Number positive	0		6	
Number negative	7		1	
Percent positive	0		86	
Percent negative	100		14	
Consensus value	negative		positive	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

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

Quantitative Valuation PCR: Sample B

No quantitative evaluation was done, because there were < 5 quantitative results.

(Quantitative) Valuation PCR: Spiking Level Sample

No quantitative evaluation was done, because there were < 5 quantitative results.

Evaluation number	Almond	Almond	z-Score Xpt_{ALL}	Method	Remarks
	[mg/kg]	[mg/kg]			
4	positive	38,0		ASU	
7	positive			ASU	
13	positive	< BG		ASU	
15	positive			GI	
6	positive			SFA	
14	positive	20,2		SFA-ID	
11	positive			SFA-Q	

Number positive	7	
Number negative	0	
Percent positive	100	
Percent negative	0	
Consensus value	positive	

Methods:

ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

Comments:
For the spiking level sample there were 100% positive results.

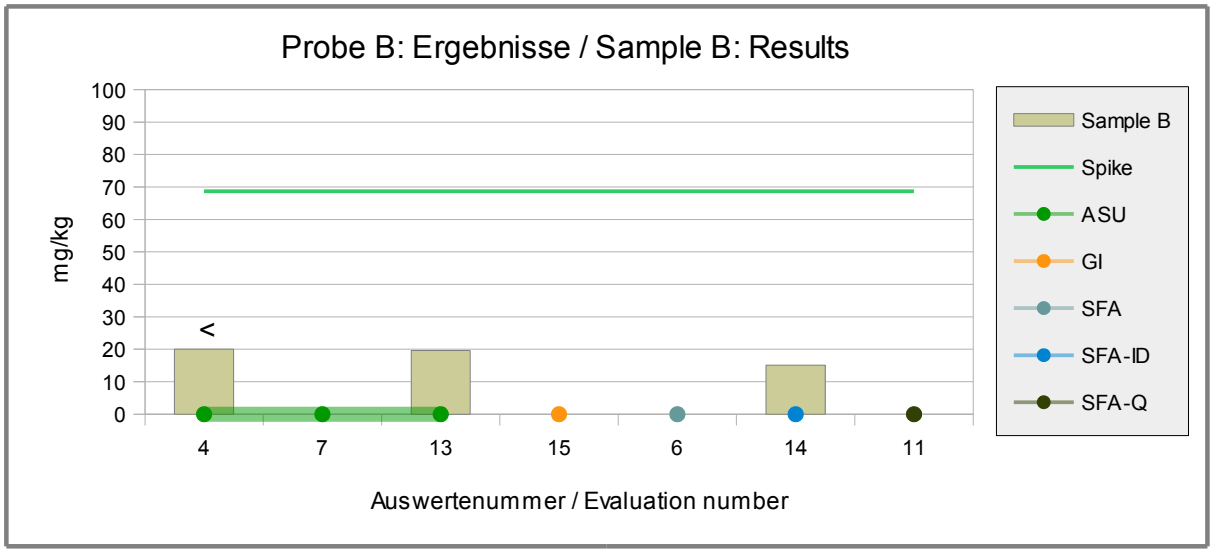


Abb./Fig. 18: PCR Results Almond Sample B
green line = Spiking level
round symbols = Applied methods (see legend)

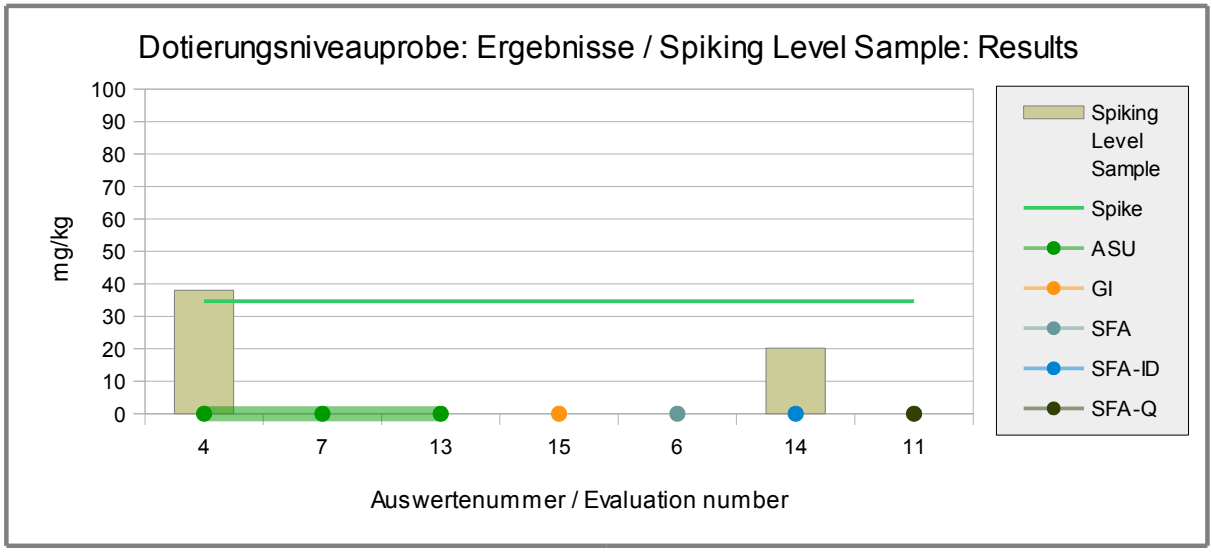


Abb./Fig. 19: PCR Results Almond Spiking Level Sample
green line = Spiking level
round symbols = Applied methods (see legend)

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

Evaluation number	Spiking Level Sample	Recovery rate*	Sample B	Recovery rate*	Method	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]		
4	38,0	**110**	< 20		ASU	
7					ASU	
13	< BG		19,6	29	ASU	
15					GI	
6					SFA	
14	20,2	**58**	15,1	22	SFA-ID	
11					SFA-Q	

RA**	50-150 %	RA**	50-150 %
Number in RA	**2**	Number in RA	**0**
Percent in RA	**100**	Percent in RA	**0**

* Recovery rate 100% relative size: Almond, s. page 5

** Range of acceptance of AOAC for allergen ELISAS

Methods:
ASU = ASU §64 Methode/method
GI = GEN-IAL First Allergen
SFA = Sure Food Allergen, R-Biopharm / Congen
SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

Comments:
Two participants obtained recovery rates for the spiking level sample by PCR methods within the range of the AOAC-recommendation of 50-150%.
For the processed spiked sample B the recovery rates were below this range of acceptance.

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 number	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
BF	3a		negative	<1	positive	50,8	positive	216		1		Peanut	MonoTrace Peanut ELISA kit, BioFront Technologies
BF	16	01.11.18	negative	0	positive	35,2	positive	118	0,24	1		Peanut	MonoTrace Peanut ELISA kit, BioFront Technologies
BK	1	03.10.18	negative	<1	positive	52,1	positive	175,1	1	1		Peanut	BioKits Peanut Assay Kit, Neogen
BK	6	24/09/ 2018, 26/09/2018	-	<LOQ	-	31	-	85		1		Peanut	BioKits Peanut Assay Kit, Neogen
IL	5	24.09.18	negative	< 0,1	positive	35	positive	71	0,1	1		Peanut	Immunolab Peanut ELISA
IL	9	30.10.18	-	<1,0	-	37,5	-	100		1		Peanut	Immunolab Peanut ELISA
IL	15	30.11.18	negative	<LOD	positive	37,6	positive	109	0,1	1	15	Peanut	Immunolab Peanut ELISA
MI-II	18	26.09.18	negative	<0,31	positive	1,3	positive	2,8	0,12	0,31		Peanut protein	Peanut ELISA Kit-II, Morinaga
RS-F	3b		negative	<2,5	positive	>20	positive	>20		2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	7	10.10.18	negative		positive	50,9	positive	103,7	2,5	2,5	34	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	10	12.10.18	-	<2,5	-	52	-	84	0,13	2,5	5,2	Peanut protein	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	11	24.10.18	negative		positive	48,8	positive		2,5	2,5	30,6	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	12	30.10.18	negative	< 0.13	positive	42,7	positive	125,3	0,13	2,5		Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	14	27.09.18	negative	<2.5	positive	48,5	positive	83,8	2,5	2,5	35,66	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
RS-F	17	10.10.18	negative	<2,50	positive	48,53	positive	79,45	0,13	2,5	12,18	Peanut	Ridascreen Fast Peanut (R6202), r-Biopharm
VT	1	02.10.18	negative	<2,5	positive	41,2	positive	73,7	2,5	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 number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredited ISO/IEC 17025	Further Remarks
		Antibody	e.g. Extraction Solution / Time / Temperature	yes/no	
BF	3a			yes	
BF	16		1:10 extraction ratio @ 62C for 10 minutes	No	Product # PA3-EK
BK	1				
BK	6	Polyconal Ab against Conarachin (Ara h1)	According to kit manual	yes	
IL	5	polyclonal			
IL	9			yes	
IL	15			yes	
MI-II	18	detects peanut proteins	According to kit manual	yes	
RS-F	3b			no	
RS-F	7		according to handbook	yes	
RS-F	10	The antibodies specifically detect peanut proteins, including the peanut allergen Ara h 1 and Ara h 2.	1 gr sample+ 20 ml extraction buffer/heating at 60oC/10min/cetrifuge 10 min 2500g/ 100µL per well	no	Further dillution 1:10 for SampleB,C
RS-F	11		As Per Kit Instructions	yes	
RS-F	12	The antibodies specifically detect peanut proteins, including the peanut allergen Ara h 1 and Ara h 2	According to kit manual	yes	
RS-F	14	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	17	Ara h 1, Ara h 2	As per kit instructions with addition of 1g skimmed milk powder	yes	
VT	1				
VT	8		PBS/15min/60°C	yes	

5.1.2 ELISA: Almond

Meth. Abr.	Evaluation number	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
AQ	2	17.10.18	negative	<0,4	positive	22,3	positive	31,6	0,2	0,4		Almond	AgraQuant ELISA Almond COKAL0748, RomerLabs
AQ	7	04.10.18	negative		positive	24	positive	34,9	0,4	0,4	16	Almond protein	AgraQuant ELISA Almond COKAL0748, RomerLabs
AQ	15	30.11.18	negative	<LOD	positive	22	positive	31	0,2	0,4	15	Almond	AgraQuant ELISA Almond COKAL0748, RomerLabs
BF	3a		negative	<1	positive	110	positive	141		1		Almond	MonoTrace Almond ELISA kit, BioFront Technologies
BF	16	01.11.18	negative	0	positive	26,8	positive	66,3	0,23	1		Almond	MonoTrace Almond ELISA kit, BioFront Technologies
IL	5	24.09.18	negative	< 0,2	positive	24	positive	27	0,2	0,4		Almond	Immunolab Almond ELISA
IL	9	30.10.18	-	<0,4	-	24,4	-	56,3		0,4		Almond	Immunolab Almond ELISA
RS-F	3b		negative	<2,5	positive	>20	positive	>20		2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	6	16/10/2018, 17/10/2018	-	<LOQ	-	30	-	30		2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	10	15.10.18	-	<2,5	-	29	-	32	0,1	2,5		Almond protein	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	11	24.10.18	negative		positive	32,1	positive		2,5	2,5	31,3	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	14	19.10.18	negative	<2.5	positive	36,52	positive	46,48	2,5	2,5	36,17	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	17	10.10.18	negative	<2,50	positive	36,6	positive	37,63	0,1	2,5	27,37	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	18	26.09.18	negative	<2,5	positive	37	positive	38	1,7	2,5		Almond	Ridascreen® FAST Almond R6901, R-Biopharm
RS-F	19	02.11.18	-	<2,5	-	42	-	37	1,5	2,5	40	Almond	Ridascreen® FAST Almond R6901, R-Biopharm
VT	1	16.10.18	negative	<2,5	positive	27,2	positive	36,9	2,5	2,5		Almond	Veratox Almond, Neogen
VT	8	18 October	negative		positive	15,3	positive	30,4	3	10	30	Almond protein	Veratox Almond, Neogen

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

Continuation ELISA Almond:

Meth. Abr.	Evaluation number	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	2				
AQ	7		according to handbook	yes	cross-reactivities to other Prunus species
AQ	15			yes	
BF	3a			yes	
BF	16	Monoclonal antibody-based assay	1:20 extraction ratio @ 62C for 10 minutes	No	Product # AP2-EK
IL	5	polyclonal			
IL	9			yes	
RS-F	3b			no	
RS-F	6	Almond protein	As Per Kit Instructions	yes	
RS-F	10	specific antibodies to almond proteins.	1 gr sample+ 20 ml extraction buffer/heating at 60oC/10min/cetrifuge 10 min 2500g/ 100µL per well	no	Further dillution 1:10 for SampleB, and 1:2 for sample C
RS-F	11		As Per Kit Instructions	yes	
RS-F	14	As Per Kit Instructions	As Per Kit Instructions	Yes	
RS-F	17	Almond protein	As per kit instructions with addition of 1g skimmed milk powder	yes	
RS-F	18	detects almond proteins	As Per Kit Instructions	yes	
RS-F	19			YES	
VT	1				Matrix effect has been observed in sample. Only diluted sample has been report.
VT	8		PBS/15min/60°C	yes	

5.1.3 PCR: Peanut

Meth. Abr.	Evaluation number	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	%		Test-Kit + Manufacturer
ASU	6	25.09.18	negative		positive		positive					Peanut-DNA	ASU §64 Methode/method
ASU	13	31.10.18	negative		positive	14	positive	27,7	5	20	50	Peanut	ASU §64 Methode/method
GI	15	29.11.18	negative		positive		positive					Peanut-DNA	GEN-IAL First Allergen, Coring System Diagnostix
SFA	3		negative	<0,4	positive	>0,4	positive	>0,4				Peanut	Sure Food Allergen, R-Biopharm / Congen
SFA	12	29.10.18	negative	< 0.4	positive	22,7	positive	78,5	0,4	1		Peanut	Sure Food Allergen, R-Biopharm / Congen
SFA	14a	26.09.18	negative	<1	positive	40,97	positive	74,09	1	1	29,12	Peanut	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	14b	26.09.18	negative	<1	positive	35,07	positive	42,42	1	1	30,56	Peanut	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	11	12.10.18	negative		positive		positive		0,4			Peanut-DNA	Sure Food Allergen Quant, R-Biopharm / Congen
div	4	02.10.18	negative		positive	6,5	positive	28	1	5	50	Peanut	andere: Ladenburger et al (2017) J AOAC Intern 101 (1), modifiziert
div	7	11.10.18	negative		negative		negative					Peanut-DNA	
div	18	26.09.18	negative		positive		positive		4			Peanut-DNA	internal method

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	6	86bp long sequence of gene for Ara h2	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
ASU	13	Ara h 2 (86 bp)	Automated extraction (Maxwell RSC), non Clean-up, 50 cycles	yes	Qualitative Test with SureFood Allergen Peanut from Congen and § 64 LFGB L-44.00-11(Peanut)
GI	15			yes	
SFA	3			yes	
SFA	12		According to kit manua	yes	
SFA	14a	As Per Kit Instructions	As Per Kit Instructions	Yes	Kit Code S3603
SFA-ID	14b	As Per Kit Instructions	As Per Kit Instructions (Quantified with Quantard 40)	Yes	Kit Code S3103
SFA-Q	11		As Per Kit Instructions	yes	
div	4	ATPase subunits 6 (atp6) 104bp	CTAB-Precipitation method, s. e.g. ASU L 18.00-22	yes	calibration/quantification by matrix standards, spiked material: peanut, roasted, defatted.
div	7	Ara h 2 L77197	Maxwell FFS Kit	yes	LOD: 0,02 ng/µl peanut DNA /PCR-vial
div	18		CTAB / Proteinase K / Promega Wizard DNA CleanUp / Real-time PCR / 45 Cycles	yes	

5.1.4 PCR: Almond

Meth. Abr.	Evaluation number	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	%		Test-Kit + Manufacturer
ASU	4	01.10.18	negative		positive	< 20	positive	38	5	20	50	Almond	ASU §64 Methode/method
ASU	7	11.10.18	negative		negative		positive					Almond-DNA	ASU §64 Methode/method
ASU	13	31.10.18	negative		positive	19,6	positive	<LOQ	10	20	50	Almond	ASU §64 Methode/method
GI	15	26.11.18	negative		positive		positive					Almond-DNA	GEN-IAL First Allergen, Coring System Diagnostix
SFA	6	26.09.18	negative		positive		positive					Almond-DNA	Sure Food Allergen, R-Biopharm / Congen
SFA-ID	14	26.09.18	negative	<1	positive	15,12	positive	20,19	1	1	30,41	Almond	Sure Food Allergen ID, R-Biopharm / Congen
SFA-Q	11	01.10.18	negative		positive		positive		0,4			Almond-DNA	Sure Food Allergen Quant, R-Biopharm / Congen

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

Meth. Abr.	Evaluation number	Specifity	Remarks to the Method (Extraction and Determination)	Method accredidet ISO/IEC 17025	Further Remarks
		Target-Sequence / -DNA	e.g. Extraction / enzymes / clean-up / real time PCR / gel electrophoresis / cycles	yes/no	
ASU	4	nsLTP-Gene; 82bp	CTAB-Precipitation method, s. e.g. ASU L 18.00-22	yes	calibration/quantification by matrix standards, spiked material: Almond, defatted.
ASU	7	PRU AV1 Genes (BQ641046)	Maxwell FFS Kit	yes	LOD: 0,004 ng/µl Almond DNA /PCR-vial
ASU	13	nsLTP Gene (82 bp)	Automated extraction (Maxwell RSC), no Clean-up	yes	§ 64 LFGB L-18.00-20
GI	15			yes	
SFA	6	characteristic sequence of almond DNA	SureFood Prep Advanced r-biopharm/ Proteinase K/ Real Time PCR/ 45 Cycles	yes	
SFA-ID	14	As Per Kit Instructions	As Per Kit Instructions (Quantified with Quantard 40)	no	Kit Code S3104
SFA-Q	11		As Per Kit Instructions	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 05-2018 Sample B

Weight whole sample	2,70	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	84	33,5
2	5,02	81	32,3
3	4,98	70	28,1
4	5,00	88	35,2
5	5,09	87	34,2
6	5,05	75	29,7
7	4,96	71	28,6
8	5,01	80	31,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	79,5	Particles
Standard deviation	6,58	Particles
χ^2 (CHI-Quadrat)	3,82	
Probability	**80**	%
Recovery rate	106	%

Normal distribution		
Number of samples	8	
Mean	31,7	mg/kg
Standard deviation	2,62	mg/kg
rel. Standard deviaton	8,28	%
Horwitz standard deviation	9,51	%
HorRat-value	**0,87**	
Recovery rate	106	%

Microtracer Homogeneity Test
DLA 05-2018 Spiking Level Sample

Weight whole sample	1,58	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	20,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	51	20,3
2	4,98	62	24,9
3	5,08	58	22,8
4	5,10	68	26,7
5	5,00	56	22,4
6	5,02	63	25,1
7	5,00	52	20,8
8	5,08	61	24,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	58,9	Particles
Standard deviation	5,55	Particles
χ^2 (CHI-Quadrat)	3,66	
Probability	**82**	%
Recovery rate	112	%

Normal distribution		
Number of samples	8	
Mean	23,4	mg/kg
Standard deviation	2,20	mg/kg
rel. Standard deviaton	9,43	%
Horwitz standard deviation	10,0	%
HorRat-value	**0,95**	
Recovery rate	112	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter (1st letter):

PT number	***DLA 05-2018***
PT name	***Allergens V : Peanut and Almond in Pastry with "Spiking Level Sample“***
Sample matrix (processing)	***Samples A + B:*** *Butter Cookies (baked at appr. 150°C) / ingredients: Wheat flour, sugar, butter, barley malt extract, skimmed milk powder, glucose syrup, baking agent ammonium carbonate, salt, emulsifier lecithins, other food additives, egg 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: Almond (Almond protein, DNA), Peanut (Peanut 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. It is the best to homogenize the whole sample.*
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 02nd November 2018***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf, PhD*

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

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		SPAIN
		Germany
		USA
		CANADA
		ITALY
		Germany
		Germany
		Germany
		Germany
		Germany
		GREECE
		Germany
		POLAND
		SPAIN
		Germany
		GREAT BRITAIN
		GREECE
		GEORGIA
		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
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Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of methods
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